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Environmental Assessment of the Alaskan Continental Shelf

Volume 10. Chemistry and Microbiology

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for the Year Ending March 1976

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1. Marine Mammals
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Environmental Assessment of the Alaskan Continental Shelf

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Fourth quarter and annual reports for the reporting period ending March 1976, from Principal Investigators participating in a multi-year program of environmental assessment related to petroleum development on the Alaskan Continental Shelf. The program is directed by the National Oceanic and Atmospheric Administration under the sponsorship of the Bureau of Land Management.

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NATURAL DISTRIBUTION OF TRACE HEAVY METALS AND ENVIRONMENTAL BACKGROUND IN THREE ALASKA SHELF AREAS

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I. SUMMARY

Baseline sample collection of water and sediment for the Gulf of Alaska and Bering Sea has been completed and some biota samples taken from these regions have also been received. Only data for Cd, Cu, Zn, Cr, and Se (and some Hg, Ni, and Pb) are currently available for a number of water and biota samples. In addition, archived sediment samples from the Beaufort Sea have been analysed, and a number of granulometric and clay mineralogy data for the surface sediment from all regions are included in this report.

The contents of trace metals analysed to data in the water show no anomolous trends and are as low (or lower) as in other reported data sets for open-ocean, uncontaminated regions. *Mytilus* and *Fucus* contents are, in general, lower than for other reported coastal areas. Some initial comments regarding the sediment grain size and clay mineralogy data are included. A detailed analysis of the heavy metal distributions in these Alaskan coastal areas cannot be given until considerably more analytical work has been completed. We would tentatively conclude that sufficient water and sediment samples have been collected in most parts of the Gulf of Alaska and Bering Sea, although more detailed collecting will be required in the coastal zones and river discharge regions. Insufficient biota samples are available at this time and the Beaufort Sea has been essentially unsampled. From our data in hand it would appear that the Alaskan OCS might well, at the present time, serve as a type example of pristine coastal waters as far as heavy metal distributions are concerned.

II. INTRODUCTION

A. Heavy Metals in the Marine Environment

The term "heavy metals" can be variously defined. In the broadn . sense it includes all those elements which are present in sea water in trace amounts; usually less than μg/l quantities. In an environmental impact study of this type we have naturally confined our attention either to those metals which have known pollutional properties or to those which may serve as index metals for petroleum geochemical reactions or functions. These heavy metals need not be insignificant components of the natural geochemical and ecological cycles; they are present in trace amounts in solution because of high reactivities in this phase which tend to partition them onto or in th: coexisting solid phases. The inorganic sediments constitute the largest repository but the contents of metals held in the biota, while being, in general, quantitatively quite minor, are of particular concern to man.

The importance of sea water in this study again lies not in the absolute amounts or concentrations of metals held in solution but in its role as a mobile phase through which, and with which, these trace constituents can migrate. Modern trace metal research is concerned with the reactions governing the partition of metals between the various phases, but also on the rates and routes of the metals between reservoirs; on the kinetics involved. One characteristic of major importance in this respect concerns the chemical form (chemical speciation) of the metal in solution. Sea water is a highly concentrated and complex solution. The heavy metals tend to form complex coordination complexes in this medium and these reactions strongly influence transfers between the liquid and coexisting solid phases. As a simplification for example, it can be considered that the major ions are bound to abiotic

solid surfaces by relatively weak coulombic forces, whereas transition metals may be adsorbed by specific (usually protolysis) substitution reactions. Such fundamental differences between the major and minor sea water components result in uptakes of the trace metals of considerably greater magnitude than would be expected from basic sorption theory.

Uptake of heavy metals onto, and incorporation into, the marine biota is clearly a considerably more complex phenomenon than sorption onto sediment and detritus. The initial removal from solution onto gill and gut surfaces may be somewhat analogous, but thereafter, transport reactions within the organism are exceedingly complex and frequently poorly understood. These intra-organism reactions are specific to each element and the "steady-state concentration" developed for each organism -- which may be many orders of magnitude greater than in the coexisting sea water -- is the difference between a complex series of assimilation and depuration processes.

Soluble inorganic complexes overwhelmingly predominate in sea water. However, considerable attention has been given in recent years to the role of organic ligands. The heavy metals should, and probably do, form a range of extremely stable organo-metallic complexes in sea water; the quantitative importance of these must, however, be very slight because of the exceedingly low concentrations of soluble organics in "average" marine waters.

This brief discussion has so far emphasized removal mechanisms of the trace metals from solution. This is a justifiable emphasis because the residence times of these elements in sea water are very short. Although the net transport is to the sediments, there are localized but important reactions acting in the reverse sense: To remobilize heavy metals from the solid phases into solution. Regeneration of these elements accompanying the oxidation and

dissolution of organic detritus must be assumed to generate spikes of anomalously high soluble contents within oxygen-minimum layers, although such have not been verified *in situ*. More importantly perhaps, such regeneration is likely to be in the form of (potentially more soluble) organometallic complexes. Enhanced concentrations of metals in upwelled, nutrient-rich waters have been recorded and organic chelation has been proposed; if this phenomenon is confirmed, a biotic source may be presumed.

The trace metal contents of sediments may be several orders of magnitude in excess of that in the sea water. The solid phase - metal bonding ranges from inert to relatively labile as noted above so that the pure or interstitial waters in intimate contact with -- and in pseudo-equilibrium with -- the sedimented material contains relatively high concentrations of many metals. This slution concentration gradient should result in a diffusional flux from the sediments into the overlying water column. This has not been conclusively identified as a general phenomenon of prime importance in the overall cycle, but again localized, near-bottom impacts have been described in several disparate, near-shore environments. This grossly attenuated outline has ignored complications imposed by sub-surface anoxic conditions. This latter may be partially balanced by a suite of reactions confined to the sediment-water boundary itself. For example, we have described (Heggie and Burrell, 1975) elevated concentrations of soluble copper at this interface, with diminishing gradients both upwards into the water and downwards into the sediment pore water. Regeneration of soluble complexes from particulate forms of the metal by microorganisms has been proposed. This type of reaction is likely to be enhanced in the case of those metals which can be converted to highly mobile complexes *via* microbially mediated reactions; for mercury, and those other metals which readily form volatile alkyl complexes, for example.

The heavy metals are added to the oceans largely *via* fresh-water run-off. The removal mechanisms outlined above are exceedingly efficient and the bulk of the continental input is scavenged from solution within the estuaries and near-shore zone so that these latter regions constitute the major sink in the system. This has been documented for the fjord-estuaries of southeast and south central Alaska by Burrell (1976). Mixing processes ensure, of course, that the soluble marine contents everywhere fall within a narrow range for each metal. Transportation mechanisms capable of transporting these elements to open-ocean, solid-phase reservoirs are quite limited; fish migrations or atmospheric input, for example, both of which are quantitatively quite minor. Transition metals may be added directly to the deep oceans *via* tectonic activity, however, and oceanic ridge activity may be important in this respect.

B. Relationship Between Oil Development and Heavy Metal Distributions

It is convenient to consider the effect of industrial activity on the shelf areas on the indigenous trace metal distributions initially in terms of the general nature of anthropogenic impingement of these metals on the natural environment -- on natural processes which are likely to be disturbed -- followed by the expected effects specifically related to oil development.

1. Heavy metal pollutional effects: Pollution effects (i.e. man-made impingements on the natural environment) of the heavy metals are of particular concern because these are the only extraneous chemicals introduced into sea water which are known to have subsequently resulted in human fatalities. In this context, the term "heavy metal" commonly includes only a quite limited number of elements, notably mercury, lead, arsenic, cadmium, selenium

and copper. But such lists largely reflect reaction to well appreciated, contemporary problems. Such elements are not necessarily the most toxic to many of the marine organisms, or the ones most likely to be concentrated in specific food species. Society has a history of alleviating one problem with another and, since it is to be supposed that other, currently more exotic, metals are likely to create pollutional problems in the future, it seems reasonable to look at broad-based impingements not specifically confined to a very narrow suite of metals.

Nearly all previous pollutional problems in the seas have been derived from terrestrial operations. It has been noted above that the heavy metals are largely immobilized in the coastal zone so that effects on open ocean areas might be expected to be negligible, and this has proved to be the case to date. Conversely, near-shore pollution *per se* has been widely documented and is likely to become an increasingly severe problem with time, not least because of the increased biotic activity within these areas. Coastal and estuarine regions must be of primary concern in any pollutional impact study. Exploitation for oil on the shelf areas, however, potentially presents a mechanism for leaking pollutants to the oceans which by-passes the natural near-shore screening processes. Since no case studies are available, it is only possible to predict likely effects based on our still incomplete knowledge of chemical and biological behaviors.

The efficient partition of the (largely) inorganic heavy metal complexes predominantly onto the coexisting solid phases has been noted above. The major quantitative anthropogenic impact, therefore, must be any process or combination of processes which arrests or reverses this trend. Potential reactions follow from the previous discussion. At the top of the list comes

solubilization of metals *via* complexation with organic ligands. Although next to nothing is known of the likely results of impacting deposited sediments in this fashion. Some speculations regarding oil spills on sediment are offered in the following section. We have also discussed previously the published evidence for enhanced solubilization of metals in sea water as a consequence of, for example, pulp-mill waste discharge.

We conceptually regard "marine pollution" in terms of the impact on man and therefore largely on deleterious effects on marine food species. Not only are organo-metallic complexes in general likely to have enhanced residence times in solution, but such chemical species are liable to be preferentially incorporated into living organisms and therefore into the natural food web. It should be appreciated that man tends to harvest higher trophic level organisms so that transfer mechanisms and efficiencies along the food chain are pertinent here.

2. Specific effects of OCS oil development: Speculation on ways that the extraction and transport of oil in the shelf areas could potentially impact the natural environment was included in the previous Annual Report (Burrell, 1975). Since no new information has appeared in the intervening months we feel justified in including this discussion again here substantially unchanged.

a. *Trace metals associated with formation waters*: Extraction of hydrocarbons will result in very large quantities of accompanying "formation water" being released from the well-head area into the immediate ocean. There is a conspicuous lack of information regarding the concentrations of heavy metals in this aqueous phase. Most such analysis programs to date have been designed with the objective of "finger-printing" the various oil

reservoirs, and the inprecise methods usually used do not permit easy comparison between published compilations. Rittenhouse *et al* (1969) and Billings *et al* (1969), for example, give ranges for several metals which differ by orders of magnitude. Iron, manganese and chromium, and nickel and copper, appear to be enhanced and depleted respectively in the formation waters studied by the former group over sea water concentrations. Billings and co-workers, however give formation:sea water ratios of 30, 40, 160 and 750 for, respectively, zinc, copper, manganese and iron, with cobalt, chromium and nickel contents below the detection limits of the analytical methods used.

More reliable evidence may be gathered from that scientific literature concerned with interstitial waters extracted from cored surface sediment samples. In general, all the common (i.e. first transition series) metals appear to have enhanced concentrations in the sediment interstitial fluids, and especially iron and manganese. Few data for trace metals in deep (10^2 m range) cores -- from the deep-sea drilling program for example -- are available, but manganese and zinc concentrations (Presley and Kaplan, 1971; 1972) do not appear to deviate substantially from ranges recorded for near surface samples. One type of interstitial fluid which could, however, differ from the "conventional" might be the highly alkaline brines occassionally found associated with petroleum deposits. Such a pH environment ($\geqslant$ 10) should result in enhanced soluble contents of those metals which form hydroxo coordination complexes. These are, predominantly, iron, manganese, titanium, nickel, zinc, vanadium and chromium (see, for example, Truedell and Jones, 1969).

From this brief discussion it might be supposed that anomalously high concentrations of heavy metals (notably, perhaps, iron, manganese and copper

TABLE I.

Trace metal contents of crude oils

	Alberta crudes[a] average concs. (ppm)	Calif. crudes[b] ranges (ppm)
Ag	–	–
As	0.10	0.06 – 1.0
Cd	–	–
Cr	0.09	0.01 – 0.02
Co	0.05	0.20 – 10.00
Cu	–	0.24 – 6.33
Fe	10.80	16.80 – 85.50
Hg	0.05	0.08 – 30.00
Mn	0.10	0.73 – 2.54
Ni	9.38	140.00 – 265.00
Pb	–	–
Se	0.05	0.40 – 1.40
V	13.60	–
Zn	0.46	7.40 – 85.80

[a] Hitchon *et al.*, 1975

[b] Shah *et al.*, 1970

or zinc) could accompany injection of formation waters into those portions of the oceans immediately adjacent to the well-heads. While this may be so, the total conceivable volume of deep water released in this "point source" fashion could only be comparable to, say, a very small river inflow. The natural reactions which buffer the soluble contents of these metals in the marine environment should prevent any discernible pollution spikes. One might, for example, expect precipitation of iron and manganese (with concomitant coprecipitation of other heavy metals) in a somewhat analogous fashion to the addition of acid mine wastes into natural water systems. This will be so also for spilled drilling mud. Barium is quite insoluble in sea water. Enhanced quantities of the sulfate may accumulate over the long term around well sites but barytes is a normal constituent of marine sediment, and is non-toxic.

b. *Trace metals associated with crude oil*: Crude oil itself contains trace quantities of certain of the heavy metals. This has lead to the erroneous supposition that it is this specific restricted suite of metals which is of sole concern in the various OCS impact programs. The actual metal content ranges are not well known; again mainly because of the poor analytical techniques which have been applied to date. Table I reproduces two recent compilations which are believed to be among the best available. It may be seen that vanadium, nickel, iron and zinc appear to occur in fairly high concentrations in these crudes and the possibility of commercially extracting vanadium for this material has been seriously suggested in recent years. Both the vanadium and nickel porphyrin complexes have been studied for many years, but it would appear that additional significant quantities of these two metals are associated also with the asphaltic fraction.

This may be so also in the case of the other listed trace elements but this is mostly speculative and some workers (see discussion in Filby, 1975, for example) have suggested that the supposed contents are associated, not with the oil *per se*, but with foreign particulate material contained within the oil.

Regardless of the actual contents of metals in the various crude fractions, this source (i.e. direct spillage of crude oil into the ocean) could only result in insignificant and probably undetectable perturbations in the water column because of dilution and mixing. The volume of foreign organic liquids likely to be added to the sea water must be even less than in the case of the previously considered formation waters. This source appears to be of potential concern in one respect only: namely, that these particular metal fractions may be held in solution as stable organo-metallic complexes. Such an enhanced solubilization phenomenon related to petroleum in natural waters has been suggested by Bugel'skii and Tsimliyanskaya (cited by Davis, 1968). And chelated soluble fractions of copper which are greater than "normal" concentrations (i.e. in excess of predictions based on inorganic equilibria) have been recorded for several disparate marine environments by, for example, Barber and Ryther (1967). Apart from maintaining the metal in the mobile aqueous phase, such organo complexation could potentially lead to preferential uptake and incorporation into the marine biota; although the opposite -- masking -- effect equally may be envisaged.

c. *The importance of trace metals associated with the sediments*: It is considered that the major threat to the normal, "buffered" oceanic trace metal regime posed by off-shore industrial activity lies in potential perturbation -- possibly acute but limited, possibly chronic and more widespread -- to the geochemical environment of the surface sediments.

It has been noted above that the deposited sediments constitute a vast reservoir for trace metals; that they are, under normal conditions, a sink for metals naturally removed from solution. The geochemical environment of the sediments differs from the overlying sea water; i.e. there is disequilibrium between the water and sediment and between various zones within the sediment itself (see e.g. Mannheim and Sayles, 1974). Considerable efforts are currently being devoted to determining the existence and magnitude of chemical gradients (both positive and negative) for the major constituents across the water-sediment interface. There is little equivalent evidence for the trace metals, although there are indications also in some environments (e.g. for copper in fjord estuarines; Heggie and Burrell, 1975) for migration from the surface sediments into the overlying water.

It is considered highly possible that contamination of the surface sediment from OCS activity may exacerbate these natural interface reactions. Only small changes to the chemical environment -- availability of organic complexing ligands or changes in the redox environment for example -- could result in large fluxes of metals from the sediment into the water column. It is important to note also that lags may occur within this sequence; the often expressed (e.g. Meadows and Meadows, 1973) fear of harmful effects appearing, long after the application of the pollutional stress, is very real. The persistence of harmful reactions beyond the time when the cause has been suppressed is an obvious corollary. Such hysteresis is dangerous because remedial actions may not be taken in time to avert considerable damage.

If these processes are, in fact, the major cause for concern with regard to industrial stress on the natural trace metal regime, then it

follows that very many individual heavy metals may be important, and not just those specifically associated with crude oil, or with paint or formation waters. The sediments contain limitless quantities of a very broad spectrum of metals. Once remobilized, the heavy metals may be incorporated into marine organisms, stored in harmful quantities, or possible passed along the food chain to those higher trophic level species which are consumed by man. An impact study of this type is ultimately concerned with such potentially harmful impacts on man. The foregoing brief discussion has attempted to demonstrate that knowledge of the latter, however, necessitates working with the total marine system. Monitoring of a few food species alone could only demonstrate, *post factum*, that the ecosystem was polluted. This would yield no clue regarding the transport rates or routes of the pollutant or how to prevent it.

C. General Nature and Scope of Study

To date this program has been largely a survey to establish baseline levels of heavy metals in the shelf and coastal environments against which the effects of any future pollution can be measured. Following on from the discussion above it was necessary to look at water, biota and sediment as follows:

1. Water: This is the reservoir which would be initially impacted. Natural concentrations of trace metals are not known for these (or most other) areas, and available analytical techniques are largely insufficiently sensitive to detect small perturbations. There has been a need, however, to determine general baseline conditions and to check for natural anomaly areas such as might be associated with the major rivers.

Sea water is the transportation conduit for metals passing to or between the major solid phase reservoirs. Future studies of transfer rates and routes will necessitate (pragmatically, for selected areas only) quite detailed knowledge of the concentrations, local gradients and chemical forms of the metals in solution.

2. Sediment: We have flagged the bottom sediments as the major trace metal reservoir and hence a major concern in this study. It is believed that oil deposited on the sediment could potentially mobilize, or increase the rate of mobilization, of a range of metals. Many such elements have known toxic properties and others would be expected to behave similarly. In addition, the metals may be solubilized in more stable (and potentially more toxic) forms, and as complexes which may be incorporated more readily, and to higher levels, in organisms. This topic has been considered in more detail above.

As explained in the work statement, we have been primarily concerned with determining, not the total heavy metal contents of the sediments, but those fractions most likely to solubilized. This latter might be variously considered the sorbed, loosely bound, or "available" fraction. None of these terms has much scientific exactitude but the intent is to isolate the labile from the structural content. In general it would be expected that the structural fraction would be quite small compared with the "extractable" so that, in practice, probably little error is introduced by performing a "whole-rock" analysis.

We have chosen, with a few exceptions, not to single out the suspended sediment for separate study. However, one initial objective, which was found to be not logistically feasible, was to specifically collect from the major river plume regions (see below).

3. Biota: Environmental assessment programs are ultimately concerned with deleterious effects on man. The end product of this investigation, therefore, is an evaluation of the actual or potential effects of heavy metals on food species; both in terms of immediate or chronic effects on food species *per se*, but also the impact of pollutant metals on the overall food web.

In keeping with the primary emphasis of this program on the sediment-water interface region, we have proposed benthic organisms as primary "index species". It has been our objective this year, therefore, to determine baseline contents of heavy metals in a range of marine organisms, but emphasizing sub- and inter-tidal benthos.

4. Sedimentological program: Uptake and release of trace metals on and from sediments is a surface phenomenon and hence a function of the nature and, more importantly, the surface area of the sediment particles. As a simplification it may be supposed that greater quantities of metals will be exchanged from finer grained sediments than from equal quantities of coarser grained material. Throughout most of the first year program we hoped to coordinate this chemical program with the complementary geological programs, but to no avail. For this current contract period, therefore, we proposed the addition of a support function within the trace metal chemistry program which would provide mineralogical and sedimentological data on splits of the samples analysed for the heavy metals.

5. Long term objectives: The long term objectives of this program are to recognize pollutional impact on the ecosystems, and to predict the effects of such pollution in terms of transport and changes in reservoir contents. This latter could only realistically apply to certain judiciously

selected sub-systems but would lead to an understanding of actual metal toxicity, or potential effects, on higher food-web organisms.

D. Specific Objectives During the Current Contract Period

1. A baseline survey of the current contents of a suite of heavy metals in the water column, in selected biota, and in the surface sediments of the study areas.

2. Ancillary mineralogical and sedimentological analyses of the sediments as needed to support (1).

3. From (1) and (2) to select those geographical areas, specific biological species and chemical fractions which should be emphasized in future studies.

4. To collect in a usable form compilations of available literature pertinent to these problem areas.

5. To develop sampling, sample treatment and analysis procedures as needed to carry out objective (1).

III. CURRENT STATE OF KNOWLEDGE

We discussed previous work on heavy metals in the Gulf of Alaska -- or rather the lack of it -- in the previous report (Burrell, 1975). For this contract period we proposed to research the available literature on the geological topics now incorporated in this progam in addition to any available information on heavy metal distributions in the enlarged study area. This work is not yet complete but some interim data may be included here. It has not been possible to include separate compilations of the citations because the required data format was received by us at far too late a date.

A. Heavy Metals in Beaufort Sea Shelf Sediments

During the past few years, considerable data on the chemical compositions of the western and central Beaufort Sea shelves have been documented. The earliest available data on the Fe, Mn, alkali and alkaline earth elements, and a selected group of heavy metals (e.g. Cu, Ni, Zn, Co, and U) in gross sediments of the Beaufort Sea shelf, were presented by Naidu and Hood (1972). These authors made an attempt to correlate the distribution of elements with water depths and lithological components, as well as organic carbon and carbonate contents in sediments. Similar investigations were further extended to the continental margin sediments of the Beaufort Sea by Naidu and Mowatt (1974a; 1975). In addition to the above, geochemical studies have been conducted by Barnes (1974), and Weiss *et al.* (1974). Weiss *et al.* (1974) were concerned with the geochemical cycle of Hg, whereas Barnes (1974) has presented semi-quantitative data on a suite of 30 elements and has documented the concentrations of some organic fractions. Thus, it is apparent that no attempt has been made to quantitatively assess the partition patterns of heavy metals in the lithogenous and nonlithogenous (relatively more "mobile" or leachable phase) components of the Beaufort Sea shelf sediments.

Compared to the western and central portions of the Beaufort Sea, only limited geochemical data are available on the shelf sediments of the eastern Beaufort Sea; the more significant work in the latter area is that of Dewis *et al.* (1972). More recently, Naidu (1976) documented the stratigraphic variations in the concentrations of major, minor and trace elements, as well as organic carbon and carbonate contents in 10 box core sediment samples taken from the Beaufort Sea shelf.

B. Mineralogical and Granulometric Studies of Alaskan Shelf Sediments

Results of granulometric analyses on the shelf sediments of the Gulf of Alaska are becoming available only now. As far as is known the only published reports on these studies are those of Gershanovich (1968), Molnia (1975), Molnia and Carlson (1975), and Sharma (1975).

Distributions of clay minerals in shelf sediments of the Bering Sea, and Gulf of Alaska have been documented by a few investigators. Moll (1970) and Matthews (1973) have described the clay mineral assemblages in bottom sediments of the Chirikov Basin of the north Bering Sea, and the Yukon Estuary, respectively. Recently, Molnia and Fuller (1975) presented preliminary data on the clay mineral assemblages of a few sediment samples from the Gulf of Alaska.

IV. STUDY AREAS

A. Northeast Gulf of Alaska

As for the previous year's work in this area (Burrell, 1975), the sampling scheme utilized has continued to be based upon the standard hydrographic grid shown in Figure 1. We have attempted to coordinate our operations as far as possible with the other participating programs, although this has become an increasingly difficult task within the context of the enlarged, second-year program. Water column samples were originally scheduled on certain of the standard GAS grid stations designated as nutrient analysis localities. With the demise of this portion of the program some of the original rationale has been lost; however, correlations between our data and the contemporaneous hydrographic parameters are still a possibility, if needed. We have coordinated our sediment sampling with the benthic biology program so that the results from both these programs should eventually provide interesting cross-correla-

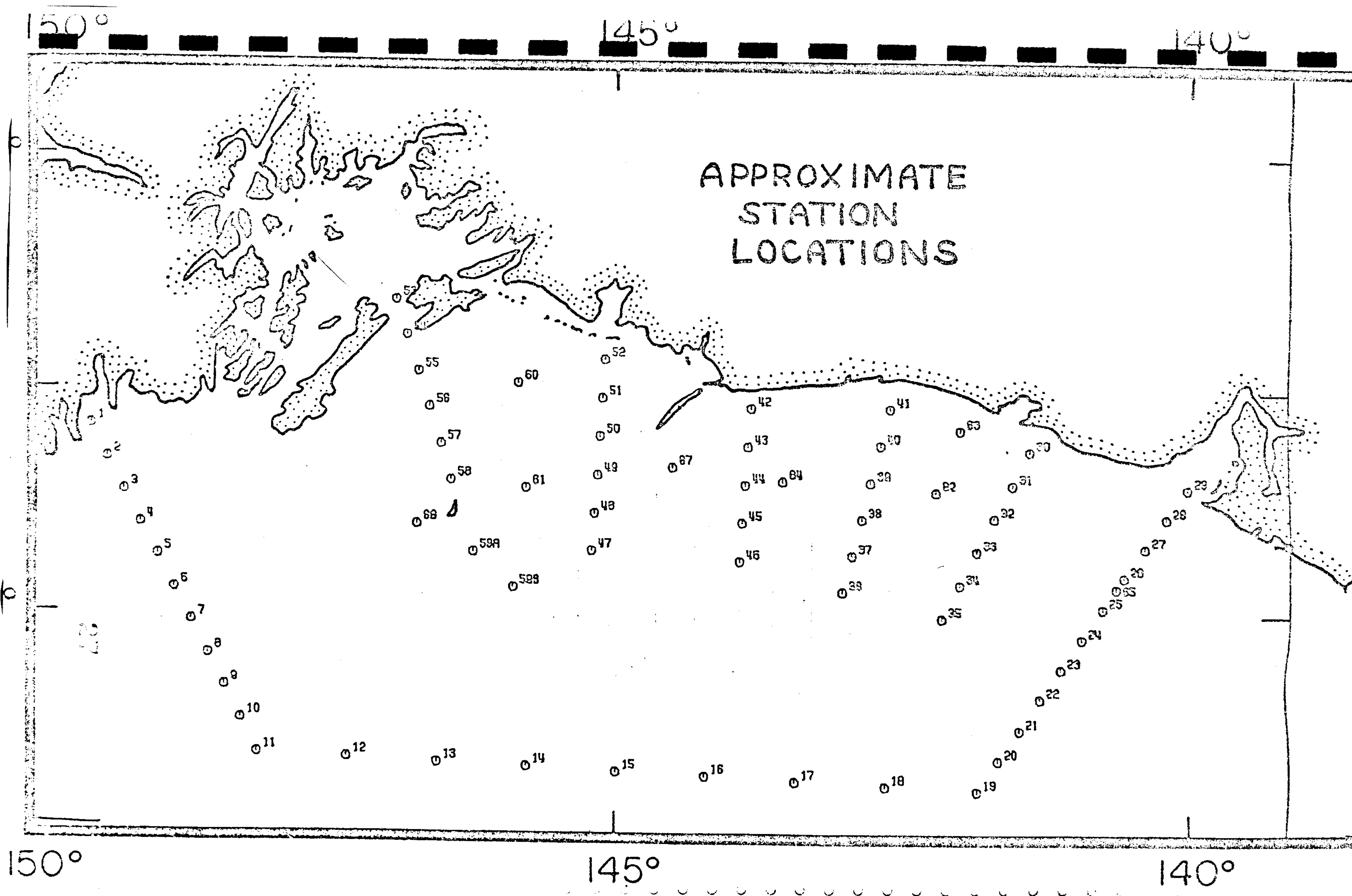

Figure 1. Standard trace metal station localities (hydrographic GAS grid) in N.E. Gulf of Alaska.

tions. As noted in the previous report cited above, the results obtained from the USGS geological program are not immediately applicable to this chemical program (or to the benthic biology program) because completely separate sampling grids have been utilized. We have, however, obtained some basic sedimentological data on our core samples as explained below.

Sediment samples have been retrieved for the shelf area only, but some deep water samples have been collected in this portion of the Gulf, mainly to function as inter-laboratory calibration samples.

As previously, biota samples were to have been collected by the various biological co-investigators. We have received some samples from the inter-tidal group and from one sub-tidal benthic trawl survey as noted below.

B. Northwest Gulf of Alaska

Sample localities for heavy metal analysis for the N.W. Gulf portion of the study area have been chosen to coincide with the physical oceanographic grid for the same reasons as discussed above. The benthic biology program has similarly opted for this grid. This station network (GASSO) consists essentially of a series of traverses normal to the peninsula and Aleutian Chain as shown in Figure 2. We have utilized only those stations on this grid located within the shelf region.

C. S. Bering Sea

No systematic physical oceanographic program has been designated for this area. At the beginning of the current contract period, therefore (in conjunction with the benthic biology and IMS geological programs), we established the "benthic grid" shown in Figure 3. Water and sediment samples

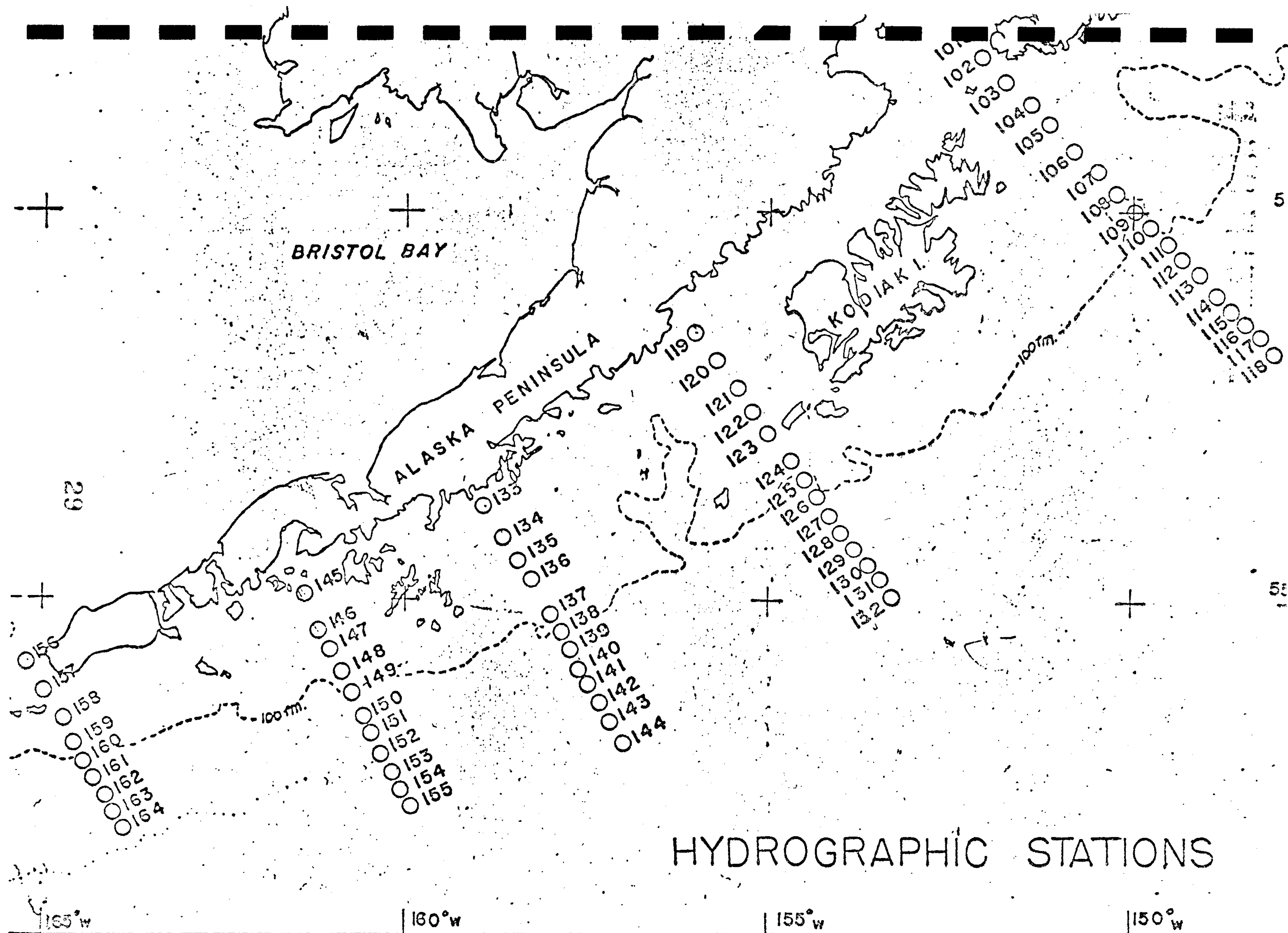

Figure 2. Standard hydrographic grid (GASSO) in N.W. Gulf of Alaska.

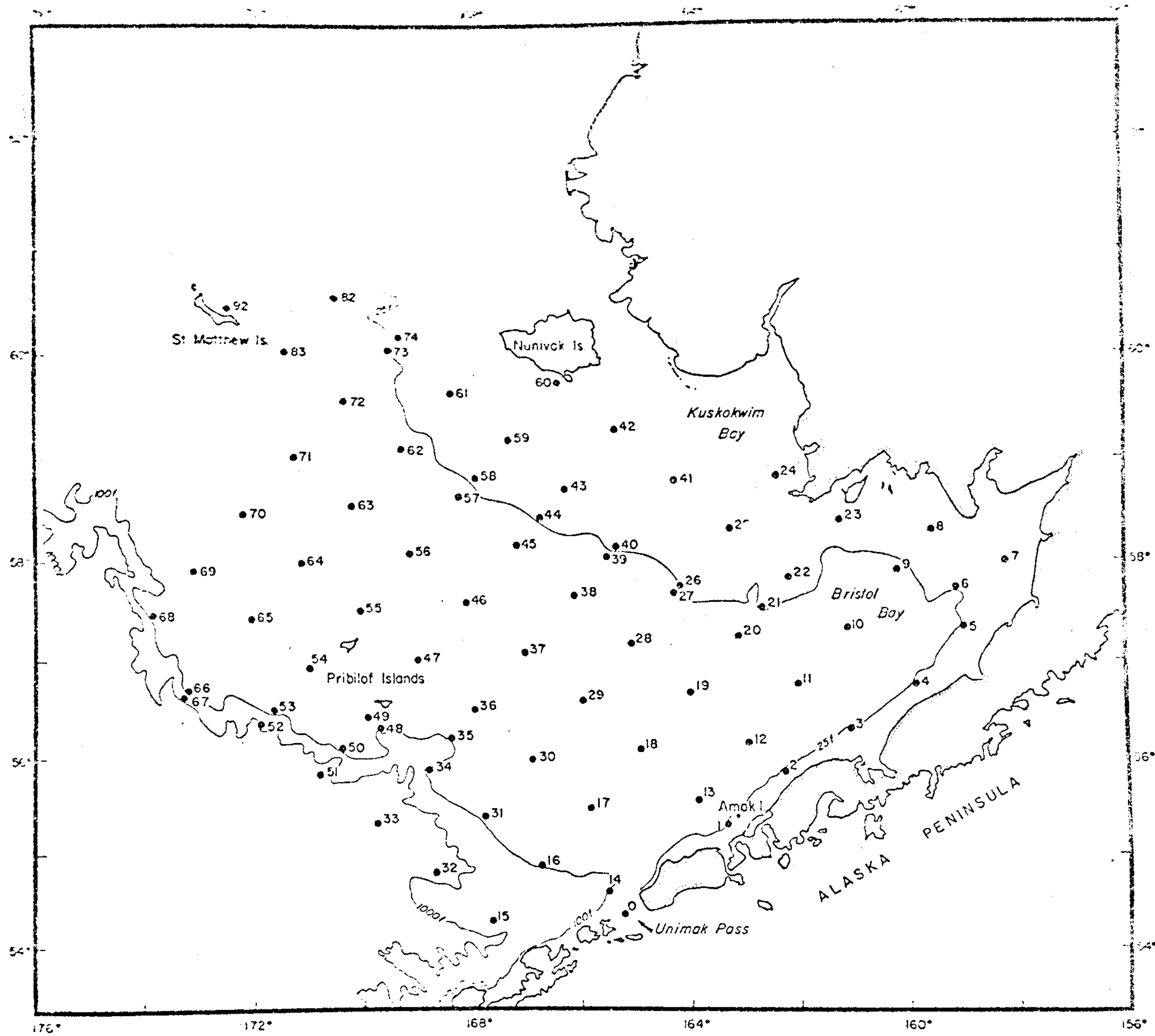

Figure 3. Standard heavy metal chemistry stations (the "benthic grid") in the S. Bering Sea.

from a large number of these stations have been obtained this year on two separate cruises. Additional samples have also been collected from Izembek and Nelson Lagoons.

Sub-tidal benthic biota samples have been collected for us on two trawl survey cruises as noted below.

D. Beaufort Sea

Station locations on the three sections normal to the coast as designated by Dr. Aagaard have been selected for this program. In addition, a number of sediment samples collected prior to the initiation of this program have been worked on. The localities of these samples are shown in Figures 4 and 5 and listed in Table II.

V. PROCEDURES

A. Types of Samples Collected

Three types of samples have been collected for this study: water, sediment, and biota. The sediment material analysed has been predominantly collected from the surface in a trace-metal free, shallow corer as described below. These samples have been obtained on the standard physical and benthic biological sampling grids. At each station individual cores have been split for trace metal and sedimentological analysis, except in the Bering Sea where the sediment size analysis work has been covered by a different program. Although these latter samples were collected contemporaneously, and at the same stations, splits from benthic van Veen grabs were used instead.

Sediment analysed from the Beaufort Sea was collected prior to this program. Sampling and storage methods are described below. At many of the water sampling stations, the particulate material collected on large-size

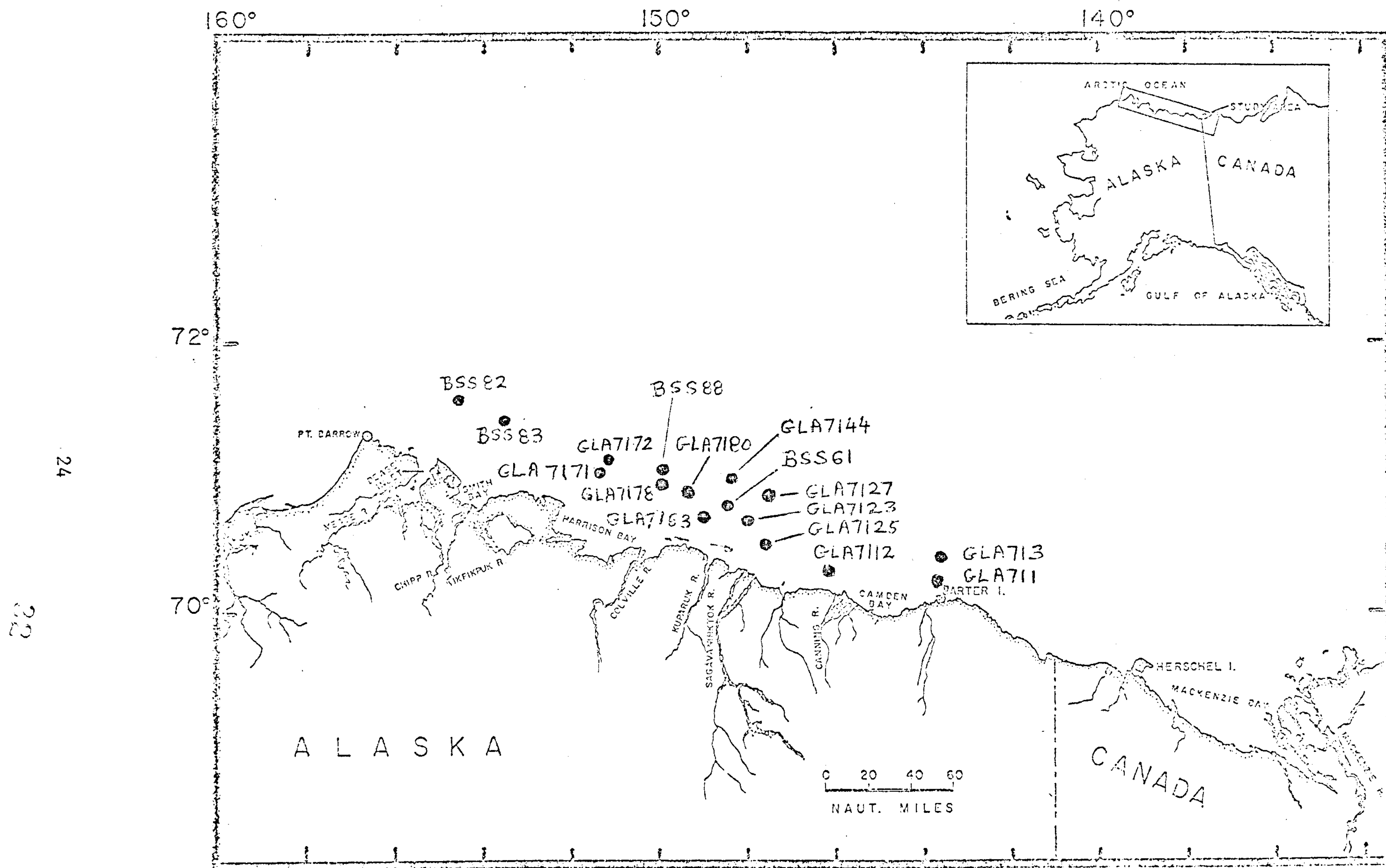

Figure 4. Map of the Beaufort Sea, showing the locations of the sediment samples taken for heavy metal analyses.

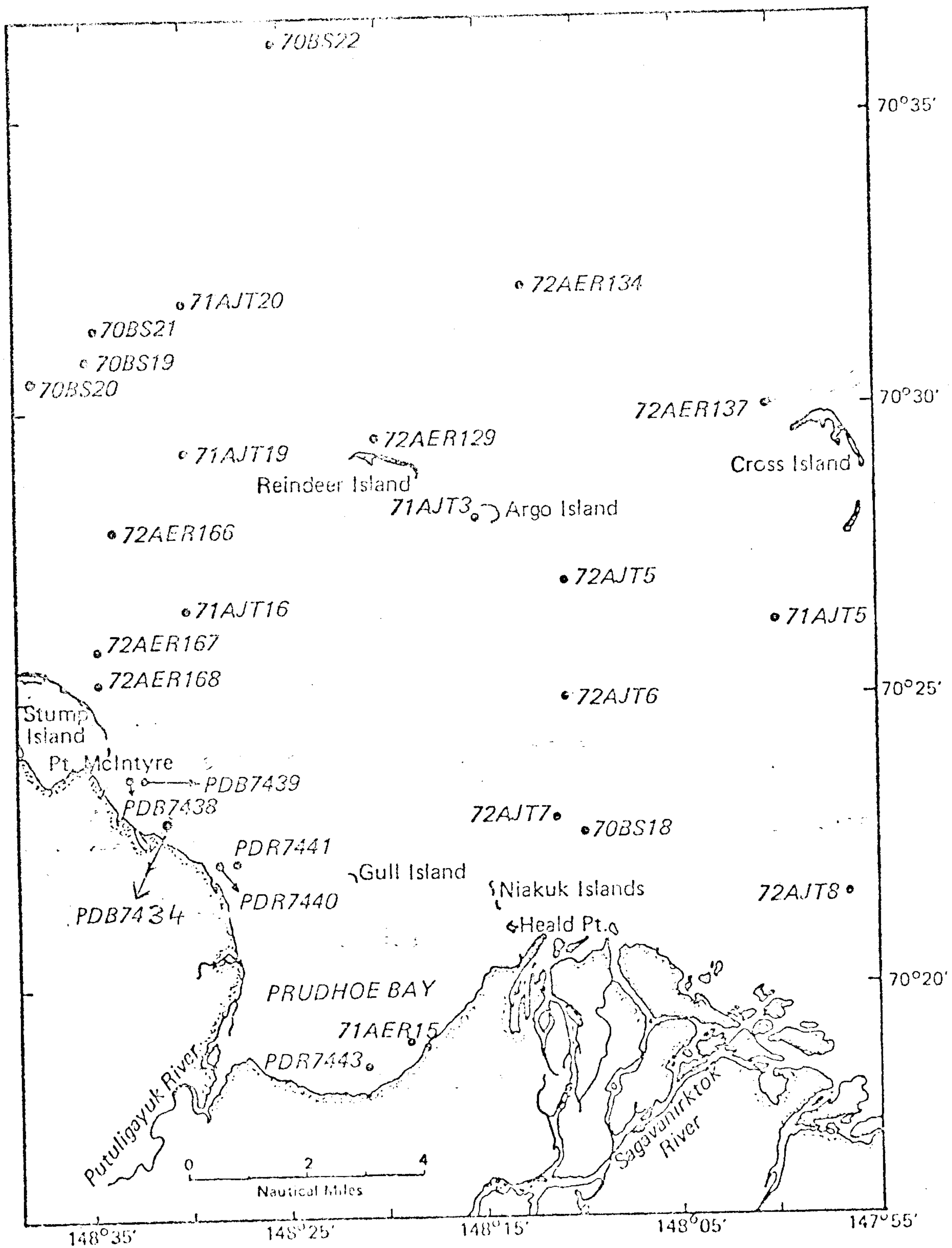

Figure 5. Locations of sediment samples in the Prudhoe Bay and adjacent shallow marine environment of north arctic Alaska.

TABLE II.

Station locations of sediment samples from the continental shelf of the Beaufort Sea, Arctic Ocean

Station No.	Lat (N)	Long (W)	Depth (m)
GLA71-1	70° 15.5'	143° 40'	26
GLA71-3	70° 27.0'	143° 33'	45
GLA72-12	70° 18.0'	146° 05'	26
GLA71-23	70° 38.4'	148° 04'	27
GLA71-25	70° 31.2'	147° 31'	26
GLA71-27	70° 54.5'	147° 26'	47
GLA71-44	71° 01.6'	148° 23'	48
GLA71-63	70° 43.0'	149° 00'	26
GLA71-71	71° 04.0'	151° 22'	21
GLA71-72	70° 11.0'	151° 14'	47
GLA71-78	70° 58.4'	149° 59'	29
GLA71-80	70° 55.7'	149° 23'	33
BSS-61	70° 50.0'	148° 28'	36
BSS-82	71° 36.0'	154° 39'	29
BSS-83	71° 27.0'	153° 39'	50
BSS-88	71° 05.0'	150° 00'	30
70BS-18	70° 22.7'	148° 10'	22
70BS-19	70° 30.9'	148° 35'	13
70BS-21	70° 31.4'	148° 34'	16
70BS-22	70° 36.3'	148° 25'	20
71AJT-5	70° 26.2'	148° 00'	7
71AJT-16	70° 26.5'	148° 30'	7
71AJT-19	70° 29.2'	148° 30'	9
71AJT-20	70° 31.8'	148° 30'	15
71AER-15	70° 19.0'	148° 19'	1
72AJT-3	70° 29.0'	149° 03'	3
72AJT-4	70° 29.5'	149° 08'	2
72AJT-5	70° 27.2'	148° 10'	7
72AJT-6	70° 25.2'	148° 10'	6
72AJT-7	70° 22.8'	148° 11'	2
72AJT-8	70° 21.4'	147° 57'	4
72AER-129	70° 29.4'	148° 20'	3
72AER-134	70° 32.0'	148° 13'	15
72AER-137	70° 29.9'	148° 00'	13
72AER-166	70° 26.6'	148° 34'	7
72AER-167	70° 25.9'	148° 35'	6
72AER-168	70° 25.3'	148° 35'	4

Nuclepore filters (0.4 μ) has been retained. Although not part of the proposed program, data for many of these samples also will be given at a later date.

Water samples have been collected at the sediment stations noted above. Because of the expense and difficulties associated with analysis of soluble trace metal contents, it has not been possible to determine detailed depth profiles. In order to maintain the geographical coverage required for this program, only samples from the surface or from close to the surface, and from adjacent to the sediment-water boundary, have been collected.

The work statement for this project listed a number of biological species for which background trace metal contents were considered to be desirable. This list was a composite of suggestions from many of the principal investigators of the associated biological programs, although there were notable omissions, such as sampling of marine mammals. For the actual sample collection we have been totally dependent on the good offices of these biological programs; particularly the intertidal and sub-tidal benthic projects. Intertidal benthic samples received to date are shown in Table II and in Figures 6 and 7, and sub-tidal benthic samples in Tables III and IV.

B. Sampling Trips

The original work statement called for one major cruise to each of the three study areas -- Gulf, Bering Sea and Beaufort Sea -- with additional sampling adjacent to the outflow of the Copper and Kuskokwim Rivers. This idealized scheme did not work out well in practice. One of the major problems encountered concerned our need to collect contamination-free samples, and hence the use of specialized equipment and facilities not available on all

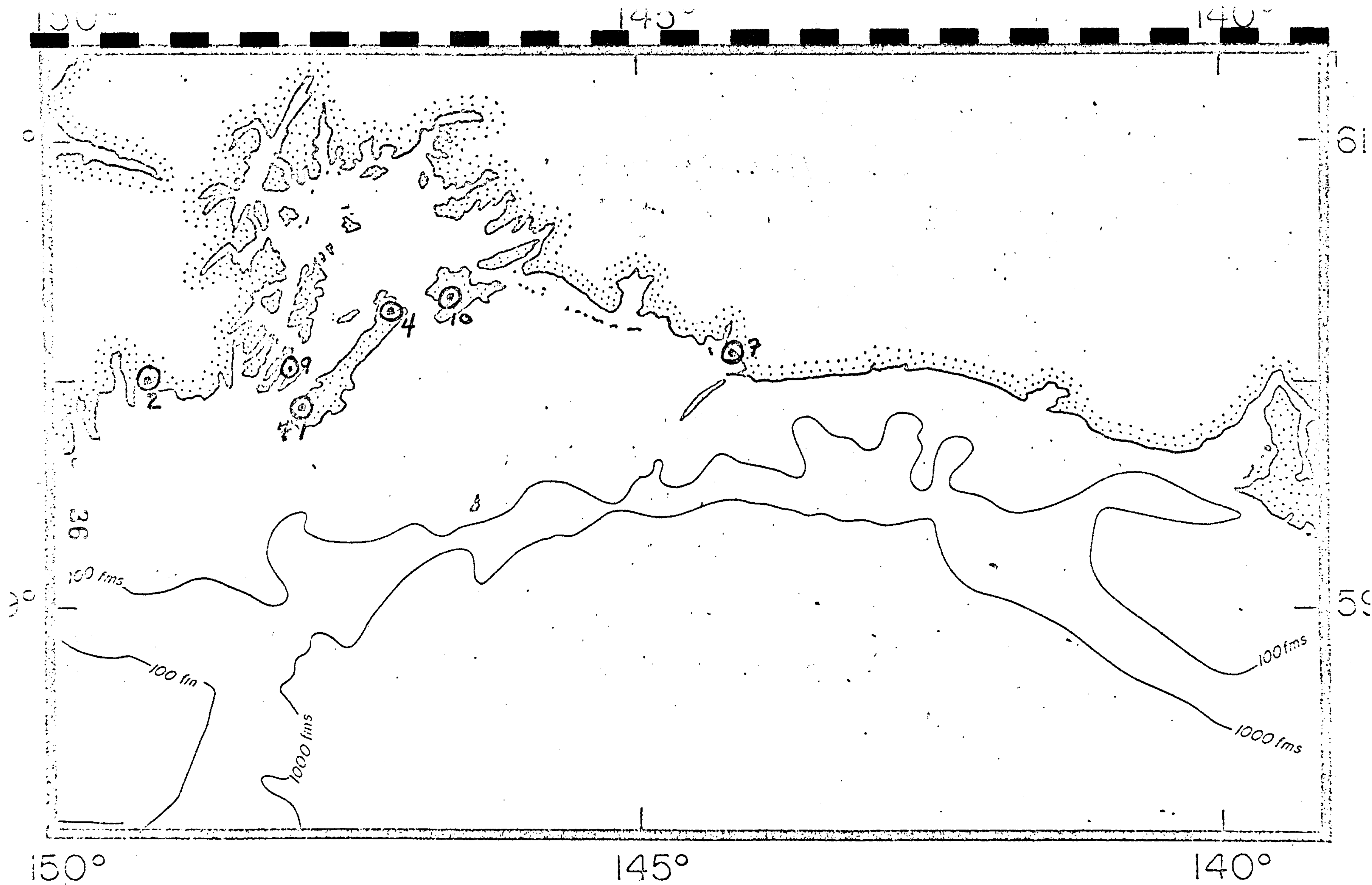

Figure 6. Intertidal sample locations in N.E. Gulf of Alaska.

Fig 6.

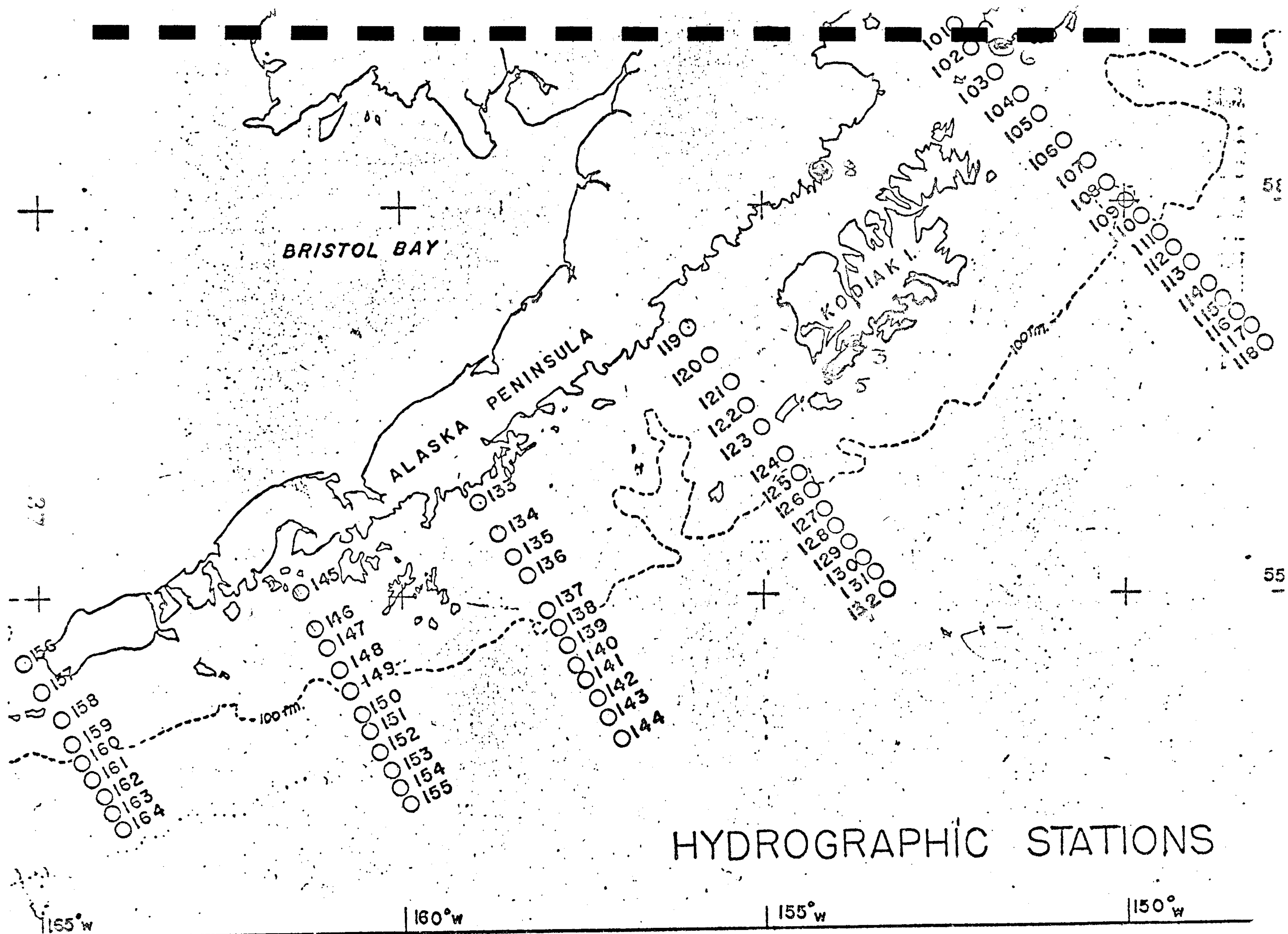

Figure 7. Intertidal sample locations in N.W. Gulf of Alaska

TABLE III.

Localities of intertidal biota samples - Gulf of Alaska

Locality	Lat. N	Long. W
McLeod Harbor	59°53'	147°47'
Anchor Cove	59°59'	149°05'
Saints Bay	57°06'	153°28'
Zarkof Bay	60°17'	147°00'
Sundstrom Island	56°41'	154°08'
Port Dick	59°18'	151°20'
Katalla	60°14'	144°31'
Cape Nukshak	58°24'	153°59'
La Touche	60°03'	147°56'
Port Etches	60°22'	146°32'

TABLE IV.

Trawl biota samples received

	Gulf	Bering		Beaufort
	"North Pacific" Cruise	"Miller Freeman" Cruises		
		#1	#2	
A. Species specified in work statement.				
Pollack	X	X	X	n.a.
Rock sole	X	X	X	n.a.
Arctic cod	n.a.	n.a.	n.a.	0
Tanner crab	X	X	X	n.a.
King crab	0	X	0	n.a.
Neptunea	X	X	X	n.a.
Scallop/Macoma	0	X	0	n.a.
B. Collected species not specified in work statement.				
Turbot	X	-	-	-
Molpodia	X	-	-	-
Spisula	-	X	-	-
Serripes	-	-	X	-

TABLE V

N.E. Gulf of Alaska

Sub-tidal benthic trawl samples

Sample No.	Species	Station/Haul Nos.	Location start/finish	Depth(m)
1	*C. bairdi*	73A/115	N60 10 W147 02/	264/
2	Turbot		N60 13 W146 59	262
3	*C. bairdi*			
4	Molpodia			
5	*C. bairdi*	73B/116	N60 63 W147 04/	142/
6	*C. bairdi*		N60 05 W147 00	138
7	Pollock			
8	Neptunea			
9	*C. bairdi*	73C/117	N59 57 W146 59/	149/
10	Turbot		N60 00 W146 56	142
11	Pollock			
12	Turbot	73D/118	N59 54 W147 00/	158/
13	Pollock		N59 57 W147 00	155
14	*C. bairdi*			
15	Neptunea			
16	Pollock	73E/119	N59 47 W147 02/	180/
17	Turbot		N59 50 W147 01	177
18	Neptunea			
19	Rock sole			
20	Pollock	74F/120	N59 38 W146 38/	93/87
21	Turbot		N59 39 W146 41	
22	*C. bairdi*	74G/121	N59 32 W146 42/	115/113
23	Pollock		N59 35 W146 45	
24	Turbot			
25	*C. bairdi*	75G/122	N59 36 W146 27/	98/95
26	Turbot		N59 34 W146 36	
27	Pollock			
28	*C. bairdi*	73H/123	N59 28 W146 57/	204/202
29	Turbot		N59 23 W146 58	
30	Pollock			
31	Neptunea			
32	Neptunea			
33	Pollock	73I/124	N59 25 W147 01/ N59 24 W147 00	198/195
34	Turbot	77I/125	N59 33 W145 59/	146/133
35	*C. bairdi*		N59 30 W145 59	
36	*C. bairdi*	77F/127	N59 50 W145 01/	95/91
37	Pollock		N59 48 W146 58	
38	Turbot			
39	*C. bairdi*	77E/128	N59 53 W146 03/ N59 52 W145 56	97/93

Sample No.	Species	Station/Haul Nos.	Location start/finish	Depth(m)
40	Neptunea		N59 43 W145 32/ N59 40 W145 31	104/100
41	Neptunea		N60 11 W146 27/	133/131
42	Rock sole		N60 10 W146 31	
43	Molpodia			
44	Neptunea	77B/133	N60 12 W146 02/	102/100
45	Rock sole		N60 12 W146 07	
46	Neptunea	77A/134	N60 17 W146 02/	55/53
47	Rock sole		N60 16 W146 07	
48	Molpodia	79A/135	N60 09 W145 33/ N60 11 W145 37	104/102
49	Rock sole	79B/136	N60 06 W145 31/ N60 04 W145 35	122/120
50	Rock sole	79C/137	N60 00 W145 29/ N60 03 W145 33	104/100
51	Rock sole	81B/138	N60 01 W144 58/ N60 02 W144 58	151/127
52	Neptunea	81F/140	N59 35 W145 00/ N59 35 W145 07	178/173
53	Molpodia	83E/142	N59 43 W144 37/ N59 41 W144 33	131/129
54	Rock sole	74D/146	N59 53 W146 57/ N59 51 W146 53	71/67
55	Molpodia	75B/147	N60 06 W146 36/ N60 08 W146 36	109

vessels. In general, of the assigned NOAA ships, only the *Discoverer* was suitable for our purposes. We found it necessary to place personnel on a large number of cruises in order to obtain all the needed samples as detailed below. The need to give first priority to field work from June through December detracted from the laboratory analysis program.

One cruise was directed to sampling in the region of the Copper River in October. Unfortunately the senior investigator who had originally proposed to work on plume sediments decided to resign from the program at about this time so that this work had to be abandoned at that time. No sampling program specifically directed to the Kuskokwim delta materialized. Also no vessels capable of supporting this trace metal program was available within the Beaufort Sea during the 1975 open-water season. In lieu of obtaining new samples we have requested permission to work on archived samples: sediment samples collected on previous ice-breaker cruises in this area.

The biological sample collecting program has been most unsatisfactory. Our work statement was based on analysis of biological specimens collected for us by other investigators and no support was requested to enable us to place our own technicians on these particular cruises. Cruises on which some biota was collected for us are noted below.

Samples for this program have been collected on the following cruises and collection trips (in chronological order):

a. *Townsend Cromwell* - May 5-19, 1975
N.E. Gulf of Alaska

Personnel: G. Landreth (IMS)

Personnel from this project participated on this cruise primarily to obtain water column samples from a number of the standard N.E. Gulf stations

and some additional sites in Prince William Sound. The total occupied stations are shown in Figure 8 and operations conducted in support of this project are noted in Table IV. Sediment samples (Table IV) were collected from grabs as reserve material for the geological support program; no sampling device suitable for retrieving samples for trace metal analysis was available for this cruise. Field procedures are given below:

b. *Discoverer* Leg II - June 2-19, 1975
South Bering Sea

Personnel: D. C. Burrell (IMS)
R. S. Hadley (IMS)
D. Cochran (Batelle, NW)

This was the first combined sampling trip in support of this project in the Bering Sea. A standard "benthic grid" was laid out and first occupied on this cruise (Figure 3). This grid was designed to permit inorganic chemistry, benthic biology and geology sediment samples to be retrieved at the same locations and, wherever possible, as splits from the same bulk sample. Water samples were recovered from one or two depths for analysis by IMS (voltammetry), Batelle N.W. (neutron activation) and NBS. Operations conducted at each of the standard stations (occupied) are summarized in Table V and details of sampling procedures are given in the following sections.

c. <u>Intertidal Sampling</u>
N.E. and N.W. Gulf of Alaska

Intertidal benthic samples have been collected for this project by Dr. Zimmerman. Localities of samples received are shown in Figures 6 and 7.

d. *Surveyor* Leg II - August 4-29, 1975
South Bering Sea

Intertidal benthic samples have been collected for our use on this cruise by Dr. Zimmerman.

e. *Silas Bent* Leg I - August 31 to September 17, 1975
N.E. Gulf of Alaska

Personnel: R. S. Hadley (IMS)

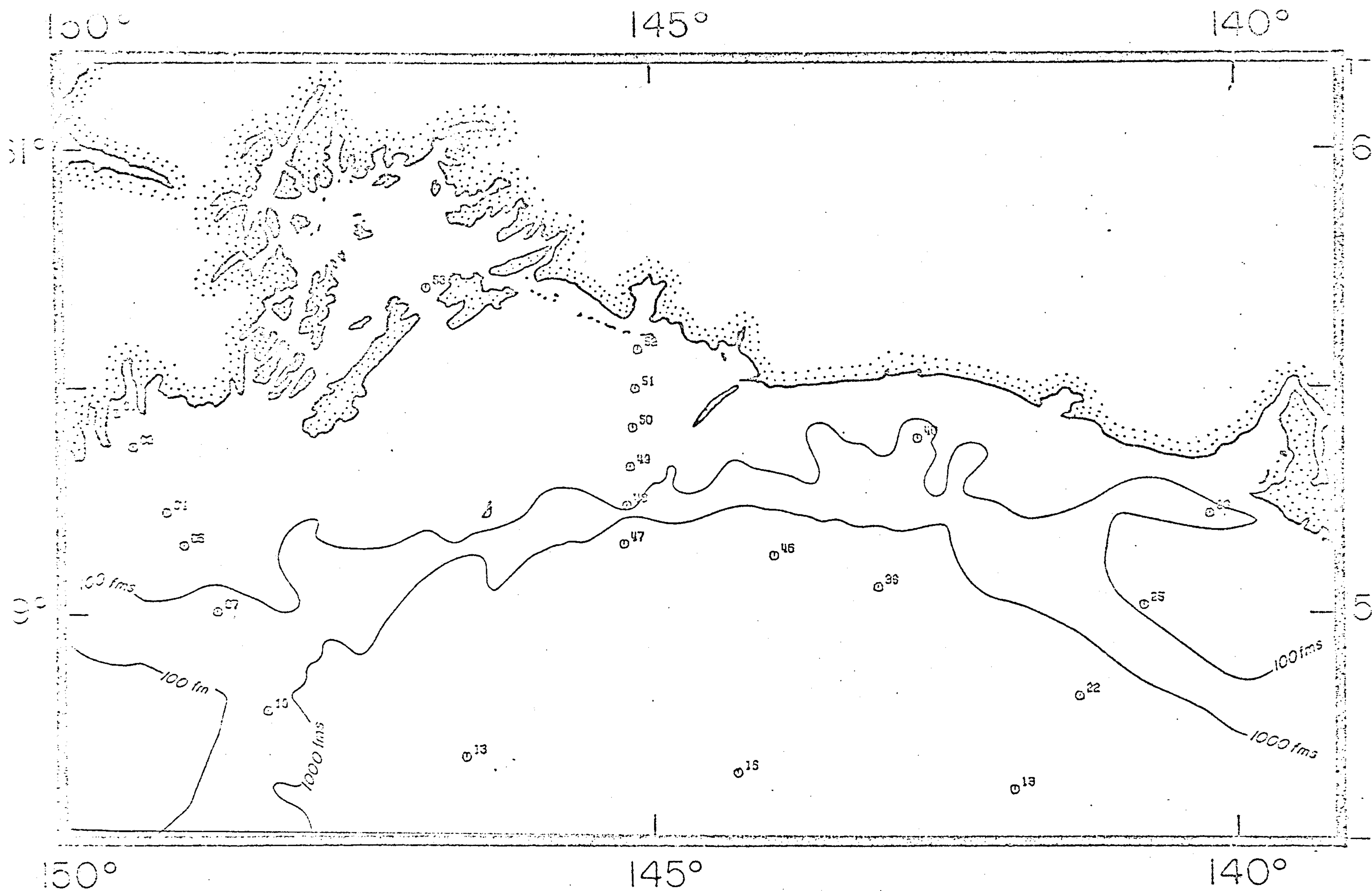

Figure 8. *Townsend Cromwell* May 5-19, 1975 - station locations.

TABLE VI

Townsend Cromwell   May 5-19, 1975 - Operations

N.E. Gulf of Alaska

Stations	Water (depths)	Sediment	Biota
01	Surf.	Grab	
02		Grab	
04	Surf.	Grab	
05		Grab	
07	0, 125, 220	Grab	
13	0, 50, 120	Boomerang	
16	Surf.	Boomerang	
17			Plankton
19	0, 100, 200		
22	0, 100, 170	Grab	
25	Surf.	Grab	
26		Grab	
27		Grab	
28	0, 130, 150		
36	0, 110, 200		
40	0, 60, 140		
46	Surf.		
47	0, 130, 180		
48	0, 150, 210		
49	Surf.	Grab	
50	Surf.	Grab	

Stations	Water (depths)	Sediment	Biota
51	0, 90		
52	Surf.		
53	Surf.		Plankton
54			Plankton
107			Plankton

TABLE VII

Discoverer June 2-19, 1975
Operations - S. Bering Sea

Station	Water depths (m)	Sediment	Biota
53	0.110		
54	0.100	Haps	
48	0.145		
34	0.175		
35			Plankt.
31	0.150		
14	0.130		Plankt.
13	0.75		
02	0.40		
06	0.40		
08	0.15	Haps	
10	0.60		
12	0.75	Haps	
19	0.65	Haps	
21	0.40	(Haps)	
24	0.40	Haps	
26	0.45		
28		Haps	
39	0.60		
41	0.25	Haps	
42	0		
43	0.30	Haps	
57	45	Haps	
59	30	Haps	
60	0.25		
62	0.45		
64	80	Haps	
65	0.100	Haps	Plankt.
69	0.105	Haps	
56	0.60	Haps	
46	0.60	(Haps)	
37		Haps	
30	0.125	Haps	
17	0.110	Haps	

We participated in this cruise in order to obtain the first batch of sediment samples for trace metal analysis from the Gulf using the Haps corer (see below). No water samples were collected because the vessel was not equipped for this operation. Stations at which Haps cores were recovered on this cruise are shown in Figure 9; duplicate cores were taken at Stations 01, 03, 06, 07, 31, 32, 62, 63, and 50.

f. *Miller Freeman* Legs I-III, August 17 to October 26, 1975
South Bering Sea

Biota samples have been collected for us from two of the trawl survey cruises conducted on this vessel (see Table III).

g. North Pacific
Gulf of Alaska

Trawled biota samples have been collected for us on one of the Gulf survey cruises (see Table III).

h. *Discoverer* Leg III - September 25 to October 3, 1975
South Bering Sea

Personnel: T. Gosink (IMS)
J. Hendee (IMS)

This cruise largely duplicated the first *Discoverer* cruise in the Bering Sea noted above. It was necessary to participate at this time because the specialized equipment required for Se and Cr analysis was not available previously. Operations conducted at stations on the standard benthic grid (Figure 3) are listed in Table VI.

i. *Discoverer* Leg IV - October 8-16, 1975
N.W. Gulf of Alaska

Personnel: G. Landreth (IMS)
T. Gosink (IMS)
J. Hendee (IMS)
K. Abel (Batelle, N.W.)

This was the first sampling cruise in support of this project to the N.W. Gulf area. The standard GASSO stations (the primary physical oceanographic

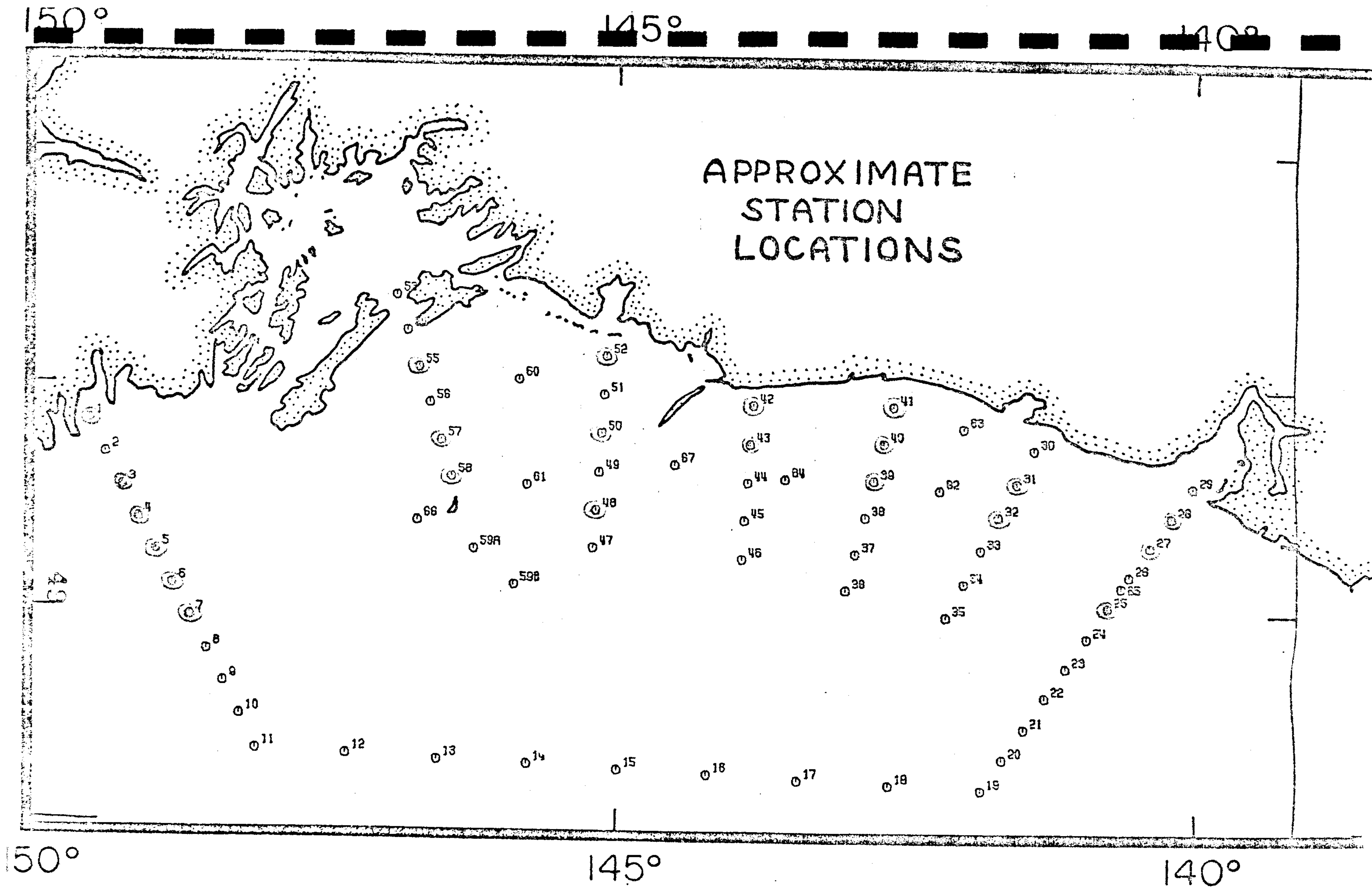

Figure 9. *Silas Bent* August 31 to September 17, 1975 - Haps core stations.

TABLE VIII

Discoverer Leg III September 25-October 3, 1975

Operations - S. Bering Sea

Stations	Water (depths)	Sediment
8	3, 18	
12	3, 76	
17	116	
19	71	
24	3, 40	
35	3, 155	
37	3, 70	
41	2, 18	
46	65	
48	3, 150	
51	3, 1500	
54	3, 98	
56	61	
59	3, 33	
66	134	

grid, Figure 2) were occupied for water column and sediment samples. Very few of the latter were obtained however. Operations conducted at each station in support of this project are summarized in Table VII; field procedures are considered below.

j. *Discoverer* Leg III - November 23 to December 2, 1975
N.E. Gulf of Alaska; N.W. Gulf

Personnel: D. C. Burrell (IMS)
J. Hendee (IMS)
H. Weiss (N.U.C.)
K. Abel (Batelle N.W.)

This was the final N.E. Gulf cruise requested in support of the current year's activities. Trace metal core samples were taken at those stations not previously covered on the *Silas Bent* cruise (above). In addition, water column samples were collected at a number of stations and sub-samples were distributed between all the laboratories participating in this program (IMS, Batelle N.W., Naval Undersea Center, and NBS). Operations are summarized in Table VIII. The final transect of the N.W. Gulf grid which was not visited on the previous *Discoverer* cruise was also occupied as shown in Table IX.

C. Field Operations

1. Water samples: Water samples for this program have been collected in 1.5, 5 and 10 liter Niskin bottles, but predominantly in 10 liter drop-top bottles attached to a rosette sampler. The standard storage bottle used for this program has been a 1 liter polyethylene bottle. These have been leached and washed in a single batch by Batelle Northwest Laboratories. Samples have been variously filtered as described below, or drawn straight from the Niskin bottle into the storage container. All samples have been

TABLE IX

Discoverer Leg IV October 8-16, 1975

Operations - N.W. Gulf of Alaska

Stations	Water(depths)	Sediment
101	1, 85	Haps[a]
102	1, 98	
104	1, 96	Haps
119	1, 206	Haps
120	1, 280	Haps
121	1, 220	Haps
122	1, 35	Haps
124	1, 105	Haps
133	1, 170	
134		Haps[b]
135	1, 141	Haps
137	1, 95	
145	10, 63	
146	1, 63	
147	1, 94	
148	1, 100	
156	1, 150	
157	1, 59	
158	1, 100	
159	1, 96	
160	1, 135	

a - IMS only
b - Battelle NW only

TABLE X

Discoverer Leg III - November 23 - December 2

Operations - N.E. Gulf of Alaska; N.W. Gulf of Alaska

Stations	Water (depths)	Sediment
2	10, 178	Haps
5	10, 162	Haps
8	10, 276	Haps
11	10,1350*	--
15	10,1500*	--
24	10, 410*	--
26	10, 136	Haps
29	71	--
30	42	--
33	10, 205	Haps
44	10, 165	Haps
48	10, 447	Haps
49	10, 120	Haps
50	10, 167	Haps
51	10, 133	Haps
52	74	Haps
53	10, 284	Haps
54	10, 202	Haps
55	10, 110	Haps
56	58	Haps
57	67	Haps
58	82	Haps
59A	10, 370	--

* Intercalibration station

TABLE XI

Discoverer Leg III November 23 - December 2

Operations - N.W. Gulf of Alaska

Station No.	Water Samples (depths)	Sediment
106	81	--
108	10, 226	--
110	10, 173*	--

* Intercalibration station

acidified as described and stored frozen prior to analysis. The specific procedures followed on each cruise are as follows:

a. *Townsend Cromwell* - May 5-19, 1975

250 ml unfiltered samples were acidified to pH 2 with reagent grade acid. No samples were collected for nickel and analysis.

b. *Discoverer* Leg II - June 2-19, 1975

Surface and bottom water samples were taken using the ships 10 liter drop-top, rosette-mounted Niskin bottles. One gallon cubetainers samples were collected unfiltered from the surface bottle, adicified to pH 4 with reagent grade nitric acid. Two 250 ml samples were collected from each depth, one of which was filtered through a 0.4 μ Nuclepore membrane in a Millepore filtering rig. All were acidified to pH 2 with reagent grade nitric acid. Five liters of water from each depth were filtered through an acid-washed 90 mm, 0.4 μ Nuclepore filter in a Wildco lucite pressure filtration apparatus. One liter was acidified with 5 ml reagent grade hydrochloric acid and stored unfrozen. The filter from this operation was retained in a cleaned petri dish.

c. *Discoverer* Leg III - September 25 to October 3, 1975

The water column was sampled at the surface and close to the sediment using the ship's Niskin-rosette system noted above. Two-liter polypropylene bottles (cleaned according to the scheme outlined below under Cr analysis) were filled directly from the Niskin bottles. Analysis was carried out at sea but is described below.

d. *Discoverer* Leg IV - October 8-16, 1975

Samples of water were taken from the surface and bottom, as described above. Eight liters of each sample were drawn into a cleaned polyethylene

transfer bottle and subsequently filtered through an in-line 140 mm, 0.4 μ Nuclepore filter. Two one-liter aliquots of the filtrate were retained, both were acidified with 5 ml Ultrex hydrochloric acid; one sample was stored unfrozen. One gallon cubetainer samples were taken as on the *Discoverer* June 2-19 cruise, acidified to pH 4 with Ultrex hydrochloric acid. Water samples were also taken for Cr and Se analysis as on the *Discoverer* September 25 to October 3 cruise above.

e. *Discoverer* Leg III - November 23 to December 2, 1975

Surface and bottom water samples were collected in ship's 10, 1 drop-top Niskin bottles mounted on the CTD rosette. Two bottles were used at each depth, and each was acid leached prior to use. At shallow stations only surface samples were collected. Except at the "intercalibration stations" the following samples were collected:

i. At or close to the surface -- four one-liter bottles filtered through a 0.4 μ in-line Nuclepore filter. One bottle was acidified to pH 4 (IMS; Ultrex acid), one had 5 ml Ultrex hydrochloric acid added (Batelle), and two were acidified with Ultrex nitric acid (NUC). All of these bottles were from the standard batch noted above. A further 100 ml sample of unfiltered water was collected at this depth and acidified to pH 2 with Ultrex hydrochloric acid.

ii. From adjacent to the sediment -- samples collected were as above with the addition of a 1 gallon cubetainer of unfiltered water, acidified to pH 4 with Ultrex hydrochloric acid.

iii. At the "intercalibration stations" samples were collected exactly as above except that additional samples were taken for NBS and other laboratories. Also multiple 10 ml centrifuge tubes were filled with filtered water for filament atomic absorption analysis.

2. Sediment samples: On early cruises sediment samples were collected from van Veen or Shipek grabs. Aliquots were taken from the middle of the sample using a plastic spoon and stored frozen in plastic bags. These samples have not been analysed during the current year's program but have been retained as back up material for the sedimentological program.

A new contamination-free (Haps) core was introduced on the *Discoverer* June 2-19 cruise. This sample recovers shallow but undisturbed surface samples. It only operates in clay-silt environments so that in many regions, e.g. most of the western Gulf, it has not been possible to obtain samples for this program. The usual mode of operation we have evolved for this operation is to take two cores serially. Both are extruded and the outer portion is trimmed off with a lucite trimmer. The trimmings of one core are retained for geological analysis and the interior portion of the core split longitudinally and stored in plastic jars for subsequent extraction analysis and for archiving. The second core is trimmed in the same fashion and then sectioned into horizontal 2 cm segments, each of which is stored frozen in high-density polyethylene jars.

3. Biota samples: All biota samples were collected by the benthic and intertidal biological programs. The instructions for collection were to handle as little as possible, to place whole samples in polyethylene bags and freeze immediately.

D. Metals Analysis

The elements studied in this program have been selected based on the following criteria:

1. Metals recognized as major pollutants affecting man: Ag, As, Cd, Ce, Hg, Pb, Sb, Se, Zn.

2. Natural petroleum "index" metals: Cr, Ni, V.

3. Indicators of environmental changes to the sediments: Fe, Mn, Co.

It is important to note that this list, selected on the basis of these criteria, includes many elements which have not been included in OCS impact programs in other areas of the U.S. These latter programs apparently assume that only metals likely to be derived directly from oil, formation waters or constructional elements (such as Pb from paint) are of importance. We believe that the major perturbation in natural trace element distributions will occur as a result of chemical stress on the sediments as explained above. Because of the large number of metals to be analysed in the various phases, a number of different analytical techniques have been used for this program (Tables XII, XIII and XIV). These are listed as follows, together with the senior investigators responsible for each procedure:

a. Cadmium, copper and zinc in filtered and unfiltered sea water by thin-film, anodic stripping voltammetry (Dr. D. C. Burrell).

b. Silver, arsenic, cobalt, mercury, iron, manganese, selenium, antimony and vanadium in water, biota and total sediment by thermal neutron activation analysis (Dr. D. Robertson).

c. Selenium and chromium in sea water, biota, sediment extracts and some particulate sediment phases by gas chromatography (Dr. T. Gosink).

d. Additional cadmium and zinc analyses in sea water by carbon filament atomic spectrometry for intercalibration purposes (Dr. D. C. Burrell).

e. Nickel in sea water by conventional flame atomic absorption (Dr. D. C. Burrell).

f. Iron, manganese, zinc, nickel and copper contents of whole-rock, archived samples from the Beaufort Sea by flame atomic absorption (Dr. A. S. Naidu).

TABLE XII

Techniques used for analysis of soluble heavy metals in sea water

	NAA	ASV	GC	FAA	SE/AA
Ag	X				
As	X				
Cd		X		(X)	
Co	X				
Cr			X		
Cu		X			
Fe	X				
Hg	X				
Mn	X				
Ni					X
Pb		X			
Sb	X				
Se	X		X		
V	X				
Zn		X		(X)	

NAA - Neutron activation
ASV - Anodic stripping voltammetry
GC - Gas liquid chromatography
FAA - Filament atomic absorption
SE/AA - Solvent extraction/flame atomic absorption

TABLE XIV

Techniques used for analysis of sediment extracts (X) and whole-rock samples (t)

	NAA	AA	GC	FAA
Ag	t			
As	t			
Cd				
Co	t			
Cr			t	
Cu		t		X
Fe	t	t		
Hg	t			
Mn	t	t		
Ni		t		X
Pb				
Sb	t			
Se	t		t	
V	t			
Zn		t		X

NAA - Neutron activation analysis
AA - Flame atomic absorption
GC - Gas-liquid chromatography
FAA - Furnace atomic absorption

g. Copper, zinc, cadmium and nickel contents of biota samples by both flame and carbon furnace atomic absorption (Dr. D. C. Burrell).

h. Copper, zinc, iron, manganese, cadmium and nickel contents of extracts from sediments by flame and carbon furnace atomic absorption analysis (Drs. D. C. Burrell and A. S. Naidu).

i. Mercury contents of whole-rock sediment and water samples by neutron activation analysis (Dr. H. V. Weiss).

j. Additional mercury contents of biota by ambient temperature atomic absorption analysis (Dr. D. C. Burrell).

Each method of analysis requires a specialized preparation treatment as outlined below.

E. Analysis Techniques

a. Water

i) Cadmium, copper, lead and zinc by DPASV (D. C. Burrell)

These metals have been determined in filtered and unfiltered sea water samples by differential pulse, anodic stripping voltammetry (DPASV) using a thin mercury film, glassy carbon electrode (GCE). Zinc has been determined at pH 8 and copper, cadmium and lead at pH 2.5.

ii) Nickel by AA (D. C. Burrell)

Nickel has been determined in acidified seawater samples (pH 4) by APDC/MIBK extraction and atomic absorption analysis (Brooks *et al.*, 1967). This is a self-compensating method in which incomplete extraction of the nickel complex is corrected for by extracting the standards from previously xtracted sea water. This is not an ideal procedure but

has the advantage of having been used in a large number of investigations so that the data should be consistent.

iii) Selenium by GLC (T. A. Gosink)

Two liters of sea water were drawn into polypropylene bottles (from the Niskin bottles described above) which had been rinsed three times with *ca*. 50 ml portions of the sea water. One liter portions were then filtered through an all-plastic filtration system using Gelman filter holders with teflon sealed magnetic clamps. A 0.45 μ Millepore filter was used in this step and this was subsequently stored in *ca*. 2 ml of 1-3 M HNO_3 in teflon lined screw-cap culture tubes for subsequent acid digestion and analysis (Nuclepore filters are too resistant to acid and char towards the completion of the digestion procedure). The filtrate was transfered to a teflon separatory funnel and pre-extracted for 1 minute with *ca*. 25 ml of nanograde benzene.

A solution of 4-nitro-o-phenylenediamine (4-NO_2) was prepared fresh every 48 hours (or sooner as required) by dissolving 0.25 g of the reagent in *ca*. 1 M HCl and extracting the solution x3 with *ca*. 25 ml portions of nanograde benzene. This solution was stored refrigerated in a plastic automatic dispenser. A 0.1 M solution of the disodium EDTA salt was prepared in deionized water and stored in a similar fashion.

5 ml each of the EDTA and 4-NO_2 solutions were added to the cleaned, filtered sea water samples. The funnels were shaken and allowed to stand for at least 2 hours after which 2 ml of nanograde benzene were added and the piaz-selenol derivative was quantitatively extracted by vigorous shaking of the separatory funnel for 5 minutes. A 2 μl portion of the benzene layer was subjected to GLC analysis immediately. See Gosink and Reynolds (1975) and Gosink (1975); the latter specifically for details of the instrumental analysis procedures.

iv) Chromium by GLC (T. A. Gosink)

Samples from the drop-top Niskin bottles were filtered as described for Se. The filtrate (225-230ml) was added to a pre-cleaned bottle containing 20 ml of Htfa and 5 ml of buffer solution. A stock solution of Hfta (trifluoro-acetylacetone) was prepared by diluting 1 ml of reagent in 100 ml benzene and stored refrigerated. The buffer solution was prepared in a teflon separatory funnel by adding 28 g reagent grade sodium acetate and 4 ml glacial acetic acid to 300 ml of deionized water. This solution was purified by adding 100 ml of the Hfta stock solution, shaking the solution and allowing it to stand overnight. The aqueous phase was washed twice with 100 ml of fresh nanograde benzene.

The samples prepared as described above were shaken periodically over a 24 hour period (the reaction is quantitative in approximately 2 hours at ambient laboratory temperatures

or in 10 minutes at 55-60°C). A 1-2 ml portion of the benzene solution was then washed twice with 2-4 ml portions of 0.1 M NaOH in a clean teflon-lined screw-cap culture tube with a 2 μl portion used for analysis (Gosink, 1975).

v) Other metals by neutron activation analysis (D. E. Robertson, and H. V. Weiss)

Details of analysis methods are not yet available.

b. Biota

i) Chromium by GLC (T. A. Gosink)

Weighed oven dried (110°C) portions of biota supplied by other OCS investigators were digested in teflon beakers using nitric and sulfuric acids. The digests were brought to a pH near 5.5 and placed in polypropylene bottles containing buffer and Hfta (see above) solutions. Digested samples were analysed by GLC as described for sea water above.

ii) Selenium by GLC (T. A. Gosink)

Samples were placed in pre-weighed teflon-lined, screw-cap, culture tubes containing 2-4 ml of 1-3 M HNO_3 on board ship. In the laboratory the acid stored samples were quantitatively transferred to Kjeldahl flasks, along with 2 ml of the acid digestion mixture and 5 ml of core HNO_3. The flask and contents were heated for 1 minute after the evolution of white fumes were evident (about 20-25 minutes). If charring occurred at any time during the digestion procedure, the sample had to be discarded because of

potential quantitative loss of selenium. After the flasks had cooled, the contents were stripped of any additional volatile interferring components by bubbling a vigorous stream of air through the digest, and then 2 ml of concentrated NH_4OH were added. The solutions were adjusted to a pH >2 with 3 M NH_4OH and then 2 ml each of EDTA, 4-NO_2 and nanograde benzene were added. After 2 hours the tubes were vigorously agitated for 1 minute to extract the piazselenol into the benzene phase. After separation in a small separatory funnel, a 2 µl portion of the benzene was subjected to immediate gas chromatographic analysis.

iii) Cadmium, copper, nickel, and zinc by AA (D. C. Burrell)

Most samples were initially rinsed with DDW, stored in polyethylene bags, and freeze dried. Where applicable, shells were removed after this step, and the remaining tissue was ground to a fine powder using a mortar and pestle. For the crab samples, only legs and claws were treated as above. Pollock and rock sole samples were partially thawed then, using a glass shard and plastic knife, the skin was removed and underlying flakes of tissue removed and freeze dried.

All glassware was cleaned with "Nochromix", 10% HCl and DDW. Approximately 1.0 g of powdered sample was weighed into a cleaned 35 ml centrifuge tube and enough DDW added to dampen the sample. Digestion was accomplished using the nitric acid, vapor phase method of Thomas and Smythe (1973).

After digestion, a volume of approximately 10 ml of solution remained. The tubes were then inserted into a custom made "furnace" which consisted of nichrome heating wires wrapped around pyrex cylinders, the whole being asbestos insulated and supplied *via* a variable power source. The solutions were slowly reduced in volume to *ca*. 1 ml and, while still warm, 50% H_2O_2 solution was added until frothing ceased to effect a final clearing of the sample solution. These latter were finally brought to volume and analysed by atomic absorption spectroscopy (AA).

All standards used for the biota analysis have been prepared using a matrix prepared from digests of the same species to be analysed.

c. Sediment

i) Cadmium, copper, nickel, and zinc in sediment extracts

(D. C. Burrell)

Sediment samples were allowed to thaw and were placed in containers for low-temperature (< 60°C) oven drying. *Ca*. 0.5 g was weighed into a bottle fitted with a teflon cap-liner. All glassware was pre-cleaned as noted above. 25 ml of mixed acid -- reducing reagent (1 M hydroxylamine hydrochloride - 25% (V/V) acetic acid as described by Chester and Hughes, 1967) was added and the sample shaken for four hours on an automatic shaker. The sample was then filtered through a Whatman #42 paper into a cleaned

35 ml centrifuge tube. 1 ml of concentrated HNO_3 was added to the filtrate which was slowly reduced in volume to fumes of HNO_3 using the multiple "furnace" device noted above. Following this step, the sample was allowed to cool, diluted to volume (*ca.* 25 ml) and analysed for the appropriate elements by AA.

ii) Chromium (T. A. Gosink)

Approximately 10 grams of surface sediment from a Haps or van Veen grab sampler was stored frozen in an all poly-propylene bottle. A weighed portion of dried sediment was prepared for solution by the method of Presley *et al.* (acetic acid and hydroxylamine) as recommended on page 3-9 in the BLM-OCS Summary Recommendations of the Trace Metal and Hydrocarbon Seminars, September 8-12, 1975. The samples prepared as described above were shaken periodically over a 24 hour period. (Reaction is quantitative in approximately 2 hours at warm room temperatures, or 10-15 minutes at 55-60°C.) A 1-2 ml portion of the benzene solution was then washed twice with 2-4 ml portions of 0.1 M NaOH in a clean teflon-lined screw-cap culture tube. A 2 μl portion was used for gas chromatographic analysis. These solutions of $Cr(fta)_3$ are quite stable for storage, but in the case of these particulate samples a 2 to 4 day period was required to destroy some of the aluminum chelate present which also formed quantitatively under the above conditions, and masked the chromium peaks.

iii) Selenium (T. A. Gosink)

1 to 5 g samples of surface sediment were immediately transfered to small pre-weighed, all polypropylene bottles containing about 10 ml of 1-3 M HNO_3. These latter were then treated and analysed as described above for the biota.

iv) Total mercury by NAA (H. V. Weiss)

Just before preparation for analyses the samples were thawed, mixed thoroughly and separate aliquots were removed for neutron activation analyses and dry weight determination. The water content was measured by weighing sediment before and after treatment at 110°C for one hour. From 2-3 grams of sediment were placed in an irradiation vial and 3 ml of concentrated nitric acid were added.

The comparator consisted of 1.0 μg of mercury as the nitrate in 10 ml of concentrated nitric acid. The nitric acid blank was comprised of three irradiation vials each filled with 14 ml of concentrated nitric acid.

Samples, comparators and blanks were irradiated for 2 hours at a flux of 10^{12} neutrons cm^{-2} sec^{-1} in a "Lazy Susan" rotated at 1 rpm about the core of the TRIGA reactor at the University of California, Irvine. The irradiations were made at 1,500 to 1,700 hour, and the following morning samples were processed to attain radiochemical purity.

Sediments were digested in a nitric- and sulfuric-acid mixture as described previously (Williams and Weiss, 1973). The sediment was prepared further for radiochemical purification by addition of 25 ml of concentrated ammonium hydroxide to the digest, and the mixture was filtered. If, at this stage, pebbles were detected in the residual sediment, they were removed and the sample was corrected for their weight.

Ten mg of mercury carrier was added to the water samples and the nitric-acid blanks. The nitric-acid blank was reduced in volume to about 10 ml. Water samples and blanks both received 9 ml of concentrated ammonium hydroxide and 10 mg each of potassium and sodium chloride (these quantities of chlorides were also added prior to the succeeding precipitations).

To the filtrate was added 2.5 ml of freshly prepared stannous chloride. The precipitated mercury metal was collected by centrifugation. The precipitate was dissolved in 5 ml aqua regia; 5 mg of copper (as nitrate) was added and the solution filtered. The reduction of mercury to the metal was repeated and the solid collected by filtration. The precipitate was again dissolved with 5 ml aqua regia (1 or 2 drops of concentrated phosphoric acid were added to the water samples), and the solution was neutralized with 5 ml of concentrated ammonium hydroxide. Hydrogen-sulfide

gas was passed through the pH-adjusted solution and the precipitate was collected by filtration. The mercuric sulfide was of sufficient purity to permit immediate measurement. The comparator was neutralized with 9 ml of concentrated ammonium hydroxide after the addition of mercury carrier, and the mercuric sulfide was precipitated and collected for measurements.

Usually, the processed mercury samples as well as mercury carrier standards (10 mg mercury) were re-irradiated for 5 sec. Through comparison of the activity level of the samples and standards, the carrier yield was computed and the counting rate in the original irradiation was corrected for this factor. Alternatively the carrier yield has on occasion also been determined by atomic absorption spectrometry.

The radioactive measurements were made with a sodium-iodide detector coupled to a 400-channel pulse-height analyzer. The counts attributable to the 77-keV radiation of ^{197}Hg were integrated by the method of Covell (1959). The standard deviation for this analysis is less than 10%.

v) <u>Total Fe, Mn, Zn, Ni, Cu in archived Beaufort Sea sediments</u>

(A. S. Naidu)

Splits of samples from an archived sediment suite were taken for heavy metal analyses. The archived sediment samples have been stored in a frozen state in acid rinsed polyvials,

and were originally collected with the specific purpose to conduct chemical analyses on them. The middle and outer shelf samples were separated, using a Teflon-coated spatula, either from the core of van Veen grabs, or from the tops of a metal-free gravity cover. A few samples represent surficial portions of short trigger core samples retrieved in plastic core liners. The sediment samples from the relatively shallow Prudhoe Bay area, were collected in the summer of 1974 by divers, who scooped a portion of undisturbed bottom surface sediments directly into acid-rinsed polyethene boxes. All these sediments were stored in a frozen state until analyses. The middle and outer shelf samples were obtained from USCGC ice breakers *Staten Island* and *Glacier* in 1969 and 1971, respectively.

Details on the techniques followed for the chemical analysis of sediments have been described by Naidu and Hood (1972). In short, gravel-free sediment samples dried at 110°C overnight were pulverized to fine powders using an agate mortar and pestle. A known weight of these powders was first ashed, and then digested in concentrated $HF-HNO_3$ acid following the procedure given by Rader and Grimaldi (1961). From the solutions thus obtained the concentrations of Fe, Mn, Cu, Ni, and Zn were analyzed in a Model 303 Perkin-Elmer atomic absorption spectrophotometer.

vi) <u>Granulometric and clay mineral composition of sediments from the Gulf and Bering Sea</u> (A. S. Naidu)

Granulometric composition of the sediments were analyzed by the conventional combined sieving-pipetting method. Prior to analysis, representative portions of each of the sediment samples were treated with H_2O_2 to remove organic matter. The mud fractions (< 62mμ) of the sediments were collected after wet-sieving the sediments through a 230-mesh sieve, and the particles in them were disaggregated into homogenous suspensions by repeated washings in double distilled water and, if necessary, by adding a few drops of Ammonium hydroxide. From these suspensions, the weight of the silt and clay sized particles were determined by following the Stoke's Law. Grain Size analyses were limited to the determination of the weight percents of gravel, sand, silt, and clay sized particles in sediments.

Clay mineral analysis was performed following the method elaborated by Naidu *et al.* (1971) and Mowatt *et al.* (1974). Briefly, it consisted of first treating each of the gross subsamples with H_2O_2 to remove organic matter. The organic-free sample was then wet-sieved using a 230-mesh sieve, and the suspensions finer than 62 mμ fraction were collected into long cylinders. From homogenized suspensions the less than 2 mμ e.s.d. (equivalent spherical diameter) particles were separated, using Stoke's Law. After centrifugation the

solids were collected and mounted with preferred orientation on glass slides, adopting the smear technique described by Gibbs (1965). X-ray diffraction patterns of each of the clay slides were obtained on a Phillips-Norelco X-ray unit having a scintillation counter. Glycol solvation, as well as fast and slow scan techniques were employed to assist in routine identification of clay minerals. From the remaining samples of the less than 2 mμ e.s.d., the less than 1 mμ e.s.d. will be separated. This work has yet to be started. Treatment of aliquots of the less than 2 mμ e.s.d. fractions with ionic solutions of Mg and K are in progress. All clay mineral quantifications in this study are based on the method given by Biscaye (1965), and is at best semiquantitative. The general precision of clay mineral analyses has been better than 10 percent.

F. Accuracy and Precision

1. Water

There are many uncertainties associated with the analysis of dissolved trace metals in sea water. These errors may be broadly grouped into those associated with sampling, handling and storage, and analysis procedures, respectively. It is generally conceded that the major problems - especially non-systematic errors - are associated with the pre-analysis steps.

We have attempted to standardize sampling and initial treatment of the water as far as possible so that all associated investigators in this program obtain their samples collected, filtered, acidified and stored in exactly the same fashion. Because there are no established techniques for

performing these operations (especially for deep samples) we have continuously modified and attempted to improve our techniques on each successive cruise through the present contract period. This has unfortunately resulted in each batch of data having been treated in a different fashion in some respect so that inter-cruise data are not strictly comparable. For example, it was noted in last years report (Burrell, 1975) that samples filtered for polarographic analysis appeared to be frequently contaminated. Consequently, during the initial cruises of this year, we elected to analyse unfiltered water. On the final cruise, however, an in-line filter-rig (designed by Batelle N.W. Laboratories) was used. Similarly water samples have acidified exclusively with Ultrex grade acids on all the later cruises, water has been collected in 10 l capacity, drop-top design Niskin bottles which have been acid rinsed prior to use, and samples have been stored in 1 liter polyethylene bottles from a single batch especially prepared for this program.

On each cruise we have collected additional samples for analysis by NBS and on the December *Discoverer* cruise a number of sample replicates were collected from four deep stations to serve as intercalibration samples for various external laboratories. Several of the elements determined in this program are also being analysed by more than one technique. All such intercalibrations are, of course, tests only of the analysis procedures used. Since there are no established standards, accuracy must be gauged *via* conformity with a mean or consenus value (within specified precision limits) for each element. We would reiterate that it is to be expected that the errors associated with the procedures prior to the final instrumental analysis will control the overall accuracy.

There are far too few reliable published values of trace metals in sea water available which might lead to designation of "mean oceanic ranges" against which data from this and similar programs could be tested. Mean values recently compiled by Brewer (1975) are given in Table XV. It should be noted that most of these values have been selected from the lowest ranges cited in the recent literature. Contamination (addition) errors are certainly very common, but adsorption and other subtraction errors are also prevalent, and the assumption that the lowest determined values are necessarily more accurate is not justified.

Precision testing for all the individual analytical procedures employed for water analysis in this program are not yet available, but some preliminary discussion follows:

a. Cd, Cu and Pb by anodic stripping voltammetry: Precision data for replicate determinations of two individual water samples are given in Table XVI. It should be noted that the percent coefficient of variation data have been improved over those cited in last year's report (Burrell, 1975). In either case, the analytical precision *via* this technique would appear to be excellent. We have not determined corresponding inter - and intra - (shipboard) sampling bottle precision ranges within this program. However, with care, these systematic errors need not be large. In a similar study, Heggie and Burrell (1975) have given values of 7.2% and 9.2% as the coefficient of variation for replicate samples from one individual Niskin bottle, and for replicate costs with a single Niskin bottle, respectively. It should not be inferred that data for the ship-board, drop-top Niskin bottles used in this program will be identical, but it is likely to be comparable.

TABLE XV

Published compilations of mean soluble contents of heavy metals in seawater and concentration factor data (x 10^{-3}) for phytoplankton and *Mytilus edulis*

Element	Seawater[a]	Phytoplankton[b]	Mytilus[c]
Ag	0.04	25	0.3
As	3.7		
Cd	0.1		100
Co	0.05	1.5	
Cr	0.3	2.4	
Cu	0.5	30	3
Fe	2.0	45	
Hg	0.03		
Mn	0.2	4	
Ni	1.7	5	14
Pb	0.03	40	4
Sb	0.24		
Se	0.2		
V	2.5	0.6	
Zn	5	26	9

[a]Brewer (1975)

[b]Lowman *et al.*, (1975)

[c]Brooks and Rumsby

TABLE XVI

Precision data for the analysis of soluble
Cd, Cu and Pb in two seawater samples
by thin-film anodic stripping voltammetry

		Cd	Pb	Cu
N.W. Gulf of Alaska sample No. 160, 0 m				
	$\bar{x}$ (µg/l)	0.03	0.075	0.25
	n	7	7	6
	σ	0.0029	0.004	0.041
	α (%)	10	5.5	16
N.W. Gulf of Alaska Sample No. 146, 0 m				
	$\bar{x}$ (µg/l)	0.03	0.08	0.24
	n	6	6	6
	σ	0.0032	0.0039	0.021
	α (%)	10.5	5	9

b. Cr by gas chromatography: The results of accuracy and precision tests for the analysis of chromium in solution *via* the procedures described above are given in Table XVII. In a replicate sampling test (five samples collected from a small boat in the N.W. Gulf of Alaska) the mean value and precision range for chromium was 0.30 ± 0.01 μg/1.

2. Biota

We have had no control over the collection or storage of the biological samples used in this program. Hence neither statistical sampling procedures, nor errors associated with collection and storage, can be considered here. Results of precision/accuracy tests for some of the analytical procedures used in this study are not yet available.

a. Cd, Cu, Ni, Zn by flame and furnace atomic absorption: New precision tests have not yet been completed but, since procedures are substantially unchanged, the data given in Burrell (1975) are closely applicable. Sure recent values obtained for these metals in the NBS Orchard Leaf Standard (No. 1577) are given in Table XVIII.

3. Sediments

No analysis results for sediment extracts are yet available but it is not, in any case, possible to determine the accuracy of such data because no standards are available.

a. Cr in sediments by gas chromatography: Table XIX gives total chromium values of a series of sediment samples, together with one standard rock, determined using both GLC (T. Gosink) and flame atomic absorption (A. S. Naidu).

b. Total Fe, Mn, Cu, Ni and Zn in Beaufort Sea sediments by flame atomic absorption: Dr. A. S. Nadu reports precision values of ± L and 12% for Fe, Mn, and Cu, Ni, Zn respectively.

TABLE XVII

Accuracy and precision tests for soluble chromium
(μg/ml)
using gas-liquid chromatography
via replicate analysis of known additions

Test	Added	Determined	n	a
1	1.12×10^{-1}	1.11×10^{-1}	4	0.1×10^{-1}
2	1.12×10^{-2}	1.21×10^{-2}	4	0.13×10^{-2}
3	1.12×10^{-3}	1.41×10^{-3}	4	0.8×10^{-3}
4	6.6×10^{-4}	8.0×10^{-4}	4	4.5×10^{-4}

TABLE XVIII

Accuracy values for biota analysis
(µg/g dry weight ± one standard deviation)
NBS Standard # 1577 Orchard Leaves

Metal	This study*	NBS certified value
Cd	<1.5	0.11 ± 0.02
Cu	12.6 ± 0.1	12 ± 1
Ni	<5	1.3 ± 0.2
Zn	23.0 ± 2.1	25 ± 3

* n = 5 replicates

TABLE XIX

Analysis intercalibration for total chromium contents of sediments (mg/kg dry weight)

Sample	G.L.C.[a]	AA[b]
G2[c]	10	13
1	48	42
2	48	57
3	77	75
4	92	74
5	87	65
6	59	59
7	55	37
8	47	53
9	64	46
10	64	47

a - gas-liquid chromatography - T. Gosink, analyst
b - Flame atomic absorption - A. S. Naidu, analyst
c - USGS Standard Rock G2, average value 9 (range 5-28) mg/kg

VI. RESULTS

The first results of the analytical procedures applied to the samples described above have now been obtained. Most of the data will, however, be generated in the latter part of the contract period.

A. Water

Voltammetric analysis has been completed on the R/V *Townsend Cromwell* cruise samples collected in the N.E. Gulf in May (Table XX). These data are for unfiltered samples and lead values were not obtained. Data for soluble cadmium, copper and lead on 0.4 μ filtered samples from the S. Bering Sea and N.W. Gulf of Alaska are given in Tables XXI and XXII. Zinc values will be provided at a later date, and nickel contents are currently available only for Bering Sea samples (Table XXI). Filtered and unfiltered chromium values for the S. Bering Sea are given in Table XXIII and for soluble selenium and chromium for the N.W. Gulf in Table XXIV. No activation analysis data are available at this time.

B. Biota

Our most complete sample collection to date is for intertidal benthos from the Gulf of Alaska. Tables XXV and XXVI give heavy metal contents of *Mytilus* and *Fucus* samples respectively. Table XXVII lists total mercury contents for a set of commercially obtained crab samples from the N.E. Gulf area and Table XXVIII other heavy metals for crab received from one of the trawl cruises, also in the N.E. Gulf of Alaska. In Table XXIX are listed metal data for *Neptunea* samples collected on the latter trawl cruise (N.E. Gulf; July 18 to August 7); the reader should note the comments on this data set in the following section.

TABLE XX

N.E. Gulf of Alaska

Soluble Heavy Metal Contents (μg/l)
Townsend Cromwell
May 6 - 21, 1975

Station No.	Depth (m)	Cd	Cu	Zn
01	0	0.20	0.24	1.1
04	0	0.07	0.36	5.8
07	0	0.05	0.27	4.6
	175	0.03	0.38	1.1
	220	0.03	0.16	1.1
10	0	0.03	0.21	2.5
	140	0.06	0.11	0.8
	250	0.08	0.30	3.6
13	0	0.04	0.22	2.2
	50	0.04	0.24	1.4
	120	0.05	0.22	1.6
16	0	0.06	0.26	3.2
19	0	0.04	0.28	2.5
	100	0.03	0.32	1.5
	200	0.04	0.32	0.9
22	0	0.03	0.49	-
	100	0.04	1.10	3.6
	160	0.05	0.37	2.6
25	0	0.12	0.65	4.0
28	0	0.03	0.40	1.8
	130	0.03	0.30	2.0
	150	0.10	0.23	0.5
36	0	0.09	0.60	-
	110	0.04	0.30	1.3
	200	0.06	0.27	0.9
40	0	0.06	0.35	1.5
	60	0.04	0.34	1.7
	140	0.02	0.28	0.6
46	0	0.03	0.16	0.6
47	0	-	0.21	0.5
	130	-	0.15	0.6
	280	-	0.16	1.9
48	0	0.08	0.24	1.7
	150	0.02	0.30	1.4
	210	0.10	0.23	2.0
49	0	0.05	0.32	4.2
50	0	0.05	0.27	2.8
51	0	0.05	0.27	2.3
	90	0.05	0.42	3.7
52	0	0.06	0.30	1.0
53	0	0.15	0.53	3.2

TABLE XXI

S. Bering Sea

Total contents of heavy metals in unfiltered water (μg/l)
Discoverer June 2-19, 1975

Station No.	Depth	Cd	Pb	Cu	Ni
02	0	0.06	--	0.45	0.65
	40	0.06	0.18	0.45	
06	0	0.03	0.17	0.32	0.45
	40	0.06	0.16	0.45	
08	0	0.03	0.22	0.75	0.95
	15	0.10	0.30	0.80	
10	0	0.03	0.20	0.50	0.60
	60	0.04	0.14	0.35	
12	0	0.03	0.12	0.32	0.60
	75	0.06	0.55	0.74	
13	0	0.04	0.07	0.36	0.60
	75	0.09	0.40	0.44	
14	0	0.04	0.14	0.35	0.70
	130	0.09	0.39	0.50	
17	0	0.04	0.31	0.39	
	110	0.11	0.16	0.58	
19	0	0.03	0.14	0.27	0.80
	65	0.05	0.16	0.36	
21	0				0.50
	40	0.03	0.16	0.38	
24	0	0.03	0.14	0.46	0.45
	40	0.02	0.20	0.46	
26	0	0.04	0.20	0.46	0.55
	45	0.03	0.15	0.35	
30	0		(0.55)	0.46	
31	0	0.06	0.15	0.32	
	150	0.10	1.28	0.24	
34	175	0.06	0.10	0.26	
39	0	0.02	0.14	0.34	0.40
	60	0.05	0.23	0.40	
41	0	0.04	0.14	0.35	0.65
	25	0.03	0.09	0.33	
42	0	0.03	0.10	0.32	0.55
43	0	0.02	0.07	0.30	
	30	0.02	[illegible]	0.32	
46	0	0.03	0.08	0.33	0.55
	60		0.20	0.34	
48	0	0.06	0.36	0.49	0.60
	145	0.06	0.11	0.23	
53	0	0.06	0.58	0.30	0.60
54	0	0.05	0.16	0.20	
	100	0.12	0.45	0.48	

Station No.	Depth	Cd	Pb	Cu	Ni
56	0	0.03	0.25	0.40	
	62	0.11	0.20	0.45	
57	45	0.03	0.16	0.40	0.40
59	30	0.02	0.07	0.33	0.35
60	0	0.02	0.13	0.35	0.70
	25	0.02	0.16	0.42	
62	0	0.02	0.09	0.30	0.65
	45	0.03	0.08	0.35	
64	80				0.60
65	0*	0.04	0.30	0.52	
	100		0.18	0.92	
69	0				
	105				0.50

*Filtered sample

TABLE XXII

N.W. Gulf of Alaska

Heavy metal contents of filtered (0.4μ) water (μg/l) *Discoverer* October 8 - 16, 1975

Sample No.	Depth (m)	Cd	Pb	Cu
101	0	0.025	0.07	0.35
	80	0.03	0.08	0.25
102	0	0.03	0.08	(0.94)
	100	0.025	0.05	0.30
104	0	0.03	0.04	0.20
	95	0.05	0.05	0.24
119	0	0.02	0.025	0.26
	240	0.03	0.035	0.16
120	0	0.04	0.13	0.42
	280	0.06	0.06	0.26
121	0	0.04	0.	0.33
	220	0.04	0.05	0.17
122	0	0.025	0.08	0.28
	40	0.03	0.07	0.23
124	0	0.025	0.15	0.28
	105	0.05	0.10	0.24
133	0	0.025	0.07	0.24
	65	0.04	0.13	0.32
135	0	0.02	0.07	0.28
	140	0.025	0.06	0.22
137	0	0.035	0.10	0.30
	95	0.025	0.05	0.19
145	0	0.035	0.07	0.26
	60	0.03	0.08	0.20
146	0	0.03	0.08	0.24
	65	0.035	0.14	0.90
147	0	0.03	0.06	0.15
	95	0.035	0.05	0.16
148	0	0.025	0.06	0.35
	100	0.04	0.06	0.20
156	0	0.035	0.17	0.26
	150	0.04	0.19	0.15
157	0	0.05	0.13	0.25
	50	0.045	0.17	0.25
158	0	0.05	0.42	0.27
	90	0.03	0.07	0.20
159	0	0.03	0.08	0.27
	90	0.02	0.05	0.33
160	0	0.03	0.08	0.25
	135	0.04	0.08	0.12

TABLE XXIII

S. Bering Sea

Soluble Cr data (μg/1) *Discoverer* Leg III
September 25 - October 3
(Filtered samples at 0.45μ)

Station No.	Depth (m)	Filtered	Unfiltered
48	3	0.29	0.69
	150		0.14
51	3		0.16
	1500	0.14	
54	3	0.15	
	98		0.07
66	134		0.03
56	61		0.04
59	3	0.02	0.14
	33	0.12	0.11
41	3	0.73	
	28	Tr	
24	3	0.37	
	40	Tr	
8	3	0.30	
	18	0.27	
12	3	0.06	
	76	0.13	
35	3		0.22
	155		0.07
19	71	0.14	
46	65	0.92	
37	3	0.59	
	70	0.08	
17	114	Tr	

TABLE XXIV

N.W. Gulf of Alaska

Soluble Se (ng/1) and Cr (μg/1) *Discoverer* Leg IV
October 8-16, 1975
(Filtered samples at 0.45μ)

Station No.	Depth (m)	Se (ng/1)	Cr (μg/1)
156	3	2.8	--
	125	4.0	
158	100	Tr	
159	3	0.8	
148	3	5.2	
	100	n.d.	
160	3	n.d.	
	135	n.d.	
137	3	4.4	
	95	n.d.	
133	3	n.d.	7.64
	70	n.d.	5.64
124	3	n.d.	n.d.
	105	n.d.	0.06
119	3	n.d.	0.23
	200	n.d.	n.d.
101	3	n.d.	
	85	n.d.	n.d.

TABLE XXV

N.E. Gulf of Alaska

Heavy metal contents of *Mytilus* samples
Intertidal collection, August 1975
(µg/g dry weight)

	Cd	Cu	Ni	Zn
Sundstrom Island	5.2	8.4	<5	
Zaikof Bay	4.3	11.1	<5	27
Saints Bay	6.1	10.2	<5	30
Cape Nukshak	4.5	12.4	<5	
Port Etches	3.8	10.0	<5	
Anchor Cove	5.9	10.8	<5	31
McLeod Harbor	4.0	9.0	<5	
La Touche	2.9	7.2	<	25

TABLE XXVI

Heavy metal contents of *Fucus* samples from the Gulf of Alaska (ug/g dry weight)

	Cu	Ni	Cd	Zn
McLeod Harbor	16.8	7.9	4.1	21.8
Anchor Cove	26.6	15.6	3.3	21.5
Saints Bay	7.5	9.5	6.1	18.3
Zaikof Bay	16.7	9.2	4.0	15.9
Point Dick	7.8	9.4	3.5	15.8
Lundstrom Island	6.8	6.1	7.0	18.8
Katecta	17.0	9.8	2.0	16.4
Cape Nukshak	6.8	6.8	6.1	--
La Touche	5.9	5.3	3.1	--
Port Etches	10.4	7.3	2.3	15.3

TABLE XXVII

Mercury content of crab samples from Gulf of Alaska
(μg/g dry weight)

Sample No.	Hg
17	0.22
20	0.23
5	0.27
16	0.25
18	0.35
10	0.55
12	0.32
15	0.24
9	0.30
13	0.44

TABLE XXVIII

N.E. Gulf of Alaska

Heavy metal contents of crab samples
Trawl cruise July 18 - August 7
(μg/g dry weight)

Sample No.	Cd	Cu	Ni	Zn
1	<1.3	45.6	<5	135
3	1.3	30.1	<5	133
5	3.4	69.8	<5	82
6	2.5	132.6	<5	118
9	2.2	46.6	<5	81
25	1.7	65.2	<5	140
28	3.2	58.2	<5	128
35	<1.3	53.8	<5	149
36	2.0	65.3	<5	125

TABLE XXIX

N.E. Gulf of Alaska

Heavy metal contents of *Neptunea* samples
Trawl cruise July 18 - August 7
(μg/g dry weight)

Sample No.	Cd	Cu	Ni	Zn
8	54.9	208.9	<5	290
18	142.3	410.4	<5	409
31	49.5	235.1	<5	357
41	85.8	245.1	<5	627

C. Sediment

The chromium contents of the coexisting particulate material for which soluble data are given in Tables XXIII and XXIV are listed in Tables XXX and XXXI for the S. Bering Sea and N.W. Gulf of Alaska respectively. Figures 10, 11, and 12 show the localities of non-contaminated Haps core samples collected for this program to date, but only data for mercury in N.E. Gulf samples are presently available (Table XXXII).

Total iron, manganese, zinc, nickel and copper values for the archived Beaufort Sea samples discussed in previous sections (localities shown in Figures 4 and 5) are given in Table XXXIII.

Figures 13, 14 and 15 show the localities of sediment samples for the N.E. and N.W. Gulf of Alaska, and for the S. Bering Sea which have been analysed up to the time of compilation of this report for some sedimentological and mineralogical parameters. The percentage of gravel, sand, silt and clay for surficial samples from the N.E. and N.W. Gulf of Alaska are given in Tables XXXIV and XXXV. Tables XXXVI, XXXVII and XXXVIII show the weighed peak area percentages of the clay minerals (after Biscayne, 1965) in the less than 2 μm e.s.d. glycolated fraction of the N.E. and N.W. Gulf of Alaska and the S. Bering Sea respectively.

VII. DISCUSSION

This section can be in the nature of an interim report only since so few data are yet available. Even some of the values cited here are potentially subject to revision. A more complete evaluation will be given at the conclusion of the contract period.

TABLE XXX

S. Bering Sea

Total chromium contents of particulate sediment (μg/ℓ co-existing water; particulate material defined by 0.45μ membrane filter)
Discoverer September 13 - October 3, 1975

Stations	Depths (m)	Cr (μg/ℓ)*
8	3	Tr
	18	n.d.
12	3	n.d.
	76	n.d.
17	3	Tr
19	3	n.d.
	71	n.d.
24	3	n.d.
	40	0.14
41	3	0.36
	28	n.d.
46	3	0.12
54	3	n.d.
59	3	0.2
	33	n.d.
PMEL 46	3	n.d.
Off Nelson Lagoon		Tr
Izembeck Lagoon		n.d.

*n.d. - not detectable above background
Tr - 0.04 - 0.10 μg/ℓ above background

TABLE XXXI

N.W. Gulf of Alaska

Total chromium contents of particulate sediment (μg/ℓ co-existing water; particulate sediment defined by 0.45μ membrane filter)
Discoverer October 8-17, 1975

Stations	Depth (m)	Cr (μg/ℓ)*
101	3	n.d.
	85	n.d.
119	200	n.d.
124	3	Tr
133	3	n.d.
	70	n.d.
137	95	n.d.
145	3	n.d.
148	100	n.d.
160	3	n.d.
	135	n.d.
164		n.d.

*n.d.-not detectable above background
Tr-0.04-0.10 μg/ℓ above background

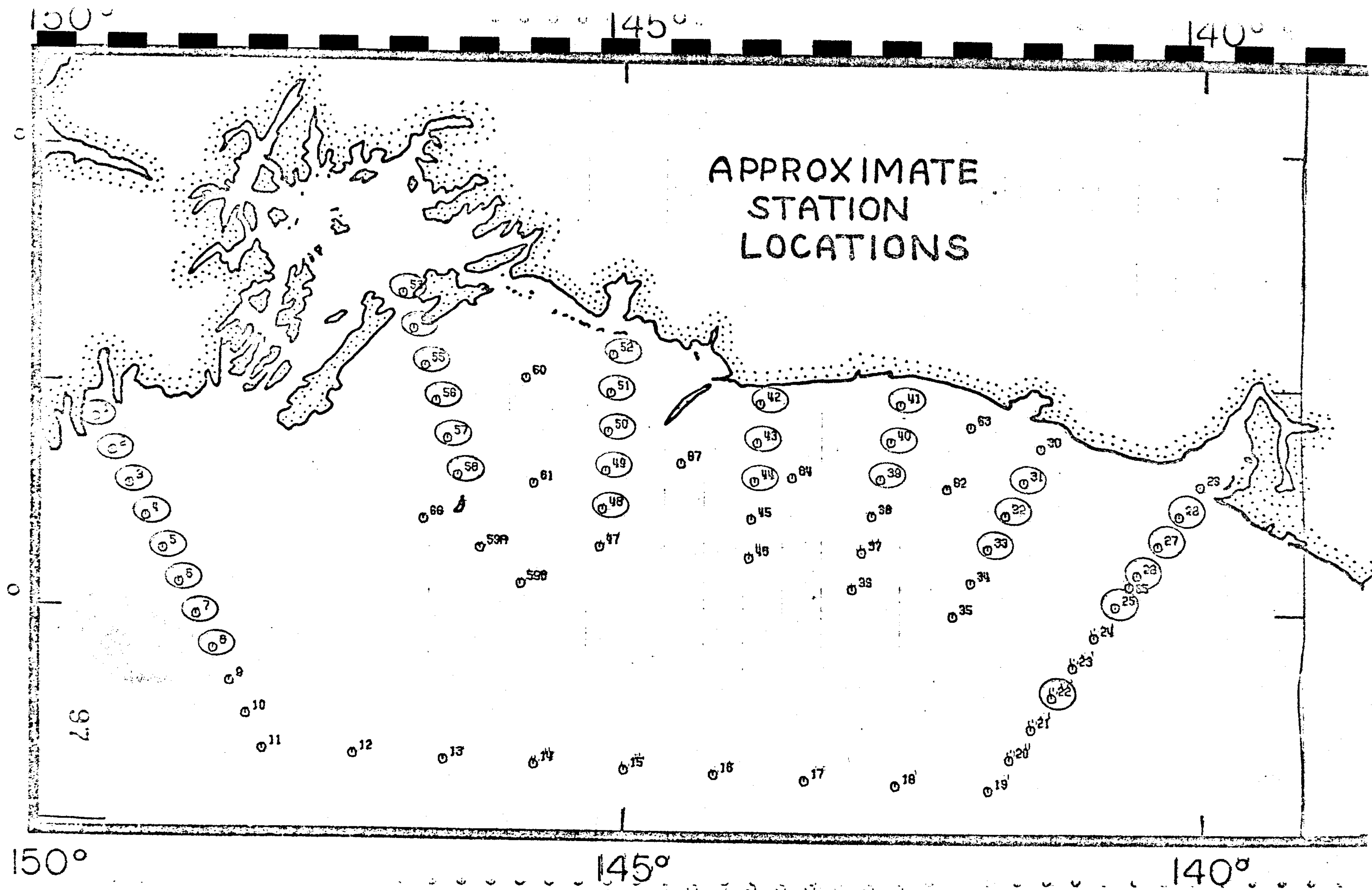

Figure 10. Haps core samples from standard stations in N.E. Gulf of Alaska.

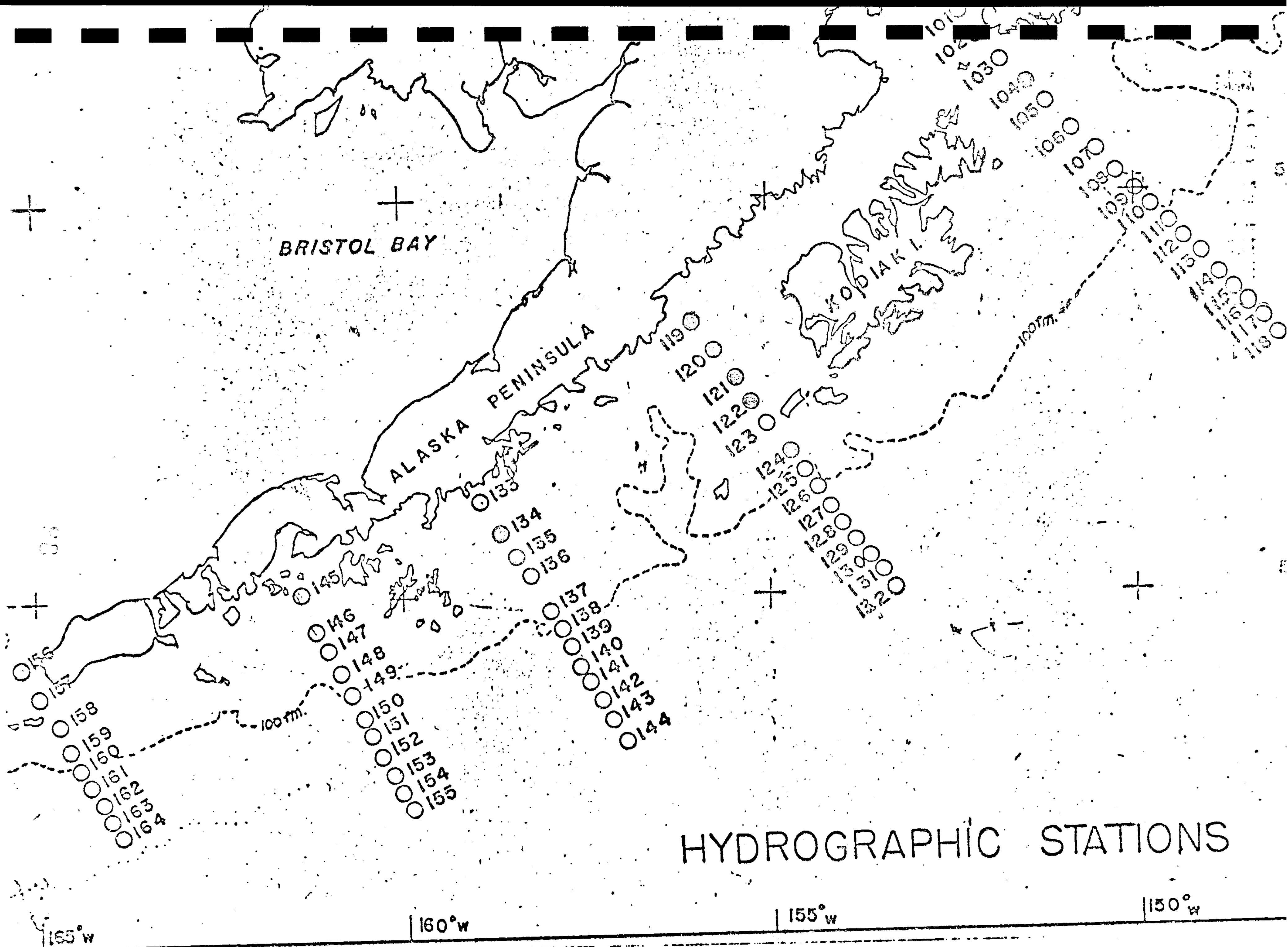

Figure 11. Haps core samples from standard stations in N.W. Gulf of Alaska.

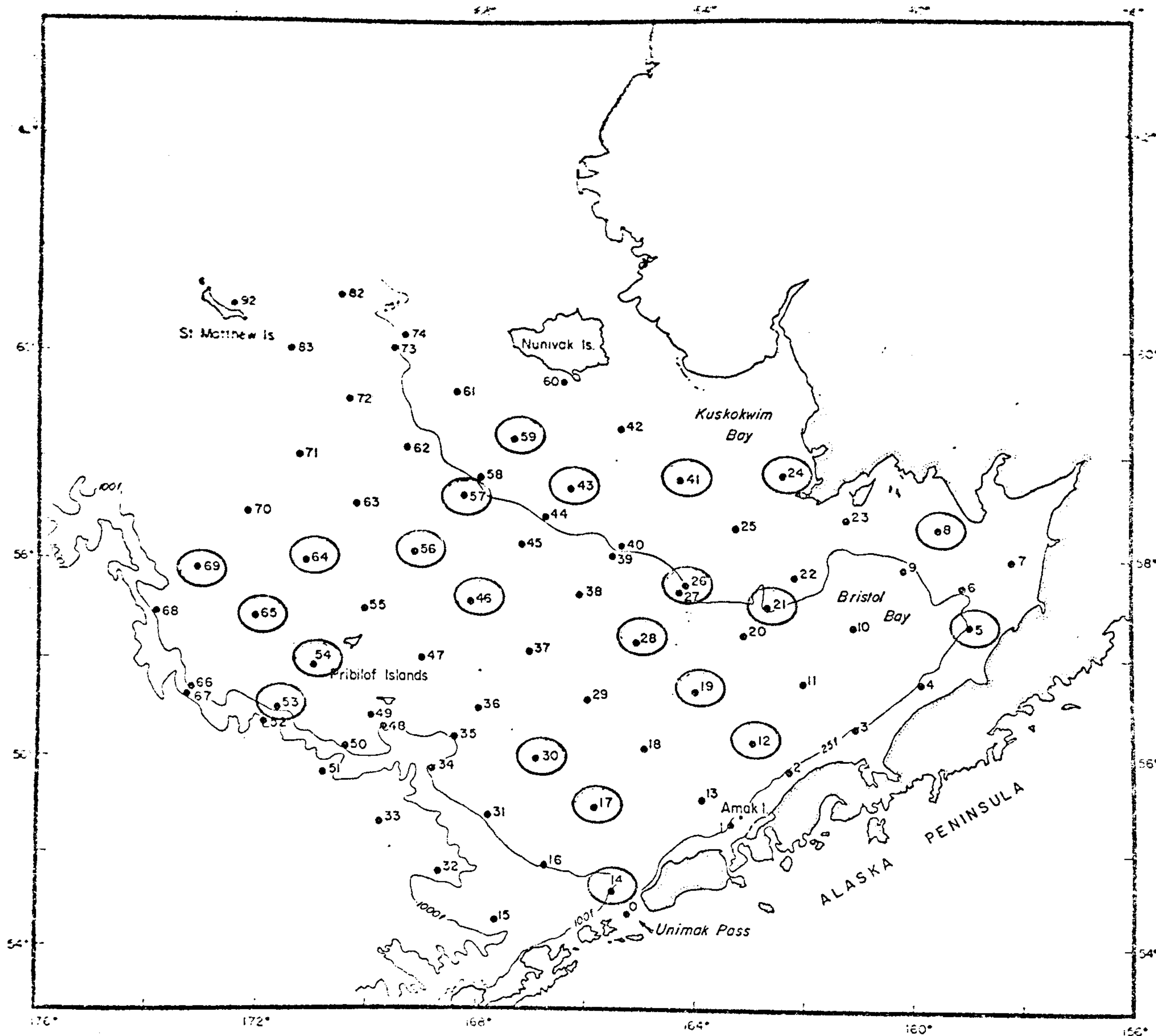

Figure 12. Haps core samples from standard stations in S. Bering Sea.

TABLE XXXII

N.E. Gulf of Alaska

Total mercury contents of surface sediment (μg/kg dry weight)
Discoverer November 23 - December 2, 1975

Stations	Hg(μg/kg)
2	67
5	33
7	49
8	35
25	31
26	30
29	23
30	11
33	39
39	65
44	33
48	46
49	57
50	60
51	50
52	52, 54
53	47
54	42
56	32
57	60
58	36
59A	59
60	57
61	45
62	28
63	49
68	55
69	52
110	19

TABLE XXXIII

Beaufort Sea

Total heavy metal contents of sediments (gravel free, dry weight)

Sample No.	Fe(%)	Mn(%)	Zn(μg/g)	Ni(μg/g)	Cu(μg/g)
Middle and Outer Shelf					
GLA71-1	2.18	0.028	102	45	28
GLA71-3	2.40	0.026	86	39	24
GLA71-12	2.80	0.034	110	53	39
GLA71-23	2.80	0.044	119	57	38
GLA71-25	2.55	0.050	111	55	35
GLA71-27	1.55	0.040	82	33	18
GLA71-44	2.22	0.059	91	45	25
GLA71-63	2.75	0.049	109	51	35
GLA71-71	2.12	0.037	97	49	60
GLA71-72	2.80	0.100	91	51	44
GLA71-78	3.55	0.063	111	66	47
GLA71-80	3.25	0.059	115	62	38
BSS-61	3.85	0.050	103	70	73
BSS-82	2.42	0.220	77	45	40
BSS-83	3.44	0.030	75	41	43
BSS-88	3.08	0.030	90	47	61
Inner Shelf					
70BS-18	1.88	0.023	93	27	11
70BS-19	1.70	0.018	30	17	7
70BS-21	2.05	0.035	93	40	24
70BS-22	1.65	0.027	84	37	24
71AJT-5	1.48	0.024	60	24	13
71AJT-16	2.15	0.026	89	40	26
71AJT-19	1.85	0.026	84	34	17
71AJT-20	1.70	0.032	100	43	26
71AER-15	1.85	0.031	91	43	24
72AJT-3	0.91	0.032	88	34	22
72AJT-4	0.90	0.033	95	40	23
72AJT-5	1.48	0.024	60	24	13
72AJT-6	1.43	0.027	60	22	10
72AJT-7	0.65	0.036	120	41	24
72AJT-8	2.28	0.042	115	51	32
72AER-129	2.40	0.031	108	45	30
72AER-134	1.20	0.025	115	52	39
72AER-137	0.98	0.015	38	19	10
72AER-166	2.58	0.024	58	40	22
72AER-167	1.33	0.020	93	32	18

Sample No.	Fe(%)	Mn(%)	Zn(μg/g)	Ni(μg/g)	Cu(μg/g)
72AER-168	2.68	0.027	108	43	28
PDB74-34	1.33	0.023	95	33	16
PDB74-38	1.85	0.028	95	32	11
PDB74-39	1.03	0.027	123	37	13
PDB74-40	1.20	0.022	79	22	10
PDB74-41	1.48	0.035	104	44	28
PDB74-43	1.26	0.029	123	25	12

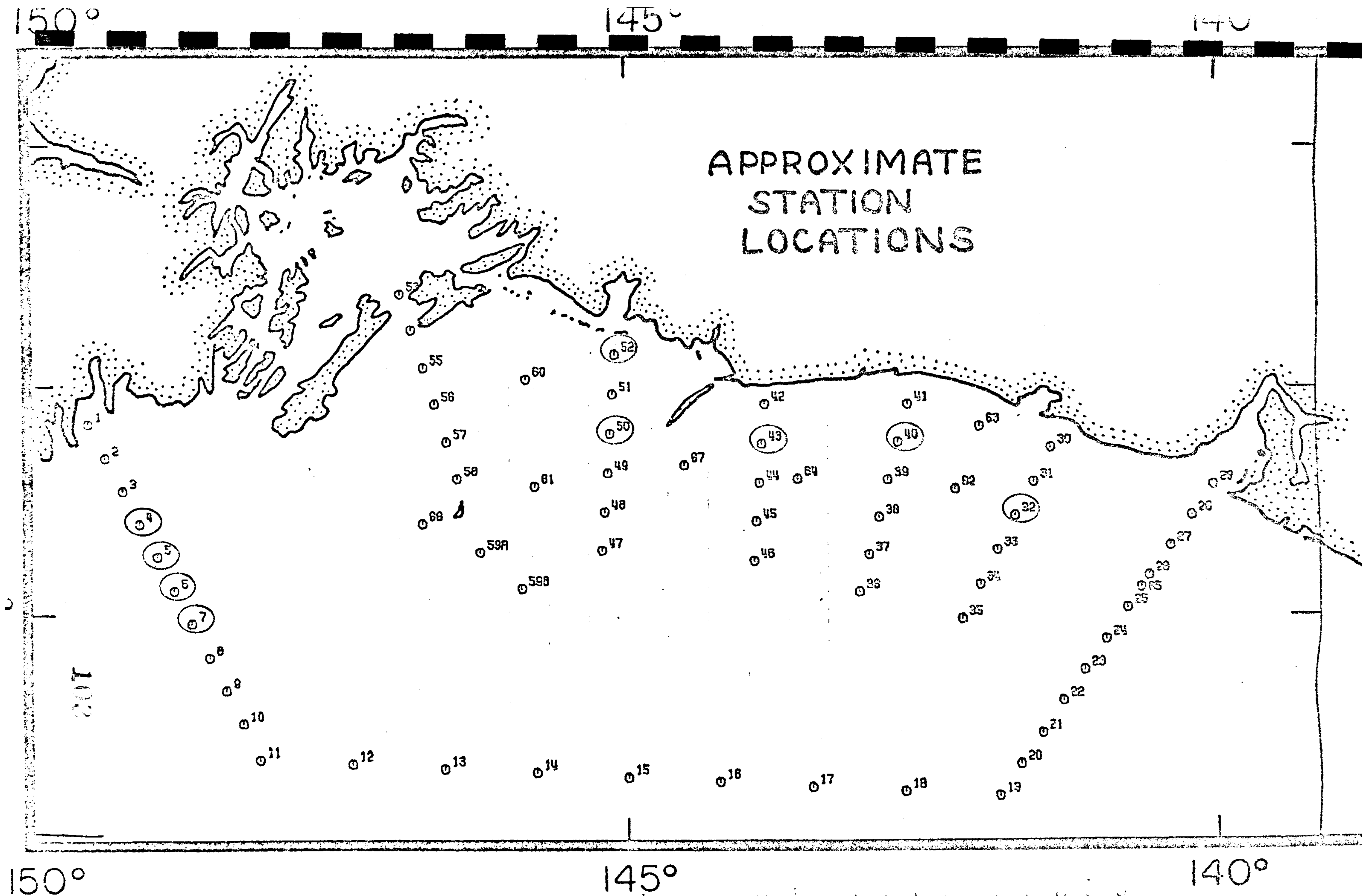

Figure 13. Map of the N.E. Gulf of Alaska, showing the locations of the sediment samples. As of this time only circled samples have been processed for clay mineral and granulometric analyses.

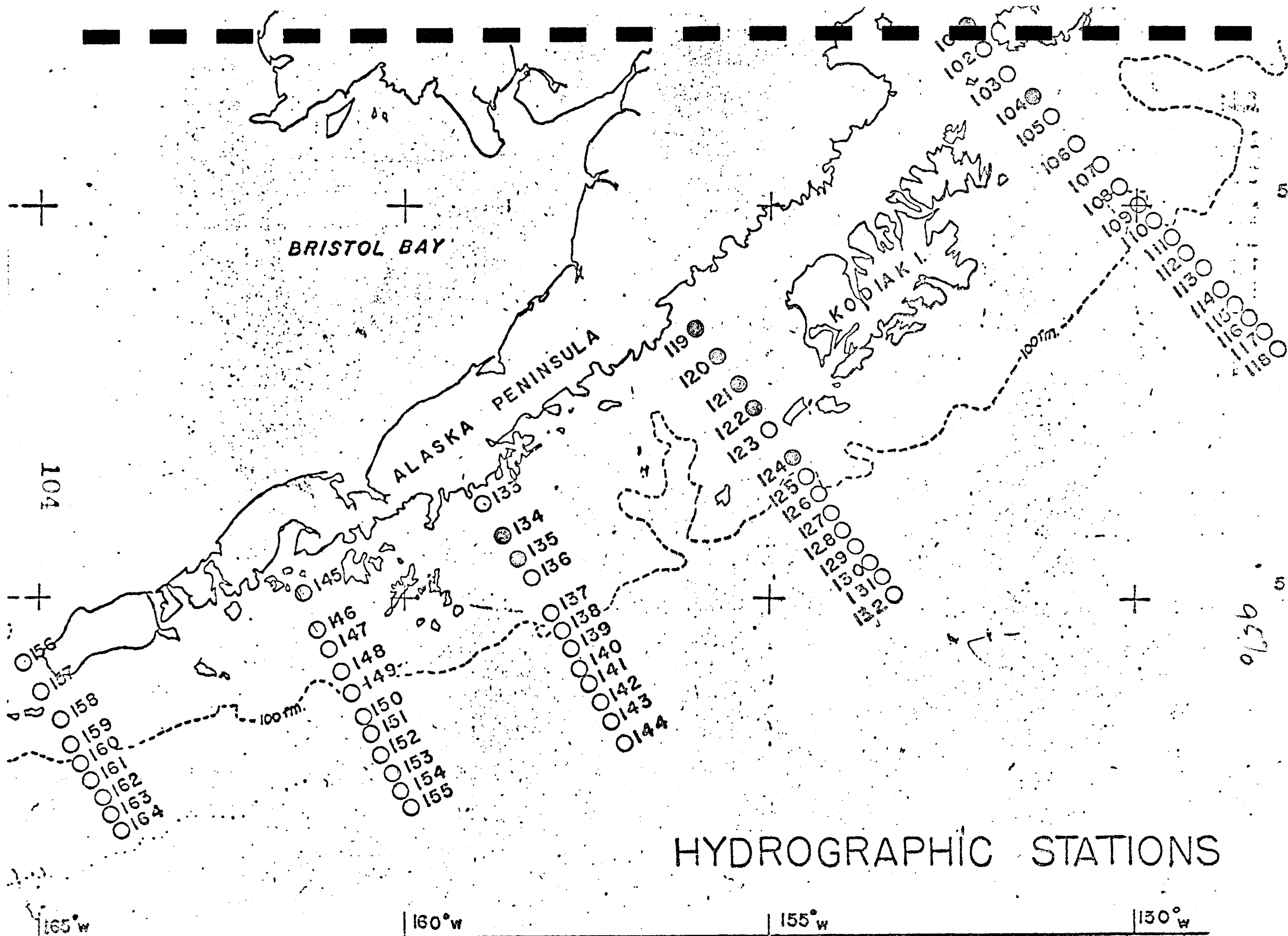

Figure 14. Map of the N.W. Gulf of Alaska, showing locations of sediment samples.

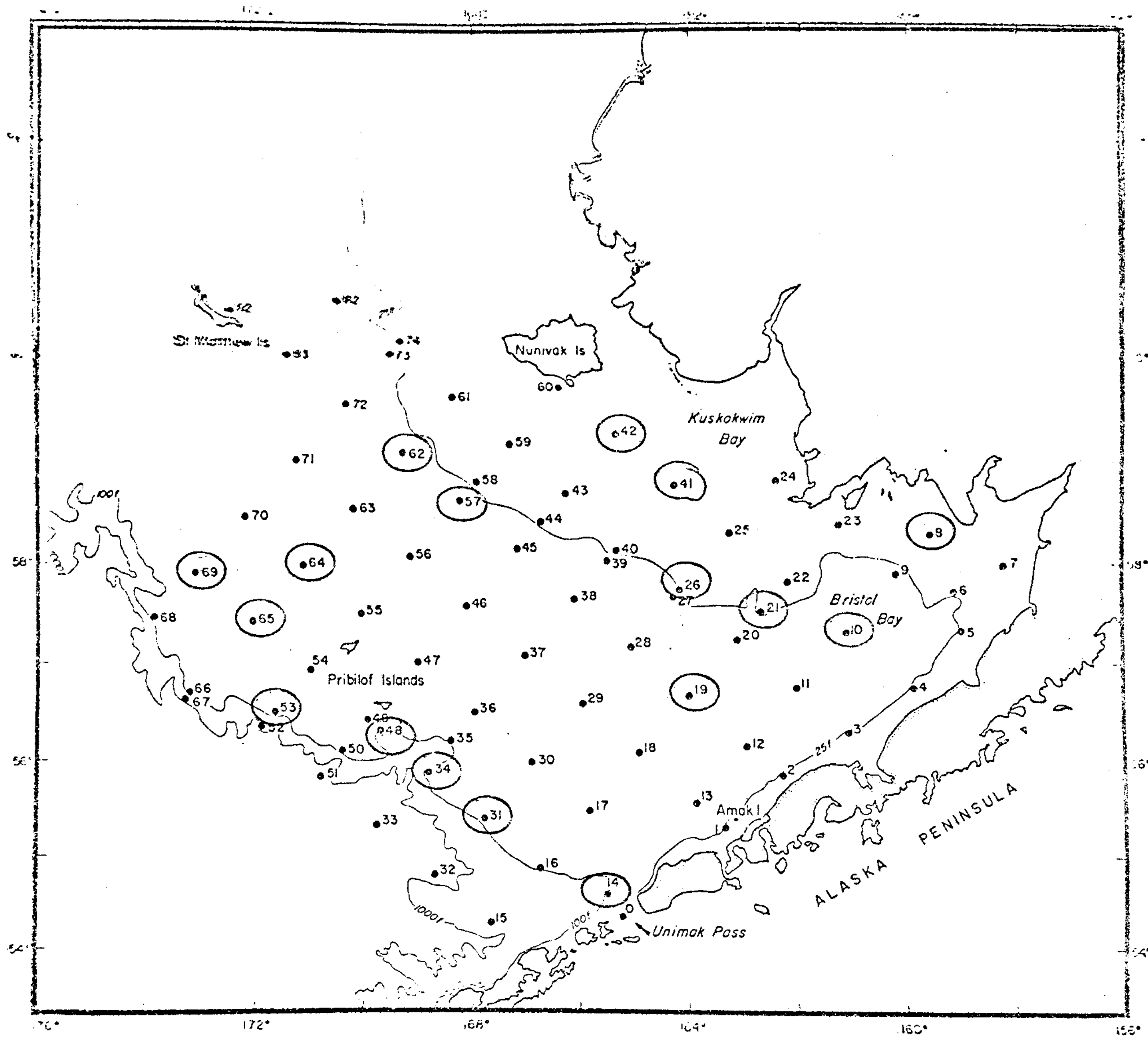

Figure 15. Locations of sediment samples from the S. Bering Sea. Only circled samples have been taken for clay mineral analyses as of this date (March 1976).

TABLE XXXIV

N.E. Gulf of Alaska

Sediment grain-size analyses (weight %)

Sample No.	Gravel	Sand	Silt	Clay
01	Tr	1.09	37.10	61.82
03	Tr	0.40	31.92	67.68
04	Tr	0.42	30.70	68.87
05	3.12	16.84	20.25	59.80
06	6.28	37.69	23.42	32.62
07	Tr	36.11	21.59	42.30
25	1.18	44.96	33.42	20.44
28	0.27	6.87	52.66	40.20
31	Tr	1.68	62.70	35.62
32	Tr	0.42	51.09	48.49
39	Tr	0.10	61.15	38.75
40	Tr	0.23	44.53	55.24
41	Tr	0.31	49.57	50.12
42	Tr	10.58	58.88	30.54
43	Tr	4.39	35.85	59.76
48	19.95	15.54	33.51	31.00
50	Tr	0.44	37.83	61.72
57	26.59	14.20	29.39	26.83
58	Tr	3.41	47.00	49.59

TABLE XXXV

N.W. Gulf of Alaska

Sediment grain-size analyses (weight %)

Sample No.	Gravel	Sand	Silt	Clay
101	10.95	89.05	Tr	Tr
104	16.48	63.32	5.54	14.65
119	21.73	20.93	27.61	29.67
120	0.19	0.33	43.19	56.29
121	Tr	1.40	55.80	42.79
122	9.32	90.68	Tr	Tr
124	9.65	57.25	20.34	12.76
131	Tr	26.26	38.01	35.73
135	1.31	63.72	18.47	16.50

TABLE XXXVI

N.E. Gulf of Alaska

Clay mineralogy of < 2μ sediment size fraction
(% weighed peak areas; see text)

Sample No.	Smectite	Illite	Kaolinite	Chlorite	Kaolinite / Chlorite	Illite / Smectite
01	Tr	41	Tr	59	0	41
04	4	41	Tr	55	0	10
05	6	40	Tr	54	0	7
06	5	39	Tr	56	0	8
07	8	38	Tr	54	0	5
32	1	37	Tr	62	0	37
40	8	41	4	51	0.1	5
43	4	38	Tr	58	0	10
50	5	36	3	56	0.1	7
52	2	39	4	45	0.1	25

TABLE XXXVII

N.W. Gulf of Alaska

Clay mineralogy of $< 2\mu$ sediment fraction
(% weighed peak areas; see text)

Sample No.	Smectite	Illite	Kaolinite	Chlorite	Kaolinite / Chlorite	Illite / Smectite
104	8	52	Tr	40	0	7
119	8	59	6	27	0.2	7
120	9	56	Tr	35	0	6
121	10	57	5	34	0.2	5
124	11	50	Tr	39	0	5
134	18	51	Tr	31	0	3
135	11	54	4	31	0.1	5

TABLE XXXVIII

S. Bering Sea

Clay mineralogy of < 2μ sediment fraction
(% weighed peak areas; see text)

Sample No.	Smectite	Illite	Kaolinite	Chlorite
8	45	10	17	28
10	43	19	12	26
14	26	31	6	37
19	26	34	10	30
21	25	33	8	34
26	24	38	10	28
31	25	35	11	29
34	28	33	12	27
41	19	37	12	32
42	33	30	8	29
48	30	35	0	35
53	26	31	11	32
57	15	41	10	34
62	25	33	10	32
64	22	45	Tr	33
65	14	52	6	28
69	23	36	12	29

A. Water

The caveats concerning the overall accuracy and precision of soluble heavy metal contents of sea water discussed above should be noted again at this point (see also the more detailed discussion given in the previous report; Burrell, 1975).

1. Cd, Cu, Zn, Ni, and Pb (D. C. Burrell)

The data presented here are directly comparable with those discussed by Burrell (1975). It should be noted that some of the values are for unfiltered and others for filtered water samples. In the latter case, particulates have been separated at the conventional 0.4 or 0.45 μm pore-size. Values for lead are instructive in this respect. Given the current state of the art, the multiplicity of techniques used and the general paucity of available data from other areas, it is not considered possible to compare these data with those from other coastal regions of the U.S. in any detail at this time. Certainly no unequivocal trends are apparent from these coarsely spaced grids. We have discarded some obviously suspect numbers, particularly from surface water samples where contaminations from the vessel might be expected. Undoubtedely other non-systematic error remains in some cases. However, these data fall well within the range of "accepted" values from open oceanic waters. There is no limit of anomolously high values and these coastal waters would appear to be quite pristine.

2. Se and Cr (T. A. Gosink)

Selenium data thus far are lower than average open mean values. Chromium values vary from non-detectable to those published for the average open ocean.

3. Other metals (D. Robertson, H. Weiss)

No data yet available.

B. <u>Biota</u>

Biota analysis to date has been confined to intertidal *Mytilus* and *Fucus* and sub-tidal crab and *Neptunea* samples; all benthic species.

It should be noted that all biota samples have been freeze-dried prior to grinding and weighing so that all data cited in this report are on a clearly defined dry weight basis. Much of the available literature data relates to wet weight and is therefore not directly comparable with our work (very approximately, wet weight values should be around an order of magnitude less than dry weight on equivalent samples).

It has not been possible to differentiate between the heavy metal contents of various functional parts of the organism. This is unfortunate since whole-organism analysis does not give a fair picture of the concentration of metals, or hence potential harm resulting from this. It was not possible to dissect the samples received for this study.

The two principle indicator species used for this study, *Mytilus* and *Fucus*, have both proved to be sensitive indicators of environmental pollution in various parts of the world. This was discussed, with examples, in last years report (Burrell, 1975). Various crab species have also been commonly used and were similarly shown to be useful from the initial data.

1. <u>Cu, Cd, Ni, and Zn</u> (D. C. Burrell)

We have improved the precision of the nickel analysis over last year and can now report contents in excess of 5 ppm. The *Mytilus* zinc data (Table XXV) are much lower than those reported for last year. The reason for this is unknown at the present time. There has been a slight change in the preparation sequence (e.g. we now remove the shell after freeze drying rather than before) but this should not cause any major deviations.

As a generalization, the heavy metal contents of the crab, *Mytilus* and *Fucus* samples are as low or lower than data reported from elsewhere. Obviously the latter values are biased in favor of polluted areas. Certainly these Alaskan samples would appear to be chemically unperturbed.

The *Neptunea* data is of interest inasmuch as high contents of Cu and Zn, and very high contents of Cd appear to be present. These data were obtained very recently and we have not yet compared them with values from other areas. The analysis have been repeated in our own laboratories but have not been checked by outside investigators. It is possible that these particular samples were contaminated by the collectors or during storage.

2. Other elements (T. A. Gosink, D. T. Robertson)

Data not yet available.

C. Sediment

So far data for the sediments from the Gulf and Bering Sea are as yet available, it is not considered to be possible to discuss this work in any meaningful fashion at this time. A few values for mercury from the Gulf and some Cr, Se numbers for particulate sediment have been given above.

1. Total Fe, Mn, Zn, Ni, and Cu in Beaufort Sea sediments (A. S. Naidu)

Generally, the inner shelf sediments have relatively smaller concentrations of Fe, Mn, and Cu. When the heavy metal data on the middle and outer Beaufort Sea shelf sediments of this study are compared with those reported earlier by Naidu and Hood (1972), broad regional variations in some of the metal concentrations are discerned. It would seem that in the western shelf of the Beaufort Sea the average concentrations of Mn and Cu are relatively higher than in the central shelf.

In the absence of statistical data showing the interelement correlations, as well as concentrations of Fe, Mn, Cu, Ni, and Zn in the nonlithogenous

sediment components (relatively more "mobile" phase), it would seem premature at this time to discuss the overall geochemistries of the metals. Organic carbon and carbonate contents have been analyzed on all sediments under a different study. This latter data will be used for understanding of the partition patterns of the heavy metals in the biogenous clastic and carbonate sediment phases.

2. Granulometric and clay mineral compositions of sediment from the Gulf and Bering Sea (A. S. Naidu)

Analyses of the selected group of random sediment samples indicated that the deposits of the central Gulf are generally silty clays, with subordinate amounts of sands and trace contents of gravels. In the N.W. Gulf, relatively higher amounts of gravels are documented. The gravel fraction of sample 120 consisted totally of shell material.

In general, the N.E. Gulf samples have relatively lower smectite and illite, and higher chorite percents than the Aleutian shelf samples (Tables XXXIV and XXXV. In addition to clay minerals, most Gulf samples contain notable quantities of quartz, feldspars, and amphibole. Sample #52, appears to have relatively higher concentrations of illite, accompained by relatively lower chlorite contents. Generally speaking, the sediments of the Gulf of Alaska have a marked paucity of kaolinite, and the kaolinite/chlorite ratios are notable lower than most low-latitude shelf sediments.

Most of the Bering Sea clays displayed poor basal peak reflections on X-ray diffraction traces, indicating possible presence of inorganic smorphous material in amounts larger than those usually encountered in other shallow-water marine deposits of Alaska. The clay mineral assemblages in these sediments consist predominantly of chlorite and illite, with subordinate amounts of smectite and kaolinite (Table XXXVIII). As of this report writing,

under the term smectite are included all expandible clay minerals, including true smectites, possible degraded chlorites and illites, and possible mixed-layered expandible clay mineral species. In addition to the said clay minerals, the southeastern Bering Sea clays have significant amounts of feldspars.

From the preliminary data, some broad trends in the distributional patterns of clay mineral assemblages in the Gulf of Alaska sediments have been deciphered. The regional difference observed between the clay minerals contents in the central and southwestern (Aleutian) Gulf of Alaska, most likely reflects the differences in the primary terrigenous source material of these clays, i.e. hinterland geology of the respective regions. The relatively higher concentrations of chlorite in the central Gulf is probably related to the abundant presence of shales and grawacke in the immediate continental provenance. Likewise, the slightly higher contents of smectite in the Aleutian area of the Gulf presumably reflect products resulting from possible interactions between terrigenous volcanic detritus and sea water. The Aleutian Islands are known to be constituted chiefly of alkaline volcanics. From the presence of relatively low "d" reflection (002) basal peaks, it would seem most of the "illite" in the Gulf of Alaska sediments is very likely a weathered product of trioctahedral mica (e.g. biotite). In addition to source, the nature of weathering prevailing in the hinterland is also reflected in the overall composition of the clay mineral assemblages in the Gulf of Alaska sediments. The near absence of kaolinite and the low kaolinite/chlorite ratios in these sediments, suggest continental source rocks that have been subjected to minimal chemical weathering. This contention by us is further supported by the detection of notable amounts of hornblende and feldspars, with relatively little quartz in the clay fraction of the sediments.

Such a mineral assemplage is considered typical of glacially weathered material, based upon studies conducted on fjordal sediments of southeast Alaska (Kunze *et al.*, 1968).

Our results agree in most cases with other studies of clay mineralogy done in the Gulf of Alaska, and tend to substantiate the scheme of latitudinal variations of clay minerals as suggested by Griffin *et al.* (1968). The notable disagreement between our results and those of Molnia and Fuller (1975) relates to the concentrations of kaolinite and smectite. The relatively larger contents of smectite documented by us most likely relates to the slight differences in the analytical techniques followed by us and Molnia and Fuller (1975). Our clay samples, unlike those of the latter authors, were not saturated with Mg solutions prior to X-ray diffraction analyses. This may have caused any such degraded chlorites and illites, and/or degraded mixed-layered chlorite and illite species present in our clays to apparently assume the "d" basal spacings characteristic of smectite, subsequent to glycolation. We should be able to confirm this, as our ongoing work calls for X-ray analyses of Mg and K saturated clays. However, the observed differences in the kaolinite concentrations between our studies and those of Molnia and Fuller (1975) can not readily be explained at this time, because we are not sure what method was followed by the latter authors to quantify kaolinite.

The poor "d" basal reflection peaks on X-ray diffractogram traces of southeastern Bering Sea sediments suggest presence of significant contents of inorganic amorphous material in those sediments. Notable amounts of diatom tests, presumably constituted of amorphous opal, have been discerned in the sand and silt-sized particles of southeastern Bering Sea sediments.

Therefore, it would seem quite reasonable to assume that significant amounts of the said test fragments most likely would also be present in the clay-sized particles of the above sediments. This would obviously tend to significantly dilute the quantity of crystallized particles.

There are significant differences between the clay mineral compositions of the southeastern Bering Sea sediments and those of the adjacent N.W. Gulf of Alaska. The latter suite of sediments have relatively larger quantities of illite, and there is a relative paucity of smectite and kaolinite. These differences obviously suggest that the clay minerals in the southeastern Bering Sea sediments are not predominantly derived from the Aleutian Volcanics; the most likely source of the said minerals rather seems to be the Kuskokwim-Nushagak basin region. On the basis of limited number of analyses available, it would seem premature at this time to make attempts to define presence of any definite distributional trends in clay mineral types in the southeastern Bering Sea.

VIII. CONCLUSIONS

Only a few tentative comments can be included here because so few analytical data are available at this intermediate stage in the investigation. For the metals analysed so far, soluble contents in the Gulf and Bering Sea waters appear to be as low or lower than in other coastal regions, and this comment applies equally to the benthic biota studied to date. As would be expected, the Alaskan shelf environments are quite pristine and any future anthropogenic perturbations should be detectable, and might seriously impact various ecosystems; for example, in no area studied has there been any mild but chronic impact which might have helped

acclimatize the biota to future industrial activity. Of the biota samples collected to date for this study, *Mytilus, Fucus, Neptunia* and crab would appear to be highly suitable index species. Future studies must focus on near-shore reactions and environments; some specific suggestions are given in the following section.

IX. NEEDS FOR FURTHER STUDY

The current year's program has largely been dictated by the available sampling programs; i.e. science has been tailored to logistics. We have obtained a good geographical coverage for water and sediment work in the lease areas, except, of course, in the Beaufort Sea. Initial results suggest that there should be no real need to continue water-column sampling in the same fashion in these open-ocean areas. Similarly it is possible that sufficient sediment samples have been collected to characterize the heavy metal distributions in both the Gulf and South Bering Sea. These analyses have not yet been completed however, and correlations with mineralogy and grain size distributions will be required before any potential anomalies can be identified.

The major need at the present time is for work related to the coastal zone. It is this area which is likely to receive the major impact from oil development activity and any impingement here might be expected to result in more obvious and economically damaging proglems that in the open-ocean regions. More importantly, from the point of view of this present study, it is these regions which form the major repository for terrestrially derived heavy metals as discussed in the introductory sections above. Figure 16, for example, documents the progressive decrease in

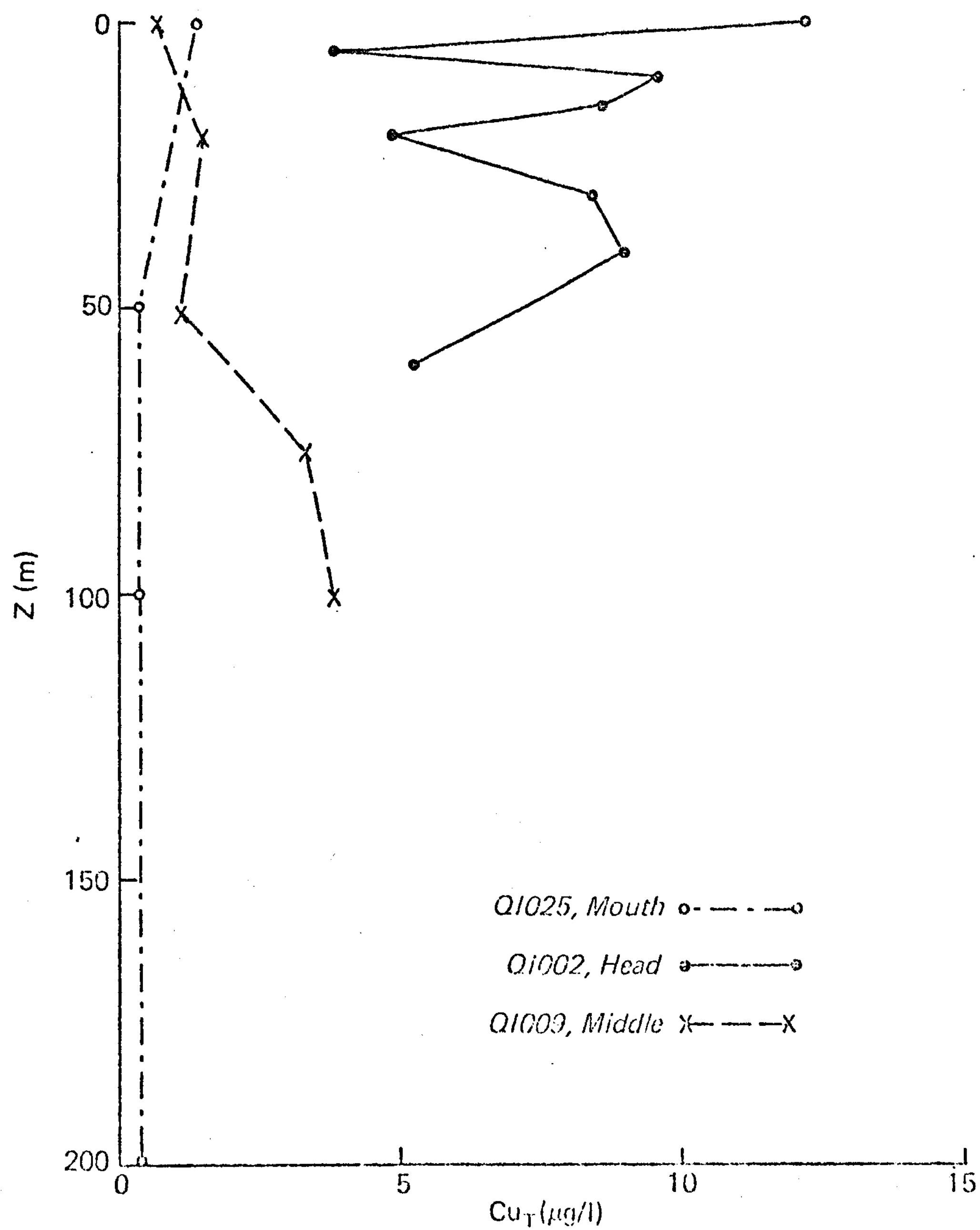

Figure 16. Progressive decrease of total copper seaward in a S. Central Alaskan fjord.

total copper seaward in an Alaskan fjord; this reflects, of course, the partioning of this metal on the suspended sediment which progressively decreases in amount away from the fresh-water source. Given the length of the coast-line under consideration - something in excess of the coastline of all the contiguous states combined - we propose work on "type areas" chosen to be representative (as far as this term can have any meaning) of each coastal "system".

Of primary importance would be disgnation of a fjord-estuary in South Central Alaska, both because of the obvious direct impact of industrial staging areas, transportation routes and potential pipelines, but - of equal or greater importance - because of the influence of open-ocean-Gulf waters on these particular estuaries. The Gulf of Alaska is dominated by the well known Aleutian low pressure system in the winter. During the summer months an intense high pressure system is frequently formed further south. Royer (1976) has shown that, during the winter, on-shore surface transport occurs which leads to down-welling conditions at the continental margins (which may be locally enhanced because the prevailing winter wind directions are down-fjord, seaward). Through the oceanographic summer period, probably as a result of the relaxation of the conditions described above, upwelling of deeper Gulf water onto the shelf occurs. We have described in considerable detail how parcels of this latter water may, periodically (particularly during late summer), penetrate directly into the fjords (Heggie and Burrell, 1973, 1974). It may be seen therefore, that there is considerable impact from open ocean waters, at various depths, on the fjords throughout the year and that pollutants discharged on the shelf could well be carried into these estuaries to remain trapped for a period

below sill depth. Fjords which open directly onto the Gulf would be prererable study sites for these processes; we recommend Resurrection Bay as a suitably located type-area.

We propose also the need for base-line data on one of the lagoon environments of the South Bering Sea; not least because of the tremendous importance of many of these to migrating water fowl. This type of large surface area, shallow coastal environment could be seriously impacted in the event of a major oil spill. We sould propose Izembek Lagoon as a highly suitable study area. Because of the intense macrophyte productivity in this locality, the sediment surface is already delicately poised between an oxic and anoxic state and oil impact could cause major environmental damage.

Two further objectives concerning the "inorganic" system should be pursued in future years. Both were proposed for the current year but problems, mainly logistic, prevented this. We believe that the river plume areas should be specifically looked at, and the obvious candidates here are the Copper and Kuckokwim Rivers (and also the Yukon when this program is extended further north). These are all major rivers by any standard; only remoteness and the diversion of limited resources to rivers in more populous areas has stymied much needed work to date. Now with the possibility of direct pollution in these plume areas, knowledge of inflow characteristics and of the geochemical bejavior of heavy metals in the mixing zone is urgently required. For this specific program we would propose first a survey of the distribution of trace metals in the suspended and deposited sediments of the outflow areas (together with sedimentological

parameters as before) and the distribution trends (i.e. the degree of "conservativeness") of selected trace metals from the fresh to marine water environments.

It is imperative to continue monitoring the heavy metal contents of the biota. This is the most important part of this overall program as far as impact monitoring is concerned. We have proposed various index species previously but have encountered various problems in obtaining the needed samples as explained above. We feel that it is probably necessary to place our own personnel on the various biota sampling excursions in order to obtain the needed samples in a form suitable for trace metal analysis. In any case this portion of the program must be continued and ought to be extended. Obviously dominant species and distributions vary widely over these vast areas and some of the species flagged by the biologists as being of prime importance have not yet been collected at all. Most of these latter have been higher trophic level food organisms. We would propose also to look at the primary producers, the plankton, since these extract chemicals directly from the water and also constitute the major biomass. It is likely that the plankton would be the most sensitive indicator of stress on the biosphere. It should be noted also that concentrations of pollutant metals are almost invariably depleted up the food chain.

Finally, as a lower order priority, it would be useful to look at temporal variations of dissolved metals in the water column, although such a study would require considerable resources and has not been attempted elsewhere. Using neutron activation or polarographic analysis for a few selected elements, the potentiality exists for obtaining precise enough data to make such a study meaningful. The goal here is to increase our

understanding of the kinetics of heavy metals in the marine environment. We would suggest initially several profiles at one standard station on the Gulf shelf seaward of Resurrection Bay. This would tie in both with the proposed fjord study and with the ongoing physical oceanographic program.

X. SUMMARY OF FOURTH QUARTER OPERATIONS

I. Field Work

No field work was undertaken during the January-March 1976 quarter. All the samples required for this program have now been obtained except for:

a. Beaufort Sea

b. Se/Cr samples from the NE Gulf

c. Sub-tidal biota samples from all areas

d. Intertidal biota samples from the Bering Sea

II. Laboratory Work

All laboratory work is progressing satisfactorily and is on schedule. Work on most of the biological samples has now either been completed or has been cancelled because of thawing of the samples noted below. We are attempting to obtain replace samples from the Bering Sea on an upcoming cruise. All the equipment required for this program has now been installed and is operating.

III. Results

All data obtained prior to March 1, 1976 have been incorporated in the Annual Report. All the work scheduled for polarographic, atomic ab-

sorption and gas chromatographic analysis is progressing on schedule. Samples have been prepared for neutron activation analysis but have not yet been analysed except for the Hg in sediment samples given in the annual report.

IV. Problems encountered

a. At the time of writing of this report, the sub-contracts external to the University of Alaska had not been finalized

b. There appear to be problems scheduling Dr. T. Gosink for Se/Cr work in the N.E. Gulf of Alaska

c. We have not yet received any inter-tidal biota samples from the Bering Sea

d. Most of the sub-tidal samples received from trawl cruises in the Gulf of Alaska and Bering Sea have been lost because of a power failure which allowed the samples to thaw. We are attempting to schedule personnel and cruises to replace these

e. No suitable logistic support has been provided to enable us to obtain samples from the Beaufort Sea.

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OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 12 R.U. NUMBER: 162/163/288/293/312

PRINCIPAL INVESTIGATOR: Dr. D. C. Burrell

Submission dates are estimated only and will be updated, if necessary, each quarter. Data batches refer to data as identified in the data management plan.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]			
	From	To	Batch 1	2	3	4
Discoverer Leg II #808	6/2/75	6/19/75	4/20/76	5/15/76	None	4/20/76
Silas Bent Leg I #811	8/31/75	9/14/75	None	None	None	None
Discoverer Leg IV #812	10/8/75	10/16/75	6/30/76	6/30/76	None	6/30/76
Miller Freeman	8/16/75	10/20/75	None	None	Unknown	None
Discoverer Leg III #810	9/12/75	10/3/75	None	None	None	None
North Pacific	4/25/75	8/7/75	None	None	Unknown	None
Intertidal Biota		1975	None	None	Unknown	None
Discoverer #816	11/12/75	12/2/75	9/30/76	6/30/76	None	6/30/76
Contract 03-5-022-34	Last	Year	4/20/76	None	None	None

Note: [1] Data Management Plan has been approved by M. Pelta, we await approval by the Contract Officer and receipt and approval by all parties of the necessary Data Format.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]			
	From	To	Batch 5	6	7	8
Discoverer Leg II 808	6/2/75	6/19/75	4/20/76	None	None	None
Silas Bent Leg I 811	8/31/75	9/14/75	None	None	None	None
Discoverer Leg IV 812	10/8/75	10/16/75	6/30/76	6/30/76	None	None
Miller Freeman	8/16/75	10/20/75	None	6/30/76	6/30/76	6/30/76
Discoverer Leg III 810	9/12/75	10/3/75	None	6/30/76	None	None
North Pacific	4/25/75	8/7/75	None	6/30/76	6/30/76	6/30/76
Intertidal Biota		1975	None	6/30/76	6/30/76	6/30/76
Discoverer 816	11/23/75	12/2/75	6/30/76	None	None	None
Contract 03-5-022-34	Last	year	4/20/76	None	4/20/76	4/20/76

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]			
	From	To	Batch 9	10		
Discoverer Leg II 808	6/2/75	6/19/75	6/30/76	5/15/76		
Silas Bent Leg I 811	8/31/75	9/14/75	6/30/76	5/15/76		
Discoverer Leg IV 812	10/8/75	10/16/75	6/30/76	5/15/76		
Miller Freeman	8/16/75	10/20/75	none	none		
Discoverer Leg III 810	9/12/75	10/3/75	none	none		
North Pacific	4/25/75	8/7/75	none	none		
Intertidal Biota		1975	none	none		
Discoverer 816	11/23/75	12/2/75	6/30/76	5/15/76		
Contract 03-5-022-34	Last	year	4/20/76	none		

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 12

PRINCIPAL INVESTIGATOR: Dr. David C. Burrell

Period April 1, 1975 - March 31, 1976* (12 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	158,353.00	71,669.21	86,683.79
Staff Benefits	26,750.00	11,963,19	14,786.81
Equipment	42,450.00	34,757.46	7,692.54
Travel	18,869.00	6,042.79	12,826.21
Other	143,228,00	118,386.28	24,841.72
Total Direct	389,650.00	242,818.93	146,831.07
Indirect	90,577.00	40,994.79	49,582.21
Task Order Total	480,227.00	283,813.72	196,413.28

* Preliminary cost data, not yet fully processed.

Following is part 2 of the quarterly report R.U.# 162 for the period ending December 31, 1975. This was received after the printing of the Quarterly Reports, July - September 1975, therefore is included here.

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NEGOA

Quarterly Report for Quarter Ending December 31, 1975

Chem & Micro. R.U. 162 163 288 293 312

Project Title: Natural Distribution of Trace Heavy Metals and Environmental Background in Three Alaska Shelf Areas

Contract Number: 03-5-022-56

Task Order Number: 12

Principal Investigator: Dr. David C. Burrell

I. Task Objectives

The primary objective of the program is to characterize the trace metal contents of sea water, sediment and selected indigenous animal and plant species in the three defined study areas: the Gulf of Alaska, the Bristol Bay Basin region of the Bering Sea and the Beaufort Basin region of the Beaufort Sea, as defined in the above referenced Task Order Work Statement. The program also incorporates sediment grainsize analysis, clay mineralogical projects and "previous work" literature searches as described in that work statement.

II. Field Activities

Field sampling has continued as a first-order priority during this quarter.

A. Bering Sea

Miller Freeman

Trawl data samples collected by personnel associated with the biological programs.

B. N. E. Gulf of Alaska

Discoverer Leg III - Nov. 23 - Dec. 2

This cruise has completed one sampling program in this area with the exception of specialized samples for Se and Cr. The specific objectives of this cruise were:

1. To collect contamination-free sediment samples from those standard GAS stations which had not been occupied on previous cruises in this area.

2. To collect contamination-free trace water samples for trace metal analysis concurrently for all the laboratories participating in the program and for NBS.

C. N. W. Gulf of Alaska

Discoverer Leg IV - October 8-16

All sections of the standard GASSO grid were occupied, except for the Cook Inlet section; this latter was cancelled due to bad weather. Water samples were collected for all the sub-projects of this program. Haps core sediment samples were retrieved from only eight stations, however, because of poor bottom conditions.

D. Beaufort Sea

No suitable logistic support was available for field sampling in this area during this quarter.

III. Results

A. Sample collection and storage

Efforts have continued to coordinate and standardize field sampling techniques. It is intended to standardize sediment sampling for subsequent trace metal analysis _via_ a Haps-style stainless steel corer. In the future, any data reports from samples not collected in this fashion will be suitably flagged. All water samples for trace metal analysis to date have been collected in NOAA-provided 10 l, drop-top Niskin bottles; pre-washed or otherwise treated. It is recognized that these samples are not entirely ideal but all other extant custom designs are presently only suitable for surface water sampling; no other means exist for retrieving water from depth. We have discussed this problem with many other laboratories nation-wide and Dr. Patterson (Cal Tech), for example, will not endorse use of this type of sample for soluble lead analyses.

Wherever possible, the outer "trimmings" of the Haps cores are being used for sedimentological and mineralogical analysis. Biota samples are collected for us by personnel associated with other OCS programs. Although handling and storage methods have been specific, we have no control over these operations.

B. Intercalibration

On the _Discoverer_ Leg III E. Gulf cruise various sets of intercalibration samples for water analysis were collected. We are continuing to collect water and sediment samples for NBS also.

C. Biota Collection

We have now presumably received all of the biota collected for us during the previous sampling season. One set (ten sites) of intertidal samples collected from the Gulf beaches during Aug./Sept. has been processed and "cuts" of each sample (_mytilus_ and _fucus_) we have supplied to the Batelle N.W. Labs. No intertidal samples from the Bering or Beaufort Seas have been received.

The status of trawl biota samples received, to date, is shown on Table I.

D. Neutron Activation Analysis Program

All sediment and water samples collected from the Gulf and Bering Sea have been processed and are ready for irradiation analysis. Not all the biota samples have yet been distributed and no samples are available for the Beaufort Sea.

E. Soluble Cd, Cu, Pb, and Zn

Samples collected on the _Townsend Cromwell_ cruise (see July - Sept., 1975 quarterly report) have been analyzed. Preliminary data are given in Table II. Initial Pb values were anomolous and all numbers for this metal have been rejected. Samples collected on subsequent cruises are currently being worked on.

F. Cd, Cu, Ni, and Zn in Biota

Data for these elements in _fucus_ from the Gulf of Alaska (see under C. above) are given in Table III. These values, as for all data in this report, are preliminary. Hg contents of the crab samples discussed in the 1975 - 76 Annual Report are given in Table IV.

G. Soluble Se and Cr

Cr values for the Bering Sea (_Discoverer_ Leg III; see previous quarterly report) are given in Table V. Due to equipment problems, it was not possible to analyse for Se at sea. These samples have been stored and will be processed if no losses have occurred. Cr and Se values for samples collected on the N. W. Gulf GASSO grid (_Discoverer_ Leg IV; see above) are given in Table VI. These samples were analyzed at sea immediately following retrieval. Filtered samples are for a 0.45u membrane.

H. Clay Mineralogical Analysis

A suite of 20 samples from the Bering Sea are now being analyzed. These samples are splits from the trace metal core samples and have also been close to tie in with ancillary benthic biology and sediment size analysis programs.

IV. Problems Encountered

A. One sub-contract has now been finalized, but another is still being negotiated. The lengthy period of time required to finalize these sub-contracts has hindered the work of the participating scientists.

B. One of the senior associate investigators resigned from the program during this quarter. This has necessitated changes to the work statement which are still being negotiated.

C. No suitable logistic support has been available for field work in the Beaufort Sea. We have requested modifications to the contract which would enable us to work on archived samples.

D. We have encountered considerable difficulty in obtaining, at short notice, the specialized technical help required for certain aspects of this program.

E. The work on this contract has been hampered because of cruise staging out of unsuitable ports. As an example, the Se and Cr analysis work suffered because of our inability to ship emergency supplies to Kodiak and some aspects of the last eastern Gulf cruise had to be cancelled because of problems in shipping supplies to Juneau and Yakutat.

TABLE I

Trawl biota samples received.

A. Species specified in work statement.

	Gulf	Bering		Beaufort
	"North Pacific" Cruise	"Miller Freeman" Cruises		
		#1	#2	
Pollack	X	X	X	n.a.
Rock sole	X	X	X	n.a.
Arctic cod	n.a.	n.a.		0
Tanner crab	X	X	X	n.a.
King crab	0	X	0	n.a.
Neptunea	X	X	X	n.a.
Scallop/Macoma	0	X	0	n.a.

B. Collected species not specified in work statement.

	Gulf	Bering #1	Bering #2	Beaufort
Turbot	X			
Molpodia	X			
Spisula		X		
Serripes			X	

TABLE II

Souble Heavy Metal Contents (ug/1)
N. E. Gulf of Alaska
Townsend Cromwell
May 6 - 21, 1975

Station No.	Depth (m)	Cd	Cu	Zn
01	0	0.20	0.24	1.1
04	0	0.07	0.36	5.8
07	0	0.05	0.27	4.6
	175	0.03	0.38	1.1
	220	0.03	0.16	1.1
10	0	0.03	0.21	2.5
	140	0.06	0.11	0.8
	250	0.08	0.30	3.6
13	0	0.04	0.22	2.2
	50	0.04	0.24	1.4
	120	0.05	0.22	1.6
16	0	0.06	0.26	3.2
19	0	0.04	0.28	2.5
	100	0.03	0.32	1.5
	200	0.04	0.32	0.9
22	0	0.03	0.49	--
	100	0.04	1.10	3.6
	160	0.05	0.37	2.6
25	0	0.12	0.65	4.0
28	0	0.03	0.40	1.8
	130	0.03	0.30	2.0
	150	0.10	0.23	0.5
36	0	0.09	0.60	--
	110	0.04	0.30	1.3
	200	0.06	0.27	0.9
40	0	0.06	0.35	1.5
	60	0.04	0.34	1.7
	140	0.02	0.28	0.6
46	0	0.03	0.16	0.6
47	0	---	0.21	0.5
	130	---	0.15	0.6
	280	---	0.16	1.9
48	0	0.08	0.24	1.7
	150	0.02	0.30	1.4
	210	0.10	0.23	2.0
49	0	0.05	0.32	4.2
50	0	0.05	0.27	2.8
51	0	0.05	0.27	2.3
	90	0.05	0.42	3.7
52	0	0.06	0.30	1.0
53	0	0.15	0.53	3.2

TABLE III

Heavy Metal Contents of *Fucus* Samples from the Gulf of Alaska
(ug/g dry wt.)

	Cu	Ni	Cd	Zn
McLeod Harbor	16.8	7.9	4.1	21.8
Anchor Cove	26.6	15.6	3.3	21.5
Saints Bay	7.5	9.5	6.1	18.3
Zaikof Bay	16.7	9.2	4.0	15.9
Point Dick	7.8	9.4	3.5	15.8
Lundstrom Island	6.8	6.1	7.0	18.8
Katecta	17.0	9.8	2.0	16.4
Cape Nukshak	6.8	6.8	6.1	---
La Touche	5.9	5.3	3.1	---
Port Eteches	10.4	7.3	2.3	15.3

TABLE IV

Mercury Content of Crab Samples from Gulf of Alaska
(ug/g dry wt.)

Sample No.	
17	0.22
20	0.23
5	0.27
16	0.25
18	0.35
10	0.55
12	0.32
15	0.24
9	0.30
13	0.44

TABLE VI

Soluble Se (ng/l) and Cr (ug/l) for N.W. Gulf of Alaska
(Discoverer Leg IV Oct. - 16)
Filtered Samples at 0.45u

Station No.	Depth (m)	Se (ng/l)	Cr (ug/l)
156	3	2.8	
	125	4.0	
158	100	Tr	
159	3	0.8	
148	3	5.2	
	100	n.d.	
160	3	n.d.	
	135	n.d.	
137	3	4.4	
	95	n.d.	
133	3	n.d.	7.64
	70	n.d.	5.64
124	3	n.d.	n.d.
	105	n.d	0.06
119	3	n.d.	0.23
	200	n.d.	n.d.
101	3	n.d.	
	85	n.d.	n.d.

TABLE V

Soluble Cr Data for Bering Sea (ug/l)
(*Discoverer* Leg III Sept. 25- Oct. 3)
Filtered Samples at 0.45u

Station No.	Depth (m)	Filtered	Unfiltered
48	3	0.29	0.69
	150		0.14
51	3		0.16
	1500	0.14	
54	3	0.15	
	98		0.07
66	134		0.30
56	61		0.04
59	3	0.02	0.14
	33	0.12	0.11
41	3	0.73	
	28	Tr	
24	3	0.37	
	40	Tr	
8	3	0.30	
	18	0.27	
12	3	0.06	
	76	0.13	
35	3		0.22
	155		0.07
19	71	0.14	
46	65	0.92	
37	3	0.59	
	70	0.08	
17	114	Tr	

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University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 12 R.U. NUMBER: 162/163/288/293/312

Submission dates are estimated only and will be updated, if necessary, each quarter. Data batches refer to data as identified in the data management plan.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]			
	From	To	Batch 1	2	3	4
Discoverer Leg II #808	6/2/75	6/19/75	3/31/76	Unknown	None	Unknown
Silas Bent Leg I #811	8/31/75	9/14/75	None	None	None	None
Discoverer Leg IV #812	10/8/75	10/16/75	6/30/76	Unknown	None	Unknown
Miller Freeman	8/16/75	10/20/75	None	None	Unknown	None
Discoverer Leg III #810	9/12/75	10/3/75	None	None	None	None
North Pacific	4/25/75	8/7/75	None	None	Unknown	None
Intertidal Biota		1975	None	None	Unknown	None
Discoverer #816	11/12/75	12/2/75	9/30/76	Unknown	None	Unknown
Contract 03-5-022-34	Last	Year	3/31/76	None	None	None

Note: [1] Estimated submission dates are contingent upon final approval of draft data management plan submitted to NOAA Nov. 20, 1975, and receipt of and agreement to the data format.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates (1)			
	From	To	Batch 5	6	7	8
Discoverer Leg II 808	6/2/75	6/19/75	3/31/76	None	None	None
Silas Bent Leg I 811	8/31/75	9/14/75	None	None	None	None
Discoverer Leg IV 812	10/8/75	10/16/75	6/30/76	6/30/76	None	None
Miller Freeman	8/16/75	10/20/75	None	6/30/76	6/30/76	6/30/76
Discoverer Leg III 810	9/12/75	10/3/75	None	6/30/76	None	None
North Pacific	4/25/75	8/7/75	None	6/30/76	6/30/76	6/30/76
Intertidal Biota		1975	None	6/30/76	6/30/76	6/30/76
Discoverer 816	11/23/75	12/2/75	6/30/76	None	None	None
Contract 03-5-022-34	Last	year	3/31/76	None	3/31/76	3/31/76

Cruise/Field Operation	Collection Dates		Estimated Submission Dates (1)			
	From	To	Batch 9	10		
Discoverer Leg II 808	6/2/75	6/19/75	unknown	unknown		
Silas Bent Leg I 811	8/31/75	9/14/75	unknown	unknown		
Discoverer Leg IV 812	10/8/75	10/16/75	unknown	unknown		
Miller Freeman	8/16/75	10/20/75	none	none		
Discoverer Leg III 810	9/12/75	10/3/75	none	none		
North Pacific	4/25/75	8/7/75	none	none		
Intertidal Biota		1975	none	none		
Discoverer 816	11/23/75	12/2/75	unknown	unknown		
Contract 03-5-022-34	Last	year	3/31/76	none		

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 12

PRINCIPAL INVESTIGATOR: Dr. David C. Burrell

Period April 1 - December 31, 1975* (9 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	158,353.00	54,078.48	104,274.52
Staff Benefits	26,750.00	8,972.35	17,777.65
Equipment	42,450.00	30,057.96	12,392.04
Travel	18,869.00	5,189.39	13,679.61
Other	143,228,00	97,862.49	45,365.51
Total Direct	389,650.00	196,160.67	193,489.33
Indirect	90,577.00	30,932.89	59,644.11
Task Order Total	480,227.00	227,093.56	253,133.44

* Preliminary cost data, not yet fully processed.

RU#190

First Annual Report

Contract Number 03-5-022-68
Research Units A-27, B-9, C-2
Reporting Period 1 May 1975 to 1 April, 1976
Number of Pages 45

Baseline study of microbial activity in the Beaufort Sea and Gulf of Alaska and analysis of crude oil degradation by psychrophilic bacteria.

SUBMITTED BY:

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Date Submitted
March 15, 1976

I. Summary of objectives, conclusions and implications with respect to OCS oil and gas development.

Our objectives are (1) to determine the microbial activity in the Beaufort Sea and the Gulf of Alaska by the heterotrophic potential technique before any accidental oil spill occurs, (2) to determine what effect crude oil might have on the microbiological picture in the sea by use of simulated _in situ_ conditions, (3) to determine the change in microbial activity in seawater when crude oil is used to perturb the ecosystem under simulated _in situ_ conditions, (4) to study the physiology of hydrocarbon degrading psychrophilic (cold loving) bacteria from the Beaufort Sea, and (5) to determine the occurrence of psychrophilic hydrocarbon oxidizers in the environment under study.

In general, the levels of the maximum potential uptake for glutamic acid (an indicator of microbial activity) in seawater samples taken behind the barrier islands in the Beaufort Sea was relatively high when compared to similar measurements made in other waters. When the relative microbial activity in the sediments was compared to that found in the overlaying seawater, it was found that in the relatively shallow waters in the study area, the microbial activity in the sediments was roughly 400 times greater than that found in the water column. The effects of crude oil on these sediments as well as their potential for biodegradation is at present unknown and should be explored further.

The effects of crude oil on the uptake and respiration (mineralization) of glutamic acid and acetate were studied. Even though these experiments were conducted under simulated _in situ_ conditions the data should be considered as being a first order approximation. There were differences found between the samples that were exposed to crude oil and those that were not. Initially there was no significant effect of crude oil on the microbial activity; however, as the incubation period was increased, the crude oil at first inhibited microbial uptake activity followed by an enhanced activity.

During the course of the above series of experiments the numbers of hydrocarbon oxidizing bacteria and the heterotrophic bacteria were monitored in the control and crude oil exposed seawater samples. In seawater exposed to crude oil the concentrations of heterotrophic and hydrocarbon oxidizing bacteria were higher than the seawater control (no crude oil present). Whether or not this shift in numbers and activity represents a change in bacterial species with time still remains to be investigated further.

Studies on microbial populations are essential to the various trophic levels in the sea because the bacterial mineralization processes are essential for primary production, and bacterial cells also serve as a food source either through coprophagy or direct ingestion. Although they may not be the main food source for many forms, their ingestion represents an excellent source of proteins, vitamins, carbohydrates and lipids. Unfortunately the microbial flora inside various marine forms has never been assessed so it is not known exactly what might happen to macroorganisms when the gut microbial flora is perturbed.

The increased activity in the presence of crude oil (an extra energy source for the indigenous microbial population) should not be taken lightly, since it can readily result in an anoxyic condition in the immediate environment. The development of an anoxyic condition can take place readily if an oil spill occurs when the ice has not melted to any degree and a thick layer of crude oil covers the surface of the water. This layer of crude oil would prevent the diffusion of oxygen into the aquatic system. An anoxyic condition will result when the utilization of oxygen is faster than the rate of oxygen replenishment.

Desulfovibrio (sulfate reducers that produce hydrogen sulfide) have been demonstrated in sediments of the Beaufort Sea that have the ability to function at low temperature. The growth of Desulfovibrio will result in the production of hydrogen sulfide which is toxic to many forms of marine life as well as being esthetically unpleasant. Dead organisms which expire due to anoxyic conditions or the presence of hydrogen sulfide will provide further energy source for maintaining the anoxyic environment. The creation and maintenance of an anoxyic environment depends on microbial activity.

Last but not least, it should be remembered that nature's way to rid the environment of natural crude oil seepage is the activity of the hydrocarbon oxidizing bacteria in the environment.

II. Introduction

A. General nature and scope of study

Our objectives are to study the baseline levels of microbial activity in the Beaufort Sea and the Gulf of Alaska during both summer and winter conditions. In addition, we were to evaluate the effects of crude oil on microbial activity and to analyze the process of hydrocarbon degradation by psychrophilic hydrocarbon utilizing bacteria isolated from the Beaufort Sea.

B. Specific Objectives

a. Determination of the relative heterotrophic potential in natural marine microbial populations (task number A-27). Studies include representitive samples of both water and sediments in different geological locations and under contrasting seasonal conditions. These studies were to be designed to give an estimate of the natural variations to be expected in microbial activity in the waters and sediments of the Beaufort Sea and the Gulf of Alaska.

b. Isolation and characterization of psychrophilic hydrocarbon utilizing marine bacteria which are capable of degrading and/or emulsifying crude oil at relatively high rates under the conditions found in the Beaufort Sea (task number B-9). Strains of bacteria having the above characteristics were to be isolated from crude oil enrichment cultures using natural samples taken from the Beaufort Sea as the inoculum. After isolation and purification, the strains were to be subjected to a number of basic

physiological studies which would determine the function of these organisms under the conditions found in the Beaufort Sea.

c. Determination of the acute effects of crude oil on the heterotrophic activity of the natural microbial populations found in the Beaufort Sea (task number C-2). These studies were to be supplemented with longer term studies which would be designed to obtain information about how crude oil affects the natural population and how, in turn, the natural population alters the crude oil.

d. Coordination of our studies with the microbial studies of Dr. Atlas and his associates to obtain the most comprehensive data possible on the role of marine bacteria in the marine ecosystems in both the Beaufort Sea and the Gulf of Alaska. To accomplish this end, our baseline studies were to be made using the same samples. In addition, a close liaison was to be established to insure that duplication of effort is minimized. In addition, we were to collect subsamples to be analyzed for inorganic nutrient concentrations by Dr. Alexander.

C. Relevance to problems of petroleum development.

Our major area of concern is the interaction between the crude oil that might accidently be spilled during the course of the development of petroleum fields and the microorganisms present in the Beaufort Sea and the Gulf of Alaska. In order to document the changes that might take place microbiologically when an oil spill occurs, a baseline knowledge of the current microbiological ecosystem must be obtained. Current studies will provide us with the natural levels of microbial activity and the composition of the microbial communities before any perturbation occurs in the environment. Currently research is underway to determine what immediate effects crude oil might have on natural populations and its rate of activity so that some educated projections may be made as to how a crude oil spill might affect the microbial ecosystem of the area in question.

There are many factors that are known to affect the rate of microbial crude oil degradation. The three most predominant are temperature, availabilitiy of fixed nitrogen and phosphorus, and the presence of crude oil degrading microorganisms. Seasonal conditions and geographical location must also be taken into consideration. An educated prediction can be made as to what might happen in the event an oil spill occurs based on baseline data accumulated, the results of various physiological studies on psychrophilic hydrocarbon oxidizers, laboratory studies on perturbing systems with crude oil, studies conducted by Dr. Atlas, and field studies employing crude oil with the water samples when the heterotrophic measurements are made.

III. Current state of knowledge

This is the first study of its kind in the Beaufort Sea. Therefore no other background materials are available at this time other than limited nutrient and hydrographic data (Alexander et al. 1974).

IV. Study area

To date, we have collected water and/or sediment samples at all Beaufort Sea stations illustrated in Figures 1 and 2. In addition, samples were taken at four stations not shown in these figures. In the Barrow area, samples were taken at stations # 11 and 12 in Elson Lagoon at 71° 19'N - 156° 16'W and 71° 15'N - 156° 00'W, respectively. In the Prudhoe Bay area, samples were taken at station #30 located south of Pingok Island at 70° 32'N - 149° 35'W and at station #40 south of Long Island at 70° 29'N - 149° 03'W.

V. Methods

1. Sampling Procedures

The water samples were taken in sterile Niskin plastic water sample bags fitted on Niskin "butterfly" water samplers. With the exception of sample number 42, all water samples were taken within one meter of the surface. Once the water sample was taken, it was placed in an ice chest for storage and transported back to the laboratory for analysis. The analyses of microbial activity were initiated within two hours after sampling was terminated.

The sediment samples were taken with a Kahl scientific mud snapper. Two or three grab samples were taken at the same time and location and combined with seawater. These samples were placed into sterile 250 ml wide mouth glass sample bottles for transport to the laboratory. All samples were maintained at or below the *in situ* temperature during sampling and transport.

Ice samples were taken from the waters adjacent to the beach at station 5b (Figure 1). The ice was removed from the water and placed into sterile sample bags and returned immediately to the laboratory. When the samples were received at the laboratory, they were allowed to melt at room temperature for 4 hours. The resulting ice melt was discarded and a portion of the remaining ice was allowed to thaw for 24 hours at 5 C. The resulting water was then used in the ice melt experiments.

2. Heterotrophic Potential Studies

The techniques used in this study were basically those of Hobbie and Crawford (1969) as further modified by Harrison, Wright, and Morita (1971). This procedure involves the addition of different amounts of U-^{14}C labeled substrate to identical subsamples. In the water and ice studies, (U-^{14}C) L-glutamic acid with a specific activity of 237 mCi/mmole (New England Nuclear) was used in a final concentration range of 0.6 μg/liter to 4.6 μg/liter. In the sediment studies, U-^{14}C glutamic acid (Amersham/Searle) with a specific activity of 10 mCi/mmole was used in the final concentration range of 10.5 μg/liter to 84.0 μg/liter. Duplicate subsamples and one control were used at each of four substrate concentrations. Initially controls were run with all samples but it soon became apparent that there was enough consistancy in the values to use a standard blank to account for abiotic absorption of the label.

After addition of the subsample, the 50 ml serum bottles that were used for reaction vessels were sealed with rubber serum bottle caps fitted with plastic rod and cup assemblies (Kontes Glass Co., Vineland, N.J.:K-882320) containing 25 x 50 mm strips of fluted Whatman #1 chromatography paper. The samples were incubated in the dark within 3 C of the in situ temperature. After the incubation period, the bottles were injected through the septum with 0.2 ml of 5N H_2SO_4 in order to stop the reaction and release the $^{14}CO_2$. After the addition of the acid, 0.15 ml of the CO_2 absorbent, β-phenethylamine, was injected onto the filter paper. The bottles were then shaken on a rotary shaker at 200 rpm for at least 45 minutes at room temperature to facilitate the absorption of CO_2. The filter papers containing the $^{14}CO_2$ were removed from the cup assemblies and added to scintillation vials containing 10 ml of toluene based scintillation fluor (Omnifluor, New England Nuclear).

The subsamples were filtered through a 0.45 μm membrane filter (Millipore). The trapped cells on the filter were washed with three 10 ml portions of seawater at 0-3 C. The filters were dried and then added to scintillation vials containing 10 ml of the above mentioned fluor. The vials were counted in a Beckman model LS-100 liquid scintillation counter located in the field laboratory.

The sediment samples contained from 30-50% sediment with the balance made up of interface water. Just prior to dilution, they were shaken by hand until all of the material was suspended. A one ml subsample was diluted 1000 times with a 32 o/oo (w/v) solution of sterile artificial seawater. After dilution, the sediment sample was treated the same as a water sample. Duplicate one ml subsamples of the sediment slurry were dried at 100 C for 24 hours and weighed to determine the dry weights. These dry weights were used to calculate the V_{max} values in terms of grams dry weight of sediment.

3. Calculations:

Calculation of the kinetic parameters was made from the relationship:

$$\frac{Cut}{c} = \frac{K_t + S_n}{V_{max}} + \frac{A}{V_{max}}$$

where c = radioactivity assimulated plus that respired as $^{14}CO_2$ by the heterotrophic population in disintegrations/min; S_n = the natural substrate concentration in μg/liter; A = the added substrate in μg/liter; $C = 2.2 \times 10^6$ μCi of ^{14}C; u = amount of ^{14}C labeled substrate added/sample bottle in μCi; t = incubation time in hours; V_{max} = the maximum velocity of uptake in $\mu g \times liter^{-1} \times h^{-1}$; and K_t = the transport constant in μg/liter. From this equation can also be calculated the time (T_t) in hours required by the natural microbial population to utilize the natural substrate in the seawater sample. For the derivation of this equation and the assumptions on which it is based, see Wright and Hobbie (1966). Saturation curves were converted to the best fitting straight line using least squares and a modified Lineweaver-Burk equation.

The percent respired was calculated by dividing the amount of labeled carbon associated with the CO_2 fraction by the total amount of substrate taken up by the cells (both cell and $^{14}CO_2$ radioactivity) and multiplying this ratio by 100.

4. Temperature studies

Water samples were taken at stations 5a and 5b which gave representative samples of both Elson Lagoon and ocean beach water. Duplicate subsamples were prepared as described above and incubated at each of the temperatures indicated in Figures 3 and 4. In cases where there were fluctuations in the incubation temperature, the temperature reported is the mean temperature (in most cases, the temperature range was less than one degree C). In order to reduce the chance of significantly altering the substrate concentrations during the course of the experiment, the incubation time for samples incubated at high temperatures was reduced. Samples at the following temperatures were incubated for the following periods: 20.5 C and 14.5 C, 4 hours; 10.0 C, 6 hours; 4 and 1.5 C, 8 hours; -2.0 C, 10 hours.

5. Acute effects of crude oil extract on the observed heterotrophic potential

Five ml of crude oil taken from Prudhoe Bay was added to 45 ml of sterilized seawater in a 150 ml separatory funnel. The mixture was shaken and allowed to separate for three hours at 5 C. The aqueous phase was removed into another separatory funnel and allowed to set for an additional ½ hour before dispensing one ml subsamples of the aqueous phase into the reaction mixtures. Each reaction vessel contained 9 ml of the seawater sample to be tested and one ml of the crude oil extract. In the controls, the one ml of crude oil extract was replaced by one ml of sterile seawater. Heterotrophic potential was measured using labeled glutamic acid as above.

6. Microbial activity changes with time in oil enrichment cultures

Water samples were taken two meters off the beach from NARL and placed into sterile gallon bottles fitted with rubber stoppers and glass siphoning tubes. Two and one half liters of water were added to each of two bottles. In the first experiment (Figures 6 and 7), 2 mg of yeast extract was added to the control and 5 ml of Prudhoe crude oil was added to the oil enrichment culture. In the second experiment (Figures 8 and 9), nothing was added to the control and 1.0 ml of Prudhoe crude oil was added to the oil enrichment culture. The cultures were incubated at 5 C. At various times, subsamples were siphoned off and used in the heterotrophic potential determinations and plate counts. The heterotrophic potential studies were conducted at 1.5 C. The incubation times for these studies ranged from 8 h to 4 h for glutamic acid and from 8 h to 1.5 h for acetate. [U-^{14}C] Acetic acid, sodium salt, with a specific activity of 59 mCi/mmole (Amersham/Searle) was used in a concentration range of 6.5 to 42 μg/liter. In the first experiment, an estimation of total number of bacteria and the number of hydrocarbon utilizing bacteria present was made by plate counts on Lib-X and crude oil plates (Atlas and Bartha, 1972), respectively.

7. Direct Cell Counts

Ten ml of seawater was fixed in the field laboratory by adding it to 0.6 ml of membrane (0.45 μm) filtered formaldehyde (37%). The vials containing the fixed water samples were sealed and stored until they could be counted in our laboratory at Oregon State University. In the sediment studies, the final dilution of the sediments in the heterotrophic potential studies was used and treated the same as the seawater samples.

From 5 to 17 ml of sample were filtered through a 0.2 μm Nuclepore filter. When a relatively high number of organisms was present, the samples were diluted with membrane filtered artificial seawater. The number of organisms per field was kept within acceptable limits and the volume filtered was kept above 5 ml. Controls were run using filtered artificial seawater with all of the reagents used in the staining and mounting procedure. These counts were no more than 5% of those found in the samples and was considered insignificant.

The staining procedure used was that of Zimmermann and Meyer-Reil (1974). This procedure involves staining the cells trapped on the membrane filter with acridine orange and then destaining with iso-propyl alcohol. The membranes were dried and mounted on microscopic slides with a mounting medium of cinnamaldehyde and eugenol (2:1).

The bacterial cells were counted using a Zeiss IV Fl epi-fluorescence condenser microscope fitted with filters KP 500, KP 490, FT 510, and LP 520. The eyepiece used was Kpt W 12.5 x and the objective was plan 100 x. Approximately 50 restriction fields were counted per sample. Representative fields were counted from the center of the membrane filter to the outside edge of the filtration circle.

Only bodies with distinct fluorescence (either orange or green), clear outline and recognizable bacterial shape were counted as being bacterial cells.

8. Isolation and characterization of psychrophilic hydrocarbon utilizing bacteria

Crude oil enrichment cultures were used to inoculate crude oil agar plates. These plates were incubated at 4 C for 6 weeks. Representative colonies were picked from these plates and streaked on Lib-X agar plates (Baross, Hanus, and Morita, 1974). These plates were incubated at 4 C for three to four days. After incubation, a representative colony was picked from each plate and was used to inoculate 10 ml of Lib-X broth. The cells were allowed to grow for three days at 4 C and were further purified by streaking on Lib-X plates. This procedure was repeated again before the strains were used for further study.

One hundred and fifty strains were screened on Lib-X agar plates for growth at both 4 and 25 C. Only those strains that grew at 4 C but did not grow at 25 C were analyzed further. Of the 150 original isolates, only 6 showed the desired characteristics. Growth-temperature profile studies were conducted by inoculating a series of tubes containing Lib-X

broth. The tubes were incubated for three days in a shaking temperature gradient incubator (Model TG1, Scientific Industries Inc.). Optical density measurements were made daily during log phase at 600 nm using a Bausch and Lomb Spectronic 20 colorimeter.

9. Isolation of sulfate reducing bacteria

One or two ml of sediment were added to sterile screw capped test tubes filled with a modified M10E medium (a differential medium for sulfate reducing bacteria). The M10E medium was similar to that described by Morita and ZoBell (1955) except the sodium sulfite and the ascorbic acid were deleted and a 0.1% (w/v) concentration of sodium formaldehyde-sulfoxylate was added. The enrichment tubes were incubated at 4 C for several weeks. Evidence of the presence of sulfate-reducing bacteria was noted by the formation of black ferrous sulfide. Sediments with the following sample numbers were tested: 3, 5, 8, 10, 16, 18, 20, 22, 25, 27, 33, 38, 41, 44, 46, 50 and 52.

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VI. Results

A. Analysis of water samples

1. Heterotrophic potential studies

The heterotrophic potential of natural microbial populations in 50 water samples was measured using ^{14}C labeled glutamic acid (Table 1). From these studies, estimates were made of the maximum velocity of uptake (V_{max}), the time in hours required by the natural microbial population to utilize the amount of glutamic acid present in the water sample (T_t), the transport constant plus the natural substrate concentration ($K_t + S_n$), and the percent of glutamic acid that was mineralized of the total glutamic acid taken up by the cells. The average values for these factors, as observed in water collected in both the Point Barrow and Prudhoe Bay areas, are summarized in Table 2a.

The V_{max} for glutamic acid uptake was higher in the water samples taken in the Prudhoe Bay area and the turnover time for glutamic acid utilization was lower than that observed in water samples taken at Barrow. In the same samples, the $K_t + S_n$ and the percent respiration values were both lower in the water samples taken in Prudhoe Bay.

Differences were also seen in the heterotrophic potential data collected within each region during a given sampling period (Table 3a and 3b). Of the water samples taken from the first 4 stations in Elson Lagoon, the samples taken at station #1 consistantly showed higher V_{max} values. In Prudhoe Bay, those stations that were the furthest from land showed the lowest V_{max} values.

2. Direct microscopic determination of bacterial concentrations in seawater

Direct microscopic determinations of bacterial concentrations in 44 seawater samples were made using epifluorescent microscopy (Table 4). The average number of organisms present per ml was 4.5×10^5 with a range of 0.1×10^5 to 11.9×10^5. A comparison of the relative concentrations of cells in the waters analyzed at Barrow and Prudhoe Bay shows no significant difference (Table 2a). The cell count data were compared with the V_{max} and plate count data taken in the same water samples; no significant correlations were found.

One factor that can be calculated from the number of organisms present is average bacterial biomass. The average mass per bacterial cell has been estimated at 2.0 and 2.2×10^{-13} grams per cell (ZoBell, 1963; Dale, 1974; respectively). More recent studies of marine bacteria using epifluorescent microscopy has led L.-A. Meyer-Reil (personal communication) to reduce this estimate to 0.7×10^{-13} grams per cell. Using this estimate and the average depth of the water at the stations studied (10.5 feet), the average bacterial biomass in a water column one meter square can be estimated at 9.5 mg.

3. Physical and chemical parameters

In most of the water samples analyzed, temperature, salinity, and pH were also measured. In addition to these factors, analyses of PO_4, NH_3, NO_3, and SiO_3 concentrations were made on the same water samples by Dr. Alexander and her associates. A summary of these findings are given in Table 2a. The average salinity in all samples was 20.5 o/oo; the lowest salinity observed was 9.0 o/oo (station 54 in Prudhoe Bay) and the highest value observed was 26.5 o/oo (station 1 in Elson Lagoon). These extremes reflect the trend in the average salinity in the two regions. The average salinity was 22.7 o/oo in the Barrow area and 16.7 o/oo in Prudhoe Bay. Both of these figures are well below the average salinity of all the world's oceans which is 35 o/oo. This reduced salinity undoubtedly results from both fresh water runoff and some ice melt, with the former being the more predominant factor during the period of this study.

The average seawater temperature (1.2 C) was the same in Barrow and Prudhoe Bay. The highest temperatures observed were measured at the beginning of the study in the Barrow area.

The average pH of the water samples tested was 7.9. This value was the same in both of the general areas studied and is slightly less than that normally found in seawater (pH 8.1 to 8.3).

Of the various ionic concentrations measured by Dr. Alexander, with the exception of phosphate, there was no significant difference in the average concentrations found in the Barrow area and in Prudhoe Bay (Table 2a). The average level of phosphate in the Barrow waters was over four times greater than that found in Prudhoe Bay.

B. Analysis of sediments

1. Heterotrophic potential studies

Microbial activity in the sediments of shallow waters has long been recognized as an important locus of microbial activity in marine ecosystems. The results of studies on heterotrophic potential in 24 sediment samples indicate that the activity was indeed very high relative to the activity found in the water column (Tables 2a and 5). The average value for the potential rate of glutamic acid uptake and mineralization was 0.52 μg of glutamic acid x gram dry weight of sediment^{-1} x h^{-1}. There is no way to directly compare this figure to potential activity in a comparable volume of water but a reasonable approximation can be made. The closest comparison between microbial activity in sediments and seawater should be made by contrasting the activity in a given volume of seawater with an equal volume of diluted sediment-seawater slurry. The average V_{max} in the diluted sediment slurries was 2.1 μg x 10^{2} x liter^{-1} x h^{-1}. This is roughly four orders of magnitude higher than the average figure of 4.2 μg x 10^{-2} x liter^{-1} x h^{-1} observed in the water samples.

Another comparison of potential microbial activity in sediments and seawater can be made by comparing the total activity in an average water column with activity in the underlaying sediment. In order to make such a comparison, at least two assumptions must be made. The first assumption is that the majority of the microbial activity is taking place in the first two cm of the sediment. This assumption is based on the findings of ZoBell (1946) and others which show that the vast majority of bacteria in sediments are typically found in the top 2 cm. The other assumption is that diluting the sediment sample with sterile artificial seawater does not significantly affect the resulting observed activity.

Keeping these assumptions in mind, the potential rate of glutamic acid utilization in the average water column (1 meter^{2} x 3 meters deep) can be compared with the same potential in the sediment below that square meter of water. The maximum potential for glutamic acid utilization in an average water column in the test area was 0.1 mg glutamic acid x water column^{-1} x h^{-1}. The sediment beneath this water column (to a depth of 2 cm) had a potential utilization of 42 mg glutamic acid x m^{2} of sediment^{-1} x h^{-1}. If our assumptions are correct, the sediments in the water columns studied had, on the average, 400 times higher activity than the entire overlaying water column. This figure should be considered an underestimation of what is probably the true value because: (1) we have assumed that there is no microbial activity in the sediments below 2 cm (2) the value was calculated in terms of a slurry which contained only 30-50% sediment with the balance consisting of seawater.

As was the case in the water samples, the level of potential activity in the sediments of Prudhoe Bay was significantly higher than in the sediments studied in the Barrow area (Table 2b). The sample with the lowest activity was taken in the Barrow area (0.2 μg x kilogram $^{-1}$x h^{-1}) and the highest value was observed in a sample taken from Prudhoe Bay (17 μg x kilogram^{-1}x hr^{-1}).

Direct counts of bacteria in the sediment were made using a modification of the same technique used to measure the bacterial concentration in seawater (Table 6). As is shown in Table 2b, the average number of cells per gram dry weight of sediment was 6.3 x 10^{8}. Unlike the cells found in the water column where the concentration of cells was approximately the same in the two areas of study, the concentration of cells found in Prudhoe Bay sediments was approximately twice that found in the Barrow area. Using the same assumptions used to estimate bacterial biomass in the water column, the biomass in upper two cm of sediment was about 0.9 gm per m^{2}.

C. Analysis of ice melt

A series of experiments was conducted to determine the level of heterotrophic potential in ice melt water and the effects of ice water melt on the heterotrophic potential observed in seawater (Table 7). Instead of finding very low levels of microbial activity in the ice melt as was anticipated, levels of activity were about the same as those found in the surrounding seawater. Furthermore, when the seawater was diluted to a salinity roughly half that found in the original sample, there was little change in the maximum velocity of uptake (V_{max}) and in the percent respiration. The turnover time in both experiments more closely resembled that found in the ice melt than that found in the surrounding seawater.

D. Changes in heterotrophic potential with incubation temperature

It has been well established that temperature is one of the most important factors affecting the biodegradation of crude oil. This series of experiments was designed to show the effects of temperature on microbial activity in seawater samples as reflected by changes in V_{max}. The effects of incubation temperature on the potential maximum velocity of glutamic acid metabolism was measured in two ocean and two lagoon water samples taken from stations 5a and 5b, respectively (Figures 3 and 4). In all samples tested, the level of potential activity increased markedly with increased temperature. The V_{max} in both ocean samples had approximately the same slope with increasing temperature (5.3 and 4.8) and the same "y" intercept.

The trends found in the lagoon samples were different. The slopes of the curves were lower than those found in the ocean samples indicating that the populations found in these waters were less affected by changes in temperature than the ocean samples. In both lagoon samples, there was an inflection in the curve at 15 C. Although more work is necessary to determine whether or not this inflection is indeed typical of the population found in the lagoon, it does open an interesting possibility.

It is quite likely that this pattern reflects a more heterogeneous population than that found in the ocean. There may be two groups of organisms present; one group that is psychrophilic in nature and is injured by temperatures above 11 C, and another group which may be either psychrotrophic or mesophilic.

E. The acute effects of crude oil extract on microbial activity

A series of pilot studies was designed to give a first approximation of the effects of crude oil on natural microbial metabolism. A portion of an aqueous Prudhoe crude oil extract was added to reaction vessels and changes in the hetrotrophic potential using glutamic acid were observed (Table 8). In none of the five experiments was there a significant change in any of the factors studied. It must be kept in mind that these experiments were conducted under artificial conditions, and that a limited number of functions were tested.

F. Microbial activity changes in crude oil enrichment cultures with time

Two studies were conducted to determine the changes that take place in natural water samples that have been enriched with Prudhoe crude oil. In the first experiment, the oil enrichment and the control were sampled periodically to determine cell numbers by plating subsamples on two types of agar media. Microbial activity was also assayed by using labeled acetate and glutamic acid. Figure 5 illustrates the changes in cell numbers as determined by plate counts using a medium designed to grow the highest number of organisms (Lib-X medium) and a selective medium designed to allow the growth of only crude oil degrading organisms (crude oil medium). In both the enrichment and the control cultures, the number of crude oil degrading bacteria was 10% or less of the total population.

The highest cell concentration levels were found in both cultures after 7 days incubation with a higher concentration of cells present in the oil enrichment culture than in the control. The relative number of oil degrading bacteria remained higher in the oil enrichment than in the control with time. These data suggest that under this highly artificial environment, the cell numbers were not adversily affected by the presence of the crude oil. In fact, the crude oil appeared to cause a relative increase in cell numbers.

The maximum velocity (V_{max}) of glutamic acid and acetate uptake were also measured (Figures 6 and 7, respectively). In general, the levels of substrate uptake reflect the same patterns that were seen in the cell number data with the highest values found on the 7th day, a large decrease by the 19th day and then a slight increase by the 29th day. The reason for the relatively low level of activity seen with acetate in the oil enrichment culture on the 7th day is not known. With this one exception the uptake patterns were similar for both glutamic acid and acetate.

A similar study was conducted over a shorter period of time in which the control contained only natural seawater (Figures 8 and 9). After 1½ days incubation, little change was seen in the either of the cultures; however, on the third day, the uptake of both glutamic acid and acetate was highest in the control. On the 5th day, the uptake of both substrates was as high or higher in the oil enrichment than in the control. As the incubation time increased, the level of uptake in the oil enrichment remained higher than in the control. In general, this is the same type of pattern that we observed in the previous experiment.

G. Isolation of sulfate reducing bacteria from Arctic marine sediments

A total of 18 sediment samples were tested for the presence of sulfate reducing forms. In all samples tested, sulfate reducing bacteria were found. In all but one sample (number 52 taken from Prudhoe Bay) there was a two to three week lag before visible sulfate reduction was noted at 4 C. Visible sulfate reduction was seen in sample #52 within 10 days at this temperature. Sulfate reducing bacteria appear to be common in Arctic marine inshore sediments.

H. Growth-temperature profiles of psychrophilic hydrocarbon utilizing bacteria isolated from the Beaufort Sea.

Oil enrichment cultures that had been incubated at 4 C were used to inoculate crude oil plates. One hundred and fifty isolates were taken from these plates, purified and screened for low temperature growth characteristics. Of the 150 isolates studied, only six which grew at 4 C were unable to grow at 25 C on agar plates. Figures 10, 11 and 12 show the growth-temperature profiles of these organisms after 42 h incubation. Three of the six strains (#52, 53, 59) had the same growth temperature profile illustrated in Figure 10. These were all gram positive, motile, short rod-shaped bacteria with a maximum growth temperature of 26 C and an optimum growth temperature of 21 C. These strains are probably very closely related and would all be classified as psychrotrophic organisms. One bacterial strain (#30) had the growth-temperature profile illustrated in Figure 11. Its maximum growth temperature was 19.5 C and its optimum growth temperature was 14.5 C. This organism is a gram positive, long slender rod which would be classified as a psychrophile. The two remaining bacterial strains (#45 and 47) had the same growth-temperature profile illustrated in Figure 12. They had a maximum growth temperature of 17.5 C and an optimum growth temperature of 9.0 C. After 67 h, growth was observed at temperatures as low as -3.0 C. These organisms were both gram negative, vibrio-shaped, motile rods and would be classified as psychrophiles. It would appear from these data, that we have at least three different strains which will be suitable for further physiological studies.

Table 1. Summary of physical and heterotrophic potential data for all water samples studied. All heterotrophic potential data was measured using ^{14}C glutamic acid. The total Vmax is the maximum velocity for both macromolecular synthesis and respiration reported as ($\mu g \times liter^{-1} \times h^{-1}$). The CO_2 Vmax is the maximum velocity of respiration (minerialization). (*) indicates ice melt samples. (NO) indicates no observation made.

Sample number	Station number	Sample temp.	Salinity 0/00	Incubation temp.	Sample pH	Total Vmax $\times 10^{-2}$	CO_2 Vmax $\times 10^{-2}$	Tt ($h \times 10^{-2}$)	Kt + Sn ($\mu g \times liter^{-1}$)	Percent Respiration	Correlation Coefficient
2	1a	3.0	26.0		NO	NO					
4	1	3.2	26.5		NO	NO					
6	1	3.0	20.1		NO	NO					
7	2	2.5	21.0	0.0	NO	8.3	4.6	0.7	6.1	56	.99
9	3	2.0	20.0	0.0	NO	9.0	5.9	0.6	5.3	67	.99
11	4	3.0	25.0	0.0	NO	9.1	4.9	0.6	5.5	57	.99
11a	7	NO	NO	4.0	NO	10.5	6.5	2.2	23.0	62	.99
12	7	3.0	31.0		NO	NO					
13	7	2.0	23.0	1.0	NO	2.5	1.7	0.7	1.7	68	.98
13a	*7	NO	0.5	1.0	NO	17.0	7.1	0.7	12.0	56	.91
14	7	3.0	23.0	1.0	NO	1.0	0.7	1.2	1.2	69	.99
15	10	-0.5	23.8	0.0	NO	1.4	0.9	0.8	1.1	71	.99
17	12	2.0	22.5	2.0	NO	11.6	4.5	0.5	5.4	51	.99
19	5b	2.0	25.0	2.0	NO	11.8	6.4	0.7	7.9	61	.99
19a	*5b	NO	0.0	1.0	NO	0.2	0.1	14.2	3.2	60	.99
21	11	2.0	17.0	2.0	NO	1.4	0.9	2.0	2.8	68	.99
23	12	1.5	21.0	2.0	NO	4.0	2.9	1.0	3.8	72	.99
24	6	2.0	25.5	2.0	NO	0.5	0.3	12.7	6.2	76	.96
24a	7	1.0	25.0	2.0	NO	2.9	1.9	1.6	4.5	67	.99
26	1	0.4	20.0	0.0	NO	5.1	3.9	1.7	8.7	54	.96
28	2	-0.5	20.5	0.0	NO	1.0	0.5	68.0	65.0	61	.99
30	3	-0.2	20.0	0.0	NO	1.5	0.1	3.2	4.8	59	.97
32	4	-0.2	20.5	0.0	NO	0.9	0.6	2.7	2.4	60	.99
34	30	1.9	12.1	4.0	NO	2.6	1.3	0.6	1.6	49	.98
36	40	1.8	18.8	4.0	NO	5.9	3.0	0.6	3.2	53	.93
37	50	1.5	20.0	4.0	NO	3.3	1.7	0.5	1.7	54	.99
38	53	-0.8	11.8	2.0	7.9	7.7	3.4	0.4	2.8	49	.97
40	55	-0.8	11.1	2.0	7.9	11.3	5.2	0.2	1.8	52	.99
42	55	-0.4	19.8	2.0	7.9	6.5	3.3	0.3	1.8	50	.98

Table 1. Continued

Sample number	Station number	Sample temp.	Salinity 0/00	Incubation temp.	Sample pH	Total Vmax $\times 10^{-2}$	CO_2 Vmax $\times 10^{-2}$	Tt (h $\times 10^{-2}$)	Kt + Sn (µg $\times$ liter^{-1})	Percent Respiration	Correlation Coefficient
43	51	-0.5	11.4	2.0	7.9	3.8	1.7	0.4	1.6	47	.99
45	54	-0.4	9.0	2.0	7.9	7.8	3.7	0.6	4.5	47	.99
47	4	-0.5	18.8	0.5	7.9	0.9	0.6	4.3	3.9	44	.99
49	3	-0.5	18.5	0.5	8.0	0.4	0.3	8.1	3.6	66	.98
51	2	-0.5	18.2	0.5	8.0	1.3	0.7	1.6	2.1	62	.99
53	1	-0.5	18.3	0.5	8.0	1.8	0.9	3.0	5.5	55	.91
55	53	1.0	14.5	1.0	7.9	6.0	3.0	0.5	3.2	51	.99
57	55	1.5	16.0	1.0	8.0	6.9	3.3	0.7	5.1	52	.97
59	56	1.5	19.5	1.0	8.0	2.8	1.3	1.4	3.8	53	.95
60	70	1.0	20.0	1.0	8.0	1.9	1.0	1.0	2.0	53	.99
62	71	0.5	21.5	1.0	8.0	2.0	1.0	0.8	1.6	52	.97
64	50	2.5	17.8	2.0	7.8	4.8	2.3	0.7	3.7	51	.98
67	52	1.9	18.1	2.0	7.8	3.2	1.5	1.3	4.2	55	.98
69	57	2.2	16.2	1.5	7.9	2.9	1.6	1.3	3.1	55	.99
71	70	1.5	18.5	1.5	8.0	1.2	0.6	1.6	1.9	55	.99
73	71	0.3	20.2	1.5	8.0	0.5	0.3	7.8	4.2	58	.97
75	72	1.9	16.0	1.5	8.0	2.0	1.0	1.1	2.1	52	.99
77	73	2.3	15.8	1.5	8.0	4.0	2.0	0.8	3.0	52	.99
79	7	2.0	25.5	1.5	8.0	2.6	1.6	5.8	15.9	68	.87
80	56	2.0	25.2	1.5	8.0	1.2	0.8	3.0	3.6	66	.99
81	56	1.5	25.0	1.5	8.0	2.4	1.5	0.5	1.1	66	.99
82	*56	NO	1.2	1.5	NO	0.1	Too low	13.0	1.7	86	.99
83	*5b	NO	0.5	1.5	NO	2.2	1.2	1.4	3.0	60	.98
84	*5b	NO	2.0	1.5	NO	0.9	0.8	2.6	2.4	75	.93
85	3	-0.5	22.2	1.0	7.9	1.1	0.6	1.9	2.1	63	.99
87	2	-0.5	22.2	1.0	7.9	1.3	0.8	2.2	2.9	64	.94
89	1	-0.5	22.0	1.0	7.8	4.7	2.4	1.0	4.5	57	.99
91	5a	-1.0	25.0	1.5	7.9	2.8	1.1	1.7	4.8	55	.90
92	5b	-1.0	26.0	1.5	7.9	4.0	2.4	2.3	9.1	55	.98
93	*5b	NO	9.0	1.0	7.7	3.4	2.5	1.8	5.9	67	.99
94	*5b	NO	6.0	1.0	7.6	6.8	4.6	2.2	15.1	64	.95
95	5b	2.0	26.0	1.0	7.9	2.8	1.7	0.7	2.0	62	.99

Table 2a. Data summary of parameters measured in seawater samples. All parameters reported as average values. *Data extrapolated from those values reported by Dr. R. Horner. @Biomass calculated for an average meter^2 with depth at 10.5 feet. °Number of bacteria estimated by direct observation using epifluorescent microscopy.

Parameter	Units	Barrow	Prudhoe Bay	Overall Average	Range From	Range To
Vmax x 10^{-2}	µg x liter^{-1} x h^{-1}	3.7	4.4	4.0	0.4	11.8
T_t x 10^2	h	5.2	1.1	3.5	0.2	68
$K_t + S_n$	µg x liter^{-1}	7.4	2.8	5.9	1.1	65
Percent respiration	%	62	54	59	44	76
In situ temperature	C°	1.2	1.0	1.2	-0.8	3.2
Salinity	0/00	22.7	16.7	20.5	9.0	26.5
pH		7.9	7.9	7.9	7.8	7.9
Depth of water column	feet	-	-	10.5	-	
°# bacteria x 10^5	# cells/ml	4.4	4.5	4.5	0.1	11.9
@Bacterial biomass	mg	-	-	9.5	-	
*PO_4	µg-at/liter	4.4	1.0	2.9	0.3	10.9
*NH_3	µg-at/liter	0.6	1.0	0.8	0.1	5.2
*NO_3	µg-at/liter	1.8	1.3	1.5	0.6	10.0
*SiO_3	µg-at/liter	9	13	10	4	40

Table 2b. Data summary of parameters measured in sediments. (1) Parameters reported in terms of grams dry weight of sediment. (2) Bacterial biomass reported for meter^2 sediment x 2 cm.

Parameter	Units	Barrow	Prudhoe Bay	Overall Average	Range From	Range To
(1) V_{max} x 10^{-1}	µg x g dry weight^{-1} x h^{-1}	3.5	7.0	5.2	0.2	17.0
Percent respiration	%	47	38	43	32	71
pH		7.2	7.5	7.4	7.2	7.7
°(1)#bacteria x 10^8	# cells x g dry weight^{-1}	4.4	9.2	6.3	0.1	41.4
(2) Bacterial biomass	mg	-	-	880	-	

Table 3a. V_{max} measurements in water samples taken at four stations in Elson Lagoon (Barrow, Alaska). Numbers given x 10^{-2} (μg x $liter^{-1}$ x h^{-1}). (NO) not observed.

Date	Station Number 1	2	3	4
8/21	NO	8.3	9.0	9.1
9/5	5.1	1.0	1.5	0.9
9/11	1.8	1.3	0.4	0.9
9/17	4.7	1.3	1.1	NO

Table 3b. V_{max} measurements in water samples taken at stations in Prudhoe Bay. *sample taken at a depth of 2 meters, all others taken at the surface

Date	Station Number	V_{max} x 10^{-2} (μg x $liter^{-1}$ x h^{-1})
9/8	53	7.7
"	55	11.3
"	55	6.5 *
"	51	3.8
"	54	7.8
9/12	53	6.0
"	55	6.9
"	56	2.8
"	70	1.9
"	71	2.0
9/13	50	4.8
"	52	3.2
9/14	57	2.9
"	70	1.2
"	71	0.5
"	72	2.0
"	73	4.0

Table 4. Number of bacteria per ml in water samples as determined by direct counts using epifluorescent microscopy.

Sample number	Number x 10^5	Sample number	Number x 10^5
2	5.8	45	0.1
4	11.9	47	3.9
6	5.3	49	1.2
7	6.6	51	6.3
9	5.3	53	1.4
11	6.5	55	5.5
15	5.9	59	7.5
17	1.2	60	6.0
19	4.5	62	6.6
21	3.7	64	0.7
23	3.7	67	4.3
24	0.3	69	5.7
26	4.8	71	7.6
28	6.7	73	0.6
30	6.7	75	0.8
32	0.8	77	0.9
34	6.3	85	0.1
37	5.8	87	0.3
38	5.3	89	6.5
40	6.0	91	4.2
42	4.7	92	6.0
43	7.0	98	5.7

Average 4.5×10^5/ml

Table 5. Summary of physical and heterotrophic potential data measured in sediment samples. The "Total Vmax" data represents the maximum velocity for both macromolecular synthesis and respiration in μg glutamate x g dry weight sediment^{-1} x h^{-1}. The "CO_2 Vmax" represents the maximum velocity of respiration only (mineralization). The temperature and salinity data was collected from the water directly above the sediment. The "Associated Water Sample Number" is the number of the water sample taken on the surface above the sediment analyzed. (S) sediment samples that were too sandy to be accurately measured using this technique. (NO) not observed.

Sample number	Station number	Sample Depth ft.	Associated Water Sample number	Water Temperature	Water Salinity	Sediment pH	Total Vmax x10^{-1}	CO_2 Vmax x10^{-1}	Percent Respiration	Correlation Coefficient
16	10	28	15	-0.5	23.8	NO	Too high			
18	12	6	17	1.5	22.8	NO	1.2	0.9	39	.82
20	11	10	21	2.0	17.0	NO	3.8	2.4	65	.99
22	12	10	23	1.5	21.0	NO	5.2	2.6	64	.82
25	1	7	26	0.4	20.0	NO	13.0	5.9	43	.99
27	2	6	28	-0.5	20.5	NO	1.1	0.5	41	.98
29	3	6	30	-0.2	20.0	NO	0.6	0.3	41	.99
31	4	6	32	-0.2	20.5	NO	0.7	0.2	37	.99
33	30	6	34	1.9	12.1	NO	7.8	2.9	39	.99
35	40	6	36	1.8	18.8	NO	S			
39	53	6	38	-0.8	19.2	7.4	11.0	4.2	35	.99
41	55	7	40,42	-0.4	19.8	7.6	6.4	2.9	36	.99
44	51	8	43	-0.2	19.8	7.5	6.5	2.1	32	.99
46	54	5	45	00.2	17.5	7.5	10.0	3.6	34	.98
48	4	24	47	-0.5	18.8	NO	2.9	1.2	44	.99
50	3	34	49	-0.5	18.5	NO	0.2	0.1	71	.99
52	2	6	51	-0.5	18.2	NO	1.1	0.5	48	.99
54	1	7	53	-0.5	18.3	NO	S			
56	53	8	55	1.0	19.5	7.6	S			
58	55	10	57	1.5	20.0	7.4	S			
61	70	9	60	1.0	21.0	NO	17.0	5.6	32	.99
63	71	23	62	0.0	22.5	NO	2.9	1.0	39	.99
65	50	5	64	2.3	17.5	7.3	4.2	1.5	35	.97
66	51	7		2.2	19.3	7.6	6.7	3.0	32	.99
68	52	8	67	2.2	8.5	7.3	5.1	1.7	32	.99

Table 5. Continued

Sample number	Station number	Sample Depth ft.	Associated Water Sample number	Water Temperature	Water Salinity	Sediment pH	Total Vmax $x10^2$	CO_2 Vmax $x10^2$	Percent Respiration	Correlation Coefficient
70	57	5	69	1.8	19.2	NO	S			
72	70	20	71	0.3	21.5	NO	S			
74	71	19	73	0.0	21.3	NO	S			
76	72	15	75	0.6	19.2	7.7	3.0	0.9	51	.99
78	73	7	77	1.5	20.2	7.6	3.2	1.0	54	.99
86	3	10	85	-0.5	22.2	7.3	Too low			
88	2	7	87	-0.5	22.2	7.2	6.5	2.2	35	.99
90	1	6	89	-0.5	22.0	7.2	5.2	2.1	40	.99

Table 6. Number of bacteria per gram dry weight sediment as determined by direct counts using epifluorescent microscopy.

Sample number	Number of Bacteria $\times 10^8$ Per Gm Dry Weight
16	0.6
18	0.6
20	4.3
22	17.0
25	4.6
27	0.1
29	0.4
31	2.5
39	10.9
41	1.5
44	5.2
46	4.6
50	3.7
52	6.1
56	0.5
65	0.4
66	41.4
68	6.7
76	11.7
78	8.8
88	12.6
90	0.7

Average 6.3×10^8 cells/gram dry weight

Table 7. Comparative heterotrophic potential measurements in samples of ice melt, associated sea water, and 50/50 percent mixtures of the two. (*) Theoretical result of mixing

Experiment Number	Sample Number	Sample Type	Vmax (μg x liter^{-1} x h^{-1})10^{-2}	Tt (h x 10^{2})	Kt + Sn (μg x liter^{-1})	Percent Respiration
25	82	ice	0.1	13.3	1.7	86
	83	ice	2.2	1.4	3.0	60
	84	ice	0.9	2.6	2.4	75
	81	seawater	2.4	0.5	1.1	66
	84 + 81	mixture	2.2	2.0	4.4	65
	*		1.6	---	---	70
29	93	ice	3.4	1.8	5.9	67
	94	ice	6.8	2.2	15.1	64
	95	seawater	2.8	0.7	20.0	62
	93 + 95	mixture	5.7	2.0	11.5	65
	*		3.1	---	---	65

Table 8. Acute effects of aqueous crude oil extract on heterotrophic potential in natural microbial populations using ^{14}C labeled glutamic acid. "Exposure Time" is the time between the addition of the extract and the addition of the labeled substrate.

Sample Number	Exposure Time (h)	Extract added	Vmax (μg x liter^{-1} x h^{-1})10^{-2}	Tt (h x 10^{2})	Kt + Sn (μg x liter^{-1})	Percent Respiration
14	0	no	1.0	1.2	1.3	69
		yes	0.7	1.8	1.3	75
24a	0	no	2.9	1.6	4.5	67
		yes	3.0	2.0	5.9	65
25a	2	no	2.6	1.9	5.0	65
		yes	3.6	2.6	9.3	66
79	0	no	2.6	5.8	14.9	68
		yes	1.9	7.9	14.4	71
98	0	no	0.7	11.2	8.1	70
		yes	0.6	11.7	6.7	70

Table 9. Comparison of microbial heterotrophic activity in seawater taken from world oceans as measured using glutamic acid.

Ocean	Vmax (μg x $liter^{-1}$ x h^{-1})	Number of Samples	Investigation
Arctic	4.0×10^{-2}	50	This paper
Antarctic	1.0×10^{-2}	23	Griffiths et al. 1976
Antarctic	1.1×10^{-2}	8	Gillespie (unpublished data)
Tasman Bay New Zealand	4.0×10^{-2}	1	Gillespie (unpublished data)
Eastern Tropical Pacific	*$.15 \times 10^{-2}$	10	Hamilton and Preslan, 1970
Yaquina Bay Newport, OR	7.3×10^{-2}	1	Griffiths (unpublished data)

*No CO_2 data included.

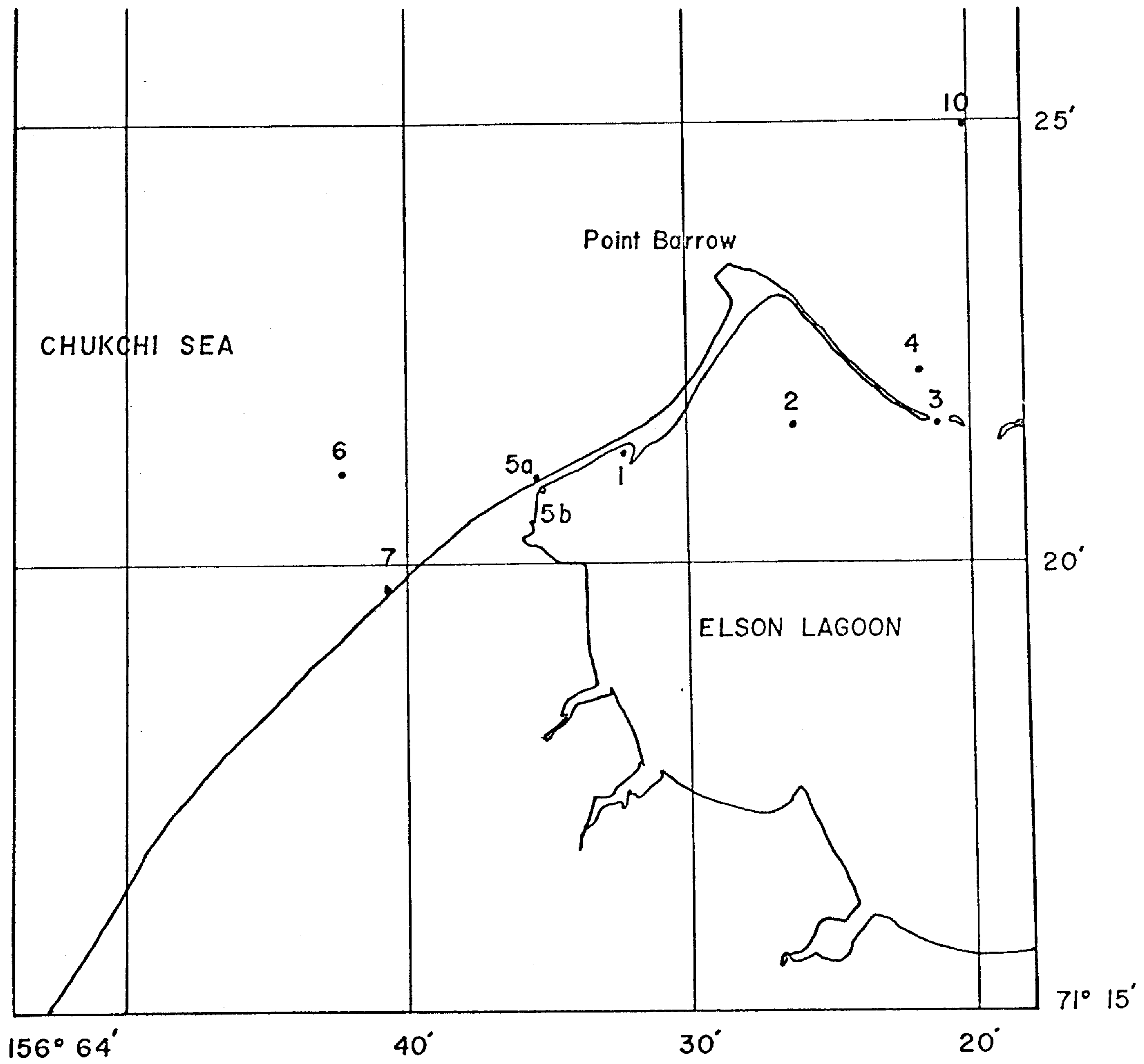


Figure 1. Stations sampled in the Point Barrow area.

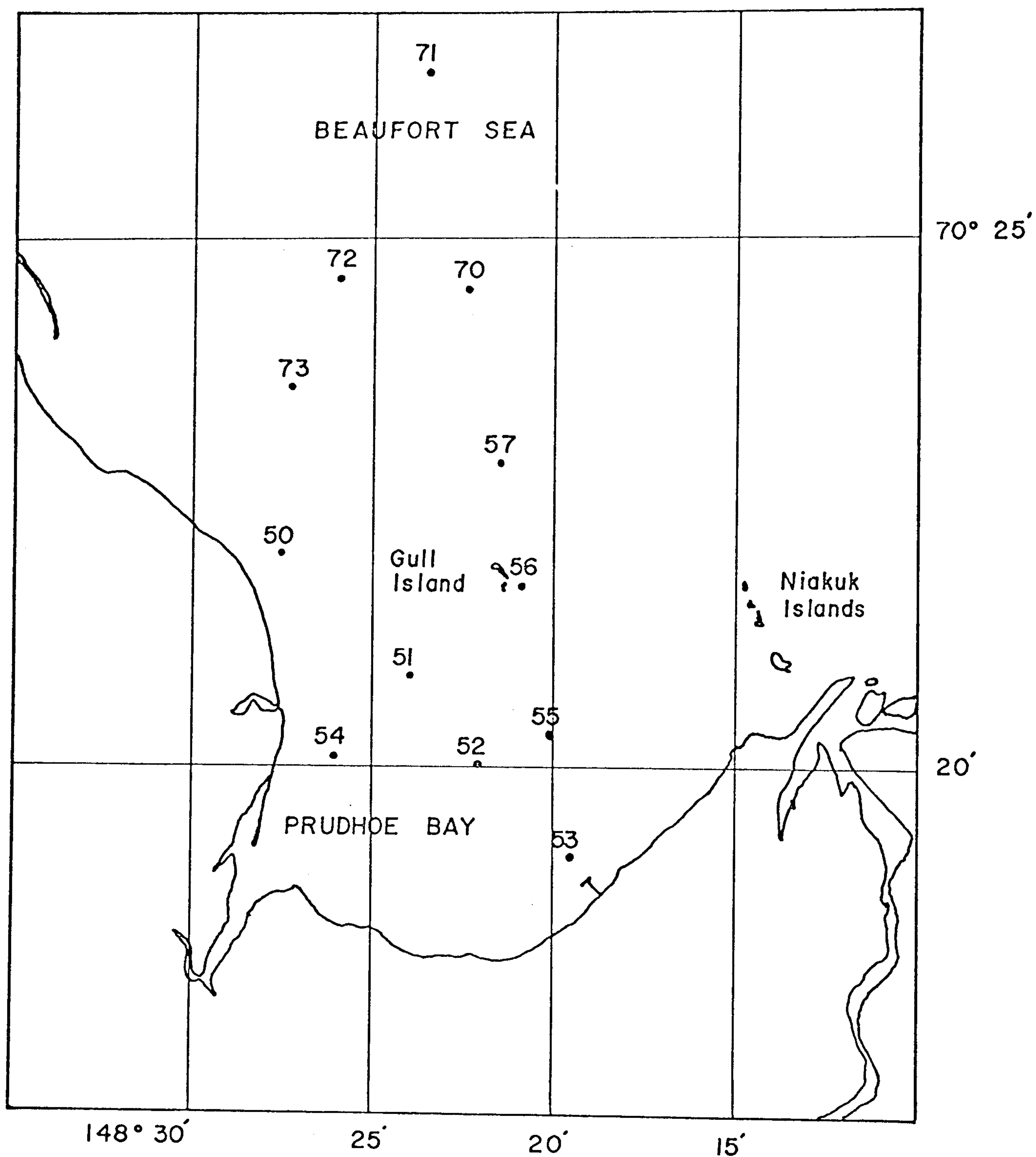


Figure 2. Stations sampled in the Prudhoe Bay area.

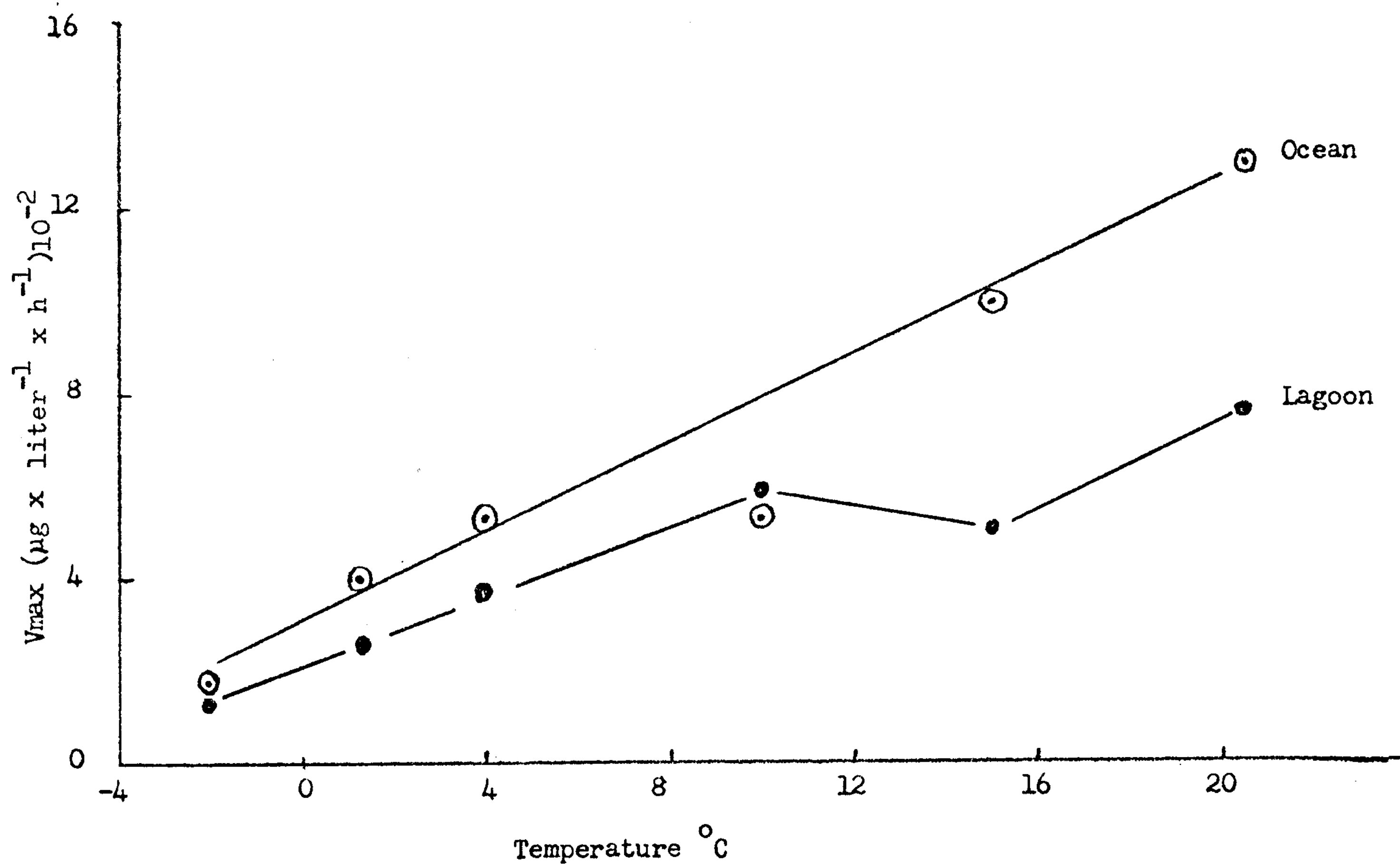


Figure 3. A comparison of the effects of incubation temperature on the maximum velocity of metabolism (Vmax) in the natural microbial populations found in an ocean (⊙) and a lagoon (●) water sample.

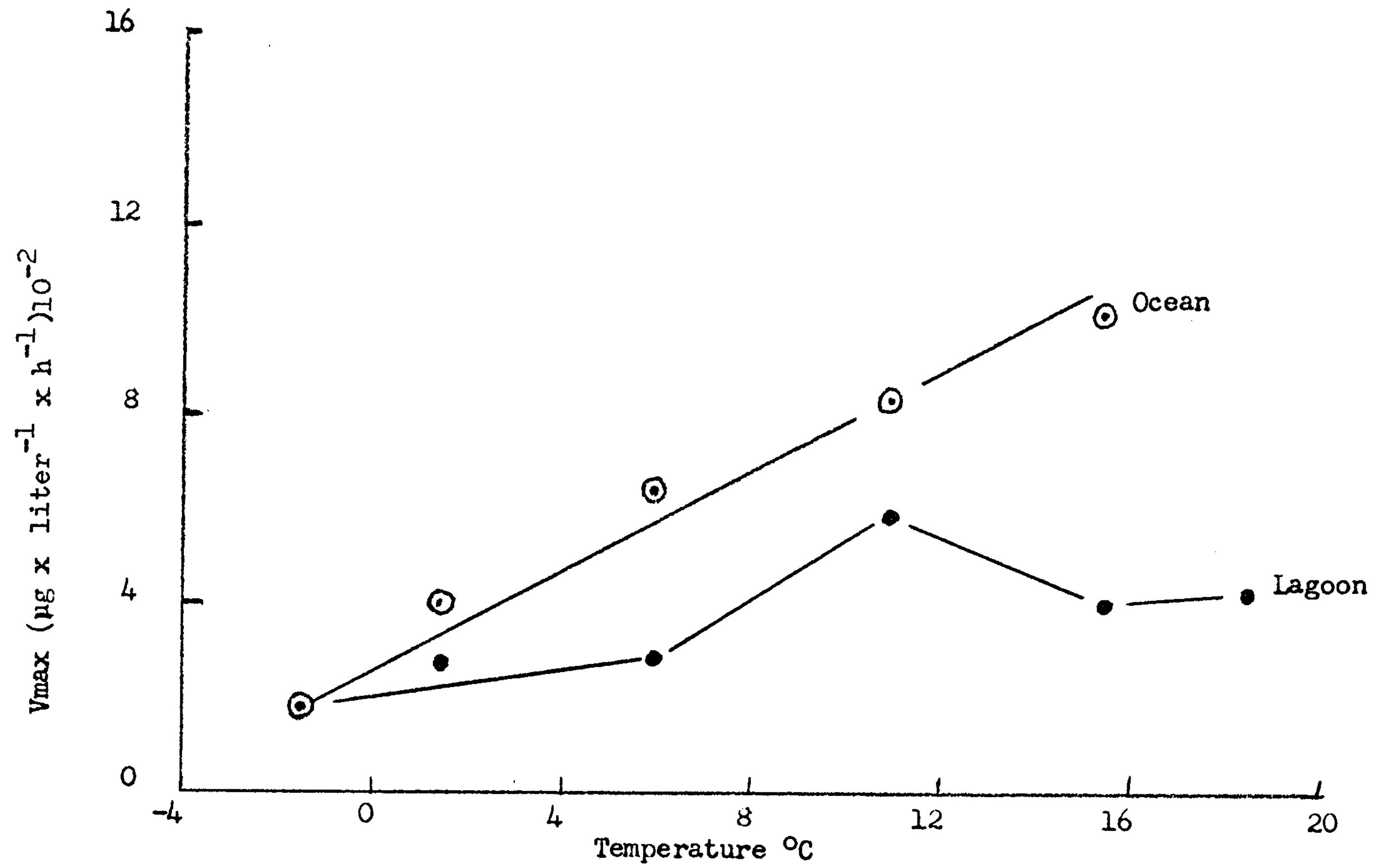


Figure 4. Another experiment using the same parameters described in Figure 3.

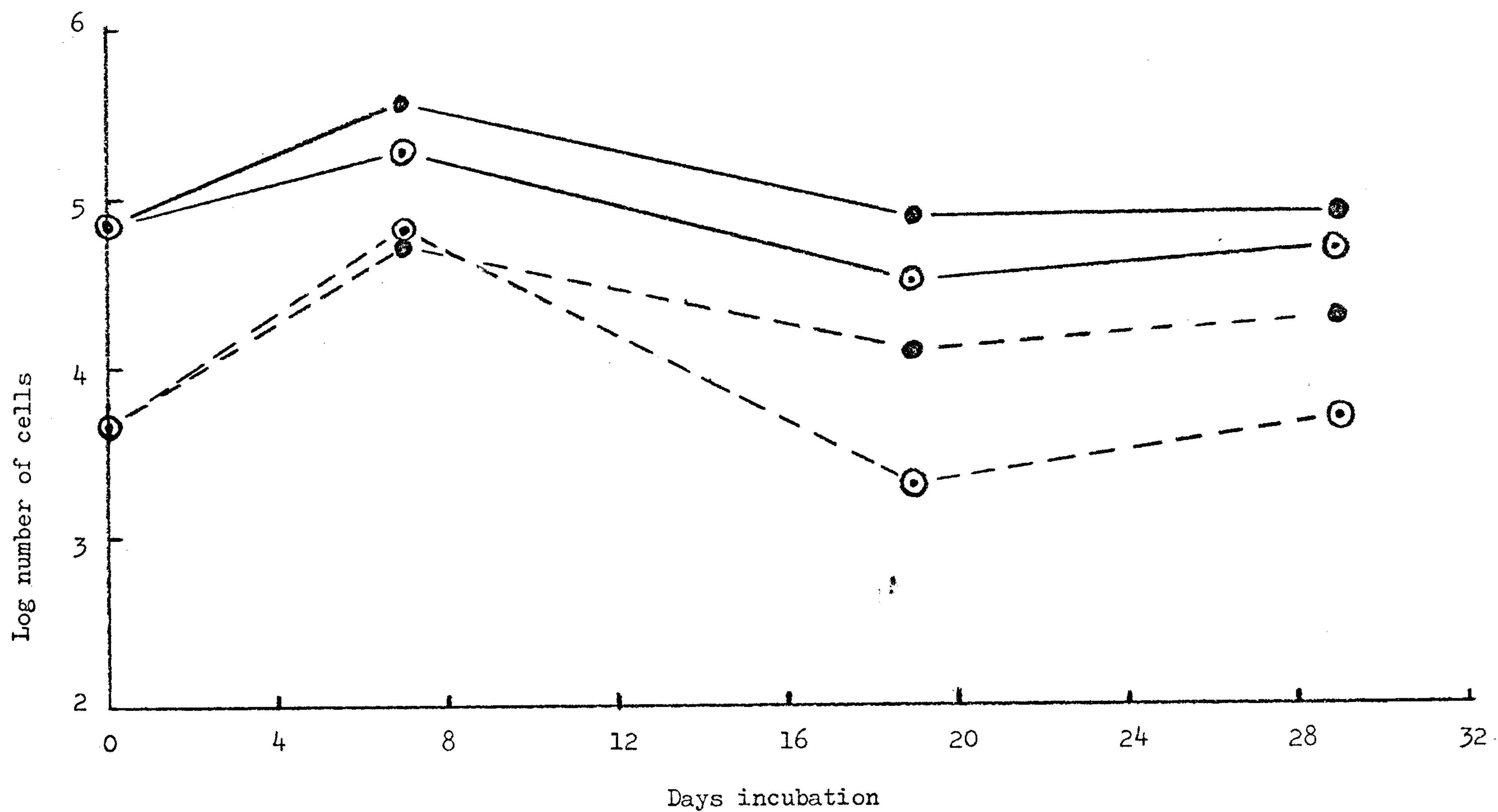


Figure 5. The concentration of bacterial cells as measured on agar plates using Lib X medium (solid lines) and crude oil medium (dotted lines). Measurements were made in a seawater sample containing crude oil (●) and an identical seawater sample containing yeast extract (⊙).

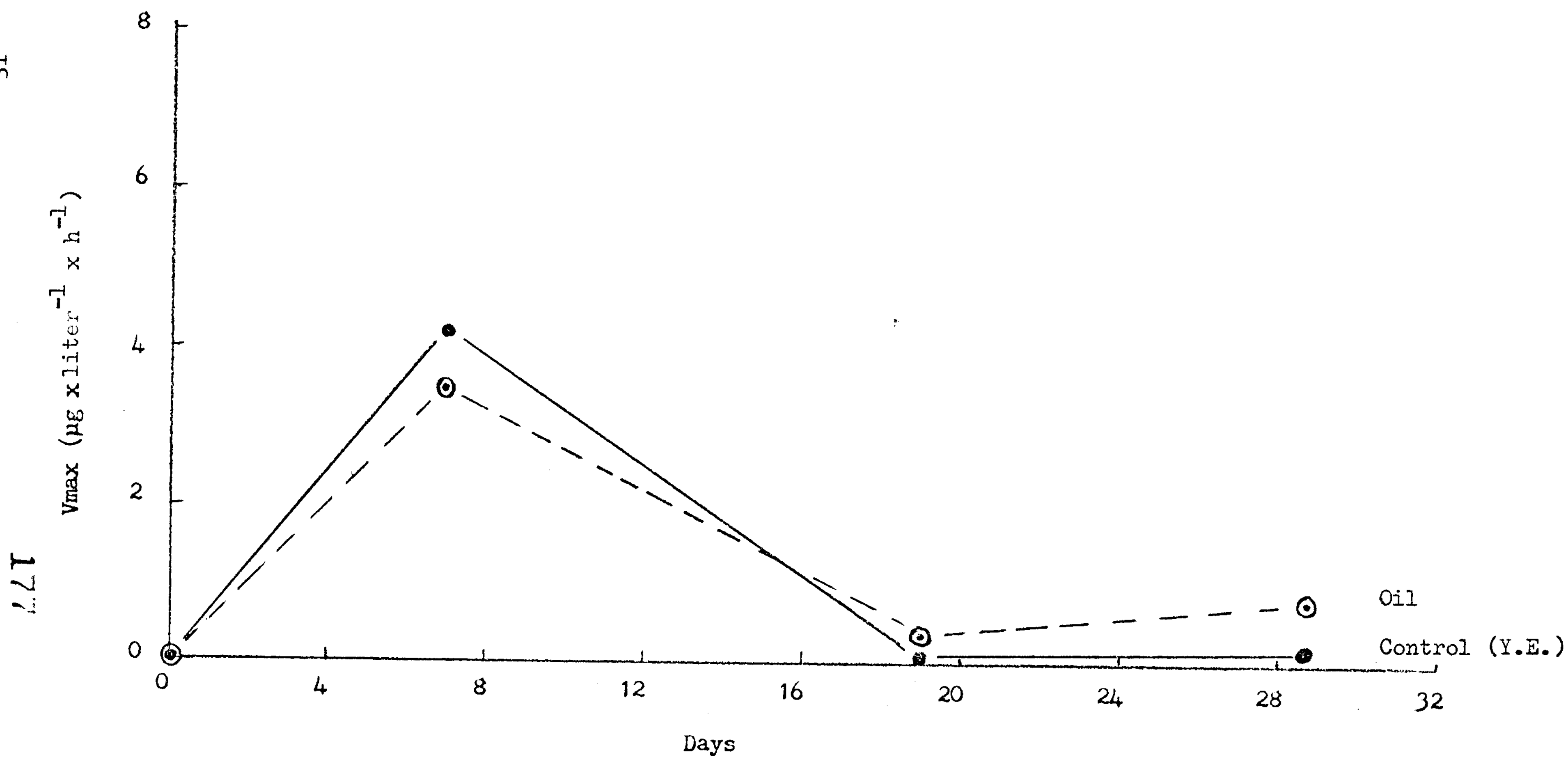


Figure 6. Changes in the maximum velocity of metabolism (Vmax) with time in a crude oil enrichment culture (⊙) and control (●) using ^{14}C glutamic acid as the assay substrate.

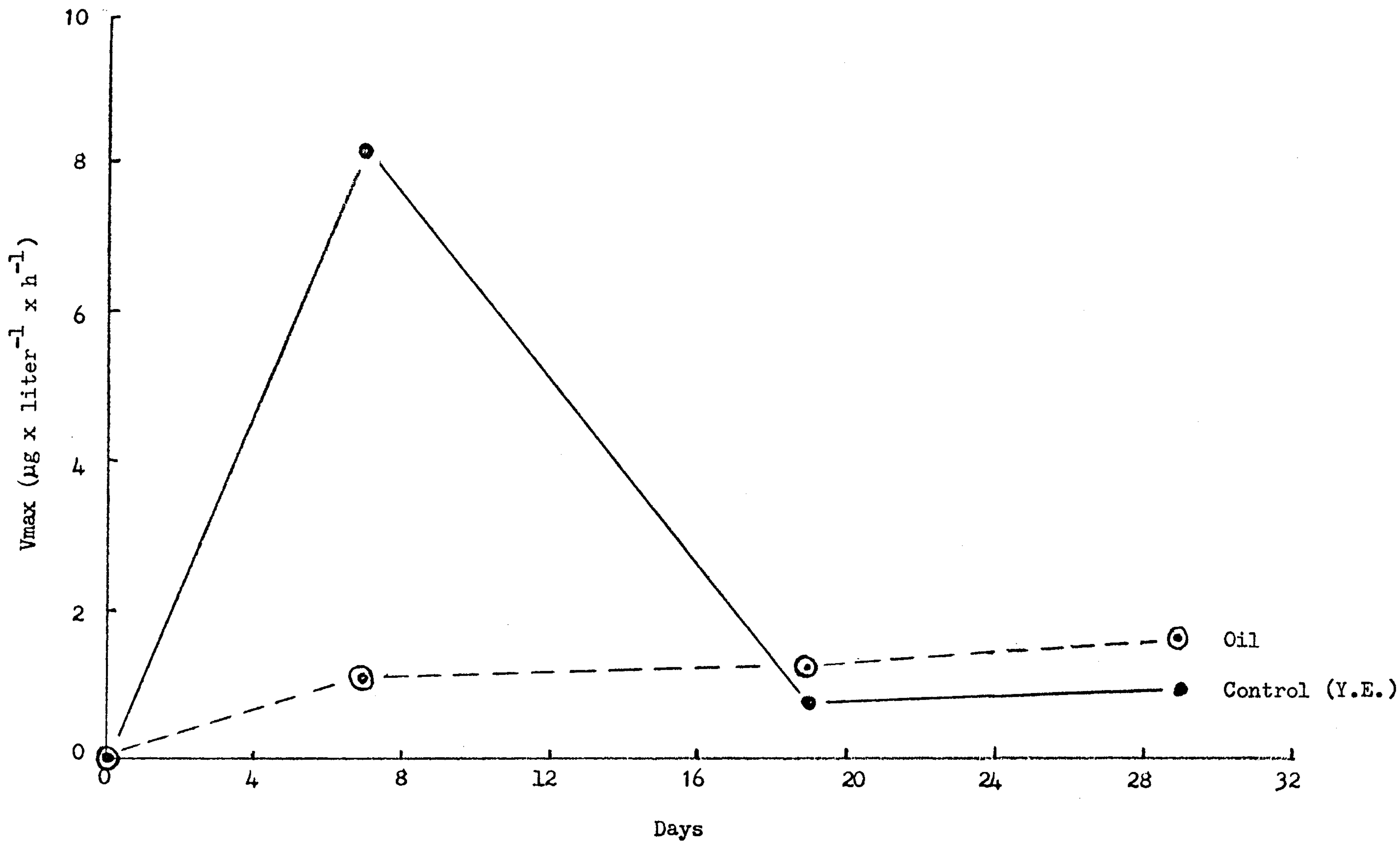


Figure 7. Same as in Figure 6. except acetate was used as the assay substrate.

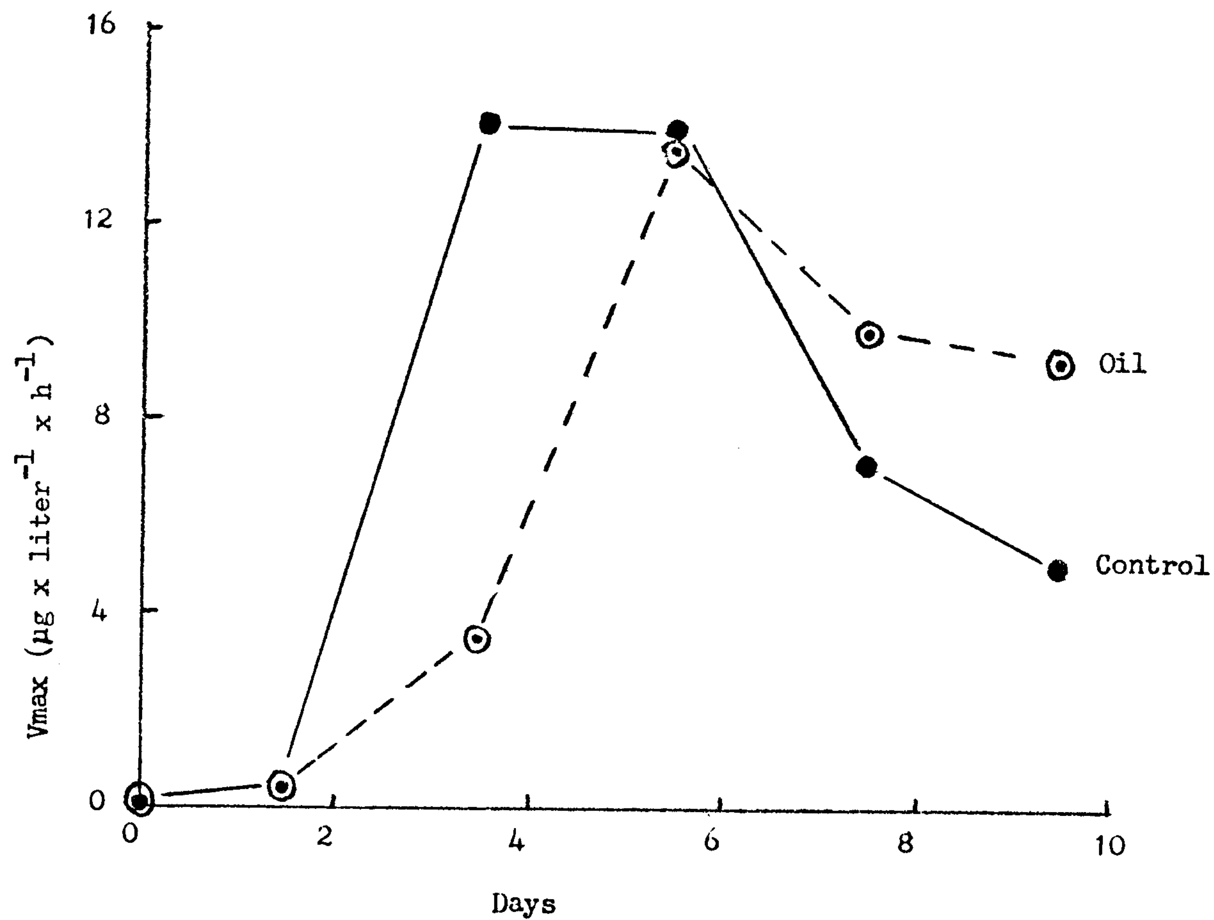


Figure 8. The same parameters as those descibed in Figure 6 except the control did not contain 0.8 mg yeast extract per liter as was the case in the previous experiment.

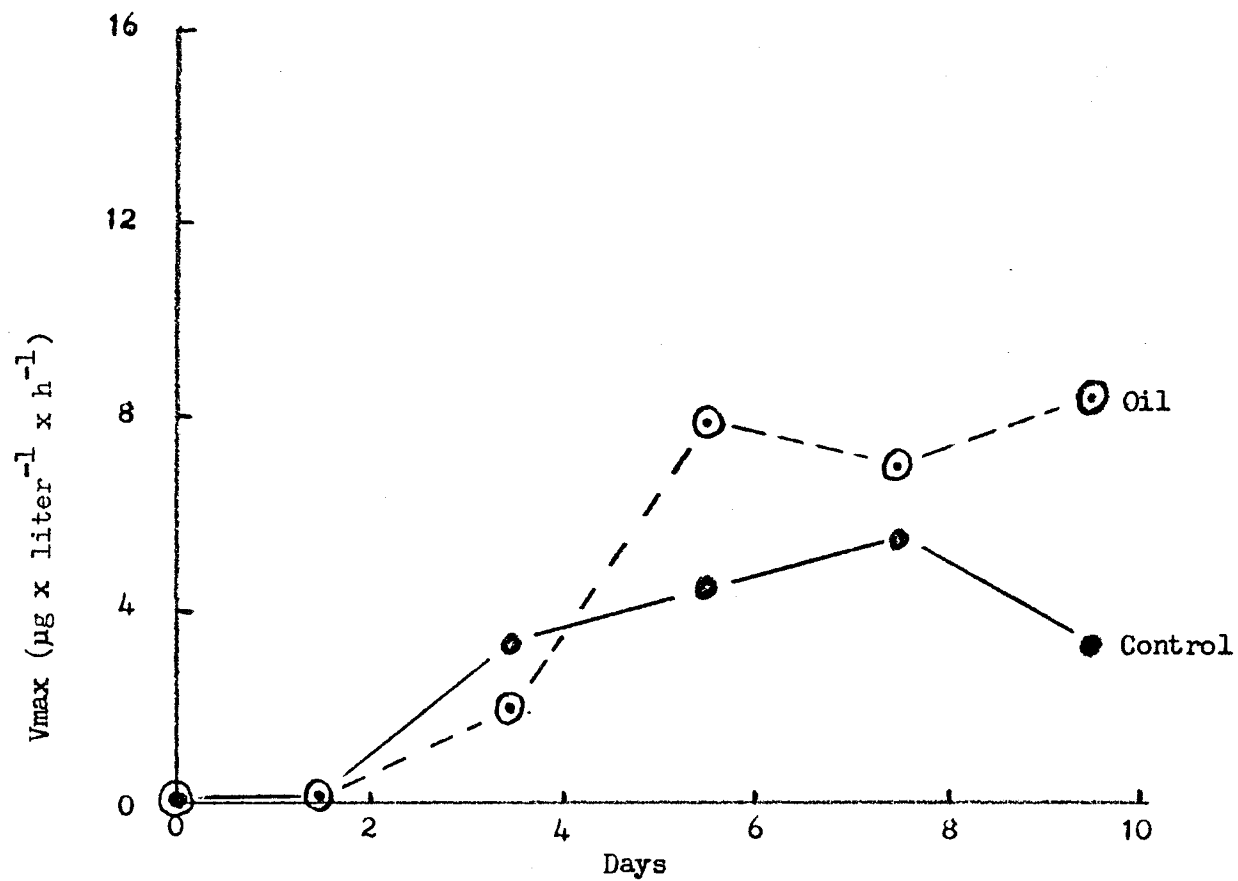


Figure 9. The same parameters as those described in Figure 7 except the control did not contained 0.8 mg yeast extract per liter as was the case in the previous experiment.

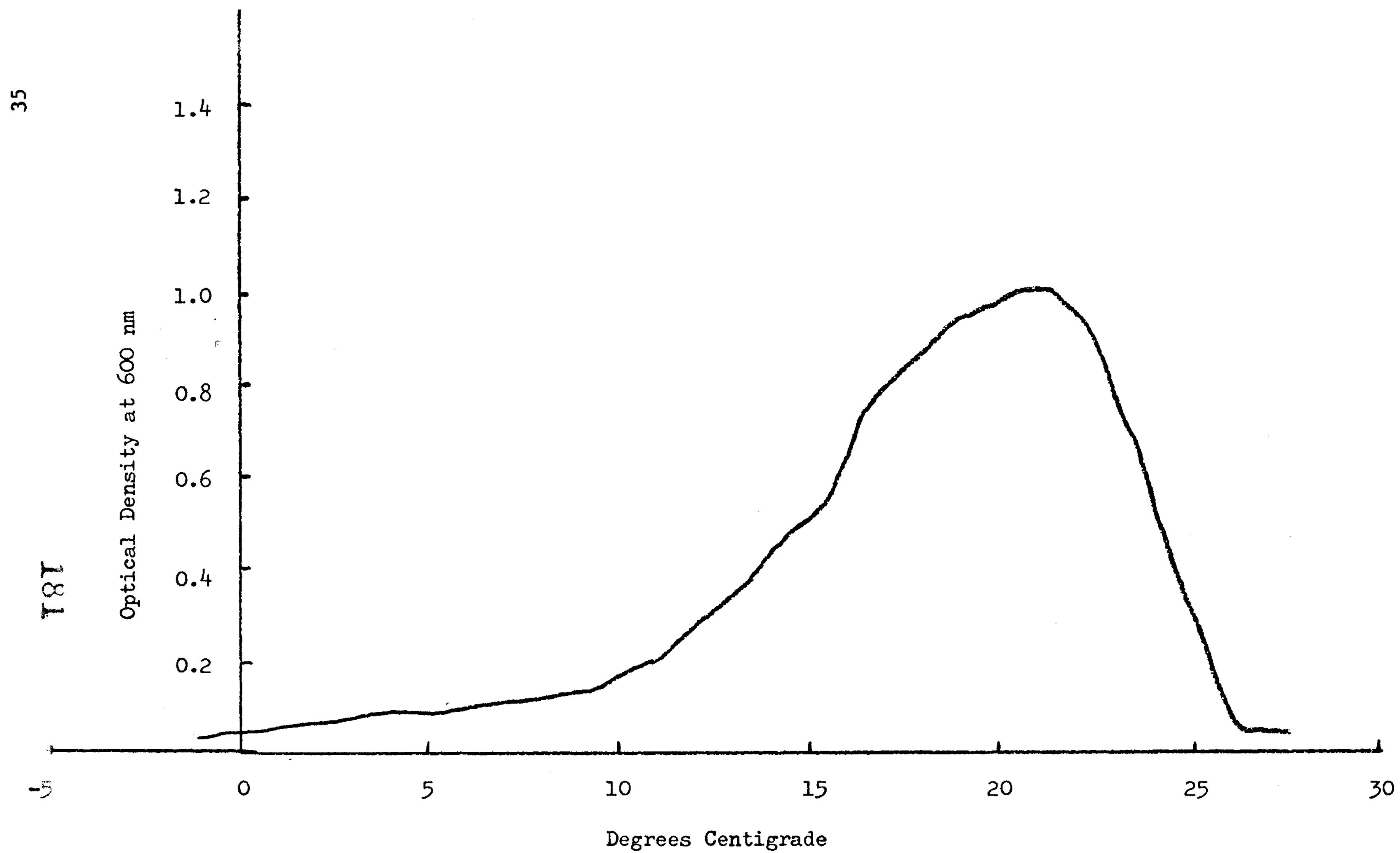


Figure 10. Temperature- growth profile of isolates number 52, 53, and 59. Optical density was measured after 42 hours incubation.

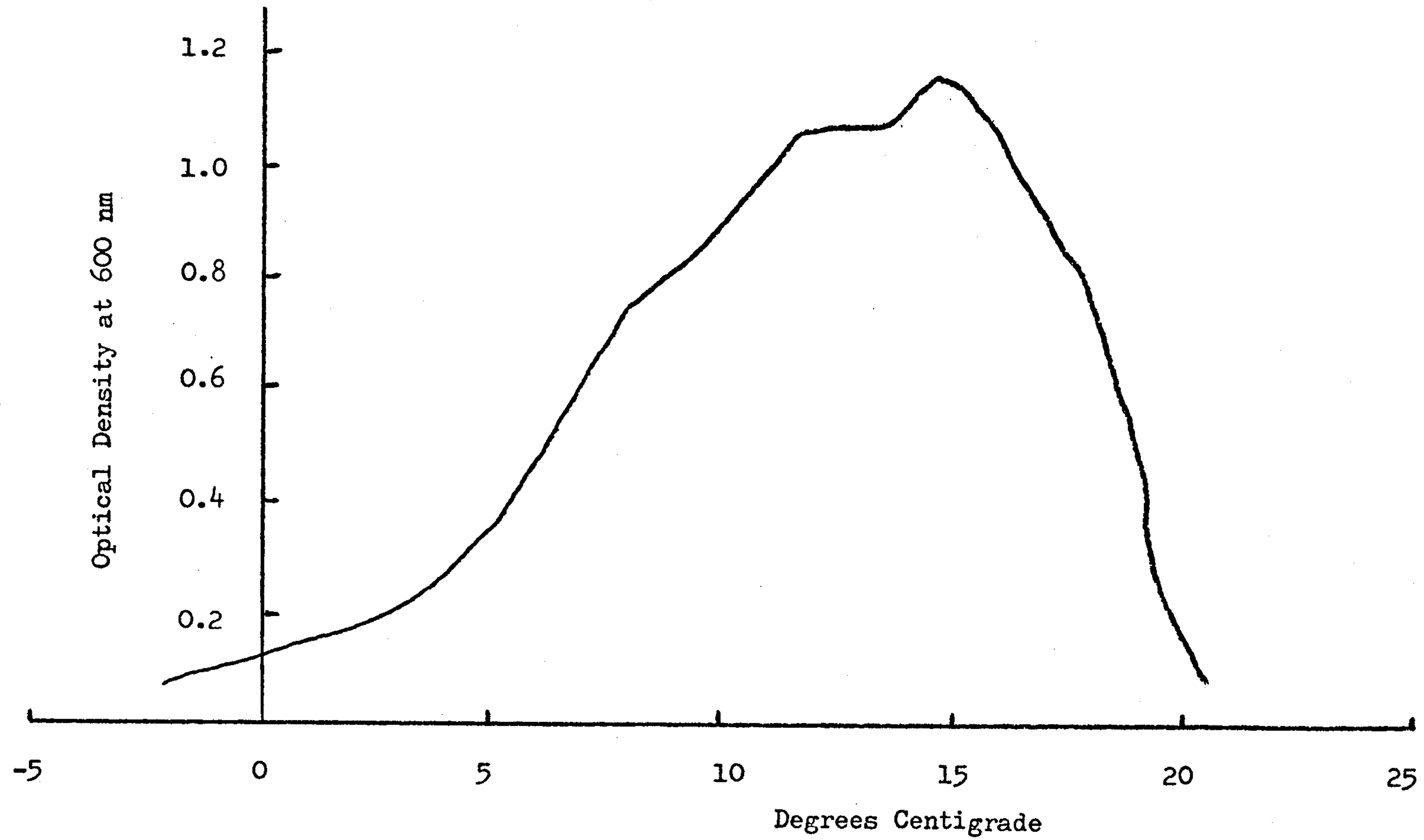


Figure 11. Temperature- growth profile of isolate number 30. Optical density was measured after 42 hours incubation.

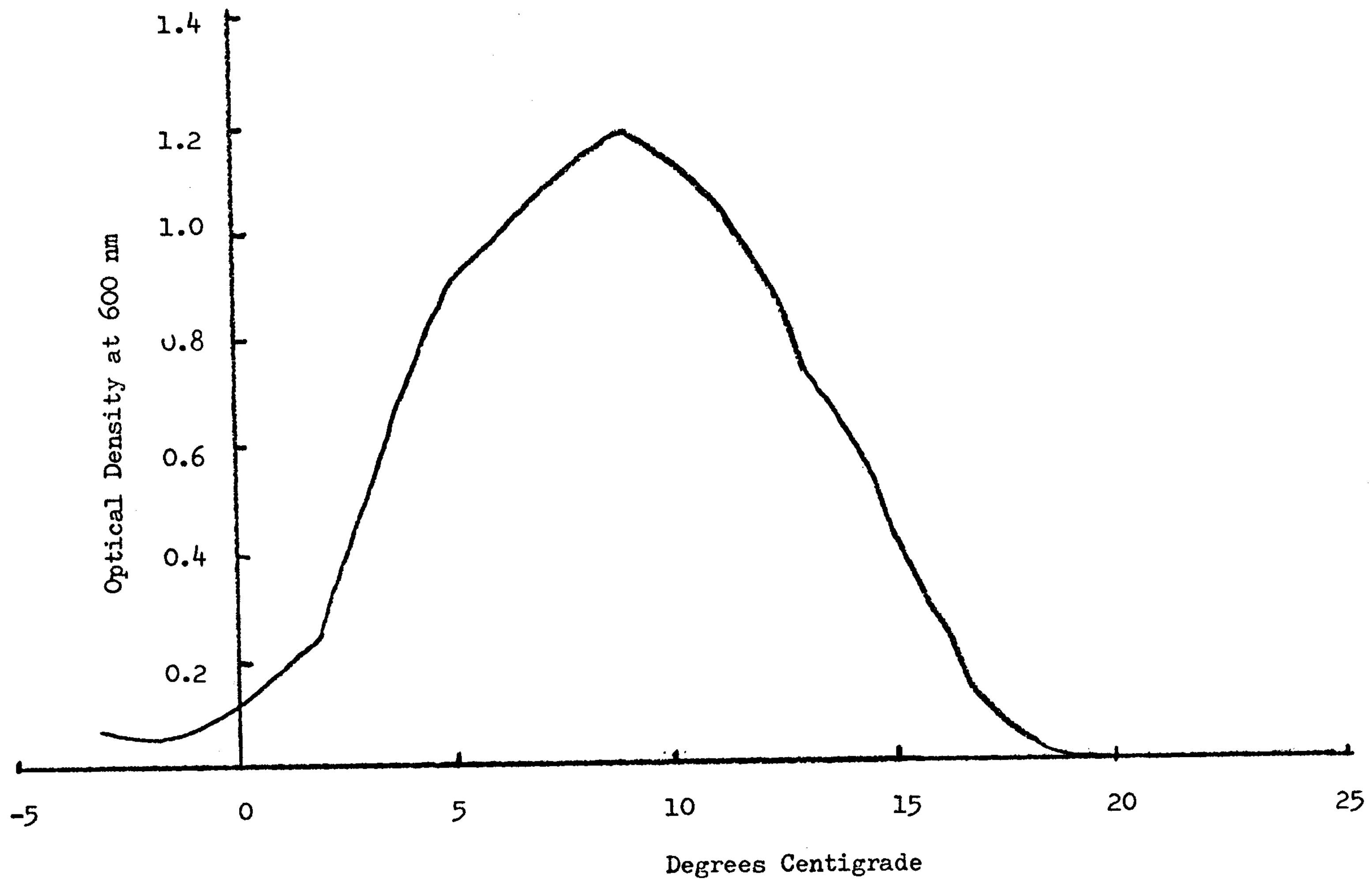


Figure 12. Temperature- growth profile of isolates number 45 and 47. Optical density was measured after 42 hours incubation.

VII. Discussion

The report covers data that was collected during one field trip to the Beaufort Sea and subsequent laboratory studies. Since there is no information of the type we have collected available as a basis for comparison, it will take several more years of field work before enough comparative data is collected from which to draw definite conclusions. The following discussion should be reviewed with this restriction in mind.

A. Heterotrophic potential studies

During the course of this study, water and sediment samples were taken from two regions; Point Barrow and Prudhoe Bay. A comparison of the data collected in these two regions will give some indication of variations which can be observed in two similar marine ecosystems along the shore of the Beaufort Sea. Of the physical and chemical factors measured, only the average salinity and phosphate concentrations showed significant differences. The difference in the salinity (20.5 o/oo at Prudhoe Bay and 26.5 o/oo at Barrow) was probably not large enough to cause significant differences in microbial activity.

The lower levels of phosphate seen in the Prudhoe Bay area did not adversely affect the levels of microbial activity as indicated by V_{max} measurements. The lower levels of phosphate observed in this region may have an indirect effect on microbial activity by affecting primary productivity but at this point not enough is known about these interactions to make a valid prediction.

There were also significant differences in the average values for all calculations made from the heterotrophic potential studies. The maximum potential velocity (V_{max}) of glutamic acid uptake was lower at Barrow than in Prudhoe Bay and the turnover time (T_t), percent respiration and transport constant and natural substrate concentrations ($K_t + S_n$) were all higher in the Barrow samples. These data suggest that the level of microbial activity in the Prudhoe Bay area is higher even though the concentration of bacteria as determined by direct counts was about the same in both areas.

The turnover time (T_t) was about one fifth as long in the seawater samples taken in Prudhoe Bay. Since T_t is the time in hours required for the natural microbial population to utilize all of the naturally occurring glutamic acid, any decrease in this quantity reflects either an increase in microbial activity, a decrease in the natural concentration of glutamic acid or a combination of both. We do not have any information on the natural concentration of glutamic acid in these waters nor do we have any information on the actual *in situ* metabolic rates thus we do not know to which extent these two factors play a part in the lowered T_t values in Prudhoe Bay.

This study was made in collaboration with a study made by Dr. Atlas and his associates. Since all measurements were made on the same samples,

our results are directly comparable to theirs. Their measurements of total cell numbers as determined by colony growth on marine agar plates incubated at 4 C show many of the same patterns that we observed in relative V_{max} determinations. They found that the average cell concentration as determined by plate counts was higher in both water and sediment samples taken in Prudhoe Bay when compared to those taken in the Barrow area (Atlas, Semi-annual Report No. 1).

Not only were there differences seen in the average V_{max} values observed in the two major regions studied, but there were other consistant differences seen within the two sample areas. Of the first four stations sampled in Elson Lagoon (Figure 1), station number one consistantly showed higher V_{max} values (Table 3a). This station was located in a "hook" along the spit. Due to its location, it is quite likely that a large amount of organic material was trapped within this area with a resulting increase in microbial activity.

In Prudhoe Bay, another trend was noted; the further from shore a water sample was taken, the lower the observed V_{max} (Table 3b). A similar trend can be seen in the cell count data reported by Atlas, i.e., the further from shore the sample was taken, the lower the cell concentration. This follows the same trend that has repeatedly been reported by others, namely, as one samples further from shore, the concentration of bacteria as determined by plate counts, decreases. This pattern is probably due to both a decreased input of terrestial organisms and reduction in the level of available nutrients.

Another factor that was measured in both water and sediment samples taken from these two areas was the percent respiration. This is the amount of glutamic acid that was mineralized to CO_2 relative to the total amount of glutamic acid taken up by the cells (more precisely the amount respired plus the glutamic acid carbons that were incorporated into macromolecules). Two trends were noted. The average percent respiration was lower in both water and sediment samples taken from Prudhoe Bay than those taken from Barrow. Secondly, the average percent respiration was lower in sediment samples than in water samples (Tables 2a and 2b). It is not known at present what has caused these trends but the underlaying factor is probably the physiological state of the cells. This will be investigated further in the laboratory. One possibility is that when cells are in a balanced growth condition, i.e. they have all the nutrients in the right proportions they need for growth, a larger percentage of the nutrient that is taken up will be utilized in biosynthesis. As the conditions for growth become less favorable, more of the nutrient will be used as an energy source and thus a greater percentage will be respired to CO_2.

There are many situations in which fixed nitrogen is an important limiting factor in the biodegradation of crude oil. At the present time there is very little information available on the nitrogen cycle in the waters of the Beaufort Sea. We plan to place more emphasis on this problem as our studies continue. There is, however, one factor that can

be extrapolated from our heterotrophic potential data which will give some estimate of potential ammonium ion release as the result of amino acid oxidation. Taking the average V_{max} and percent respiration data from the seawater samples we analyzed, we can calculate the maximum potential release of ammonium ion into the seawater due to substrate oxidation by the microbial population. This figure is 0.02 mg-at/m^3/hr or 0.02 µg-at/liter/hr. The average concentration of ammonium ion in the seawater samples tested was 0.8 µg-at/liter/hr. If enough glutamic acid was made available to the natural microbial population, the concentration of ammonium ion found in these waters could be replaced every 40 hours.

Although we have no information on the natural concentration of ammonium in the sediments, we can calculate the potential input of ammonium due to the oxidation of glutamic acid. Again, using the average V_{max} and percent respiration figures in sediments, we can calculate that the maximum rate of ammonium input would be 1.6×10^{-3} µg-at/ g dry weight/hr. This figure can be extrapolated to 0.38 mg-at ammonium ion released per square meter of sediment per day. These figures are all maximum potential rates and should not be considered as actual *in situ* rates. Only with further study will we be able to estimate actual *in situ* rates of ammonium ion production.

One factor that should be kept in mind while interpreting the results of the heterotrophic potential studies is that the technique used in preparing the samples for the radioactivity assay removes a significant amount of the labeled substrate from the cells (Griffiths, Hanus, and Morita, 1974). A further investigation into this phenomenon indicated that cells that had been treated as they have in this study (acidification prior to assay) retain only that label that was associated with the substrate that was incorporated into macromolecules (Baross et al., 1975). Subsequently, all substrate that was bound to the surface of the cell and/or contained as pooled material within the cell was lost. Thus all of the V_{max} calculations should be considered as conservative estimates of the potential amount of substrate that could be taken up by the cells.

B. Incubation temperature studies

Temperature has traditionally been cited as one of the most important factors affecting levels of metabolic activity in biological systems. The importance of this parameter in the biodegradation of crude oil in marine environments has been pointed out by several investigators (Atlas and Bartha, 1972; Mulkins-Phillips and Stewart, 1974; and Gibbs, Pugh, and Andrews, 1975). Until now, nothing has been reported on how temperatures above that found *in situ* might affect the function of natural microbial populations in the Beaufort Sea. Local warming during the summer months in relatively shallow waters such as those found in Prudhoe Bay have been observed (R. M. Atlas, personal communication). This phenomenon could be further intensified locally by the addition of a solar radiation absorbing slick of crude oil on the water's surface.

The effect of incubation temperature on the observed V_{max} values in a given population was studied (Figures 4 and 5). With the exception of the deflection in the V_{max} observed in the lagoon samples at about 15 C, there appeared to be very little indication of functional changes due to thermal injury within the temperature range studied. This is in contrast to the observations made by Griffiths, Hayasaka and Morita (1976) in a similar study conducted on seawater samples taken from the Antarctic Ocean. In that study, two of the four samples studied showed a large reduction on the V_{max} values as the samples were heated to 16 C. Even though there was little evidence of thermal injury in the uptake and respiration of glutamic acid, data reported by Atlas in his first semi-annual report suggest that there is probably a significant psychrophilic population present in these waters. His data show that the number of bacteria that would grow on plates incubated at 4 C was, on the average, greater than those capable of growth at 20 C.

In both studies in which ocean and lagoon samples were compared, there was a difference in the V_{max} patterns observed with increasing incubation temperature. Both ocean samples showed greater increases in V_{max} values than did the lagoon samples. The response in the ocean samples was also more linear than in the lagoon samples. These trends probably indicate a basic difference in the microbial populations found in these two waters. More work will have to be done to substantiate these findings and to determine what, if any, application the temperature profiles of bacteria might have in characterizing water masses. One factor that was brought out by these studies was that even though the observed V_{max} might be the same in two water samples, it is not possible to predict what the V_{max} will be when the water temperature is raised.

C. Effects of crude oil on natural microbial populations.

One of the objectives of this study was to determine what the immediate effect of crude oil might be on a natural microbial population in marine waters. There is no one technique which will enable the observer to measure all possible functions under *in situ* conditions. The technique used involved adding an aqueous extract of crude oil to the reaction vessels used in the measurement of heterotrophic potential. When the subsamples that contained the crude oil extract were compared with the controls, no consistant differences could be seen. These results suggest that the transport, respiration and the incorporation of glutamic acid into macromolecules were not adversely affected by the presence of crude oil. We do not know how closely the experimental conditions imitate those found *in situ* during an actual oil spill nor do we know what other functions may be affected.

Another series of experiments was conducted which was designed to determine the effects of crude oil itself on glutamic acid uptake over a longer period of time. In these experiments, natural seawater samples were exposed to crude oil and incubated at 4 C. Subsamples were taken periodically to determine the levels of glutamic acid uptake and to determine the number of organisms capable of forming colonies on two

agar media. In both experiments, the V_{max} values were lower in the oil enrichment cultures than in the controls near the beginning of the experiment. This was the case when either acetate or glutamic acid was used as the labeled substrate. In a similar study, which is currently in progress, the same trend was observed. Plate counts made during the first experiment indicated that at the beginning of the experiment, even though the crude oil apparently had an adverse effect on the V_{max} values, there was no adverse effect on the number of organisms that were able to form colonies on either of the media utilized. As the incubation time was increased, the V_{max} as measured using either acetate or glutamic acid was higher in the oil enrichment culture and the cell counts on both media increased as well. It would appear from these data, that after several day's incubation, there may be some adverse effect caused by exposure to crude oil, but soon after that time, the crude oil itself or byproducts of crude oil degradation may act as a substrate for cell growth.

One important factor must be kept in mind when interpreting the results of these experiments. By the very experimental design of these studies, significant errors have been introduced. Namely the drastic changes which are known to occur in any seawater sample that is confined to a vessel. ZoBell (1946) was one of the first to observe that the number of bacteria present in a given water sample markedly increases after a relatively short period of confinement. Since the conditions of confinement are much different than that found *in situ*, it is quite likely that after a relatively short period of time, shifts in the composition of the population occur, thus the resulting population is no longer representative of the original.

VIII. Conclusions

1. The levels of potential glutamic acid uptake (V_{max}) observed in water samples taken from the Beaufort Sea were as high as those observed in other relatively productive marine waters.

2. The methods used to measure heterotrophic potential in water samples were modified for sediments. The microbial activity in the sediments was considerably higher than that found in the water column. In the relatively shallow waters that we studied, the sediments accounted for about 400 times the activity found in the overlaying water column. Direct bacterial counts of sediments and water samples showed that, when compared on a volume to volume basis, there were roughly 1,000 times more bacteria in the sediments than in the water column.

3. Basic differences were found in the water and sediment samples analyzed in the Barrow area and in Prudhoe Bay. Both the salinity and the concentration of phosphate were lower in Prudhoe Bay. The maximum potential velocity of glutamic acid uptake was higher in both the water and sediments of Prudhoe Bay than those of the Barrow area. The percent of glutamic acid respiration was lower in Prudhoe Bay.

4. In both Barrow and Prudhoe Bay there were locations which consistantly showed higher levels of potential microbial activity. In Barrow the highest levels were seen in the water samples taken at station #1 and in Prudhoe Bay, the water samples taken closest to shore gave the highest levels.

5. When the effects of incubation temperature on the maximum potential velocity of glutamic acid uptake (V_{max}) was studied, it was found that there was a marked increase in V_{max} with increasing incubation temperature. These data suggest that over the temperature range that might be expected from localized warming, no loss of activity should result during short warming periods. These data also showed that there were basic differences between the response of open ocean water and lagoon microorganisms to heating. This phenomenon should be explored further to determine if this might be a useful method for differentiating microbial populations.

6. On the average, there was roughly a 10% increase in the observed V_{max} values with each degree increase in the incubation temperature above that found _in situ_.

7. Studies on the effects of melted ice water on heterotrophic potential data suggest that when melting ice water is released into the surrounding seawater, there is little effect on the observed microbial activity or the percent respiration. The level of potential microbial activity found in the ice water melt was close to that found in the surrounding seawater.

8. The acute effects of crude oil on the uptake and respiration of glutamic acid was studied by adding an aqueous crude oil extract to the reaction bottles used to determine heterotrophic potential. No consistant alteration in function was observed when the extract was added.

9. Changes in the heterotrophic potential with time were studied in a natural microbial population exposed to crude oil using both labeled glutamic acid and acetate. In the initial stages of incubation, the levels of activity were lower in the crude oil enrichment than in the control. As the incubation progressed, the levels of activity increased in the crude oil enrichment until they were higher than in the control.

10. There was a different pattern of uptake with time in the two labeled substrates indicating that there might have been shifts in the relative function of various groups of organisms within the original population. Further study must be made to determine the significance of these shifts.

11. In the crude oil enrichment studies, there was also evidence that within the aqueous phase, the concentration of crude oil utilizing bacteria increased faster in oil enrichment culture than in the control.

12. Sulfate reducing bacteria appear to be very common in the inshore sediments of the Beaufort Sea.

13. Psychrophilic crude oil degrading bacteria are probably quite rare in the waters of the Beaufort Sea. Of the 150 crude oil degrading bacterial strains isolated from this region, only three strains have a maximum growth temperature below 20 C. Hence most of bacteria degrading crude oil are probably psychrotrophic.

IX. Needs for further study

a. The heterotrophic potential studies should be continued to include representative water, sediment and ice samples from more geographical locations. These studies should be expanded to extend our knowledge of the natural temporal and spacial variations in microbial activity. The location of sample sites should be expanded to include respresentative shoreline environments as well.

b. Studies on the effects of crude oil on natural microbial populations both in water and in sediments should be continued using improved techniques. The range of functions monitored should be expanded by using different substrates.

c. Whenever possible, sampling should continue to be coordinated with Dr. Atlas with supportive nutrient data provided by Dr. Alexander.

d. Since fixed nitrogen is so important to the biodegradation of crude oil, some estimate should be made of the rates of nitrogen fixation and nitrification in the sediments of shallow waters.

e. The basic physiology of the psychrophilic marine hydrocarbon utilizing bacteria should be studied further to determine how they function under simulated _in situ_ conditions as found in the Beaufort Sea. (The physiological studies of hydrocarbon degradation by these organisms have not progressed as planned because of the time delay in acquiring the gas

chromatograph. This instrument should be operational by June, 1976.) These studies will give a better understanding of the potential function of these organisms in the event of an actual oil spill and will give some estimate of their potential usefulness as seeding organisms.

f. The usefulness of incubation temperature profiles for differentiating certain populations of microorganisms should be explored further.

g. The results of the oil enrichment culture experiments indicate that the measurement of heterotrophic potential using more than one substrate may, under certain conditions, give useful information about the function of more than one group of organisms within a given sample. These possibilities should be explored further.

X. Summary of 4th quarter operations.

A. Laboratory activities at Oregon State University.

During this period, much of our effort was directed toward compiling, analyzing and reporting the data collected during our summer field study period. In addition, we have isolated and purified over 150 strains of hydrocarbon utilizing bacteria. Of these strains, 6 were selected which were either psychrotrophic or psychrophilic in nature. Studies were initiated which were designed to determine their growth - temperature profiles and identity. A crude oil enrichment study was initiated using a natural water sample taken from Yaquina Bay, Oregon. This study was designed to give a better understanding of the changes that take place within a natural microbial population when it is exposed to this pollutant.

B. Ship activities

During this period, we will have initiated our first study in the Gulf of Alaska. We will be participating in a two week cruise on board the Discoverer. After the cruise, we will proceed to NARL to continue the studies that we initiated there last summer.

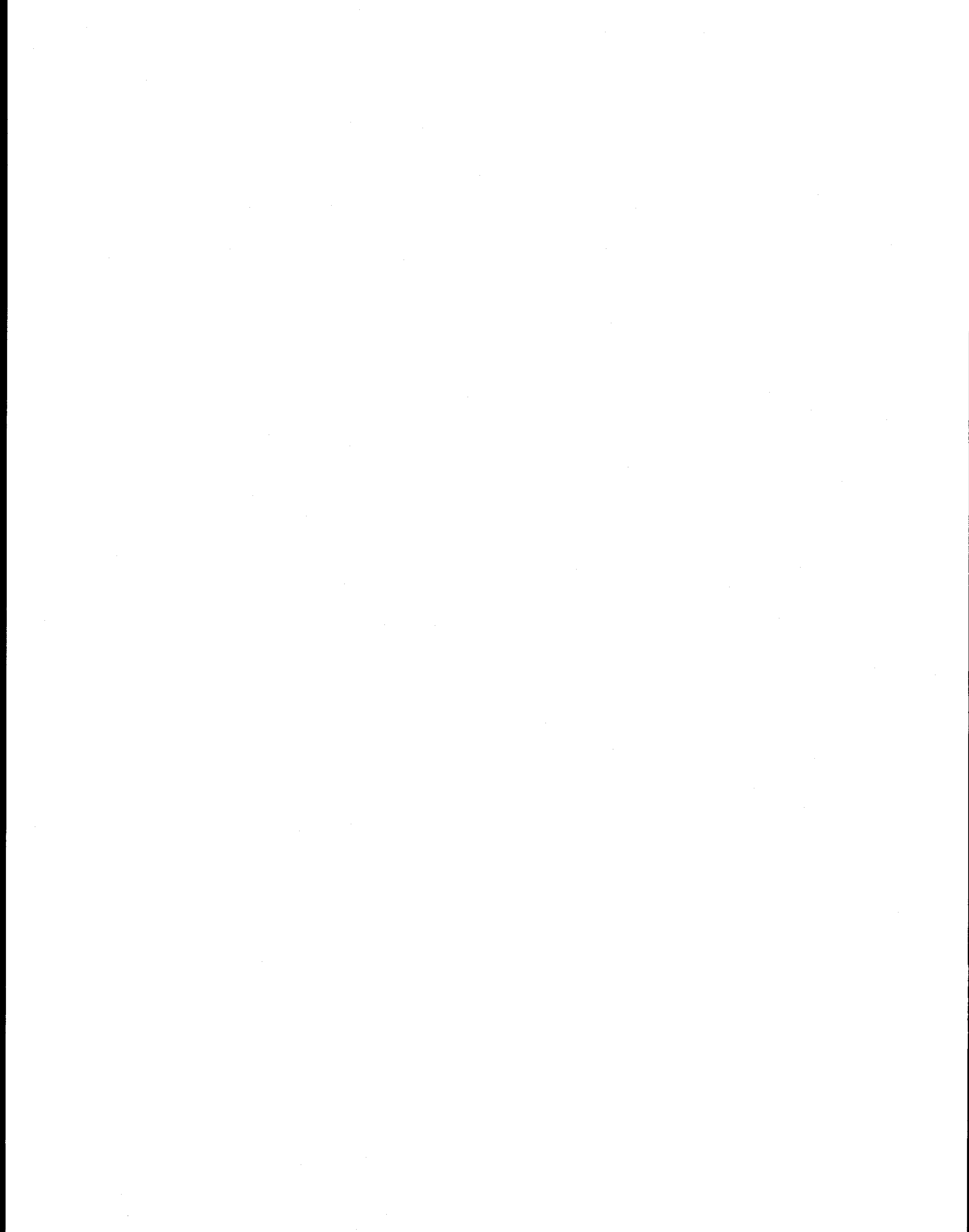
1. We will be on board the Discoverer from 16 March to 3 April, 1976.

2. Dr. Robert P. Griffiths of the Department of Microbiology, Oregon State University, will be the sole investigator on board from our group.

3. The same methods will be used as were described earlier in this report.

4. A select number of stations will be sampled along the standard sampling grid for the NE Gulf of Alaska; see reference to leg II of the Discoverer Project Instructions dated January 30, 1976 from Dr. Herbert E. Bruce.

5. Heterotrophic potential data will be collected on approximately 20 seawater and sediment samples.



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HYDROCARBONS: NATURAL DISTRIBUTION AND DYNAMICS ON THE ALASKAN OUTER CONTINENTAL SHELF

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I. Summary

The objectives of this continuing work are to determine the kinds and amounts of hydrocarbons in water, biota, sediment, and seston in the Alaskan OCS environments and to determine the extent to which hydrocarbons are adsorbed by suspended sediments of the Gulf of Alaska. These measurements provide the link between impacts, such as the decline of a biological population, and suspected causes of those impacts, such as the addition of petroleum to the system.

Based on work so far completed, it appears that the levels of hydrocarbons in the Alaskan OCS environments are as low as, or lower than other areas of the world ocean not subject to obvious petroleum pollution. Information is still lacking about how hydrocarbons are dispersed and degraded in these environments.

II. Introduction

The general goal of this project is to measure the ambient kinds and amounts of hydrocarbons in various components of the Alaskan OCS environment and to study key processes by which added hydrocarbons are transported and degraded in this environment. The primary focus of this work has been the Gulf of Alaska. Work has also begun in the Bering Sea. Proposed work in the Beaufort Sea has not yet begun because of lack of logistic support.

Measurements of ambient levels of hydrocarbons are relevant to petroleum development because these measurements show that the Alaskan OCS presently has hydrocarbon levels comparable to other areas of the world ocean free of obvious hydrocarbon contamination. Thus, these measurements provide reference "baseline" data for future comparison. The study of transport and degradation processes will lead to the ability to predict the fate and effects of petroleum which enters the Alaskan OCS.

This project has several interrelated specific objectives:

1. Collection and analysis of biological materials from the Gulf of Alaska and Bering Sea.
2. Collection and analysis of surface water from the Gulf of Alaska.
3. Collection and analysis of floating tar from the Gulf of Alaska and the Bering Sea.
4. Collection and analysis of benthic sediments from the Gulf of Alaska, Bering Sea and Beaufort Sea. This work has been carried out by a sub-contractor and will be reported on separately by him.
5. Investigation of the interaction of petroleum hydrocarbons with suspended sediments from the Gulf of Alaska under laboratory conditions.

III. Current State of Knowledge

The methodology for the analysis of environmental materials for hydrocarbons is in a state of evolution; considerable controversy exists among analysts as to which of the available procedures are most suitable for this kind of work. In order to proceed with this project, it has been necessary to select specific (but undoubtedly not universally favored) analytical procedures. This has been done after comparison of several of the methods in current use. To facilitate comparison of the results obtained here with those of other studies, we present a detailed description of our method and provide comparative results which relate it to other methods.

The results of this study, reported here and in our previous annual report, (Shaw, 1975), are the first measurements of hydrocarbons on the Alaskan OCS. There is no historical data base to draw on.

IV. Study Area

In the Gulf of Alaska this project's study area includes that part of the continental shelf from Yakutat Bay on the east to Unimak Pass on the west. In the Bering Sea, the study area is that part of the southeastern Bering including Bristol Bay bounded on the northwest by St. Matthew Island and on the southwest by Unalaska Island. In the Beaufort Sea, the study area includes that part of the continental shelf between Point Barrow on the west and Demarcation Point on the east.

V. Methods and Rationale of Data Collection

1. Sampling.

Our plans for collecting samples of materials to be analysed were formed in consideration of the need for geographic and temporal coverage as well as the limitations of the total number of samples to be analysed and the logistic support available. Water samples have been collected quarterly in the hope of obtaining data in sufficient detail to allow it to be related to the movement of water masses and to seasonal biological fluctuations. To facilitate such comparisons, water collections were made at stations selected from those being used for collection hydrographic, zooplankton and benthic fauna data. The details of water collections are set forth in Tables 1, 2, and 3.

Collection of seston to be examined for floating tar has been carried out on an opportunity basis; the specifics are enumerated in Tables 4 - 7.

Biological materials have been collected by other investigators associated with this program as they collected materials for their own programs. This has provided a wide range of species and collection sites. Based on analyses of these materials, decisions can be made about the most desirable species for continued study. The collections of biological materials are presented in Tables 8 and 9.

2. Methods

Biota

Biological collections have been made by several cooperating investigators. Each has been given pre-cleaned bottles, tongs and the following instructions:

"Samples must not be touched directly or allowed to come in contact with plastics or other non-environmental materials. They should be handled only with tongs or other metal implements which have been cleaned just prior to use by heating in a flame (propane torch is suitable for this). If possible, place an entire animal (or several) in a bottle. If a fish is too large, cut the body a few inches forward of the tail at right angles to the backbone and bottle the tail portion. The insides of the jars and their aluminum cap liners have been specially cleaned to prevent contamination. Open and close the lids firmly and gently to prevent damage to the liners. If a liner is damaged, do not use that bottle. Do not touch the interior of the bottle or its liner. Label each jar with location, depth, date, species (if known) and length (if the sample is less than a whole animal). Freeze

the samples immediately and label the sample cases "Keep Frozen". Carry out the sampling in an area free of obvious contamination (oil soaked deck, smell of fuel, downwind of engine exhaust, etc., are common sources of contamination)."

Before the procedure for the analysis of biological materials described below was adopted, a laboratory comparison of several possible methods was made. This comparison study is described in Appendix I.

In the laboratory specific handling prior to extraction is dependent on material being analyzed. For viscera, gills, meat, or other tissue samples we routinely macerate with a virtis homogenizer with 100 ml methanol prior to saponification. This basic procedure is modified for some samples. Ground up shell of invertebrate samples cause bumping during saponification and increases emulsion problems during liquid:liquid extraction. Crab legs are therefore cut into pieces (approximately 1 cm) and saponified directly, without maceration. Fish bones can cause similar problems. If a fish sample is cut into small pieces ($<1\ cm^3$) extraction is still complete in 24 hours.

After the sample is cut into pieces and/or homogenized, 100 ml 1N KOH in methanol and 50 ml H_2O (total 250 ml solvents) are added and the sample is saponified for 24 hours. The pH is then checked to assure that it is greater than 10. The sample is then liquid:

liquid extracted three times with 100 ml hexane. The combined hexane extracts are washed with 150 ml saturated aqueous NaCl then dried overnight with Na_2SO_4. The hexane extract is concentrated to 10 ml. A 100 µl aliquot is air dried and weighed to determine total non-saponifiable lipid.

A column of 10 ml alumina over 20 ml silica is packed in a 50 ml buret. This makes a column of 11 mm x 35 cm with a pore volume of approximately 10 ml. Both alumina and silica are Soxhlet extracted for 48 hours with 1:1 methanol:benzene prior to use. Alumina is activated at 250° for 24 hours and then partially deactivated with 6% H_2O. Silica is activated at 120° for 24 hours and partically deactivated with 5% H_2O (Farrington, et al., 1972). A column:lipid ratio of approximately 1,000:1 is used. Enough sample to yield approximately 20 mg lipid is concentrated to less than 1.0 ml and charged to the column. Two eluates are collected: 40 ml hexane and 40 ml benzene, at 1 to 2 ml per minute. The eluates are concentrated to approximately 1.0 ml for gas chromatographic analysis. For small samples (<18 mg total lipid) smaller columns are used.

Gas chromatographic (G.C.) analysis is done on a Hewlett Packard model 5710A with flame ionization detectors. The colums are 1/8" x 12' stainless steel packed with 3% OV 101 on Chromosorb W 100-120 AW-DMCS. The temperature program is 2 minutes at 80° followed by 80° - 280° at 8° per minute. The carrier gas is helium at 50 ml per minute.

Quantation is done with a Hewlett Packard 3380A integrator using an external standard procedure wherein the area under the curve for the sample is compared to the area under the curve for a standard of known concentration. The efficiency of the entire sample preparation procedure is regularly measured and taken into consideration in the quantation calculations.

Water

Water samples for hydrocarbon analysis are collected from near the bow of the ship immediately upon reaching the selected station. Samples are taken in one gallon glass bottles which have teflon cap liners. These glass bottles are cleaned prior to use by rinsing 4 times with tap water, twice with distilled water and then firing in an oven at 500°C for about 5 hours. The cap liners are cleaned by boiling in methanol, then benzene, and twice in hexane. They are then rinsed and placed by flamed tweezers in the bottle cap. After water collection by submergence of the sample bottle, some of the water is poured off and 25 ml CCl_4 is added to the sample. Reagent grade CCl_4 has been distilled by us. The quality is checked by concentrating 100 ml of CCl_4 with a rotary evaporator to 0.5 ml and analysing 5 μl of this concentrate by G. C. using the conditions at which the samples are run. The surface water sample is now sealed, shaken, and stored in a refrigerator until it can be analyzed in the laboratory. The samples range in size from 3000-3800 ml.

The sample is weighed before the extraction is begun. It is then liquid:liquid extracted once by the 25 ml CCl_4 which was added during collection and once more with 25 ml CCl_4. The sample is extracted the first time in the sample bottle and the second time in another bottle which has been through the same cleaning procedure. Each bottle is additionally rinsed with 5 ml CCl_4. The extracts are combined in a 250 ml separatory funnel for removal of water. The CCl_4 extract is concentrated to about 2 ml with a rotary evaporator. In order to saponify the sample the solvent is changed from CCl_4 to benzene. This is accomplished by adding 10 ml benzene to the concentrated CCl_4 extract and concentrating via a rotary evaporator to 1 ml. This procedure is repeated twice (benzene is added a total of 3 times). The sample is then at a volume of 1.0 ml and the solvent is primarily benzene. The sample is saponified by refluxing for 2 hours with 10 ml benzene, 10 ml 1.0 N KOH in methanol, and 5 ml water. After saponification, the sample is extracted 3 times with 10 ml hexane, the extracts are combined and dried overnight with anhydrous Na_2SO_4.

A 7 cm column of 6% deactivated Al_2O_3 over 5% deactivated SiO_2 (1:2, v:v) is packed and the sample, which has been concentrated to 0.5 ml is placed on the column. Two 2.5 ml fractions are eluted, and collected in clean vials. The first fraction is eluted with hexane and the second is eluted with benzene. Each fraction is concentrated to 0.3 ml with a stream of ultra high purity nitrogen.

The samples are analyzed on a Hewlett Packard 5710A gas chromatograph with dual flame ionization detectors. The analytical columns are 12' x 1/8" stainless steel packed with 3% OV-101 on 100-120 chromosorb W (AW DMCS). Five microliters of the sample are injected and recorded on a 3380A Hewlett Packard integrator.

The carrier gas is helium flowing at 50 ml/minute. Both the injector and detector are held at 300°. The column temperature program includes 2 minutes at 80° followed by a linear temperature rise to 280° at 8°/minute and then 16 minutes at 280°.

Tar

Collections of tar were made using a surface sampler contructed to the design of Sameoto and Jaroszynski (1969) using 363 mesh nets. The sampler was rigged so that it rode outside the ship's bow wake. Each tow was for one nautical mile (1.85 km), most commonly obtained by towing at four knots for 15 minutes. In this way, the 0.4 m wide mouth of the sampler swept through 740 m^2 of sea surface. A sample was washed into a glass bottle, frozen, and returned to the laboratory for visual inspection. Tar lumps were dried in a dessicator for at least 48 hours before weighing. Hexane extracts of selected tar lumps were subjected to gas chromatography on a Varian model 1520 instrument using a 12 foot by 1/8 inch stainless steel column of 3% OV-101 on 100-120 mesh Chromosorb W(AW-DMCS). Chromatograms were temperature programmed from 60° to 290° at 8° min^{-1}.

3. Interaction of Hydrocarbons and Suspended Sediment

Petroleum introduced into the marine environment is dispersed and degraded by a large number of biological, chemical and geological processes. These include evaporation, solution, ingestion, metabolism, and sedimentation to mention only a few of the more important classes. The relative efficency of these processes and the selectivity of each of them for various chemical constituents of petroleum, determines what happens to oil in the marine environment. For example, if petroleum is introduced at or near the surface and the processes which carry that oil to the sea bed are very inefficient compared to other dispersion processes, then there is relatively little reason to be concerned about the effect of that oil on benthic biota. On the other hand, if processes that sediment oil are very efficient, then the effect of the oil on benthic biota should be a major concern.

If hydrocarbons in seawater were adsorbed on suspended sediment particles, these hydrocarbons would be transported to the bottom as the sediments settle out. However, the efficiency with which hydrocarbons are adsorbed by suspended sediments is poorly understood in general and completely unknown for the conditions that prevail in the Gulf of Alaska and other Alaskan OCS waters. In view of this, we have set out to design and execute experiments to determine the extent of interaction between representatives of various chemical

classes of hydrocarbons present in petroleum and suspended sediments from the Gulf of Alaska. At the time of this writing (March 1976) we are beginning a matrix of experiments in which hydrocarbons will be allowed to partition between water and suspended sediments from the Gulf of Alaska. The experimental variables that will be investigated include:

A. Molecular structure of the hydrocarbons -- an alkane, cycloalkane, aromatic, and naptheno-aromatic will be tested.

B. Hydrocarbon concent ation.

C. Presence or absence of other non-petroleum hydrocarbons that might be present under natural conditions (surfactants).

D. Salinity.

E. Temperature.

F. Mixing turbulence.

G. Sediment size.

H. Sediment mineralogy.

Suspended sediments were collected by centrifugation at two stations near the mouth of the Copper River during a cruise of the R/V Acona 6-10 October 1975. The locations of the stations at which these collections were made, CR1 and CR2 are given in Table 5. The samples were frozen and returned to the laboratory.

The grain size distribution of the sediments has been determined. Both suspended sediment samples passed completely through a 230 mesh screen (4 ϕ or 62.5 μm). Size analysis was done according to the pipetting procedures of Folk (1968). Times and depths of withdrawal of aliquots were also determined after Folk.

The one liter cylinders used in the analysis were put in a water bath at 27° C and allowed to equilibrate overnight. Approximately 20 g of sample were used in each analysis. Equal aliquots were withdrawn at the appropriate depths and times with a 20 ml pipette and pipette bulb. The beakers were allowed to dry in an oven overnight at 95° C and were cooled at least one hour prior to weighing. The beakers were weighed using an analytical balance.

VI. Results

The results of hydrocarbon analyses of biota are presented in Table 10. Concentrations of hydrocarbons are reported in the units of μg/g (ppm) based on wet weight of the material analyzed for two hydrocarbon fractions, referred to as "fraction 1" and "fraction 2". The first of these, "fraction 1", refers to the hydrocarbons eluted by hexane in the column chromatography step of the sample work up. This includes saturated hydrocarbons (alkanes and cycloalkanes) and some unsaturated hydrocarbons (alkenes and cycloalkenes).

The "fraction 2" includes larger and more extensively unsaturated hydrocarbons, aromatic hydrocarbons and possibly some non-hydrocarbon, non-saponifiable organic compounds of low polarity. In addition to the total concentrations of Table 10, annotated gas chromatograms are presented in Appendix 2.

The results of analyses of water for hydrocarbons are presented in Table 11. The concentration units are μg/kg (ppb) and the meaning of "fraction 1" and "fraction 2" are the same as for biota. Tar was found on only three of the 51 tows reported in Tables 4-7. The weights of tar and the locations at which they were found are given in Table 12.

The grain size distributions of suspended sediments from the mouth of the Copper River are shown in Figure 1.

VII. Discussion

Biota

Hydrocarbons present in biota may be the result of indigenous biosynthetic processes or petroleum pollution. Distinguishing between these two sources can be difficult and time consuming, particularly if the pollution levels are less than overwhelming. Criteria for making this distinction have been discussed by Blumer

and Sass (1972) and by Clark (1974). In this work we have taken the following features to be indications of the presence of petroleum.

1. The presence of phytane at concentrations comparable to pristane: These two isoprenoid alkanes are typically both present in petroleum at similar concentrations. However, among biosynthetic hydrocarbons phytane is very rare while pristane is often a major constituent.

2. The presence of a homologous series of n-alkanes without a marked odd, even predominance: Such a series is found in most petroleums. Organisms, on the other hand, typically synthesize only one or a few n-alkanes, more often with an odd number of carbon atoms.

3. The presence of an unresolved envelope in the gas chromatogram: This is characteristic of a mixture with the complexity of petroleum. The limited number of biosynthetic pathways typically leads to less complex mixtures.

4. The presence of aromatic compounds as determined by mass spectrometry: This is not equivalent to the presence of peaks in the gas chromatogram of "fraction 2" (sometimes called the "aromatic fraction"), because many biogenically produced olefins also appear in this fraction.

Because of the variability of indigenous hydrocarbons and of the hydrocarbon composition of petroleums from different sources, none

of the criteria above are foolproof. However, when carefully used together, they provide a reliable guide for recognizing materials in which more than half of the hydrocarbons are of petroleum origin.

For this project, 22 analyses of tissue from 9 species of marine biota have been performed (Table 10). In several cases replicate analyses have been made to provide an indication of variability, in other cases different tissues from an individual have been analyzed separately. The gas chromatograms of these materials are reproduced in Appendix 2. The legs of Chionoecetes bairdi and C. opilio (both commonly known as snow crab or tanner crab) showed quite low hydrocarbon concentrations in fraction 2. Body soft parts of C. opilio consistently contained pristane as a major peak in fraction 1. Flesh and skin of three individuals of Lepidopsetta bilineata (rock sole) were analyzed separately. All three showed a single large peak at the retention time of n-octacosane in fraction 2. Two of the three showed very little in fraction 1. Sample NP8 showed a much more complex array of peaks in fraction 1. This array is strikingly similar to that found in NP12, the soft parts of the shrimp Pandalopsis dispar. It is unclear whether the similarity of fraction of NP8 and NP12 is real or an artifact. These samples were collected and analyzed separately. The chromatograms of the other two analyses of P. dispar are qualitatively similar although the absolute concentration of fraction 2 for NP3 is considerably higher than for NP9.

The two analyses of soft parts of Pecten caurinus (scallop) were qualitatively similar. In particular both had a characteristic high molecular weight group of peaks in fraction 2. Viscera, skin and flesh, and gills were analyzed separately for a sample of Theragra chaleogramma (pollock). The viscera gave very high concentrations and a very complex pattern, especially in fraction 1. For all three tissues, pristane is the major peak in fraction 1, accounting for roughly three fourths of the total aliphatic hydrocarbons. For the gills and the flesh and skin the fraction 2's are qualitatively similar, but the fraction 2 of the viscera is considerably more complex. Shrimps of the species Pandalopsis borealis, Spirontocaris synderi, and Pandalus jordani were each analyzed. Fraction 1's of the latter two species showed large pristane peaks.

Water

Performing the task of measuring the concentration of hydrocarbons in seawater takes one to the limits of present day analytical methodology. The accuracy of these measurements is poorly defined since reliable standards are not available. Precision, especially between laboratories, is also questionable. Thus the 18 surface water hydrocarbon values reported here (Table 11) are probably best regarded order-of-magnitude approximations. Viewed in this way, the present results can be compared with our previous results from the Gulf of Alaska (Shaw, 1975) and with results from other parts of the world ocean.

A compilation of reported values of hydrocarbons in seawater has recently appeared (National Academy of Sciences 1975 p. 56). Concentrations (μg/liter) range from a high of 1,000 for a sample of Baltic surface water to a low of <<1 for 2,000 meter deep water near Bermuda. Values within an order of magnitude of 1 μg/liter have been found by several workers for water from locations expected to be free of pollution. The values reported in Table 11 are also generally within this range. Table 11 also indicates that several samples were lost to laboratory contamination subsequent to collection. We now believe that we have identified and removed these sources of contamination. But we also now believe that some of the higher values reported in our previous report (Shaw, 1975) are due to contamination. The data records transmitted to the National Oceanographic Data Center contain the appropriate corrections.

Tar

The occurrence of pelagic tar in various parts of the world ocean is a well-documented fact that has been the subject of recent reviews (National Academy of Sciences 1975 and Butler, Morris and Sass 1973). Evidence that the tar's origin is associated with petroleum transport includes the findings that its spatial distribution corresponds to major tanker routes and the chemical similarity of the tar to material in tank washings of oil tankers (Butler, Morris and Sass 1973). Further evidence of this association is the elevated iron content of pelagic tar which indicates contact with steel structures (Attaway, unpublished, cited in National Academy of Sciences 1975 p. 49).

Most measurements of pelagic tar have been made in areas where high concentrations are to be expected such as the north Atlantic and the Mediterranean Sea. Measurements made in more remote areas such as the southwest Pacific show significantly lower concentrations (Wong unpublished, cited in National Academy of Sciences 1975 p. 53). The tar measurements reported here are from a region, the Gulf of Alaska and the Bering Sea, which might a priori be expected to be relatively free of this material. Some coastal oil seeps are known in the Gulf of Alaska and crude oil moved through the area from fields in Cook Inlet to Japan and the west coast of the United States. But these crude oil inputs seem to be minor compared to global levels. There is no history of tar being washed up on Gulf of Alaska beaches as there is for instance in some parts of southern California. The total production of oil fields in the Cook Inlet area was about 62 million barrels ($9.9 \times 10^6 m^3$) in 1974, (International Petroleum Encyclopedia 1975). Only part of this was shipped by sea. Neither the Gulf of Alaska nor the Bering Sea is an area of heavy shipping.

These expectations of low tar abundance were confirmed by the results of this project. The arithmetic mean abundance for the 51 tows made during this project is $6.6 \times 10^{-4} mg/m^2$. This is lower than any mean value reported by the National Academy of Sciences (1975). The only comparable value is $<0.01 mg/m^2$ for the southwest Pacific. Mean values for various parts of the north Atlantic, the Mediterranean, and the Kuroshio Current system in the Pacific fall in the range 0.1 to 10 mg/m^2. Wong, Green and Cretney (1974) have

reported tar concentrations in the north Pacific. These workers found 3.8 mg/m^2 in the west, 0.4 mg/m^2 in the east along 35^oN and a complete absence of tar along 125^oW.

If the results of tar collections made during the previous year of this study (Shaw 1975) are included, the grand arithmetic mean tar abundance for the Gulf of Alaska and Bering Sea in the period October 1974 to October 1975 is 3.3×10^{-3} mg/m^2. Even this latter value is low by global standards.

Gas chromatograms of three tar lumps collected over the two-year course of this project are reproduced in Figure 2. Chromatogram A has the appearance of a relatively fresh crude or fuel oil. It was collected at EBBS 12 and was a sticky, semi-solid material which flowed on standing in a vial. Identified n-alkane peaks extend from heptadecane to dotriacontane. The value of $N_{\frac{1}{2}}$ for this tar is 18.5. $N_{\frac{1}{2}}$, the equivalent normal paraffin carbon number having a retention index equal to that at which the unresolved envelope reaches half its maximum height, is an indication of the extent of weathering of a tar lump (Butler and Harris 1975 and references therein). Tar lumps from the north Atlantic analyzed by Butler and Harris showed $N_{\frac{1}{2}}$ values in the range 15-20. Tars represented by chromatograms B and each C have an $N_{\frac{1}{2}}$ of 24 which is indicative of more highly weathered oils. The tar of chromatogram B was collected at GASS 30 a near shore station off Icy Bay, an area where several coastal oil seeps have been observed. The tar of chromatogram C came from GASS 48 a station at the edge of the continental shelf.

These two lumps were physically quite different from that shown in chromatogram A, being more solid and less sticky. The biomodal distribution of chromatogram B is typical material discharged by oil tankers in their tank washing procedures, but would be highly unusual in a crude oil. Thus, although this tar was collected adjacent to a natural oil seep area, it probably did not come from a seep. Chromatogram C shows a highly weathered material as evidenced by its $N_{\frac{1}{2}}$ value and by the almost complete lack of normal alkanes.

Suspended Sediments

The results of the grain size analysis as shown in Figure 1 indicate that for each sample 96% of the material is smaller than 22.1μm (5.5ϕ). CR2 is a bit finer than CR1 with median grain sizes of 3.5μm (8.15ϕ) and 4.8μm (7.7ϕ). With this basic characterization of the suspended sediments now complete, the adsorption experiments described in Section V are beginning. A mineralogical characterization is also planned.

VIII. Conclusions

Our general conclusion is that the Gulf of Alaska and the Bering Sea appear to have hydrocarbon levels as low or lower than other areas of the world ocean free of obvious petroleum contamination. This is evidenced by the hydrocarbon concentrations in the surface

water in the low parts per billion range; by hydrocarbon concentrations in the biota in the low parts per million range; by the absence of indications of petroleum in the gas chromatograms of biota; and by the extremely low abundance of floating tar in these waters.

In evaluating our analyses of surface water for hydrocarbons, we conclude that these measurements are of insufficient precision to allow correlation of the values obtained with other environmental variables such as water mass movements or biological processes. These measurements can best be regarded as in aggregate giving a semi-quantative estimate of the average hydrocarbon concentration in the Gulf of Alaska.

From our survey of hydrocarbons in subtidal biota we find that several of the species examined have quite low concentrations of biogenic hydrocarbons. Since sensitive biological monitoring materials are ones with low natural hydrocarbon levels but which can be expected to rapidly take up petroleum hydrocarbons several of the materials analyzed show monitoring potential. Thus, pollock gills, crab body soft parts and shrimp soft parts are probably worthy of further study. However, pollock viscera (high natural hydrocarbons) and crab legs (probable slow uptake) appear to be less valuable as indicator materials.

From the chromatographic analyses we conclude that the pelagic tar of the Gulf of Alaska is more highly weathered than tar from the north Atlantic. The chromatographic evidence also indicates that this tar's origin was probably tank washings rather than an oil seep.

As yet we have no direct knowledge of the processes by which added hydrocarbons would be dispersed and degraded in the Gulf of Alaska.

IX. Recommendations for Further Study

Both baseline measurements and process related studies of hydrocarbons in the Alaskan OCS environment need to be continued. Considerable effort has already been put into the making of baseline measurements in the Gulf of Alaska, but this work has barely begun in the Bering Sea and has yet to begin in other areas. Work on the process by which petroleum interacts with suspended sediments is beginning and studies of biological processes are underway in other laboratories. But only when considerably more data is in hand about ambient hydrocarbon concentrations and about the processes by which added hydrocarbons are degraded and dispersed, can be predictive ability be developed to forecast the fate and effect of added oil with any certainty.

Emphasis in water analysis should shift from quarterly measurements in the Gulf of Alaska to less frequent measurements over a wider geographical area. Quarterly measurements were introduced in the

hope of obtaining correlation with other environmental parameters. But, since the lack of precision of the results of these water measurements has made such correlations impossible, less frequent sampling should be adequate to establish average concentrations.

Analysis of biota should continue and be expanded both geographically and to include intertidal as well as subtidal organisms.

The collection of floating tar also should be continued. This activity is much less expensive on a per sample basis than the sophisticated analyses that are being used for water and biota. Thus tar abundance provides a very economical indicator of petroleum burden.

In the Bering Sea and particularly in the Gulf of Alaska our offshore baseline measurements of hydrocarbons have proceeded at a much faster rate than work in coastal and estuarine environments. These latter areas need greater emphasis since they probably show greater natural diversity and could be more severly affected by petroleum.

Considerably more effort needs to be directed to process studies. These studies provide the link between our knowledge of the present environmental conditions and our ability to predict changes.

X. Activity Summary for the Quarter Ending 31 March 1976

A. Field Activities

Samples of seston and surface water were collected on a cruise of the R/V *Mauna Wave*, 21 February to 5 March. Twelve seston and twenty-four surface water samples were collected from stations of the standard hydrographic grid in the northeast Gulf of Alaska.

B. Laboratory Activities

1. Analysis of biota, water and seston are all presently proceeding at rates more than adequate to reach the goals set in our work statement.
2. Our investigation of hydrocarbon sediment interactions is proceeding as described in the main body of this report.
3. The GC-MS-data system has been installed and is now semi-operational.
4. Our use of self-made glass capillary columns for G. C. is now in the experimental stage.

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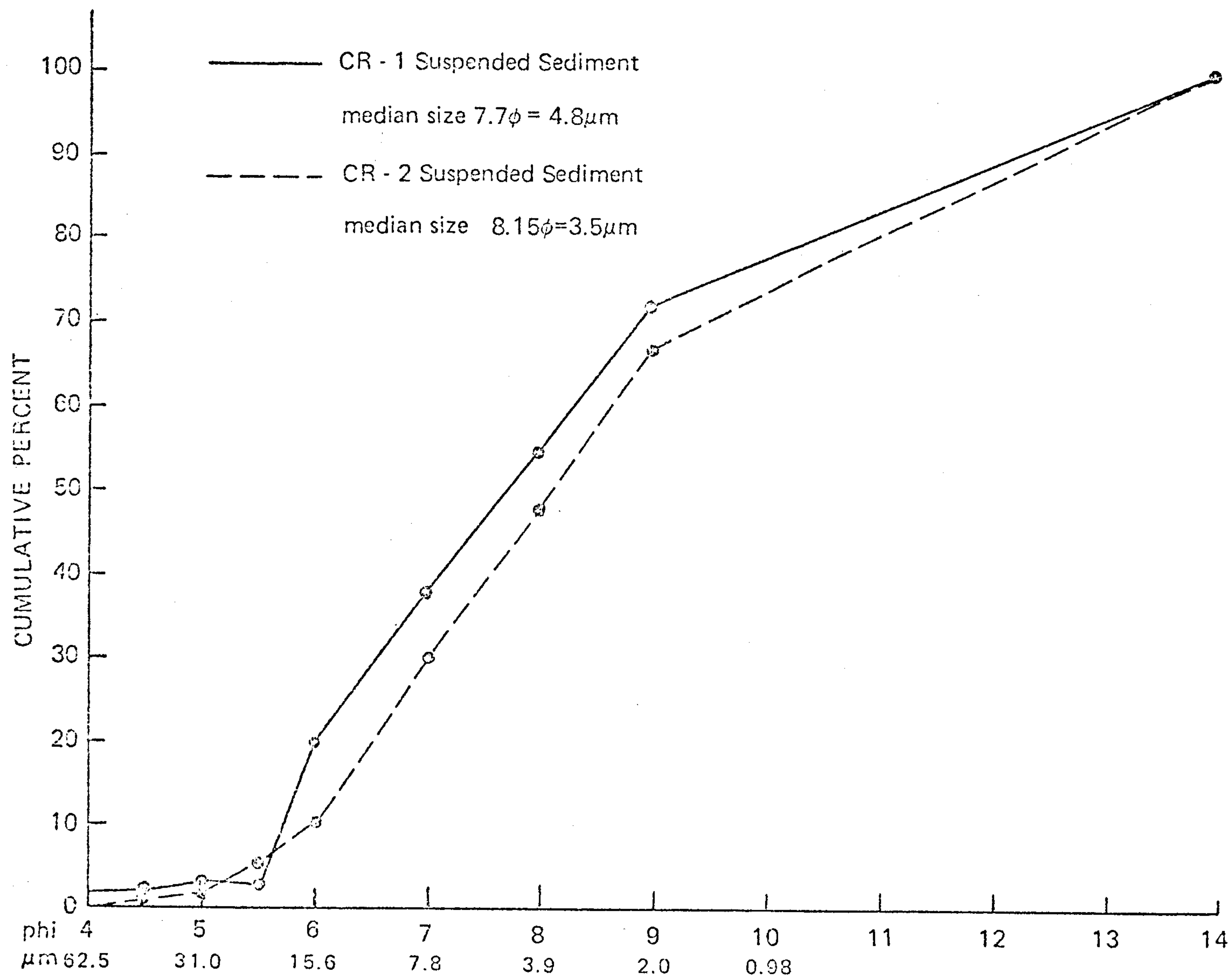


Figure 1. Grain size distribution of suspended sediments from the mouth of the Copper River.

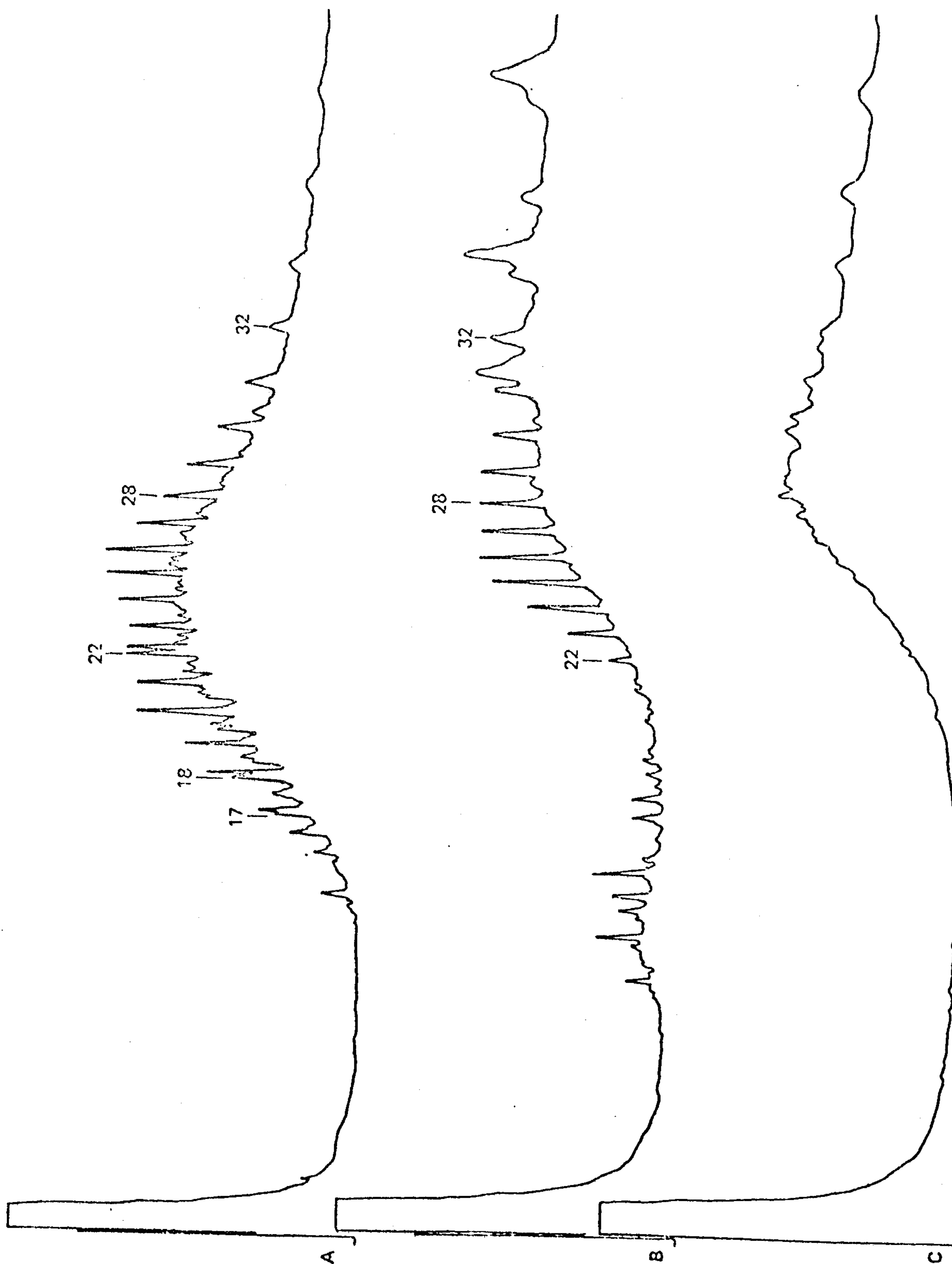


Figure 2. Gas chromatograms of hexane extracts of tar lumps. A - tar collected at EBBS 12, $N_{½}$ = 18.5; B - tar collected as GASS 30, $N_{½}$ = 24; C - tar collected at GASS 48, $N_{½}$ = 24.

TABLE 1

STATION LOCATIONS OF HYDROCARBON SURFACE WATER SAMPLING
USNS SILAS BENT
8/3/75 - 9/14/75

	Station	Data Taken	Position Latitude (N)		Position Longitude (W)	
GASS	Ø1	9/14	59°	50.2'	149°	30.5'
	Ø6	9/13	59°	07.2'	148°	47.5'
	Ø9	9/12	58°	41.1'	148°	21.6'
	24	9/2	58°	54.3'	141°	00.5'
	29	9/1	59°	34.6'	140°	06.0'
	37	9/5	59°	16.2'	142°	59.2'
	39	9/5	59°	35.7'	142°	49.5'
	41	9/4	59°	55.1'	142°	39.5'
	47	9/7	59°	17.5'	145°	13.0'
	50	9/7	59°	47.7'	145°	09.0'
	52	9/8	60°	07.6'	145°	06.5'
	53	9/8	60°	23.0'	146°	54.0'
	107	9/19	58°	18.6'	150°	28.0'
	115	9/19	57°	20.6'	148°	38.7'
	121	9/21	56°	43.2'	155°	27.9'
	125	9/21	55°	58.9'	154°	28.5'
	135	9/23	55°	20.3'	158°	25.1'
	141	9/22	54°	19.2'	157°	25.2'
	147	9/24	54°	36.2'	161°	00.7'
	153	9/24	53°	37.0'	160°	09.9'
	156	9/26	54°	29.2'	165°	11.2'
	160	9/26	53°	43.3'	164°	25.6'
	164	9/25	53°	08.7'	163°	52.0'

TABLE 2

STATION LOCATIONS OF HYDROCARBON SURFACE WATER SAMPLING
USNOSS SURVEYOR
10/28/75 - 11/17/75

	Station	Data Taken	Position	
			Latitude	Longitude
GASS	Ø1	11/3/75	59° 50.2'	194° 30.5'
	Ø6	11/3/75	59° 07.2'	148° 47.5'
	15	11/2/75	58° 18.1'	145° 00.5'
	23	11/2/75	58° 46.1'	141° 11.3'
	29	11/1/75	59° 34.6'	140° 06.0'
	30	11/1/75	59° 44.1'	141° 36.8'
	32	11/1/75	59° 26.3'	141° 54.8'
	34	11/1/75	59° 08.5'	142° 03.8'
	47	10/31/75	59° 17.5'	145° 13.0'
	51	10/30/75	59° 57.6'	145° 07.8'
	53	10/30/75	60° 23.0'	146° 54.0'
	101	11/5/75	59° 19.8'	152° 24.1'
	107	11/5/75	58° 18.6'	150° 28.0'
	115	11/4/75	57° 20.6'	148° 38.7'
	125	11/6/75	55° 58.9'	154° 28.5'
	135	11/10/75	55° 20.3'	158° 25.1'
	153	11/11/75	53° 37.0'	160° 09.9'
	156	11/12/75	54° 29.2'	165° 11.3'
	160	11/12/65	53° 43.3'	164° 25.6'
	164	11/13/75	53° 08.7'	163° 52.0'

TABLE 3

STATION LOCATIONS OF HYDROCARBON SURFACE WATER SAMPLING

R/V MOANA WAVE 2/21/76 - 3/5/76

STATION	DATE	POSITION LATITUDE	POSITION LONGITUDE
GASS Ø1	2/21/76	59° 50.2'	149° 30.5'
Ø2	2/21/76	59° 41.5'	149° 22.0'
Ø3	2/21/76	59° 33.0'	149° 13.2'
Ø4	2/21/76	59° 24.5'	149° 04.9'
Ø5	2/22/76	59° 16.0'	148° 56.0'
Ø6	2/22/76	59° 07.2'	148° 47.5'
Ø7	2/22/76	58° 58.7'	148° 38.7'
Ø8	2/22/76	58° 49.7'	148° 30.0'
Ø9	2/23/76	58° 41.1'	148° 21.6'
10	2/23/76	58° 32.3'	148° 13.2'
11	2/24/76	58° 23.2'	148° 04.8'
15	2/24/76	58° 18.1'	145° 00.5'
24	2/25/76	58° 54.3'	141° 00.5'
29	2/26/76	59° 34.6'	140° 06.0'
37	2/27/76	59° 16.2'	142° 59.2'
39	2/27/76	59° 35.7'	142° 49.5'
41	2/27/76	59° 55.1'	142° 39.5'
47	2/29/76	59° 17.5'	145° 13.0'
50	2/27/76	59° 47.7'	145° 09.0'
52	2/28/76	60° 07.6'	145° 06.5'
53	2/29/76	60° 23.0'	146° 54.0'
57	2/29/76	59° 45.6'	146° 31.0'
58	2/29/76	59° 36.2'	146° 25.5'
75	2/29/76	59° 07.6'	145° 53.4'

TABLE 4

SESTON COLLECTIONS FOR FLOATING TAR MADE DURING A CRUISE OF USNS SILAS BENT 9/1/75 - 9/14/75

	Station	Date Taken	Position	
			Latitude	Longitude
GASS	Ø1	9/14/75	59° 50.2'	194° 30.5'
	Ø6	9/12/75	59° 07.2'	148° 47.5'
	Ø8	9/9/75	59° 49.7'	148° 30.0'
	24	9/2/75	58° 54.3'	141° 00.5'
	29	9/1/75	59° 34.6'	140° 06.0'
	37	9/5/75	59° 16.2'	142° 59.2'
	39	9/5/75	59° 35.7'	142° 49.5'
	41	9/4/75	59° 55.1'	142° 39.5'
	47	9/7/75	59° 17.5'	145° 13.0'
	52	9/8/75	60° 07.6'	145° 06.5'
	53	9/8/75	60° 23.0'	146° 54.0'

TABLE 5

SESTON COLLECTIONS FOR FLOATING TAR MADE DURING A CRUISE OF
R/V ACONA
10/8/75

Station	Date Taken	Position	
		Latitude	Longitude
CR 1	10/8/75	60° 11.0'N	145° 10.0'W
CR 2	10/8/75	60° 14.9'N	145° 34.0'W

TABLE 6

SESTON COLLECTIONS FOR FLOATING TAR MADE DURING A CRUISE OF USNOSS DISCOVERER
10/10/75 - 10/14/75

Station	Date Taken	Position	
		Latitude	Longitude
103	10/15/75	59° 00.0'	151° 45.1'
119	10/14/75	57° 06.9'	156° 00.0'
120	10/13/75	56° 55.0'	155° 44.1'
121	10/13/75	56° 43.2'	155° 27.9'
122	10/13/75	56° 31.3'	155° 12.0'
124	10/13/75	56° 07.1'	154° 39.4'
133	10/12/75	55° 46.3'	158° 51.0'
134	10/12/75	55° 33.4'	158° 38.3'
135	10/12/75	55° 20.3'	158° 25.1'
137	10/12/75	54° 54.3'	157° 59.0'
145	10/11/75	55° 00.9'	161° 20.5'
146	10/11/75	54° 49.4'	161° 12.5'
147	10/11/75	54° 36.2'	161° 00.7'
148	10/11/75	54° 23.5'	160° 49.1'
156	10/10/75	54° 29.2'	165° 11.3'
158	10/10/75	54° 04.5'	164° 46.2'
159	10/10/75	53° 51.9'	164° 34.0'
160	10/10/75	53° 43.3'	164° 25.6'

TABLE 7

SESTON COLLECTIONS FOR FLOATING TAR MADE DURING A CRUISE OF USNOSS DISCOVERER
9/14/75 - 9/28/75

Station		Date Taken	Position	
			N. Latitude	W. Longitude
EBBS	Ø8	9/19/75	58° 17'	159° 32'
	12	9/22/75	56° 09'	162° 09'
	17	9/28/75	55° 26'	165° 50'
	19	9/23/75	56° 40'	163° 57'
	24	9/18/75	58° 46'	162° 29'
	28	9/24/75	57° 10'	165° 04'
	35	9/14/75	56° 13'	168° 20'
	37	9/26/75	57° 06'	167° 01'
	38	9/24/75	57° 40'	166° 06'
	40	9/17/75	58° 08'	165° 16'
	41	9/17/75	58° 47'	164° 15'
	43	9/17/75	58° 42'	166° 17'
	46	9/25/75	57° 35'	168° 04'
	48	9/14/75	56° 19'	169° 42'
	51	9/14/75	55° 49'	170° 47'
	54	9/15/75	56° 56'	170° 56'
	56	9/15/75	58° 06'	169° 05'
	59	9/16/75	59° 12'	167° 18'
	65	9/15/75	57° 25'	172° 05'
	66	9/15/75	56° 45'	173° 12'

TABLE 8

BIOLOGICAL MATERIALS COLLECTED FOR HYDROCARBON ANALYSIS DURING A CRUISE OF
M/V NORTH PACIFIC
6/26/75 - 7/15/75

Sample #	Species	Location	Depth	Date
1.	*Pandalus jordani*	N59° 25' W141° 45' - N59° 28' W141° 47'	176 m	6/27/75
2.	*Chioncecetes bairdi*	N59° 22' W141° 40' - N59° 23' W141° 45'	182 m	6/27/75
3.	*Pandalopsis dispar*	N59° 16' W141° 12' - N59° 13' W141° 12'	295 m	6/28/75
4.	*Pecten caurinus*	N59° 39' W140° 46' - N59° 39' W140° 52'	73 m	6/30/75
5.	*Chionoecetes bairdi*	N59° 39' W140° 36' - N59° 39' W140° 31'	73 m	7/2/75
6.	*Pecten caurinus*	N59° 39' W140° 36' - N59° 39' W140° 31'	73 m	7/2/75
7.	*Chionoecetes bairdi*	N59° 39' W140° 36' - N59° 39' W140° 31'	73 m	7/2/75
8.	*Lepidopsetta bilineata*	N59° 39' W140° 36' - N59° 39' W140° 31'	73 m	7/2/75
9.	*Pandalopsis dispar*	N59° 32' W140° 44' - N59° 34' W140° 49'	270 m	7/4/75
10.	*Chionoecetes bairdi*	N59° 32' W140° 44' - N59° 34' W140° 49'	270 m	7/4/75
11.	*Crangonid* sp.	N59° 40' W141° 00' - N59° 43' W141° 05'	47 m	7/5/75
12.	*Pandalopis dispar*	N59° 33' W141° 01' - N59° 35' W140° 55'	209 m	7/5/75
13.	*Spirontocaris snyderi*	N59° 30' W141° 03' - N59° 32' W140° 58'	254 m	7/5/75
14.	*Pecten caurinus*	N59° 43' W141° 32' - N59° 45' W141° 37'	74 m	7/6/75
15.	*Lepidopsetta bilineata*	N59° 43' W141° 32' - N59° 45' W141° 37'	74 m	7/6/75

Sample #	Species	Location	Depth	Date
16.	Chionoecetes bairdi	N59° 44' W141° 14' - N59° 45' W141° 20'	55 m	7/6/75
17.	Lepidopsetta bilineata	N59° 44' W141° 14' - N59° 45' W141° 20'	55 m	7/6/75
18.	Chionoecetes bairdi	N59° 28' W141° 33' - N59° 28' W141° 27'	160 m	7/8/75
19.	Pandalopsis borealis	N59° 37' W141° 35' - N59° 35' W141° 29'	120 m	7/8/75
20.	Chionoecetes bairdi	N59° 37' W141° 35' - N59° 35' W141° 29'	120 m	7/8/75
21.	Pandalopis dispar	N59° 37' W142° 01' - N59° 34' W141° 58'	165 m	7/9/75
22.	Pandalopsis borealis	N59° 43' W142° 04' - N59° 41' W141° 59'	152 m	7/9/75
23.	Pandalopis borealis	N59° 43' W142° 04' - N59° 41' W141° 59'	152 m	7/9/75
24.	Lepidopsetta bilineata	N59° 52' W141° 58' - N59° 52' W141° 05'	68 m	7/10/75
25.	Lepidopsetta bilineata	N59° 52' W141° 58' - N59° 52' W141° 05'	68 m	7/10/75
26.	Lepidopsetta bilineata	N59° 52' W141° 58' - N59° 52' W141° 05'	68 m	7/10/75
27.	Pandalopsis hypsinotus	N59° 45' W143° 03' - N59° 43' W143° 03'	167 m	7/12/75
28.	Pandalopsis dispar	N59° 42' W143° 28' - N59° 40' W143° 23'	298 m	7/13/75

TABLE 9

BIOLOGICAL MATERIALS COLLECTED FOR HYDROCARBON ANALYSIS DURING A CRUISE OF FR/V MILLER FREEMEN 8/17/75 - 10/24/75

Sample #	Species	Location	Depth	Date
1.	*Chionoecetes opilio*	N57° 00' W167° 02.9' - N47° 58.0' W167° 05.2'	72.8 m	8/18/75
2.	*Theragra chalcogramma*	N57° 00' W167° 02.9' - N47° 58.0' W167° 05.2'	72.8 m	8/18/75
3.	*Theragra chalcogramma*	N57° 01.0' W167° 36.3' - N57° 02.0' W167° 41.5'	75.7 m	8/18/75
4.	*Chionoecetes opilio*	N57° 01.0' W167° 36.3' - N57° 02.0' W167° 41.5'	75.7 m	8/18/75
5.	*Chionoecetes opilio*	N57° 19.5' W168° 53.0' - N57° 20.0' W168° 57.2'	69.6 m	8/19/75
6.	*Chionoecetes opilio*	N57° 40.4' W169° 35.1' - N57° 42.2' W169° 34.8'	68.3 m	8/19/75
7.	*Chionoecetes opilio*	N58° 18.8' W179° 20.5' - N58° 20.1' W170° 18.0'	71.0 m	8/20/75
8.	*Chionoecetes opilio*	N59° 39.6' W170° 59.0' - N58° 42.0' W171° 02.8	81.0 m	8/20/75
9.	*Chionoecetes opilio*	N59° 20.0' W171° 46.8' - N59° 19.9' W171° 50.5'	80.1 m	8/20/75
10.	*Chionoecetes opilio*	N59° 59.7' W171° 56.1' - N60° 0.2' W171° 56.1'	65 m	8/21/75
11.	*Theragra chalcogramma*	N60° 21.1' W170° 40.0' - N60° 19.6' W170° 39.8'	61 m	8/22/75
12.	*Chionoecetes opilio*	N60° 21.1' W170° 40.0' - N60° 19.6' W170° 39.8'	61 m	8/22/75
13.	*Chionoecetes opilio*	N60° 20.0' W170° 01.5' - N60° 20.9' W169° 59.0'	50 m	8/22/75
14.	*Theragra chalcogramma*	N59° 59.8' W169° 15.2' - N60° 00.8' W169° 19.0'	44 m	8/23/75
15.	*Theragra chalcogramma*	N59° 40.8' W169° 16.0' - N59° 40.1' W169° 12.4'	46 m	8/24/75

Sample #	Species	Location	Depth	Date
16.	*Theragra chalcogramma*	N59° 40.1' W168° 38.2' - N59° 40.0' W168° 34.0'	36.4 m	8/24/75
17.	*Theragra chalcogramma*	N59° 40.2' W169° 59.1' - N59° 40.0' W167° 55.1'	34.7 m	8/24/75
18.	*Theragra chalcogramma*	N59° 40.0' W167° 19.9' - N59° 39.8' W167° 16.3'	32 m	8/24/75
19.	*Chionoecetes opilio*	N57° 20.0' W166° 28.9' - N57° 18.4' W166° 27.1'	71 m	10/3/75
20.	*Theragra chalcogramma*	N57° 20.0' W166° 28.9' - N57° 18.4' W166° 27.1'	71 m	10/3/75
21.	*Theragra chalcogramma*	N56° 39.0' W165° 48.0' - N56° 37.7' W165° 48.8'	73 m	10/3/75
22.	*Chionoecetes opilio*	N56° 39.0' W165° 48.0' - N56° 37.7' W165° 48.8'	73 m	10/3/75
23.	*Chionoecetes opilio*	N57° 40.3' W163° 59.0' - N57° 39.2' W163° 56'	52 m	10/4/75
24.	*Theragra chalcogramma*	N57° 20.2' W163° 23.3' - N57° 19.0' W163° 22.1'	54 m	10/3/75
25.	*Chionoecetes opilio*	N57° 20.2' W163° 23.3' - N57° 19.0' W163° 22.1'	54 m	10/3/75
26.	*Theragra chalcogramma*	N57° 39.4' W160° 56.0' - N57° 40.7' W160° 53.1'	56.4 m	10/5/75
27.	*Chionoecetes opilio*	N57° 39.4' W160° 56.0' - N57° 40.7' W160° 53.1'	56.4 m	10/5/75
28.	*Theragra chalcogramma*	N57° 59.2' W160° 15.2' - N58° 00.5' W160° 12.5'	53 m	10/5/75
29.	*Chionoecetes opilio*	N57° 59.9' W162° 09.7' - N58° 00.5' W162° 07.0'	38.2 m	10/7/75
30.	*Theragra chalcogramma*	N58° 14.7' W162° 03.2' - N58° 13.3' W162° 05.7'	46.6 m	10/7/75
31.	*Theragra chalcogramma*	N58° 40.8' W167° 12.1' - N58° 39.5' W167° 10.7'	45 m	10/13/75
32.	*Chionoecetes opilio*	N58° 00.0' W167° 08.1' - N57° 58.4' W167° 05.9'	66 m	10/13/75
33.	*Chionoecetes opilio*	N58° 38.8' W166° 34.2' - N58° 37.0' W166° 34.9'	44 m	10/14/75
34.	*Theragra chalcogramma*	N58° 20.8' W166° 32.8' - N58° 18.9' W166° 32.6'	47.3 m	10/14/75

TABLE 10

HYDROCARBON CONCENTRACTIONS IN BIOTA OF THE GULF OF ALASKA AND BERING SEA

Sample	Species	Hydrocarbon Concentration μg/g	
		Fraction 1	Fraction 2
NP 2	*Chionoecetes bairdi* legs	.23	9.8
NP 5	*Chionoecetes bairdi* legs	.03	.33
NP 7	*Chionoecetes bairdi* legs	.09	1.3
NP 10	*Chionoecetes bairdi* legs	.08	4.2
MF 7	*Chionoecetes opilio* (M) body soft parts	1.2	14.
MF 7	*Chionoecetes opilio* (F) body soft parts	6.7	18.
MF 12	*Chionoecetes opilio* legs	.27	3.6
MF 22	*Chionoecetes opilio* (F) body soft parts	2.1	20.
NP 8	*Lepidopsetta bilineata* flesh & skin	15.	5.6
NP 15	*Lepidopsetta bilineata* flesh & skin	.06	3.1
NP 17	*Lepidopsetta bilineata* flesh & skin	.21	--
NP 3	*Pandalopsis dispar* soft parts	.11	8.6
NP 9	*Pandalopsis dispar* soft parts	.21	1.6
NP 12	*Pandalopsis dispar* soft parts	15.	3.6
NP 6	*Pecten caurinus* soft parts	1.2	7.6
NP 14	*Pecten caurinus* soft parts	.72	15.

TABLE 10 (continued)

Sample	Species	Hydrocarbon Concentration µg/g Fraction 1	Fraction 2
NP 3	*Theragra chaleogramma* viscera	170.	25.
NP 3	*Theragra chaleogramma* flesh & shin	2.9	6.9
NP 3	*Theragra chaleogramma* gills	6.4	15.
NP 19	*Pandalopsis borealis* soft parts	1.2	9.5
NP 13	*Spirontocaris snyderi* soft parts	15.	4.7
NP 1	*Pandalus jordani* soft parts	3.7	13.
Blank*	11-17-75	0.04	0.15
Blank	1-12-76	0.04	0.37
Blank	2-9-76	0.06	0.07
Blank	2-11-76	0.02	0.04

*Blank concentractions are calculated on the basis of 50.0g wet sample weight.

TABLE 11

HYDROCARBON CONCENTRACTIONS IN WATER FROM THE GULF OF ALASKA AND BERING SEA

GASS	Date	µg/Kg Fraction 1	µg/Kg Fraction 2	Date	µg/Kg Fraction 1	µg/Kg Fraction 2
Ø1	9/14	ND	ND	11/3	C	C
Ø6	9/13	C	C	11/3	C	C
Ø9	9/12	C	C			
15				11/2	0.32	0.87
23				11/2	ND	ND
24	9/2	C	C			
29	9/1	ND	0.86	11/1	C	C
30				11/1	C	C
32				11/1	C	C
34				11/1	C	C
37	9/5	ND	1.16			
39	9/5	C	C			
41	9/4	C	C			
47	9/7	ND	4.25	10/31	0.04	0.45
50	9/7	ND	<7.0			
51				10/30	0.57	2.45
52	9/8	C	C			

TABLE 11 (continued)

GASS	Date	μg/Kg Fraction 1	μg/Kg Fraction 2	Dat	μg/Kg Fraction 1	μg/Kg Fraction 2
53	9/8	ND	ND	10/30	0.46	0.33
101				11/4	C	C
107	9/19	*(<10)	**(<5)	11/5	C	C
115	9/19	*(<10)	**(<5)	11/4	C	C
121	9/21	C	C			
125	9/21	ND	1.82	11/6	C	C
135	9/23	1.12	2.49	11/10	3.2	9.3
141	9/22	ND	1.77			
147	9/24	*(<10)	**(<5)			
153	9/24	C	C			
156	9/26	C	C	11/12	0.79	0.88
160	9/26	*(<10)	**(<5)	11/12	0.27	ND
164	9/25	C	C	11/12	0.93	5.0

μg/Kg -- μg of sample detected per Kg surface water extracted
ND -- None detectable
C -- Contamination
< -- Less than
* -- Large unresolved envelope with high shifting bleed line
** -- Peaks have poor resolution

TABLE 12

FLOATING TAR COLLECTED IN THE GULF OF ALASKA AND BERING SEA

Station	Date	Tar (mg)
GASS 121	10/13/75	9.1
GASS 124	10/13/75	9.2
EBBS 12	9/22/75	6.7

APPENDIX 1

COMPARISON OF BIOLOGICAL METHODS

Introduction

Before carrying out analyses of biological materials for hydrocarbons we made a comparative study of several techniques for this analysis. We compared the efficiency of Soxhlet extraction followed by saponification with extraction by alkaline digestion (direct saponification). For the latter extraction procedure, we compared the effectiveness of 2 hour and 24 hour reaction times. Each of the procedures was performed on approximately 60 g of soft parts of *Saxidomus gigantea* (Butter clam) and repeated with a similar weight of tissue spiked with a mixture of aliphatic and aromatic hydrocarbons (Appendix - Table 1). Each of the extracts was split and subjected to column chromatography on alumina packed over silica using both fully activated and partially deactivated surbents. This experimental scheme is shown in Appendix - Table 2.

Methods

For each analysis, approximately 60 gm of tissue (3 clams) was cut into small pieces and macerated in 100 ml methanol with a Virtis homogenizer. Before Soxhlet extraction of a sample, Whatman cellulose thimbles were extracted for 24 hours with 300 ml 1:1 methanol:benzene. This solvent was discarded and replaced with fresh solvent. After another 24 hour extraction, the solvent was concentrated on a rotary evaporator to less

than 1.0 ml. This concentrate was checked by gas chromatography (G. C.). The macerated samples were added to the clean extraction thimbles with 50 ml methanol and 150 ml benzene (total solvent volume 300 ml). The Soxhlet extraction was run for 48 hours.

Either directly after maceration, or after Soxhlet extraction, 100 ml 1N KOH in methanol and 50 ml H_2O were added for saponification which was run for either two or twenty-four hours, after reflux was attained. After cooling, the pH of the saponified sample was checked to be sure it was greater than 10 (it was 12 in all samples). In other samples run by this procedure, the Soxhlet extract has been concentrated to approximately 100 ml before saponification. An oily residue formed when the *S. gigantea* samples were concentrated, so nearly the entire 300 ml was saponified.

After saponification the samples were extracted three times with 100 ml hexane. The combined hexane extracts were washed with 150 ml saturated aqueous NaCl. The hexane extract was dried overnight with Na_2SO_4. The hexane extract was then concentrated to 10 ml. A 100 μl aliquot was air dried and weighed on an electrobalance to determine total weight of non-saponifiable lipid.

Columns of 10 ml Al_2O_3 packed over 20 ml SiO_2 (10 gm:10 gm) were used. The column dimensions were 11 mm x 35 cm. The alumina and silica were prepared according to Farrington, et al., (1972) for partially deactivated sorbents. For fully active columns the deactivation step was omitted. An adsorbent to lipid ratio of approximately 1000:1 was

used. For these samples that was 20% of each sample. The final volume of samples charged to the column was adjusted to less than 1.0 ml. A 40 ml hexane eluation volume and a 40 ml benzene elution volume were collected (four pore volumes of each solvent). The eluates were concentrated to approximately 1.0 ml and the volume precisely measured.

A Hewlett Packard model 5710A with flame ionization detector was used for G. C. analysis. The columns were 1/8" x 12' stainless steel packed with 3% OV 101 on Chromosorb W 100-120 AW-DMCS. The temperature program was 80° to 280° at 8° per minute. The carrier gas was Helium at 50 ml per minute.

Mass spectral analyses of several of the extracts were performed with a Hewlett Packard model 5930/5933 gas chromatograph-mass spectrometer-data system.

Results and Discussion

From these experiments we have obtained information about the efficiency of the various procedures, about the completeness with which esters are removed by 2 hour and 24 hour saponification, about the separation characteristics of fully activated and partially deactivated column sorbents, about the extent of contamination introduced by the various procedures and about the relative convenience of performing the procedures.

The percentage recovery of hydrocarbons added to samples of clam meats is shown in Appendix - Table 3. These spiked samples were subject to

the complete analytical procedures and the recoveries determined by measuring peak heights in the resulting gas chromatograms. The recoveries of the added aromatic hydrocarbons could not be determined because, even using chromatograms of unspiked samples for comparison, the size of the added peaks was obscured by superposition of peaks from the sample. The bottom two lines of data in Appendix - Table 3 labeled "Chromatography of Spike Solution" give the recoveries of the spike solution from column chromatography alone.

Appendix - Table 3 indicates that similar recoveries of added alkanes are obtained by the three extraction methods. The table also shows that fully activated columns give slightly better recoveries of both alkanes and aromatics than do the partially deactivated columns. It is note worthy that the recoveries of alkanes from column chromatography alone are no better than the recoveries from the full extraction procedures. We reject the explanation that all other steps of the extractions are quantitative; instead we suspect that in the presence of clam lipids the recoveries from column chromatography are higher.

Appendix - Table 3 shows that a total of eight samples were subjected to saponification for two hours (either directly or following Soxhlet extraction). The benzene eluates from column chromatography of five of the eight were analysed mass spectrally. Of this five, three showed the presence of methyl esters. Among the four samples subject to saponification for 24 hours, the benzene eluates of two were given mass spectral analysis.

Neither show evidence of esters. The absence of esters has generally been confirmed in subsequent analyses of extracts that have been saponified for 24 hours.

Comparison of gas chromatograms of samples which had been liquid chromatographed on fully active and partially deactivated columns showed that several peaks which appeared in the hexane eluate using partially deactivated columns appeared completely or in part in the benzene eluate if fully active columns were used. Mass spectral analysis of some of these peaks indicated that they were hydrocarbons with 25 to 30 carbon atoms and six to eight units of unsaturation. In one analysis the dotriacontane spike appeared in the benzene eluate.

Contamination introduced during analysis was satisfactorially low for all procedures. Blank analyses indicated that background from all sources was always at concentrations less than one percent of the sample total hydrocarbon concentrations.

In terms of laboratory convenience, the procedure of Soxhlet extraction followed by saponification is inherently slightly inferior to direct saponification since the former requires two separate operations. However, this consideration is minor compared to others.

A major drawback to the Soxhlet extraction procedure is the lengthy thimble cleaning step. We are aware that some investigators reduce the need for this step by re-using thimbles without intermediate cleaning.

Our limited experience with re-using thimbles has indicated that memory effects can be a problem unless only samples of approximately uniform hydrocarbon concentration are extracted. The cleaning requires both time and solvents. Since we have never found a totally reliable commercial supplier of "contamination free" solvents, we re-distill all solvents and analyse every one gallon batch. Thus, the use of 600 ml of solvents to clean each thimble requires a significant supporting distillation effort. The added time of this cleaning further means that the entire Soxhlet extraction procedure requires at least one week.

Another important difference between the procedures is the tendency of extract solutions to form an emulsion when extracted with hexane. By far the worst emulsions were formed when the two hour saponification was used. These were sometimes stable for days and drastically increased the time and effort required for liquid:liquid extraction. The 24 hour saponification and Soxhlet extraction followed by 2 hour saponification each gave much less troublesome emulsions than the 2 hour saponification. The Soxhlet extraction was slightly superior to the 24 hour saponification. Subsequent work has indicated that if the 24 hour saponification is performed on diced but non-macerated tissue, hydrocarbon recovery is not affected but the emulsion problem is further reduced.

Conclusions

We judge that 24 hour saponification and column chromatograph on partially deactivated columns constitute the best procedure tested.

Soxhlet extraction followed by 2 hour saponification did not completely remove methyl esters and was the most laborious technique. Direct 2 hour saponification was also questionable in the removal of esters and led to severe emulsion problems. The recoveries of hydrocarbons by the three was substantially the same. Although the recoveries from fully active colums were slightly superior to those from partially deactivated columns, the latter were selected to avoid the possibility of alkene isomerization.

The column chromatography step was originally designed for use with samples heavily contaminated with petroleum and thus having a low percentage of olefinic materials among their hydrocarbons. When this step is applied to extracts of unpolluted organisms, considerable caution is necessary because of the intermediate behavior of the olefins.

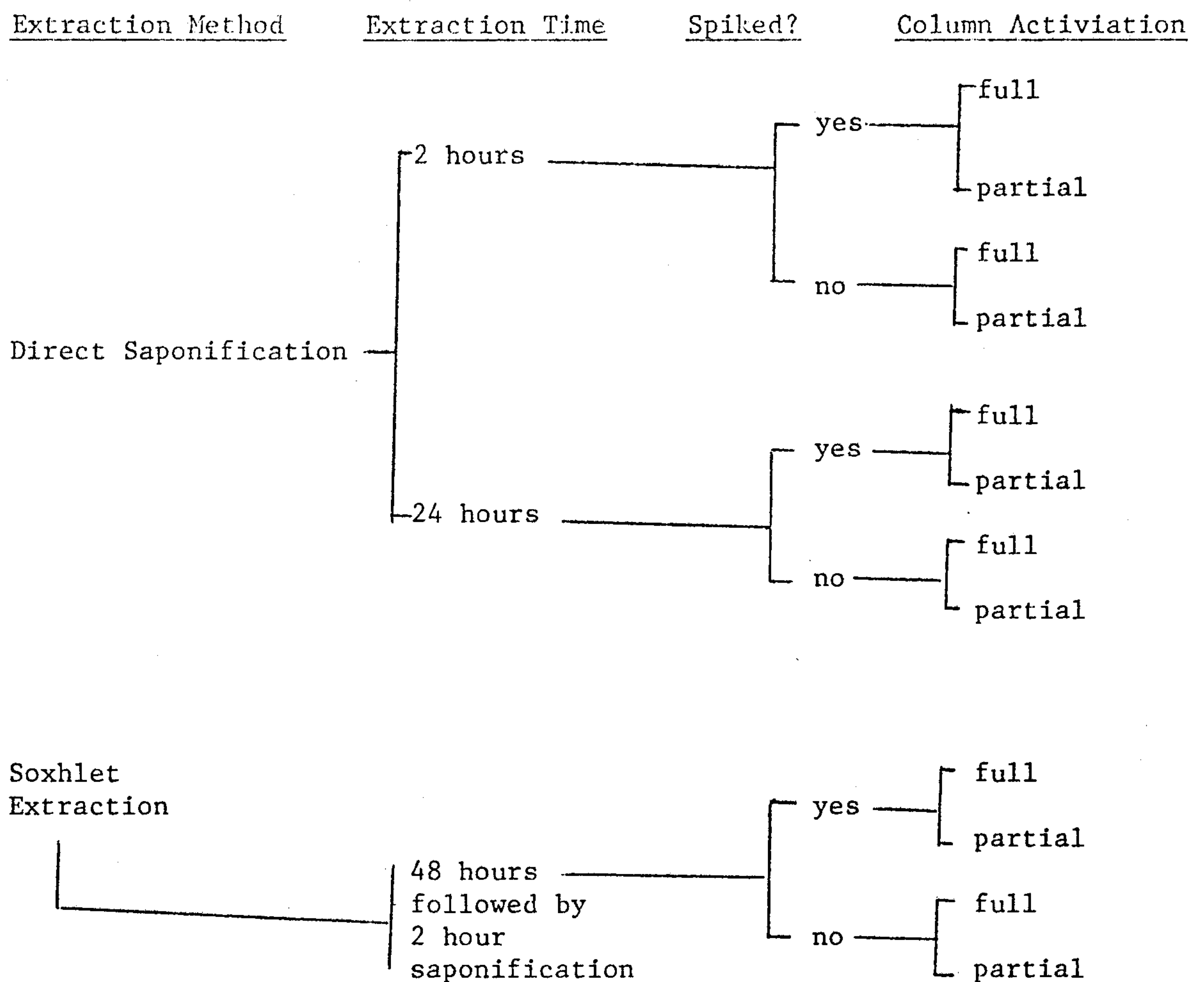
APPENDIX - TABLE 1

Concentrations of hydrocarbons in hexane spike solution.

COMPOUND	CONCENTRACTION Mg/Ml
hexadecane	0.046
docontane	0.038
dotriacontane	0.028
naphthalene	0.044
anthracene	0.034
chrysene	0.048
perylene	0.042
TOTAL HYDROCARBONS	0.280

APPENDIX - TABLE 2

EXPERIMENTAL SCHEME FOR BIOLOGICAL METHODS COMPARISON



APPENDIX - TABLE 3

RECOVERY OF ADDED HYDROCARBONS

Extraction Method	Column[1] Type	Percentage Recovery						
		hexadecane	docosane	dotriacontane	naphthalene	anthracene	chrysene	perylene
2 hour saponification	PD	53	55	68	---	---	---	---
	FA	[2]13	80	79	---	---	---	---
24 hour saponification	PD	72	[3]100+	65	---	---	---	---
	FA	92	81	[4]120	---	---	---	---
Soxhlet	PD	79	64	69	---	---	---	---
	FA	109	104	[4]124	---	---	---	---
Chromatography of spike solution	PD	77	80	81	04	45	40	44
	FA	93	92	96	26	53	51	63

1 FA = fully active, PD = partially deactivated
2 Sample evaporated to dryness after column chromatography
3 Peak appears as shoulder, baseline is uncertain
4 Spike peak superimposed on sample peak, baseline is uncertain

APPENDIX 2

ANNOTATED GAS CHROMATOGRAMS OF BIOTA

The gas chromatograms are in the same order as the data on Table 10, with the daily standards at the end. Annotations used on the chromatograms are:

1. The first date in the sample identification is the date the sample was analyzed by gas chromatography. The corresponding standard (SAM) will have the same date.
2. The sample is then identified by species - in some cases this is by common name, e.g., pollock, instead of T. chaleogramma.
3. The reference number for the sample is then given, e.g., NP#2 is sample #2 from the North Pacific cruise in the Gulf of Alaska, June 26 to July 15, 1975.
4. If there is a date in the sample identification, it is extraction date and is for in-house reference.
5. The liquid column chromatography elution fraction is then identified: fraction 1 is hexane, fraction 2 is benzene.
6. The volume of sample injected is always given. For all of these samples the volume is 5 µl.
7. Beside the integrator listing, the corresponding Blank is identified.

8. To simplify calculations, the printout of data is also annotated. Peaks which are not "real" in the sample are lined out and explained:

   A. MeOH: peaks in solvent check of methanol used

   B. S: peaks wich result from septum bleed

   C. B (or cont.): peaks in corresponding Blank

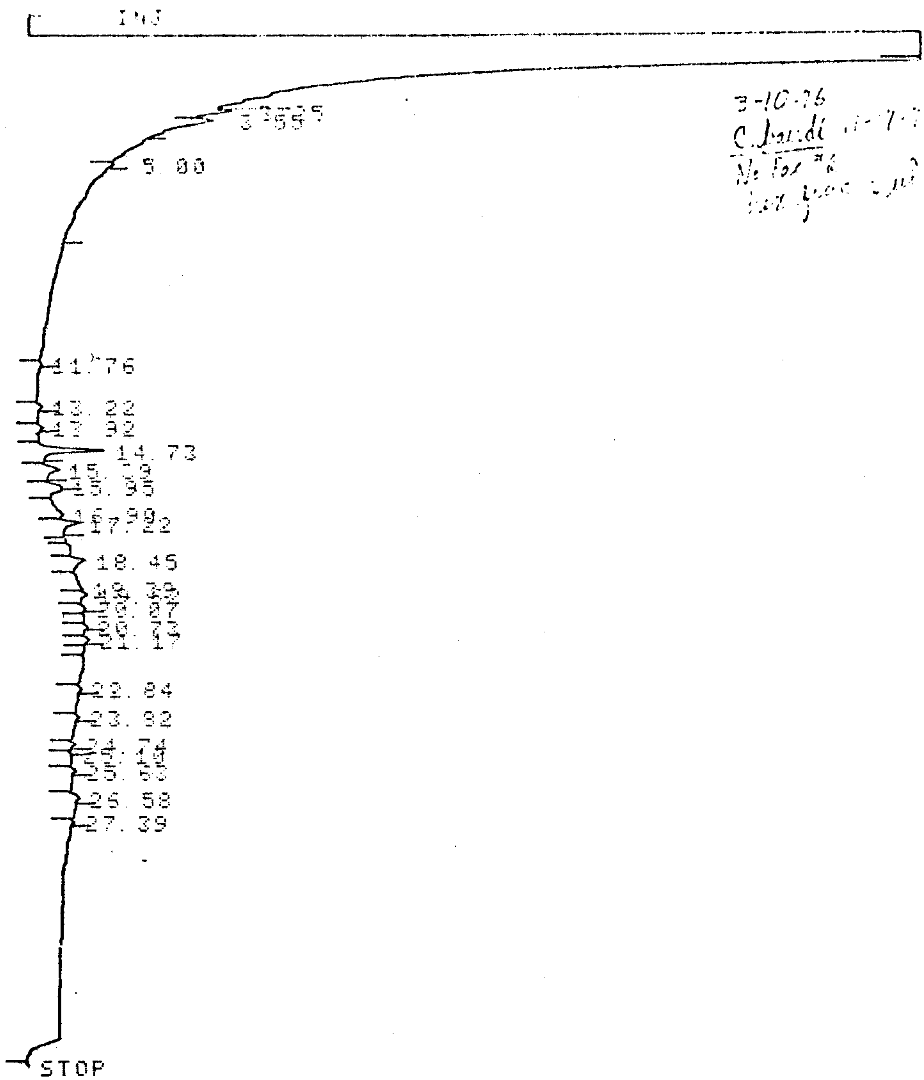
   D. peaks with areas of less than 100 are rejected

   E. the last peak in some runs occurs when the oven door opens at the end of the program.

9. SAM = Standard Alkane Mixture. This is the standard which is run daily to calculate the response factor (RF) and measure relative retention times. It is made up of:

n - tetradecane	0.0140 mg/ml
n - heptadecane	0.0100 mg/ml
n - octadecane	0.0102 mg/ml
n - docosane	0.0151 mg/ml
n - octacosane	0.0110 mg/ml
n - dotriacontane	0.0140 mg/ml

10. The large peaks in some of the Blanks are the spike which is added at the beginning of the extraction procedure to calculate recovery rates.



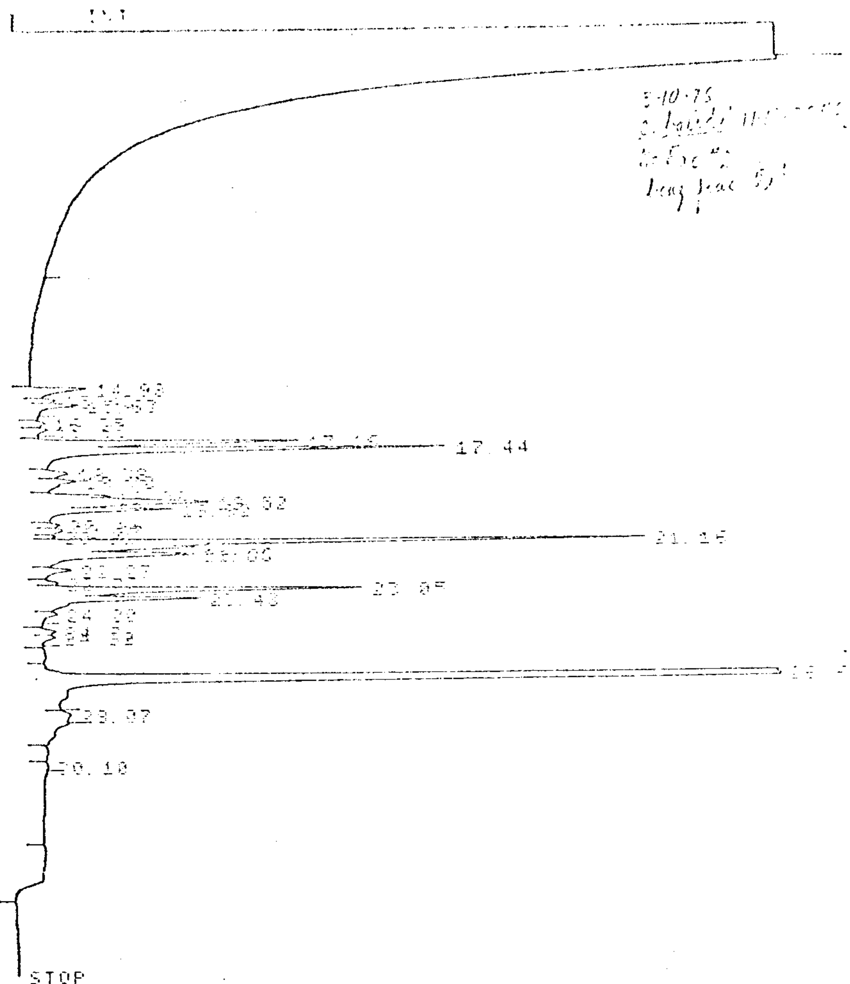
AREA %

	RT	TYPE	AREA	
	~~2.25~~	~~T~~	~~[illegible]~~	~~5.727~~
MeOH	~~3.55~~	~~TH~~	~~[illegible]~~	~~12.11~~
	~~5.00~~	~~T~~	~~144~~	~~1.092~~
	11 76		111	.841 9
	13.22		280	2.124
	13 92		165	1.252
	14.73		3149	23.89
S	~~15.[illegible]~~		~~[illegible]~~	~~6.44~~
	~~15.95~~		~~74~~	~~.561 3~~
	16.90		709	5.378
	17 22	M	1079	8.184
	18 45		841	6.379
	19.39	M	798	6.053
	19.52	M	608	4.612
	20.07	M	219	1.661
	20.73		149	1.13
	21 17		236	1.79
B	~~22.[illegible]~~		~~[illegible]~~	~~1.[illegible]~~
B	~~23.[illegible]~~		~~[illegible]~~	~~1.[illegible]~~
B	~~24.[illegible]~~		~~[illegible]~~	~~1.[illegible]~~
	~~25.[illegible]~~		~~[illegible]~~	~~.[illegible] 2~~
C	~~25.[illegible]~~		~~[illegible]~~	~~1.[illegible]~~
C	~~26.[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~27.[illegible]~~		~~[illegible]~~	~~1.[illegible]~~
			8344	

Blank 11-17-75

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

251

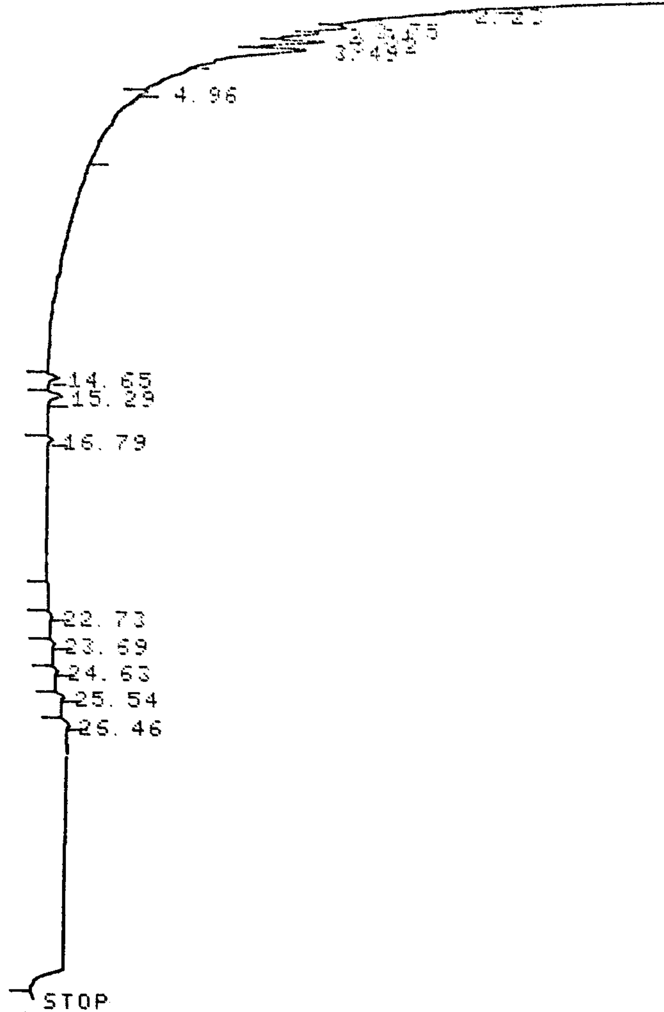


AREA %

	RT	TYPE	AREA	
	14.98		3516	.937 8
S	~~15.43~~	~~T~~	~~620~~	~~.165 1~~
	15.63	M	2047	.546
	16.35		452	.120 6
S	~~16.88~~	~~M~~	~~563~~	~~.150 2~~
	17.16		13408	3.576
	17.44	M	25933	6.917
	18.38	T	1495	.398 7
	18.70	TM	2505	.668 1
	19.28	TM	2238	.596 9
	19.52	TM	13217	3.525
	19.82	TM	7732	2.062
	20.44	TM	817	.217 9
	20.67	TM	1249	.333 1
	20.91	TM	542	.144 6
	21.16	M	30301	8.082
	21.38	M	7456	1.989
	21.66	M	11441	3.051
	22.27	M	1974	.526 5
	22.77	M	839	.223 8
	23.05	M	19765	5.272
	23.43	M	11191	2.985
B	~~24.00~~	~~M~~	~~1057~~	~~.281 9~~
	24.71		879	.234 4
	24.92	M	998	.266 2
	26.36		211856	56.51
	29.07		549	.146 4
	30.10		292	.077 88
			372692	

Blank 11-7-7

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



3-10-76

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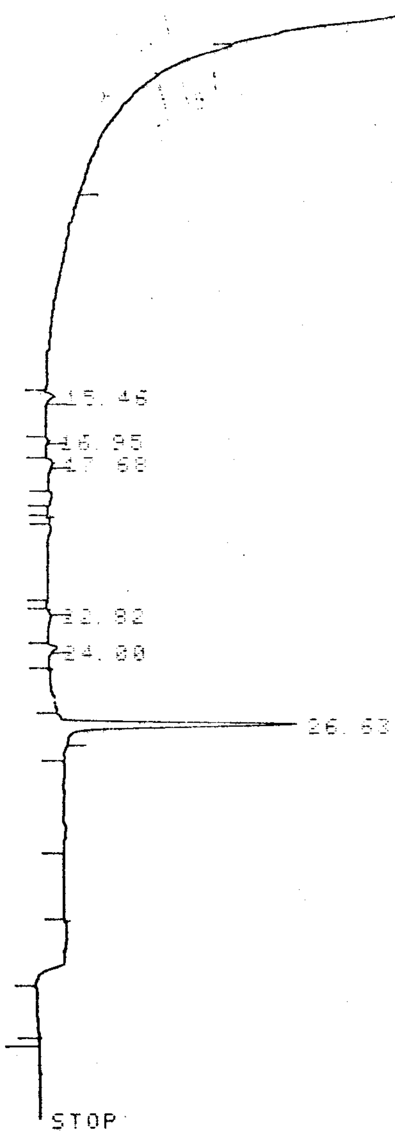
AREA %

	RT	TYPE	AREA	AREA %
MeOH	~~[illegible]~~	[illegible]	~~[illegible]~~	~~[illegible]~~
	~~[illegible]~~	[illegible]	~~[illegible]~~	~~[illegible]~~
	~~[illegible]~~	[illegible]	~~[illegible]~~	~~[illegible]~~
	~~[illegible]~~	[illegible]	~~[illegible]~~	~~17.55~~
	~~[illegible]~~	[illegible]	~~[illegible]~~	~~[illegible]~~
	~~4.96~~	[illegible]	~~[illegible]~~	~~[illegible]~~
	14.65		580	5.531
S	~~15.29~~		~~[illegible]~~	~~[illegible]~~
	16.79		326	3.109
B	~~22.73~~		~~[illegible]~~	~~[illegible]~~
C	~~23.69~~		~~[illegible]~~	~~[illegible]~~
12	~~24.63~~		~~256~~	~~[illegible]~~
8	~~25.54~~		~~276~~	~~[illegible]~~
13	~~26.46~~		~~[illegible]~~	~~[illegible]~~
			906	

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



5-10-[illegible]

AREA %

RT	TYPE	AREA	
~~15.46~~		~~[illegible]~~	~~[illegible]~~
~~16.95~~		~~[illegible]~~	~~[illegible]~~
17.68		308	2.406
~~22.82~~		~~[illegible]~~	~~.593~~ 6
~~24.00~~		~~[illegible]~~	~~[illegible]~~
26.63		11421	89.21
		11729	

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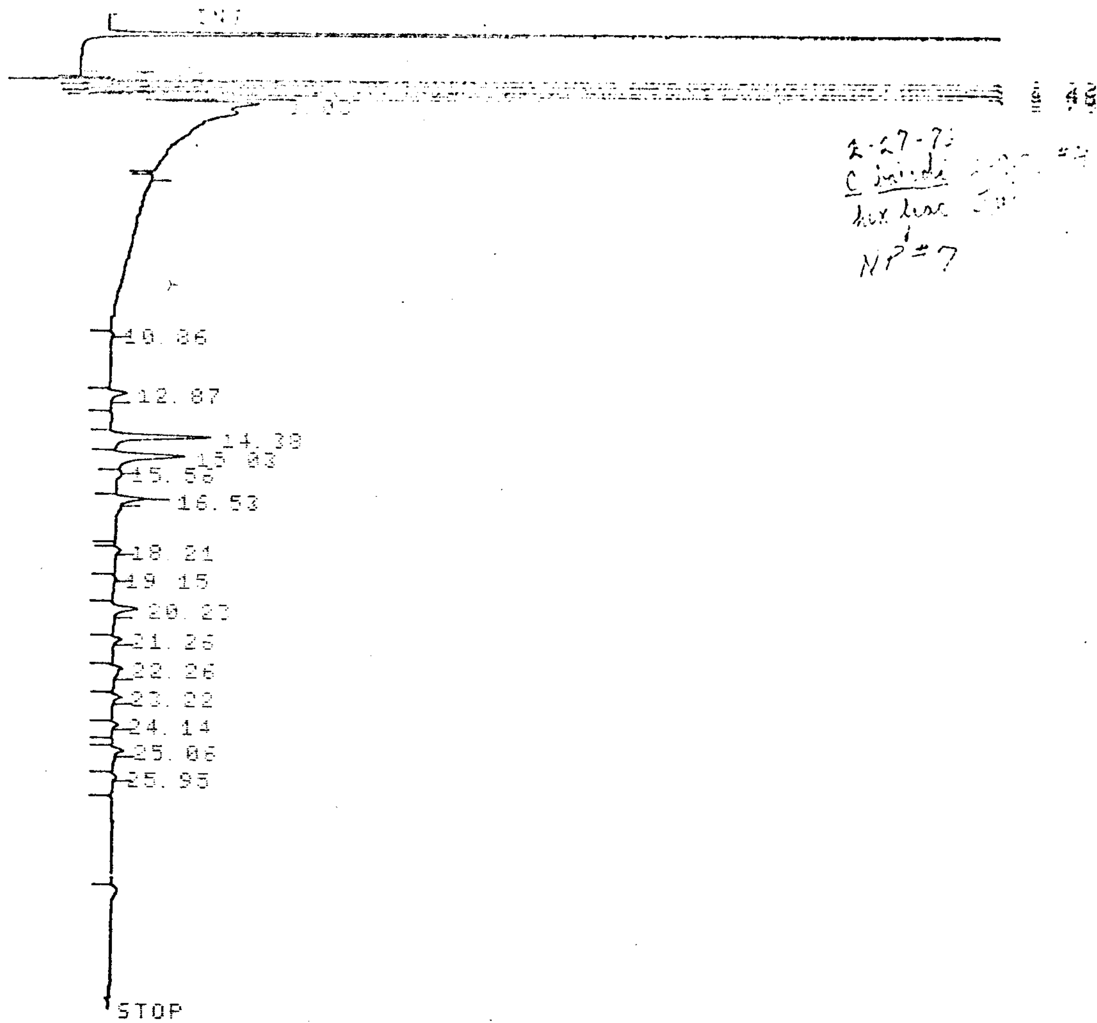
[illegible], but smaller

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M 10 ATTN 4



AREA %

	RT	TYPE	AREA	AREA %
	~~2.16~~		~~910~~	~~.071 24~~
	~~2.28~~	~~M~~	~~[illegible]~~	~~16.[illegible]~~
	~~2.46~~	~~M~~	~~[illegible]~~	~~27.07~~
	~~2.54~~	~~M~~	~~[illegible]~~	~~15.17~~
	~~2.70~~	~~M~~	~~[illegible]~~	~~27.~~
	~~3.03~~	~~T~~	~~[illegible]~~	~~1.921~~
	~~10.86~~		~~85~~	~~.037 794~~
S	~~12.87~~		~~341~~	~~.077 00~~
	14.38	PRISTANE	4337	.397 5.
S	~~15.03~~	~~M~~	~~[illegible]~~	~~.251 2~~
B	~~15.56~~	~~M~~	~~64~~	~~.005 566~~
S	~~16.53~~		~~1294~~	~~.118 5~~
S	~~18.21~~		~~237~~	~~.018 37~~
B	~~19.15~~		~~171~~	~~.012 04~~
B	~~20.23~~		~~1333~~	~~.122 2~~
B	~~21.26~~		~~337~~	~~.026 29~~
B	~~22.26~~		~~634~~	~~.058 11~~
B	~~23.22~~		~~434~~	~~.039 36~~
B	~~24.14~~		~~282~~	~~.025 85~~
S	~~25.06~~		~~491~~	~~.045~~
B	~~25.95~~		~~282~~	~~.025 66~~
			4337	

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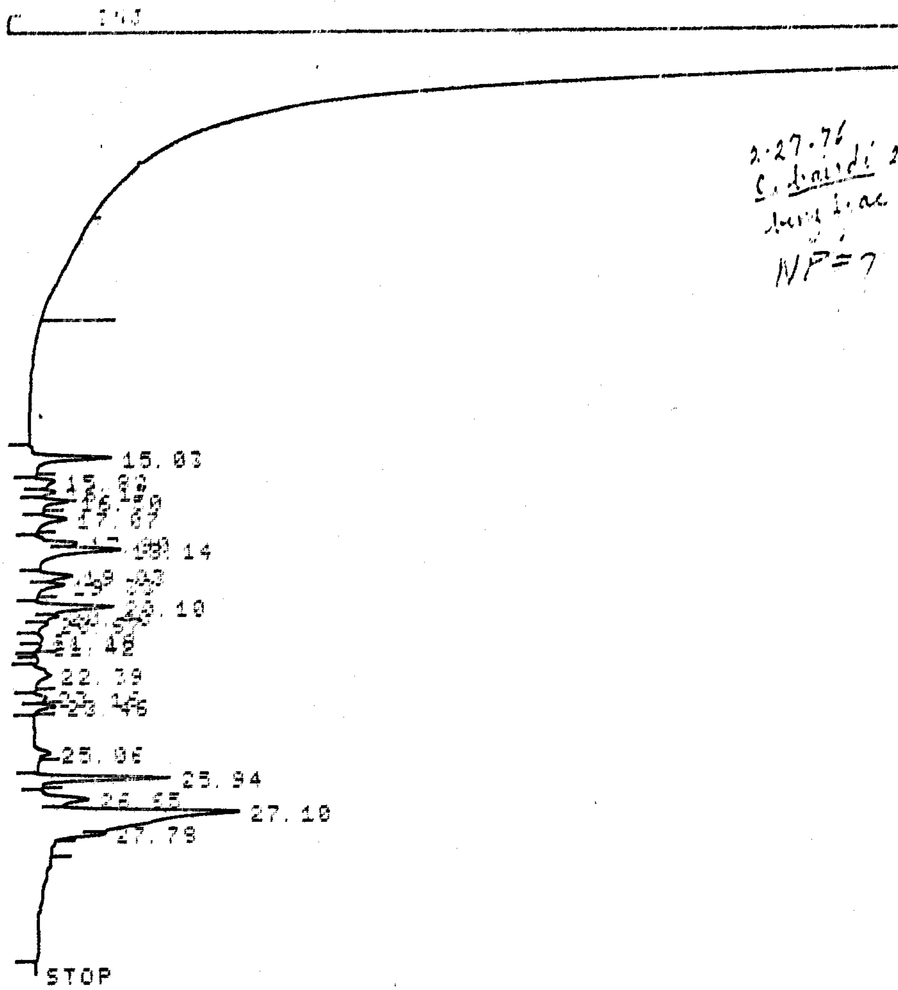
HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA	AREA %
S	~~15.03~~		~~4336~~	~~6.451~~
	15.82		1102	1.639
	16.17	M	725	1.079
S	~~16.50~~	~~M~~	~~1211~~	~~1.802~~
	17.07		1363	2.028
	17.90		2151	3.2
	18.14	M	5657	8.416
	19.03	M	2390	3.556
B	~~19.33~~	~~M~~	~~1637~~	~~2.435~~
	20.10		4284	6.373
B	~~20.40~~	~~M~~	~~1226~~	~~1.827~~
	20.67	M	733	1.09
	21.18	M	582	.865 9
B	~~21.42~~	~~M~~	~~274~~	~~.407 6~~
	22.39		1651	2.456
	23.16		294	.437 4
B	[illegible]		[illegible]	[illegible]
B	[illegible]		[illegible]	[illegible]
	25.94		5389	8.017
	26.65		3099	4.61
	27.10	M	28028	41.7
	27.78	T	225	.334 7
			57673	

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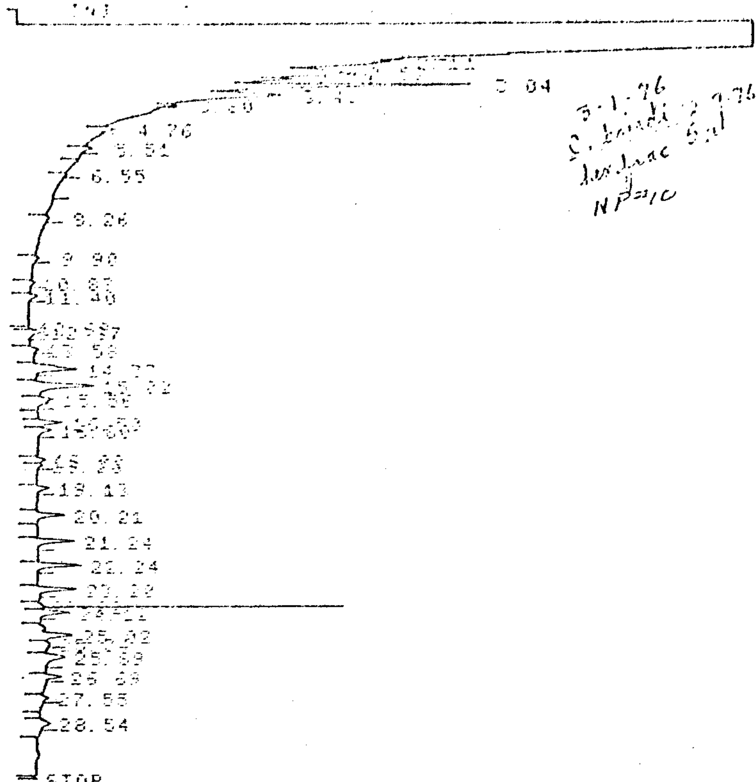
HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M 10 ATTN 4



AREA %

RT	TYPE	AREA	
~~[illegible]~~			
9.90		218	.463 5
10.83		208	.442 2
11.40		452	.961
13.58		309	.657
14.37		2862	6.085
16.80	M	725	1.541
23.82		1578	3.355
27.55		339	.720 8
28.54		621	1.32
		7312	

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

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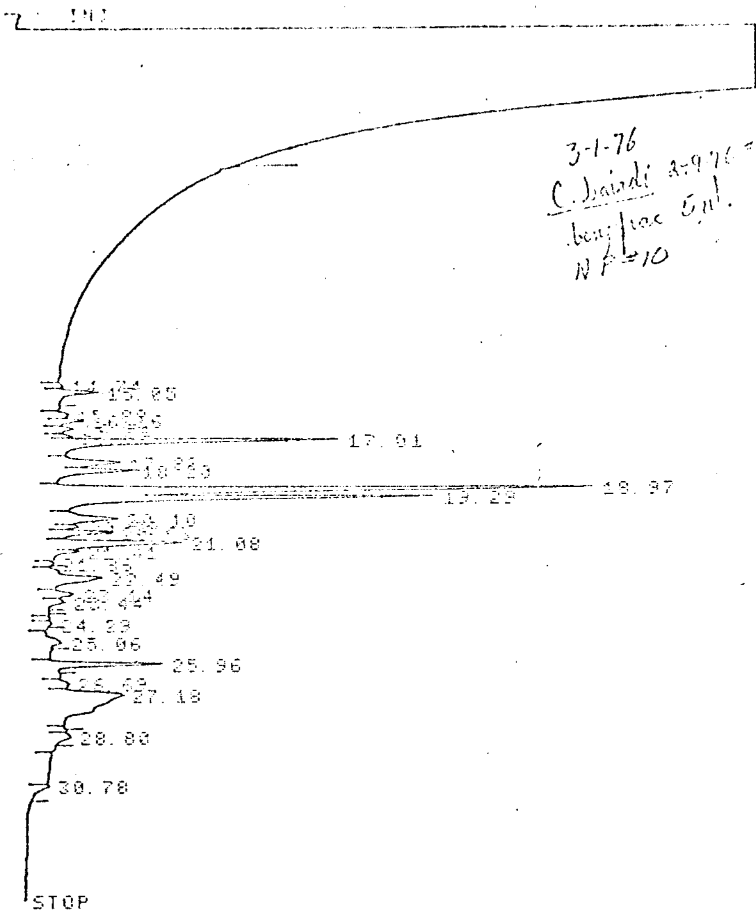
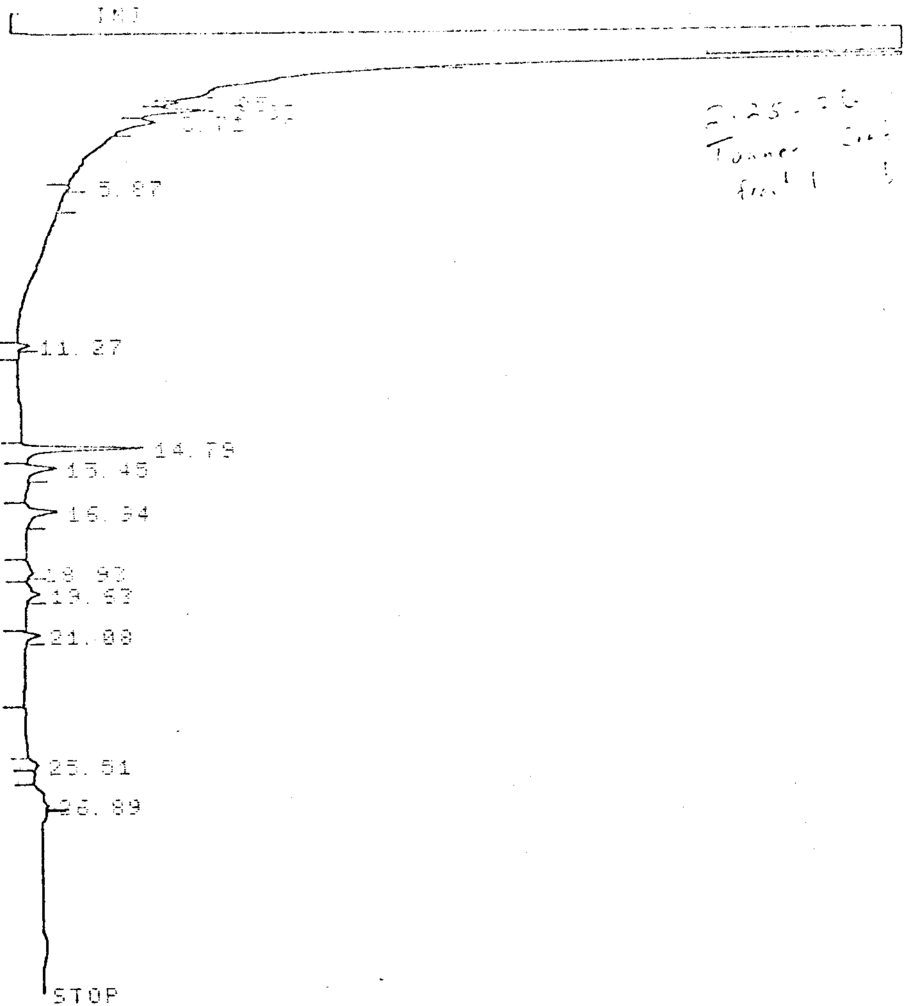


AREA %

	RT	TYPE	AREA		
	14 74	T	258	.183	2
s	[illegible]	[illegible]	[illegible]	[illegible]	
	15 89	T	672	.477	1
	16 16	TM	1438	1.021	
s	[illegible]	[illegible]	[illegible]	[illegible]	
	16.82	TM	685	.486	4
	17.01	TM	15991	11.35	
	17.88	TM	5437	3.86	
	18.20	TM	5451	3.87	
	18.97	TM	32760	23.26	
	19.29	TM	22506	15.98	
	20.19	TM	6046	4.293	
	20 39	TM	1384	.982	7
	20 63	TM	3915	2.78	
	21 08	TM	9815	6.969	
B	[illegible]	[illegible]	[illegible]	[illegible]	
B	[illegible]	[illegible]	[illegible]	[illegible]	
	22 49	T	5509	3.912	
	23.14	TM	1961	1.392	
B	[illegible]	[illegible]	[illegible]	[illegible]	
	24.29		178	.126	4
	25.06		1011	.717	8
	25 96		5388	3.826	
	26.69		611	.433	8
	27 18	M	12678	9.002	
	28 80		705	.500	6
	[illegible]		[illegible]	[illegible]	
			139399		

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA	
cont	[illegible]		[illegible]	[illegible]
cont.	[illegible]		[illegible]	[illegible]
cont.	[illegible]		[illegible]	[illegible]
cont.	[illegible]		[illegible]	[illegible]
	11.27		463	2.798
	14.79		5314	32.11
	15.45	M	1808	10.93
	16.94		2121	12.82
	18.93		689	4.164
	19.63		977	5.904
	21.08		787	4.756
septum	[illegible]		[illegible]	[illegible]
cont	[illegible]		[illegible]	[illegible]
			12159	

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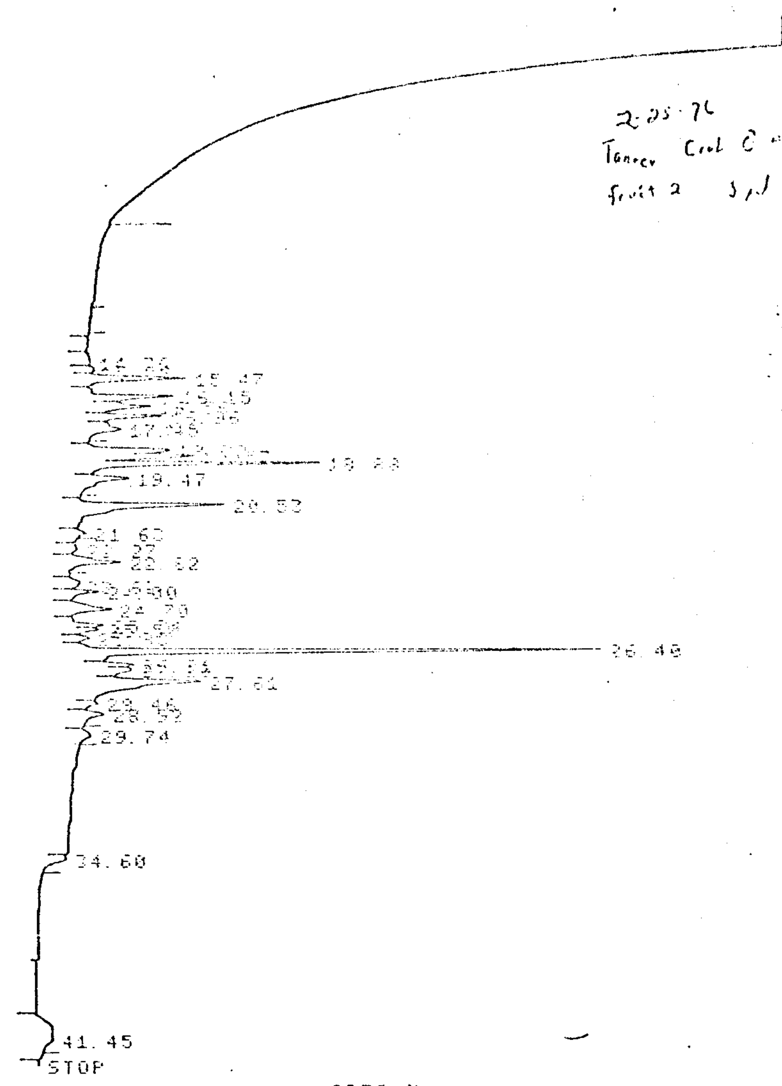
HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M 10 ATTN 4



AREA %

	RT	TYPE	AREA	
	14.76		695	.480 5
septum	[illegible]	M	[illegible]	[illegible]
	15.47	M	6106	4.283
	16.15	M	5430	3.809
	16.56	M	5378	3.772
	16.96	M	4313	3.025
	17.45	M	4022	2.821
	18.32		5524	3.875
	18.57	M	7026	4.928
	18.88	M	12324	8.644
	19.47	M	4105	2.879
	20.53		10498	7.363
cont.	[illegible]	M	[illegible]	[illegible]
	22.27		565	.396 3
	22.92	M	3957	2.775
cont.	[illegible]		[illegible]	[illegible]
	24.00	M	1549	1.086
	24.70	M	3228	2.264
	25.34	M	1570	1.101
	25.50	M	1514	1.062
	25.93	M	1030	.722 5
	26.40	M	26781	18.78
	26.91	M	3338	2.341
	27.10	M	5664	3.973
	27.61	M	15829	11.1
	28.46	M	1534	1.076
	28.92	M	2073	1.429
	29.74		1050	.736 5
alc	[illegible]		[illegible]	[illegible]
oc	[illegible]		[illegible]	[illegible]
			135418	

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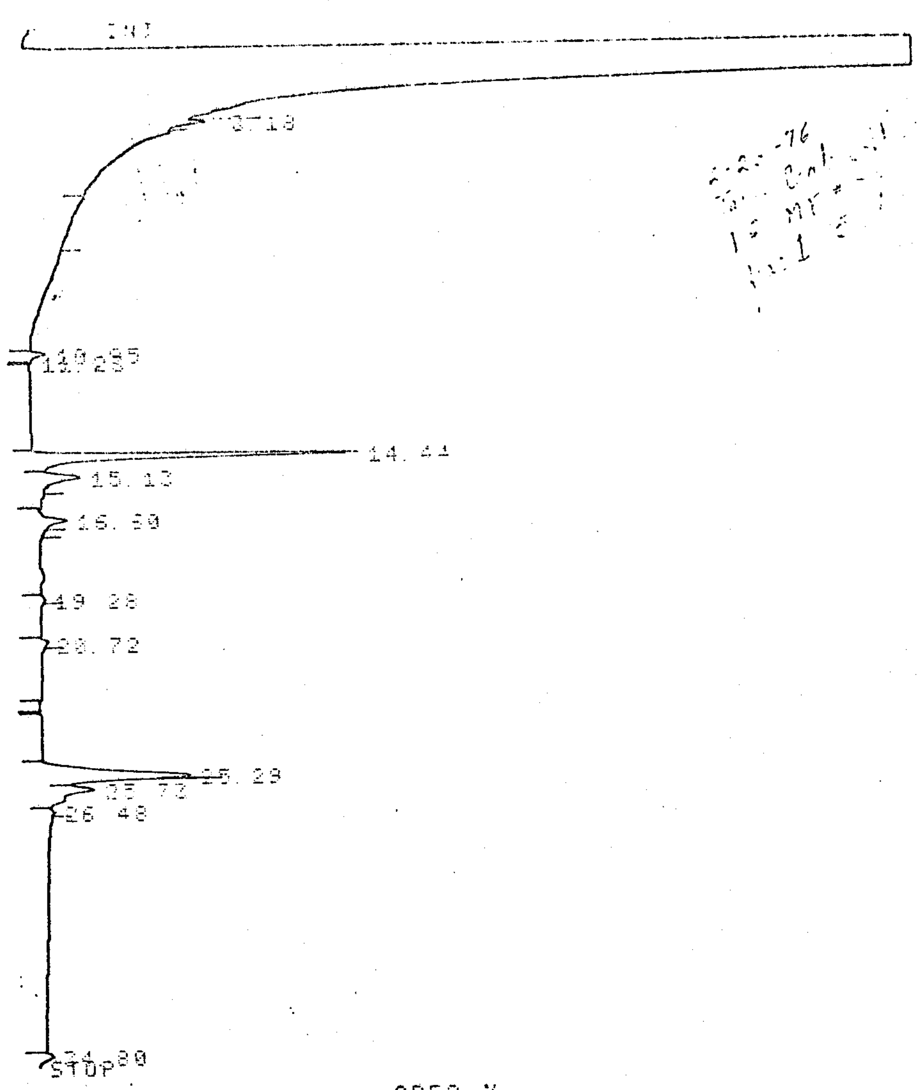
HP 3380A

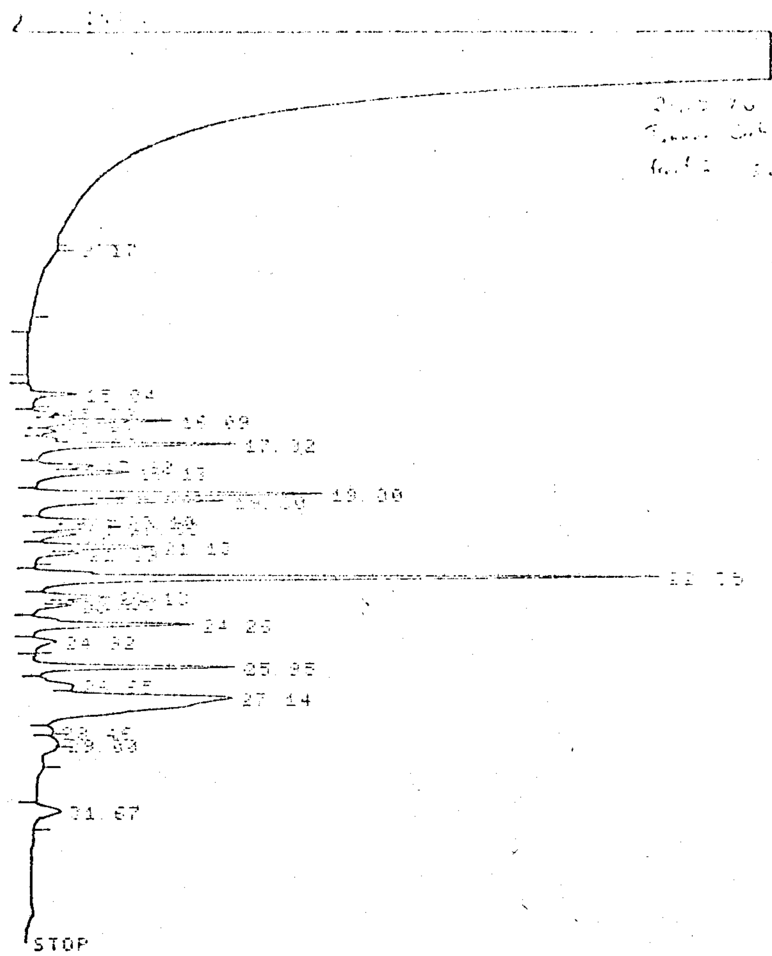
260



AREA %

	RT	TYPE	AREA		
cont.	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
	10.95		770	1.779	
a.ts	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
	14.44		15669	36.11	
septum	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	19.28		160	.369	7
	20.72		310	.716	3
	25.29		15063	34.81	
	25.73	M	4374	10.11	
	26.48	M	204	.471	4
oc	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
			36346		

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



AREA %

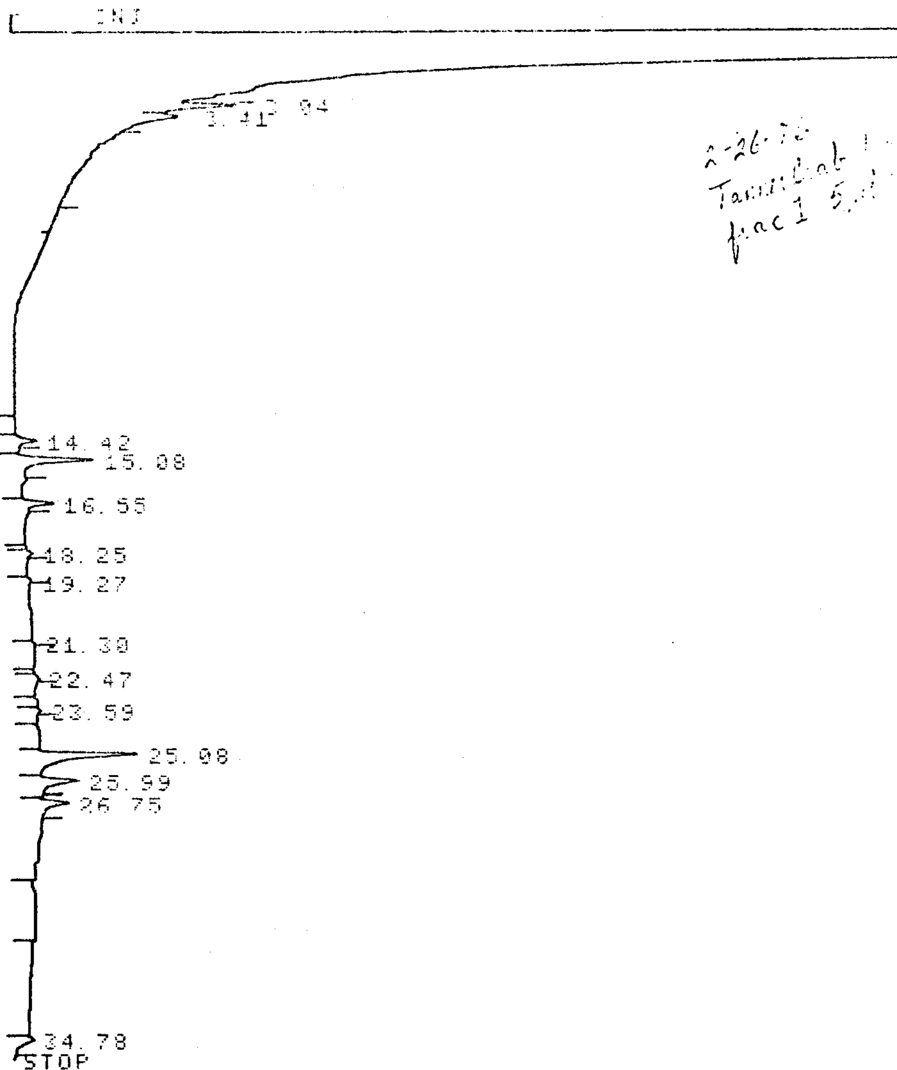
	RT	TYPE	AREA	AREA %	
[illegible]	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	15.79		550	.262	E
	16.09		5620	2.683	
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	17.02	M	11298	5.394	
	17.88	T	3405	1.626	
	18.13	TM	6800	3.247	
	19.00	M	17227	8.225	
	19.38	M	11713	5.592	
	20.10	M	4702	2.245	
	20.33	M	5225	2.495	
	20.65	M	2699	1.289	
	21.13	M	8158	3.895	
	21.39	M	2549	1.217	
	22.25		36635	17.49	
	23.13	M	5112	2.441	
	23.33	M	1984	.947	?
	23.47	M	2063	.985	
	24.26	M	10781	5.147	
	24.92	M	1759	.839	9
	25.95		11290	5.39	
	26.65	M	4096	1.956	
	27.14	M	47490	22.67	
	28.45	T	337	.160	9
	29.03	T	474	.226	3
	31.67		3800	1.814	
			205757		

Black

HP 3380A

DLY 2. STOP 45 REJECT OFF

MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA	
cont.	~~2.04~~	~~T~~	~~[illegible]~~	~~[illegible]~~
cont.	~~3.41~~	~~TM~~	~~1595~~	~~7.[illegible]~~
	14.42		905	4.504
septum	~~15.08~~		~~[illegible]~~	~~[illegible]~~
septum	~~16.55~~		~~[illegible]~~	~~[illegible]~~
septum	~~18.25~~		~~[illegible]~~	~~1.[illegible]~~
	19.27		180	.895 8
ats.	~~21.30~~		~~[illegible]~~	~~[illegible]~~
	22.47		152	.756 5
	23.59		150	.746 5
	25.08		5424	26.99
	25.99	T	2029	10.1
	26.75		1531	7.62
cc	~~34.78~~		~~[illegible]~~	~~1.[illegible]~~
			10724	

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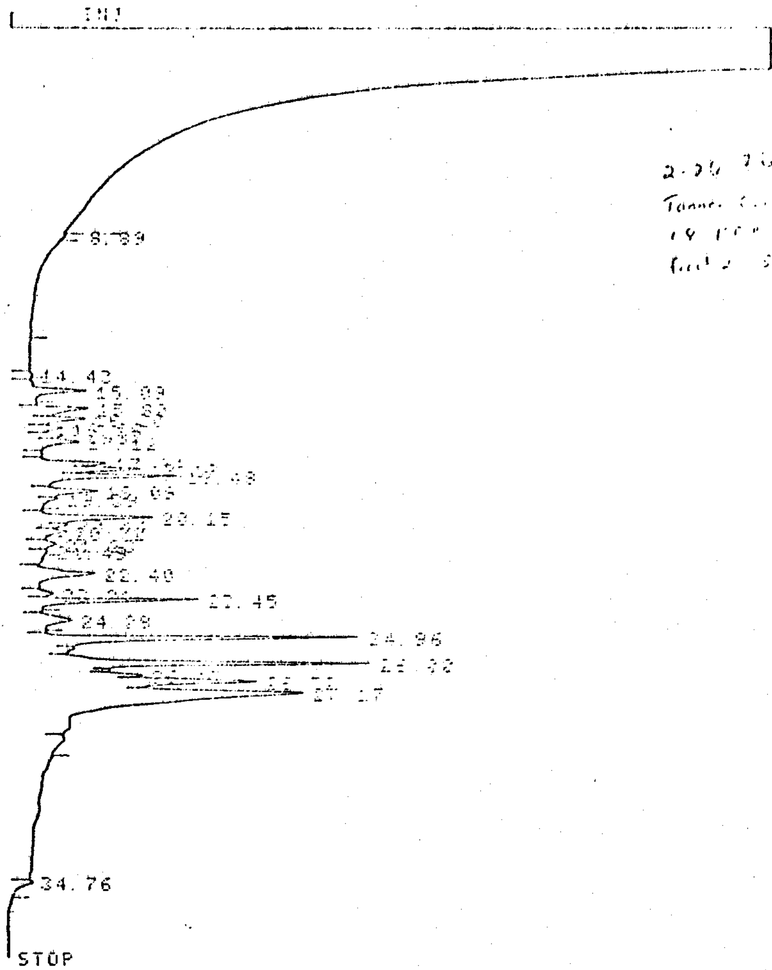
HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA		
	8.89	T	197	.142	
	14.43		169	.121	1
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	15.85		3500	2.523	
	16.20	M	2622	1.89	
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	16.87	M	1306	.941	6
	17.12	M	2517	1.815	
	17.94		3834	2.764	
	18.19	M	6395	4.611	
	18.48	M	8114	5.85	
	19.09	M	4093	2.951	
	19.39	M	1062	.765	7
	20.15		9219	6.647	
	20.44	T	200	.144	2
	20.72	TM	724	.522	
	21.23	T	817	.589	
	21.49	TM	210	.151	4
	22.40		5196	3.746	
	23.21	T	792	.571	
	23.45	M	7517	5.419	
	24.29		2621	1.89	
	24.96		16057	11.59	
	26.00	M	13407	9.666	
	26.47		1559	1.124	
	26.72	M	12232	8.819	
	27.17	M	29604	21.34	
	~~[illegible]~~		~~[illegible]~~		
			133763		

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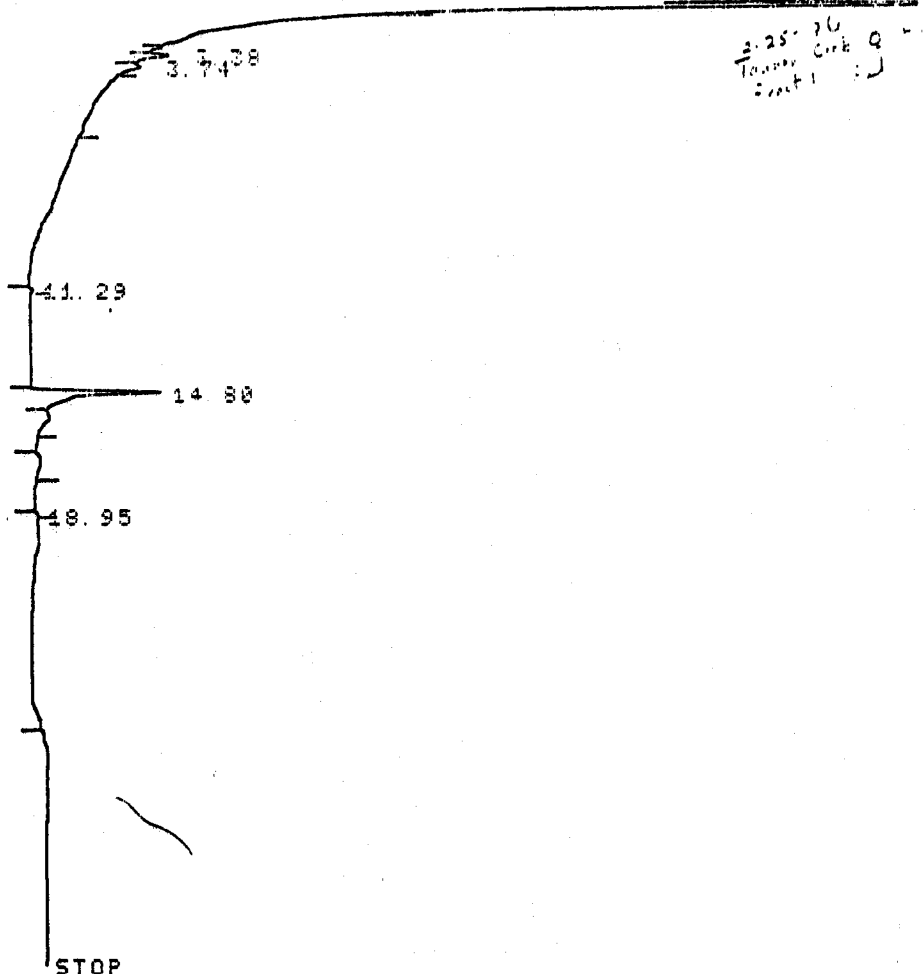
HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

264

MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA	
cont.	~~3.[illegible]~~		~~[illegible]~~	~~[illegible]~~
cont.	~~3.[illegible]~~		~~[illegible]~~	~~[illegible]~~
	11.29		204	1.675
	14.60		10523	86.38
alt	~~18.95~~		~~[illegible]~~	~~[illegible]~~
			10727	

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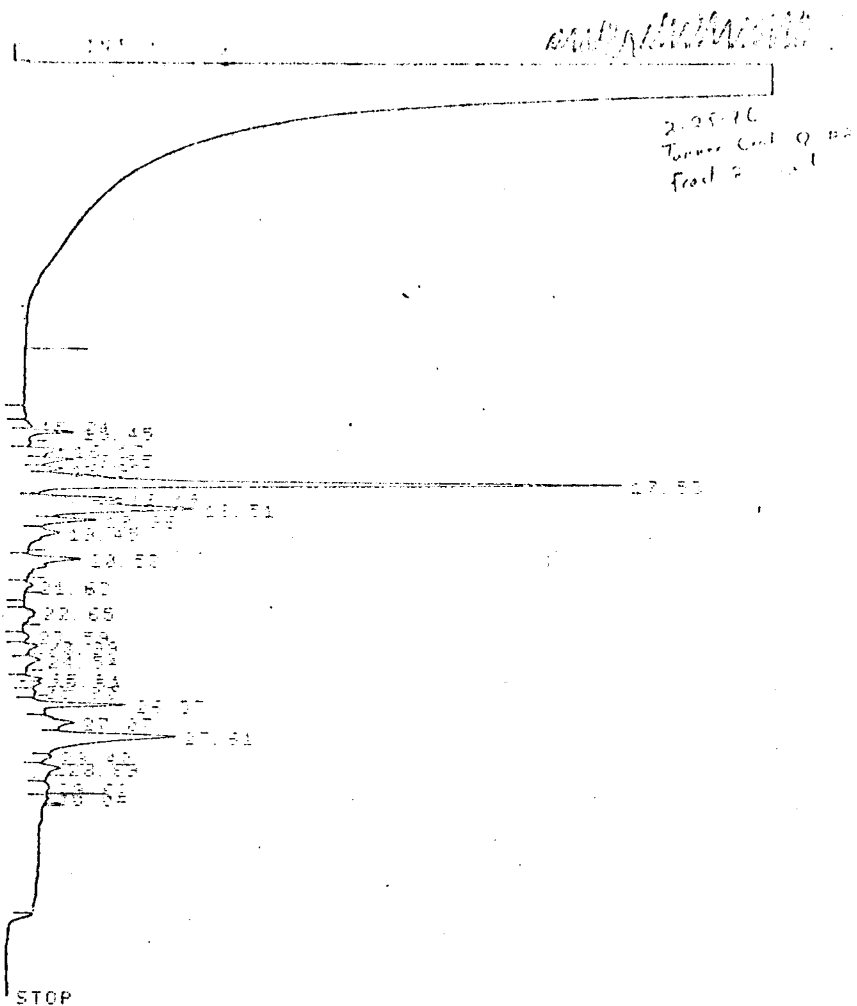
HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

265



STOP

AREA %

RT	TYPE	AREA	
15.21		465	.355 8
15.45	M	2264	1.729
16.17		2238	1.709
16.55	M	3480	2.657
16.93	M	2124	1.622
17.53	M	43804	33.44
18.12	M	4845	3.699
18.27	M	3666	2.799
18.51	M	15951	12.18
18.96	M	4870	3.718
19.45	M	3914	2.988
20.53		5239	4
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
22.65		1252	.955 9
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
23.99	M	859	.655 8
24.54		754	.575 7
25.31		612	.467 3
25.50	M	654	.499 3
25.82	M	259	.197 7
26.37		4717	3.601
27.07	M	5211	3.979
27.61	M	20155	15.39
28.42	T	321	.245 1
28.89	TM	1169	.891 8
29.62	T	504	.384 8
30.04	TM	687	.524 5
		130014	

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M 10 ATTN 4

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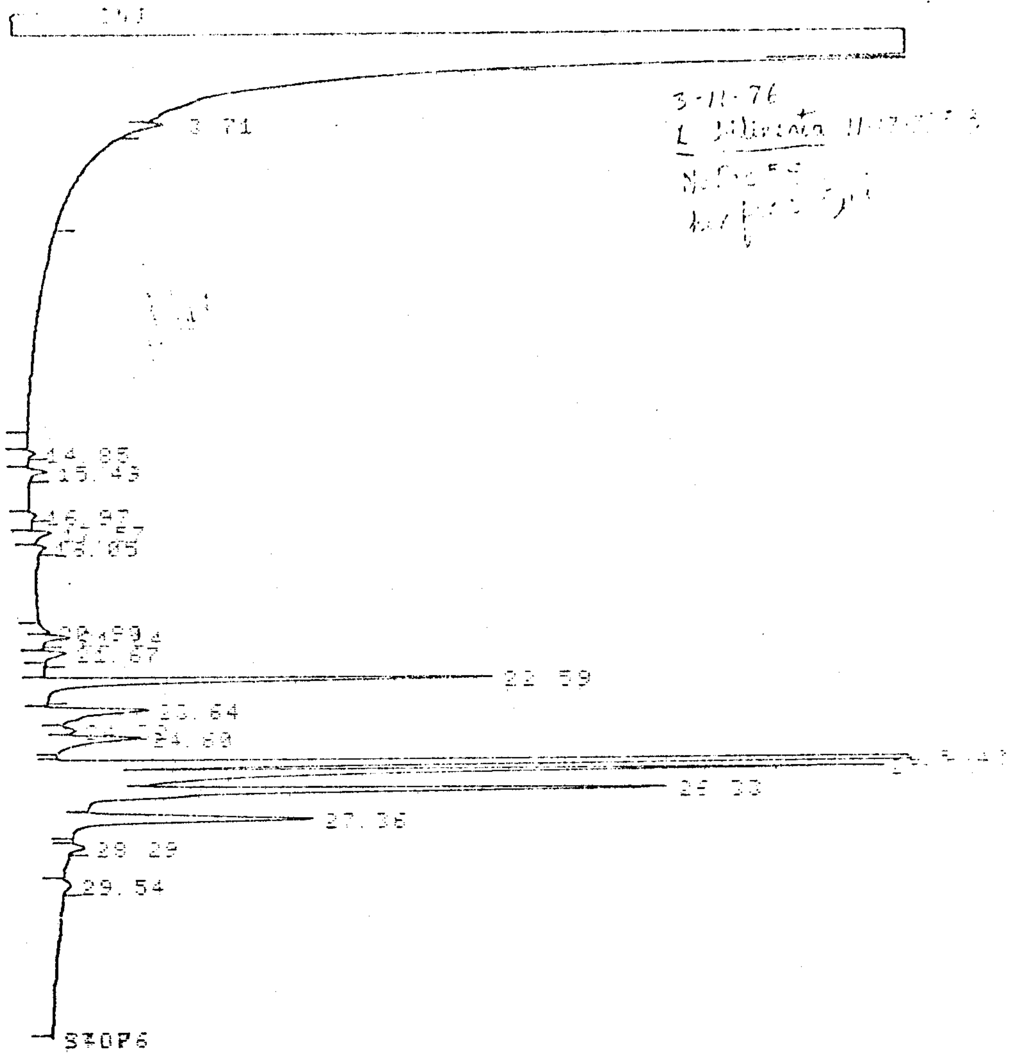

AREA %

RT	TYPE	AREA	
3.71	T	1041	.535 4
14.85		389	.200 1
~~15.43~~		[illegible]	[illegible]
~~16.97~~		[illegible]	[illegible]
17.57		971	.499 4
18.05	M	486	.25
20.90		415	.213 4
21.14	M	1441	.741 2
21.67		1467	.754 5
22.59		19468	10.01
23.64		7183	3.694
24.32	T	703	.361 6
24.50	M	4865	2.502
25.43	M	62730	32.26
25.71	M	39267	20.2
26.33	M	34600	17.8
27.36	M	16683	8.581
28.29		697	.358 5
29.54		634	.326 1
34.76	I	~~65~~	033 43
		194,360	

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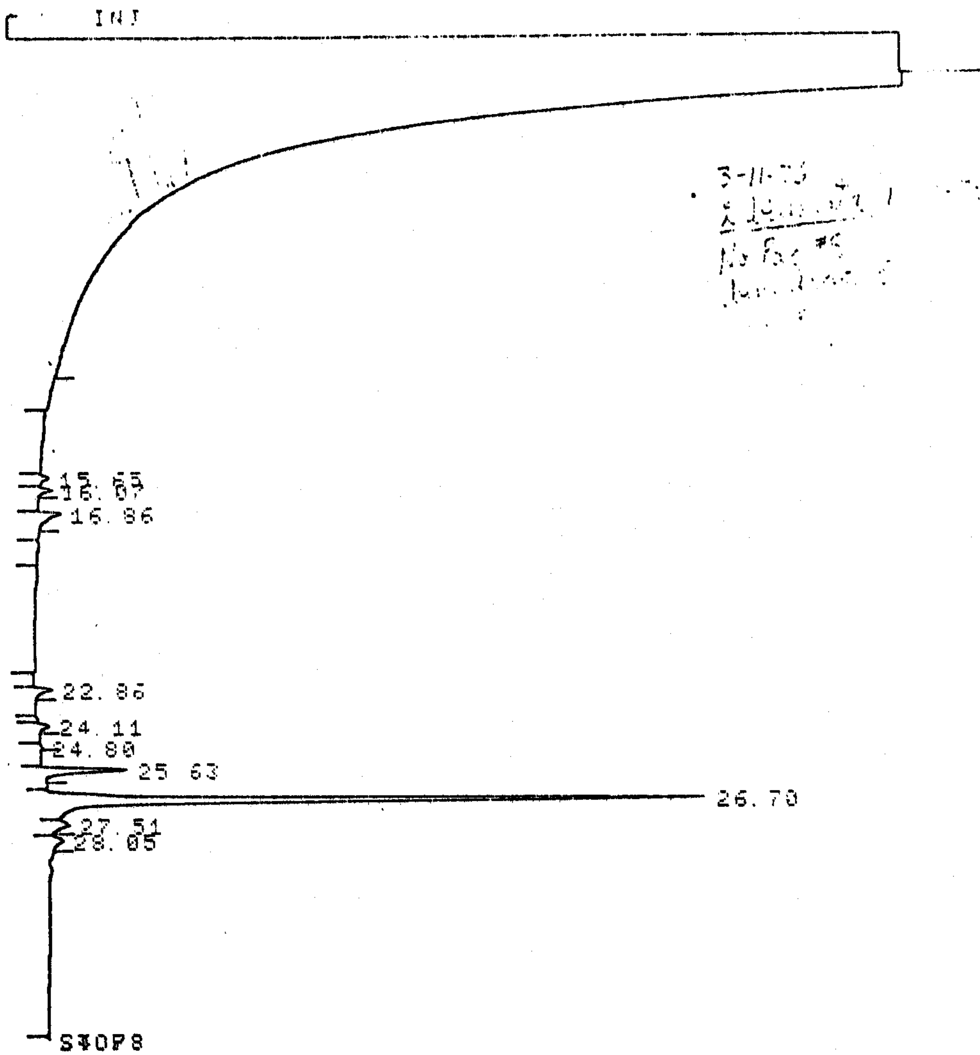
HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

267



AREA %

	RT	TYPE	AREA	
S	~~15.65~~		~~[illegible]~~	~~[illegible]~~
	16.07	M	580	1.342
	16.86		1692	3.914
	22.86		863	1.996
B	~~24.11~~		~~[illegible]~~	~~[illegible]~~
	24.80		151	.349 3
	25.63		3770	8.72
	26.70		32829	75.94
	27.51	M	1366	3.16
	28.05		779	1.8
	~~[illegible]~~	~~I~~	~~[illegible]~~	~~[illegible]~~
			42029	

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HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

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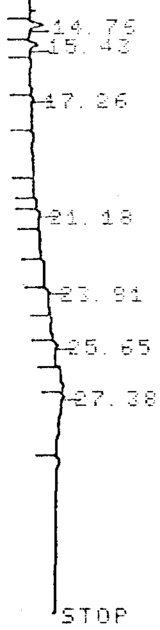
3-10-76

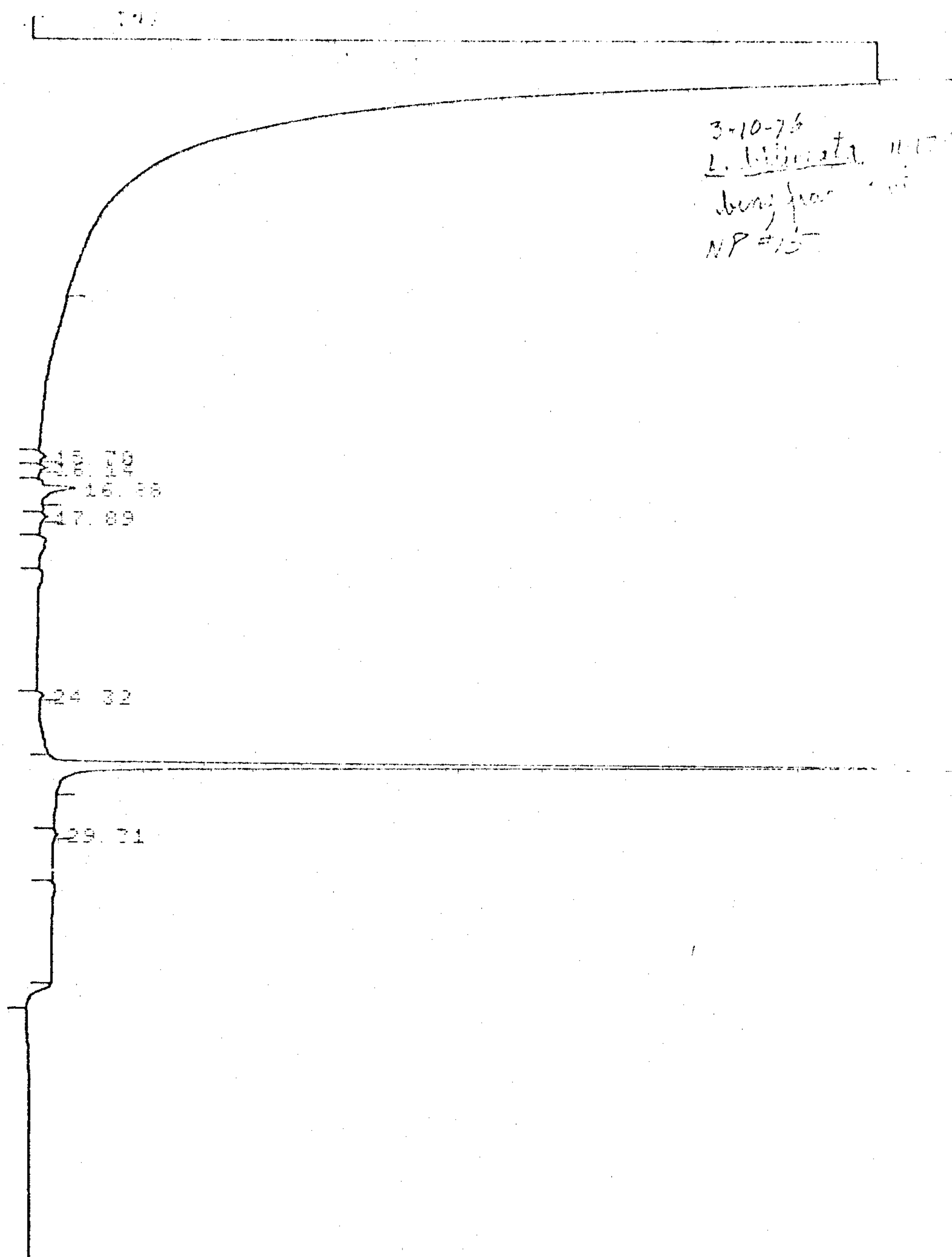


AREA %

RT	TYPE	AREA	
14.76		698	34.57
S ~~15.43~~		~~[illegible]~~	~~[illegible]~~
17.26		153	7.578
21.18		181	8.965
23.81		140	6.934
B ~~25.65~~		~~[illegible]~~	~~[illegible]~~
B ~~27.38~~		~~[illegible]~~	~~[illegible]~~
		1172	

Blank 11-17-75

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



STOP

AREA %

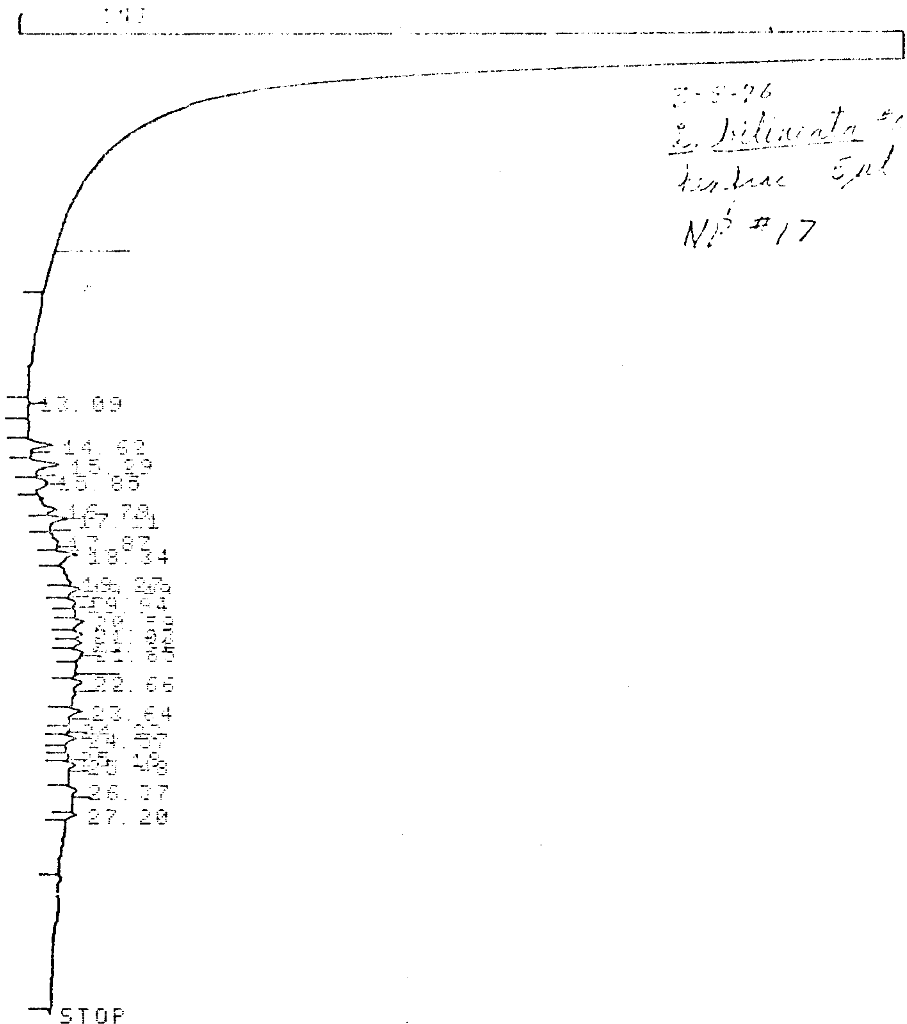
	RT	TYPE	AREA		
5	~~15.70~~		~~427~~	~~.901~~	~~3~~
	15.14		326	.688	1
	16.88		2442	5.154	
	17.89		297	.626	9
B	~~24.32~~		~~245~~	~~.517~~	~~1~~
	26.90		43369	91.54	
	29.31		272	.574	1
			46726		

Blank 11-17-75

also in Blank, but much smaller

HP 3380A
DLY 2 STOP 45 REJECT OFF
AV/M 10 ATTN 4

270



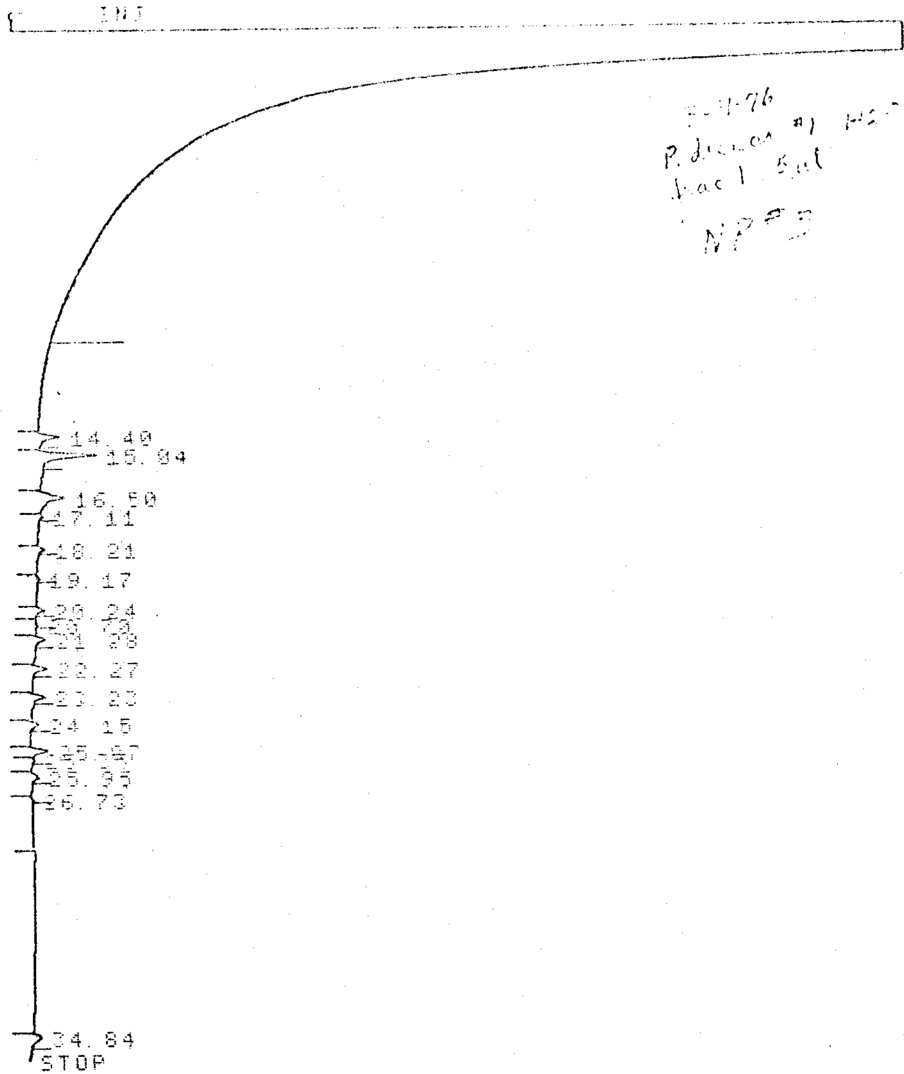
AREA %

	RT	TYPE	AREA		
	-13.09		113	.918	5
	-14.62		1155	9.389	
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	-15.85		88	.715	3
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	-17.11	M	1083	8.803	
	-17.87		363	2.951	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	-19.27	M	804	6.536	
	-19.49	M	718	5.836	
	-19.94		261	2.122	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	-21.03	M	460	3.739	
	-21.31	M	355	2.886	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
F	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	-24.57		399	3.275	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	-25.49		438	3.317	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	-27.20		[illegible]	1.626	
			6318		

Blank 1.12.7?

HP 3380A
DLY 2. STOP 60 REJECT OFF
MV/M 10 ATTN 4

271

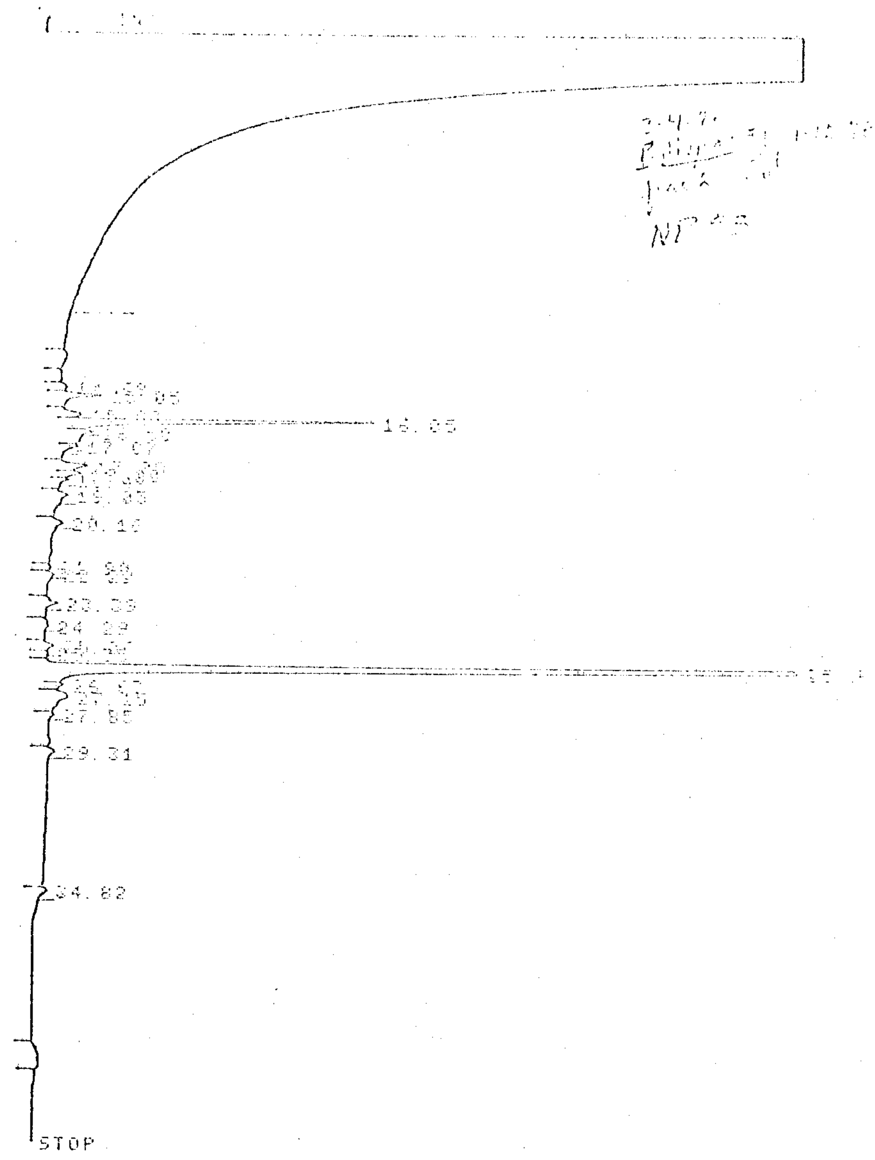


AREA %

	RT	TYPE	AREA	
	14.40		1099	9.108
S	[illegible]		[illegible]	[illegible]
S	[illegible]		[illegible]	[illegible]
	17.11	M	205	1.715
S	[illegible]		[illegible]	[illegible]
	19.17		166	1.389
B	[illegible]		[illegible]	[illegible]
	20.70		211	1.765
B	[illegible]		[illegible]	[illegible]
B	[illegible]		[illegible]	[illegible]
B	[illegible]		[illegible]	[illegible]
B	[illegible]		[illegible]	[illegible]
S	[illegible]		[illegible]	[illegible]
	25.35	M	209	1.748
S	[illegible]		[illegible]	[illegible]
B	[illegible]		[illegible]	[illegible]
S	[illegible]		[illegible]	[illegible]
			1880	

Blank 1-12-76

HP 3380A
PLV [illegible] STOP 45 REJECT OFF
MV/M .10 ATTN 4

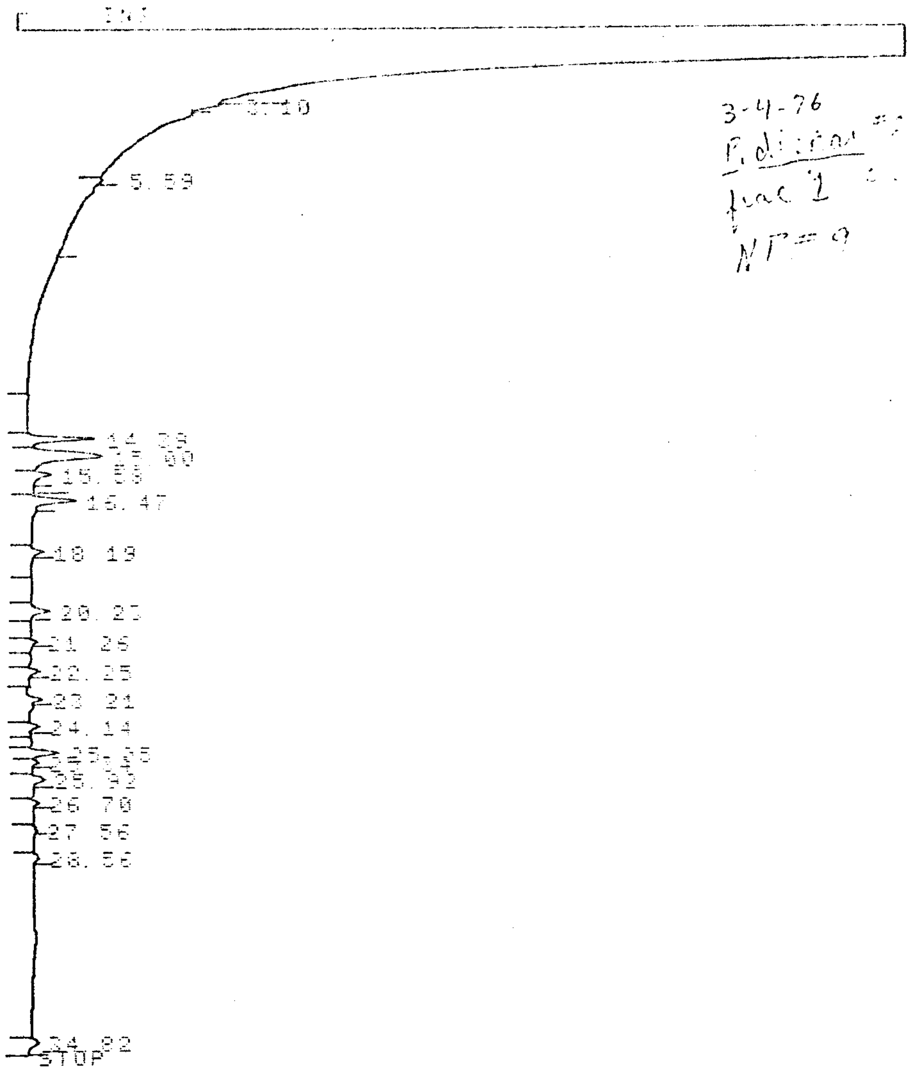


AREA %

	RT	TYPE	AREA	
—	14.68		420	.296
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
—	15.80	M	1670	1.169
—	16.05	M	21675	15.17
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~ 4
—	17.07	T	260	.184 1
—	17.38		2072	1.45
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
—	18.44	M	560	.397 5
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
—	20.16		550	.387
—	21.90		115	.080 48
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
—	25.35	M	181	.126 7
—	25.96		101377	70.94
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
—	27.15	M	3664	2.564
—	27.85	M	425	.297 4
—	29.31		580	.408
S	~~34.82~~		~~[illegible]~~	~~[illegible]~~
			133565	

Blank 1-12-76

HP 31809
DLY 2   STOP 45   REJECT OFF

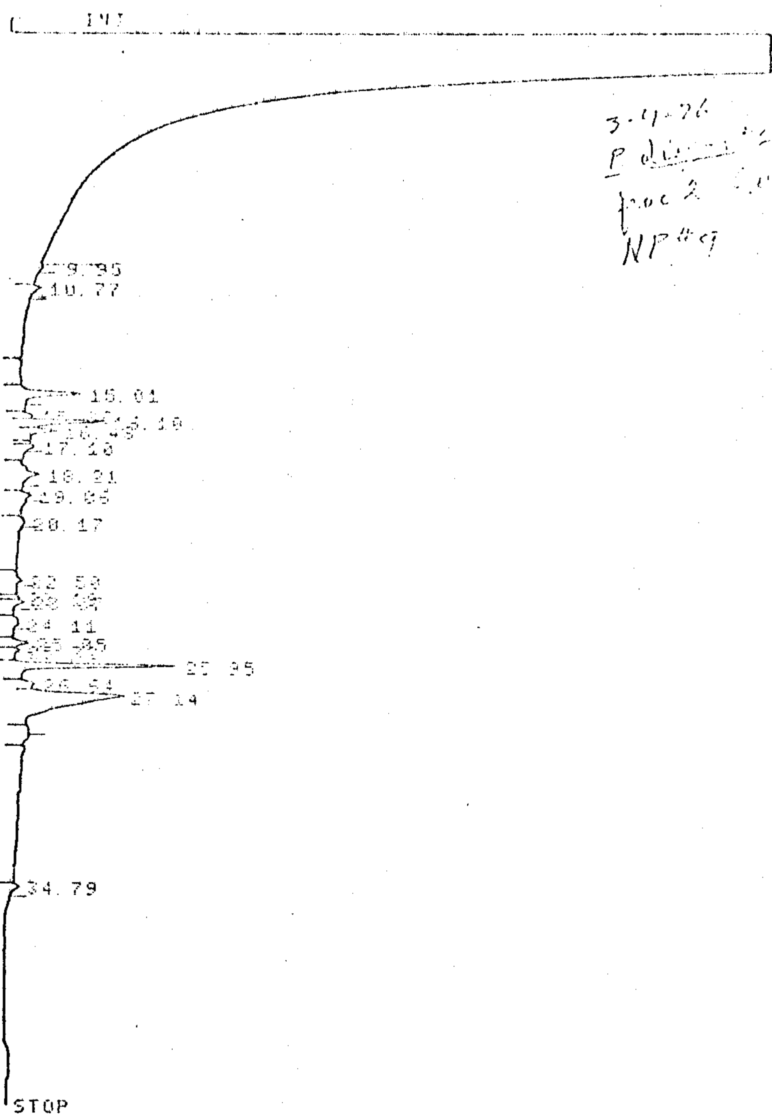


AREA %

	RT	TYPE	AREA		
	~~3.10~~	~~T~~	~~[illegible]~~	~~.947~~	~~1~~
	~~5.59~~	~~T~~	~~[illegible]~~	~~[illegible]~~	~~7~~
	14.39		2895	14.51	
S	~~15.00~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	
	15.58	M	1081	5.417	
S	~~16.47~~		~~[illegible]~~	~~[illegible]~~	
S	~~[illegible]~~		~~551~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	25 34	M	362	1.314	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	27 56		188	.942	1
	28.56		379	1.899	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
			4905		

Blank 1-12 76

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

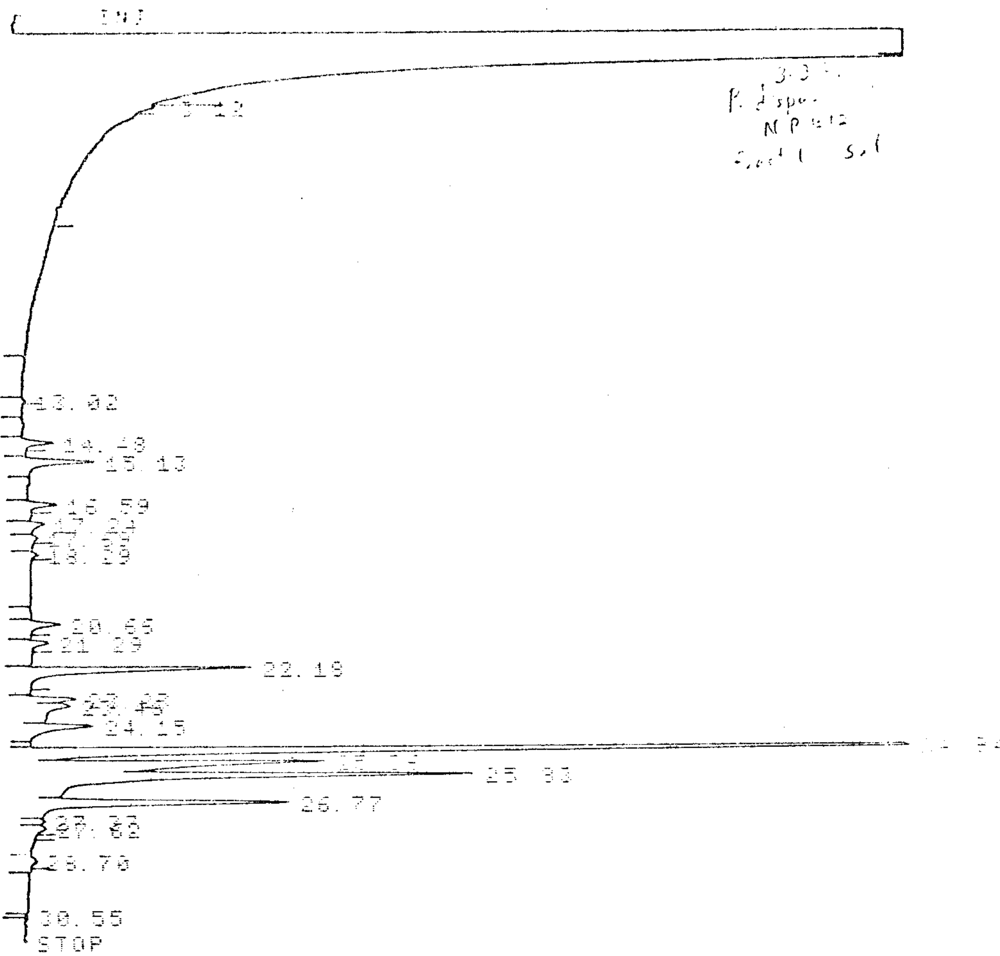


AREA %

RT	TYPE	AREA	
~~9.95~~	~~T~~	~~[illegible]~~	~~[illegible]~~
10.77	T	599	1.326
S ~~15.01~~		~~[illegible]~~	~~[illegible]~~
15.86		479	1.06
16.10	M	4802	10.63
S ~~[illegible]~~	~~T~~	~~[illegible]~~	~~[illegible]~~
F ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
S ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
20.17		414	.916 3
22.50		641	1.419
23.19		206	.455 9
B ~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
C ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
S ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
25.32	M	250	.553 3
25.95		8283	19.33
26.64	M	1436	3.178
27.14	M	17859	39.53
S ~~[illegible]~~		~~[illegible]~~	~~1.177~~
		34969	

Blank 1-12-76

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



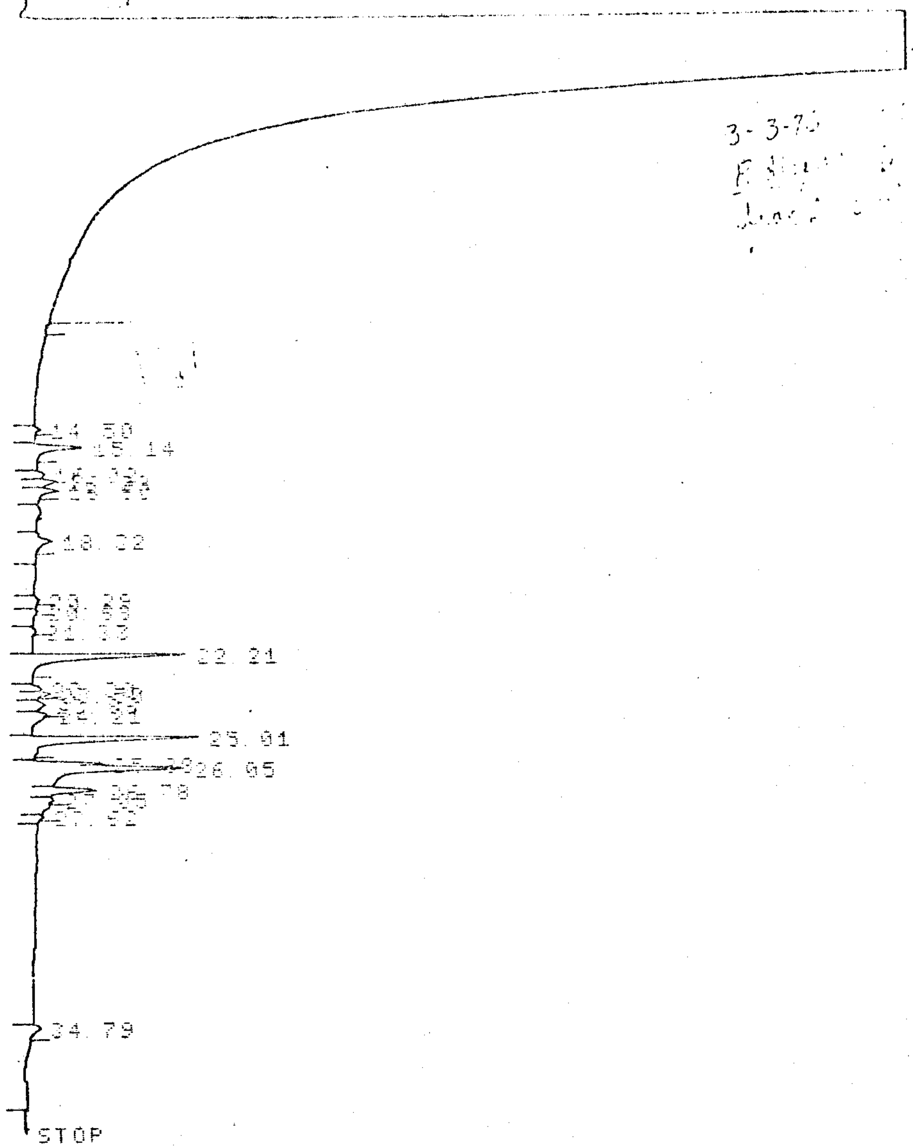
AREA %

	RT	TYPE	AREA	AREA %
cont.	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
	13.02		120	.096 65
	14.48		1315	.949 5
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	17.24		727	.524 9
	17.69	M	302	.218 1
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	20.66		1483	1.071
	21.29		845	.610 2
	22.18		9959	7.191
	23.23		2094	1.512
	23.46	M	4285	3.094
	24.15	M	3710	2.679
	24.94	M	54672	39.48
	25.36	T	15895	11.49
	25.83	TM	24668	17.81
	26.77	TM	12234	8.834
	27.37	TM	183	.132 1
	27.62	TM	424	.306 2
	28.70		437	.315 5
als	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
			133353	

Blank 2 [illegible] 76

HP 3390A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

276



AREA %

RT	TYPE	AREA	
14.50		302	.797 2
~~septum [illegible]~~		~~[illegible]~~	~~[illegible]~~
16.02		403	1.064
~~septum [illegible]~~		~~[illegible]~~	~~[illegible]~~
~~cont. [illegible]~~		~~[illegible]~~	~~[illegible]~~
~~septum [illegible]~~		~~[illegible]~~	~~[illegible]~~
20.29		309	.815 7
20.68		118	.311 5
21.33		198	.522 7
22.21		6998	18.47
23.30		328	.865 8
23.50	M	566	1.494
23.86		384	1.014
24.21	M	230	.607 1
25 01		7846	20.71
25 88		1716	4.53
26 05	M	9351	24.68
26.78	M	2672	7.053
27.05	M	322	.85
27.62		142	.374 9
34.79		522	1 378
		32407	

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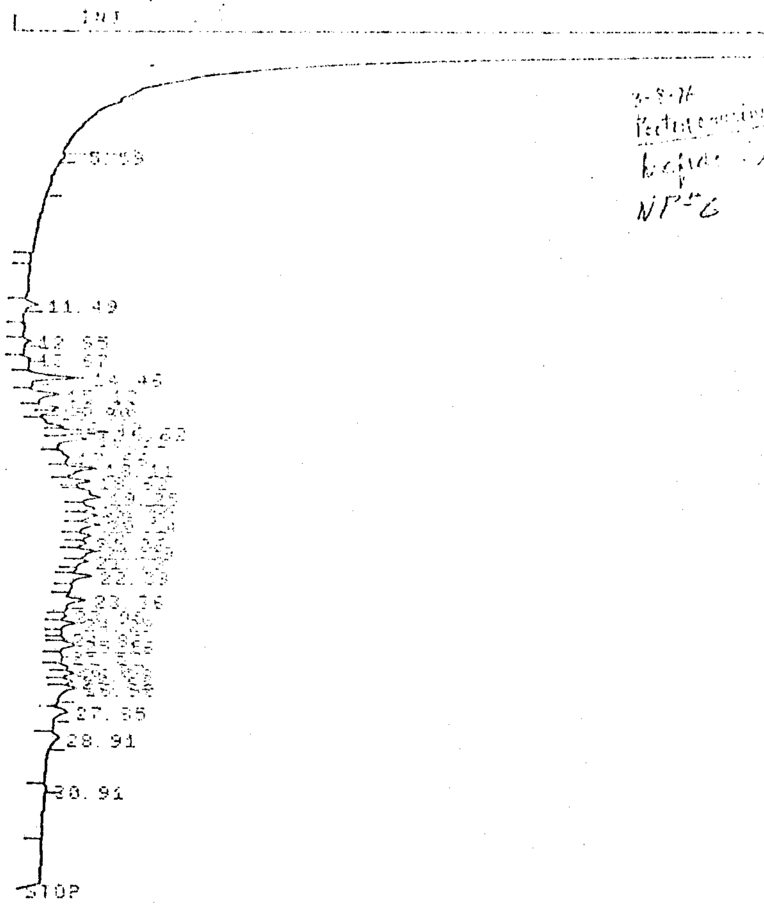
HP 3380A
DLY 1 STOP 45 REJECT OFF
MV/M 10 ATTN 4



NP-6

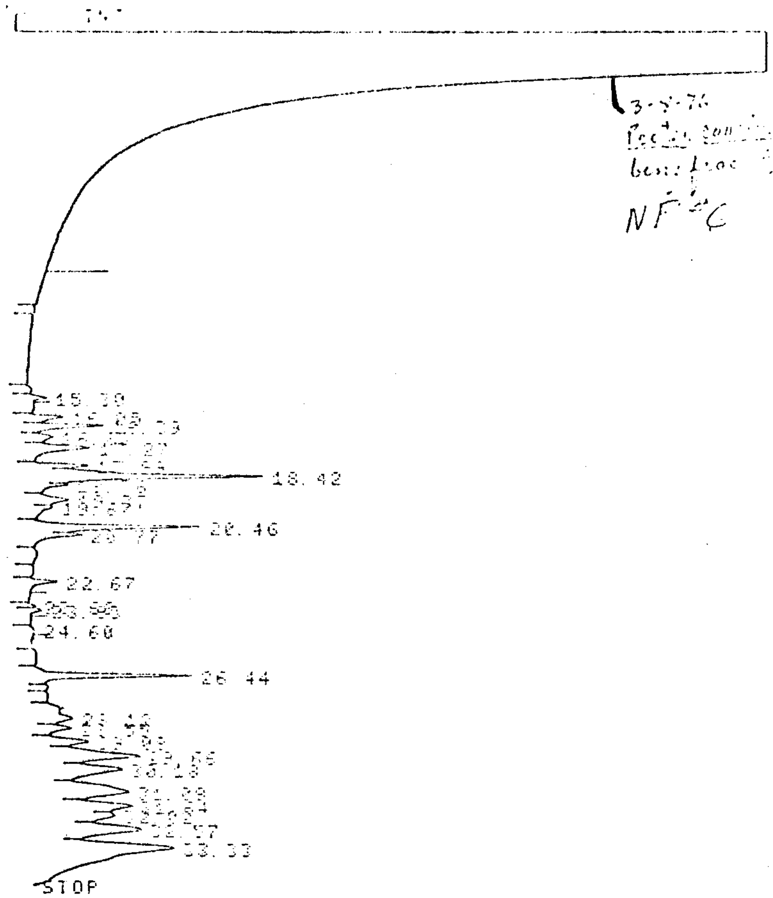
AREA %

RT	TYPE	AREA	
~~5.5[illegible]~~		~~[illegible]~~	~~[illegible]~~
11.49		889	2.401
12.95		418	1.129
13.67		146	.394 4
14.46		3189	8.614
S ~~15.1[illegible]~~		~~[illegible]~~	~~[illegible]~~
15.66	M	1062	2.869
15.80	M	679	1.834
S ~~1[illegible]~~		~~[illegible]~~	~~[illegible]~~
16.62	M	3184	8.601
16.91	M	2158	5.829
17.66	M	643	1.737
18.11		1837	4.962
18.62	M	641	1.731
19.25		1788	4.83
19.70	M	1145	3.093
19.97	M	1243	3.358
20.34	M	1336	3.609
20.77	M	781	2.11
21.06	M	560	1.513
21.39	M	1306	3.528
21.79	M	556	1.502
22.39		972	2.626
23.36		938	2.534
23.95		165	.445 7
24.29	M	643	1.737
24.88		118	.318 7
25.16	M	1012	2.734
25.57	M	95	.256 6
26.07		530	1.432
26.29	M	969	2.618
26.62	M	960	2.593
26.90	M	1486	4.014
27.85		1152	3.112
28.91		1066	2.88
30.91		292	.790 7
		33861	

Blank 1-12-76

HP 3380A

278



AREA %

	RT	TYPE	AREA	AREA %	
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
—	16.05		1741	.894	1
—	16.39	M	3645	1.872	
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
—	17.27	M	4243	2.179	
—	17.99	M	2871	1.474	
—	18.42	M	18488	9.494	
	18.76	M	2242	1.151	
	19.37	M	3217	1.652	
	19.67	M	1581	.811	3
	20.46	M	10518	5.401	
	20.77	M	3157	1.621	
	22.67		1545	.793	4
	23.66		293	.150	5
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	28.12		4410	2.265	
	28.55	M	2321	1.192	
	29.08	M	4014	2.061	
	29.66	M	13140	6.748	
	30.18	M	12194	6.262	
	31.08	M	16465	8.456	
	31.64	M	12177	6.253	
	32.02	M	9925	5.097	
	32.57	M	16631	8.541	
	33.33	IM	39150	20.11	
			183968		

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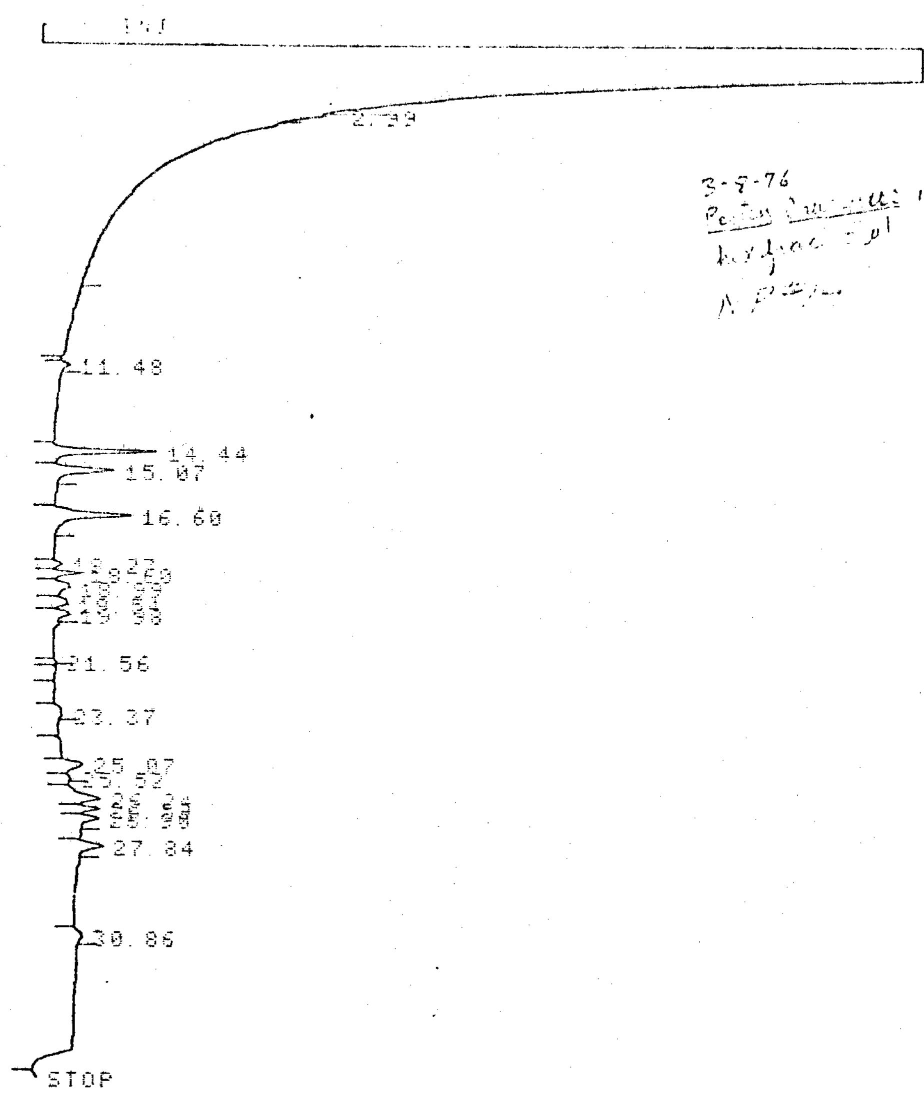
HP 3380A
DLY 2. STOP 60 REJECT OFF
MV/M .10 ATTN 4

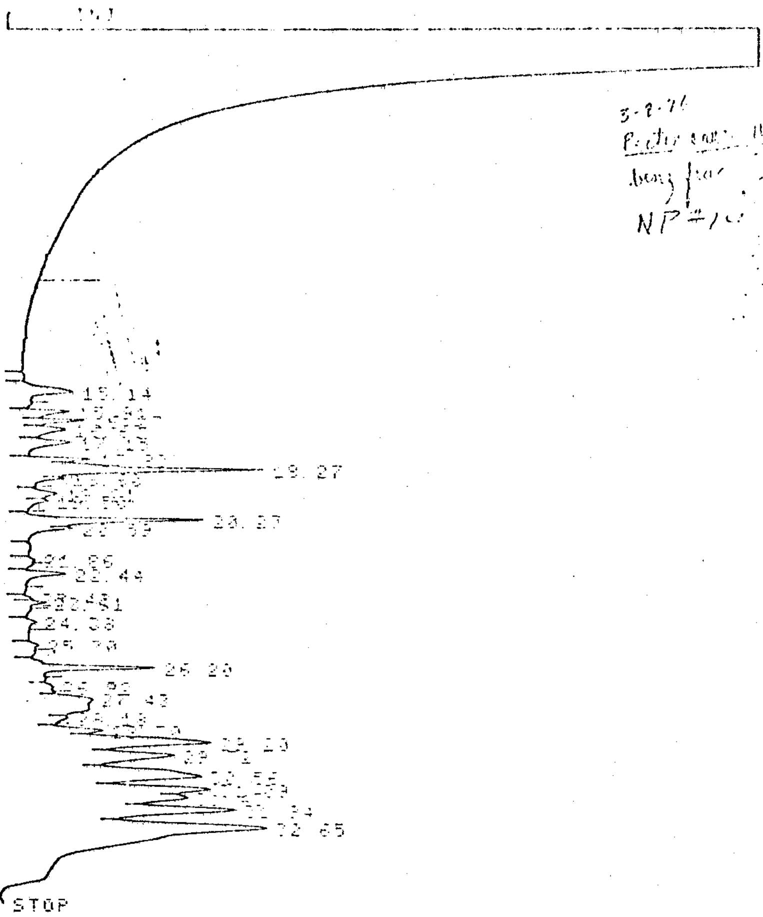


AREA %

	RT	TYPE	AREA		
	~~2.99~~	T	268	.966	4
–	11.48		452	1.63	
–	14.44		4603	16.6	
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
S	~~18.27~~		~~[illegible]~~	~~1.[illegible]~~	
	18.60	M	1032	3.721	
	18.99	M	1431	5.16	
	19.54	M	962	3.469	
	19.98	M	811	2.924	
	~~21.[illegible]~~		~~[illegible]~~	~~[illegible]~~	
C	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
F	~~25.[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	25.52	M	193	.695	9
	26.24		2155	7.771	
	26.59	M	1222	4.406	
	26.90	M	1284	4.63	
	27.84		1545	5.571	
	30.86		663	2.391	
			16353		

Blank 1-12-76

HP 3380A
DLY 2. STOP 60 REJECT OFF
MV/M .10 ATTN 4

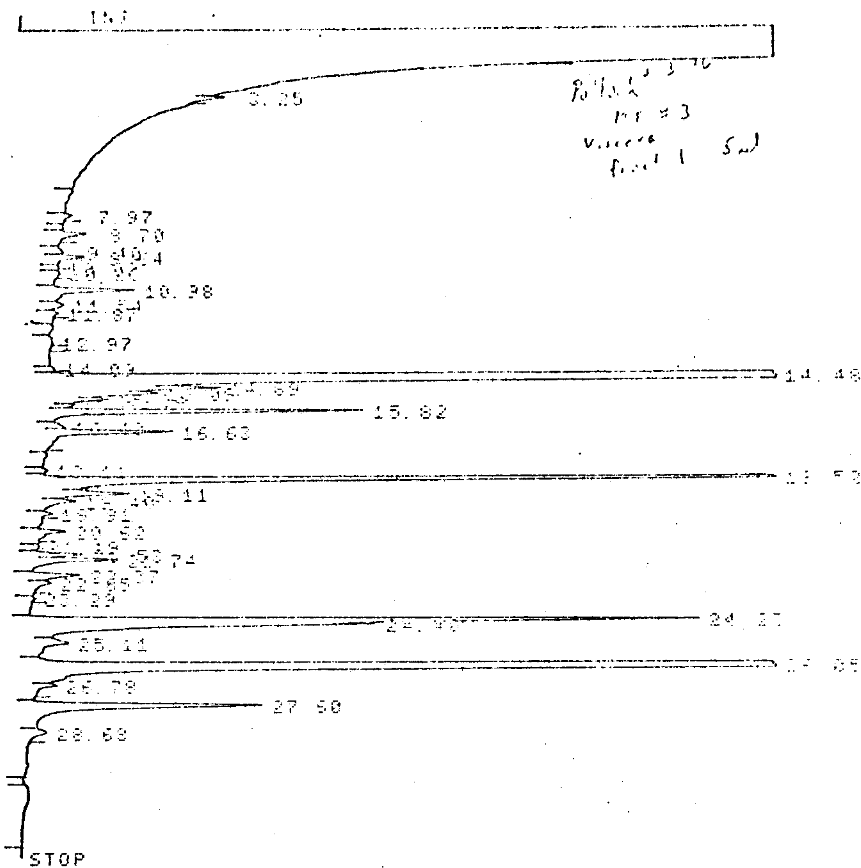


AREA %

Blank

	RT	TYPE	AREA	
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
–	15.91		2658	.890 6
–	16.27	M	3103	1.036
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
–	17.15	M	3694	1.233
–	17.83	M	3183	1.063
–	18.27	M	18840	6.289
	18.60	M	1596	.532 8
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	19.50	M	1188	.396 6
	20.27		11324	3.78
	20.59	M	2890	.964 7
	21.86		161	.053 74
	22.44		2597	.866 9
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	27.43		8308	2.773
	28.18	T	252	.084 12
	28.70		3493	1.166
	29.20	M	22055	7.365
	29.72	M	18922	6.316
	30.56	M	28202	9.414
	31.09	M	20241	6.757
	31.43	M	18392	6.139
	31.94	M	29768	9.937
	32.65	M	[illegible]	26.84
			281307	

HP 3380A
DLY 2. STOP 60 REJECT OFF
MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA	
cont.	[illegible]	[illegible]	[illegible]	[illegible]
	7.97	T	422	.039 17
	8.70	T	1352	.125 5
	9.40	T	378	.035 09
	9.64	TM	1320	122 5
a.l.s	[illegible]	[illegible]	[illegible]	[illegible]
	10.26	T	238	.022 09
	10.98	T	4594	.426 4
	11.54	TM	805	.074 72
	11.87	TM	365	.033 88
	12.97	T	344	.031 93
	14.09		107	.009 932
	14.48		✓654452	60.75
	14.89	M	9207	.854 6
a-pin	[illegible]	[illegible]	[illegible]	[illegible]
	15.34	M	3239	.300 6
	15.56	M	1212	.112 5
	15.82	M	14923	1 385
	16.40	M	1250	.116
	16.63	M	7179	.666 4
	18.11		.242	.022 46
	18.52	M	56999	5.291
	19.11	M	6134	.569 4
	19.40	M	2427	.225 3
	19.91	M	542	.050 31
	20.62		1612	.149 6
a.l.s	[illegible]	[illegible]	[illegible]	[illegible]
	21.53		2109	.195 8
	21.74	M	4791	.444 7
	22.37	M	2816	.261 4
	22.55	M	1323	.122 8
	23.29		204	.018 94
	24.23		37511	3.482
	24.40	M	20923	1.942
	25.11	M	4632	.429 9
	26.05	M	205066	19.03
	26.78	M	1635	.151 8
	27.60		18215	1.691
	28.68	T	1485	.137 8
			1069378	

HP 3380A

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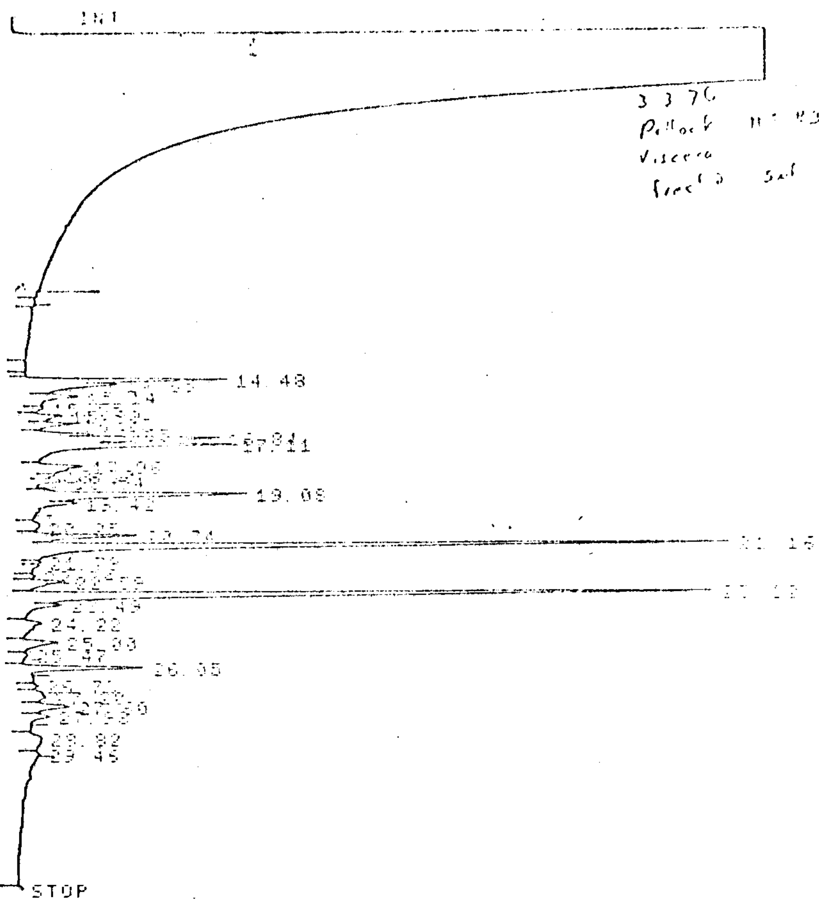
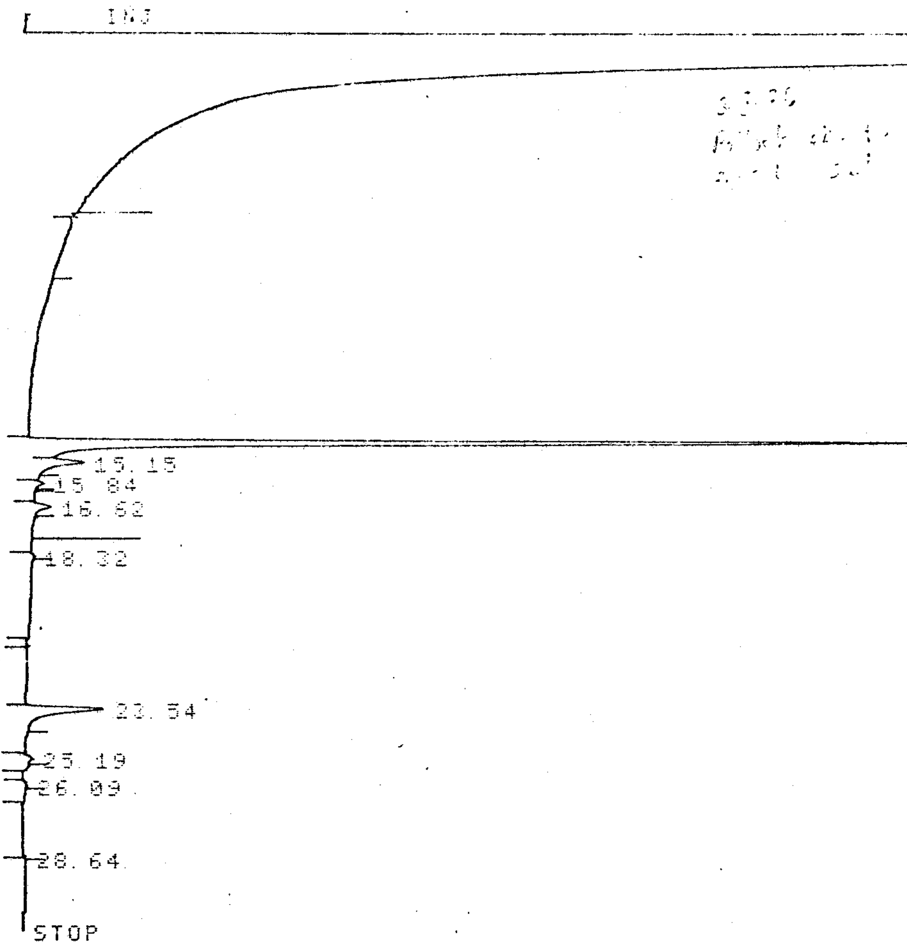


AREA %

RT	TYPE	AREA	
14.48		17943	10.59
14.65	T	805	.474 9
septum ~~[illegible]~~			
15.55	TM	289	.170 5
15.89	TM	1510	.890 8
16.23	TM	2053	1.211
septum ~~[illegible]~~			
16.84	M	10747	6.34
17.11	M	13058	7.704
17.96	M	3744	2.209
septum ~~[illegible]~~			
18.54	M	1438	.848
19.08		12733	7.512
19.42	M	2582	1.523
20.25		624	.366 1
20.74	M	5439	3.209
21.16	M	35578	20.99
a.t.i. ~~[illegible]~~			
22.34		167	.098 52
22.59	M	2209	1.303
23.12	M	34162	20.15
23.49	M	2120	1.251
24.22	T	1692	.998 2
25.00	M	2453	1.447
25.47	M	334	.197
26.05		5566	3.284
26.73		178	.105
27.20	M	1448	.854 2
27.60	M	2667	1.573
27.98	M	1264	.745 7
28.82		1341	.791 1
29.46	M	[illegible]	.155 2
		169407	

Blank [illegible]

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA	
	14.48		43745	83.17
septum	~~[illegible]~~	~~T~~	~~[illegible]~~	~~[illegible]~~
	15.84	T	315	.598 9
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
cont.	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
a.t.s.	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
			44060	

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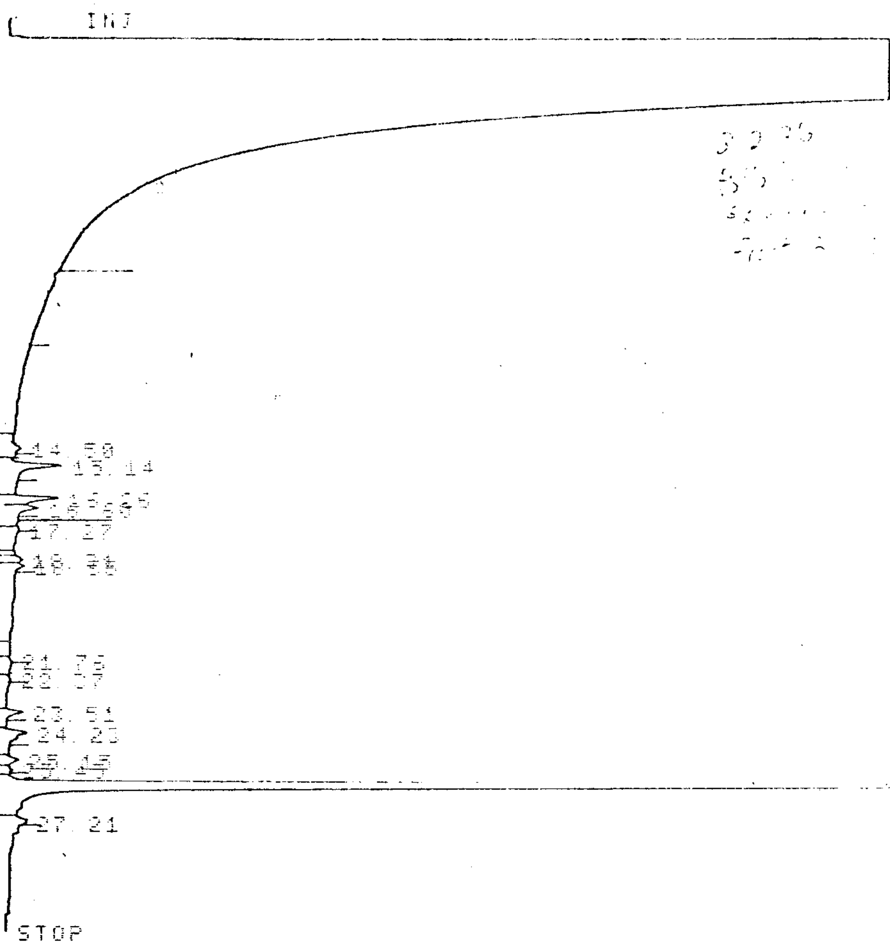
HP 3390A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

284



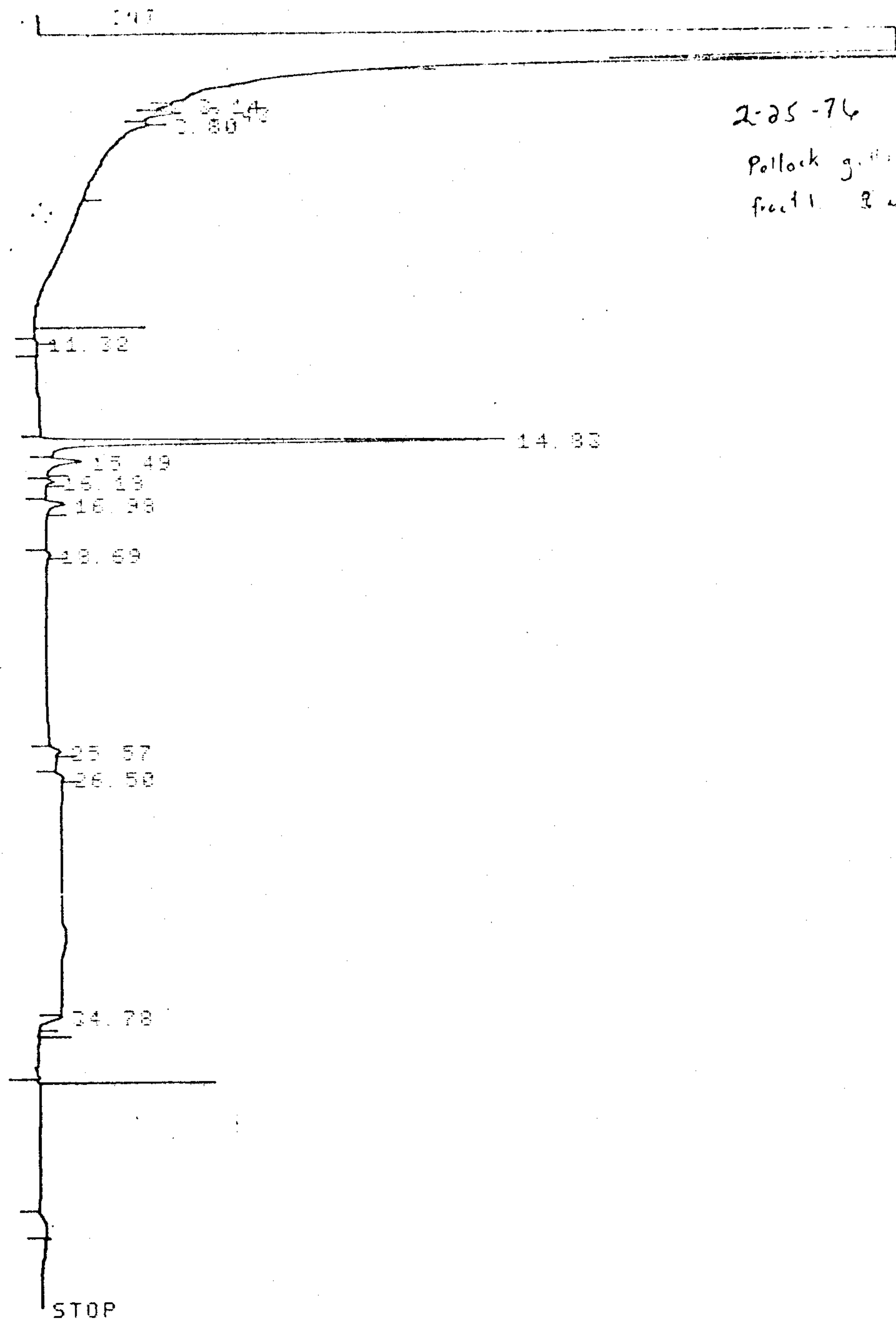
AREA %

	RT	TYPE	AREA	
	14.50		332	.280 9
septum	~~15.14~~		~~[illegible]~~	~~1.[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~1.[illegible]~~
cont.	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~ 1
a.t.s	~~17.27~~		~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	18.55	M	406	.343 5
a.t.s	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	22.37		143	.121
	23.51		667	.564 2
	24.23		1319	1 116
cont.	~~25.15~~		~~[illegible]~~	~~[illegible]~~
	25.45	M	164	.138 7
	26 05		108308	91.62
	27.21	M	544	.460 2
			111883	

Black

HP 3390A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

285



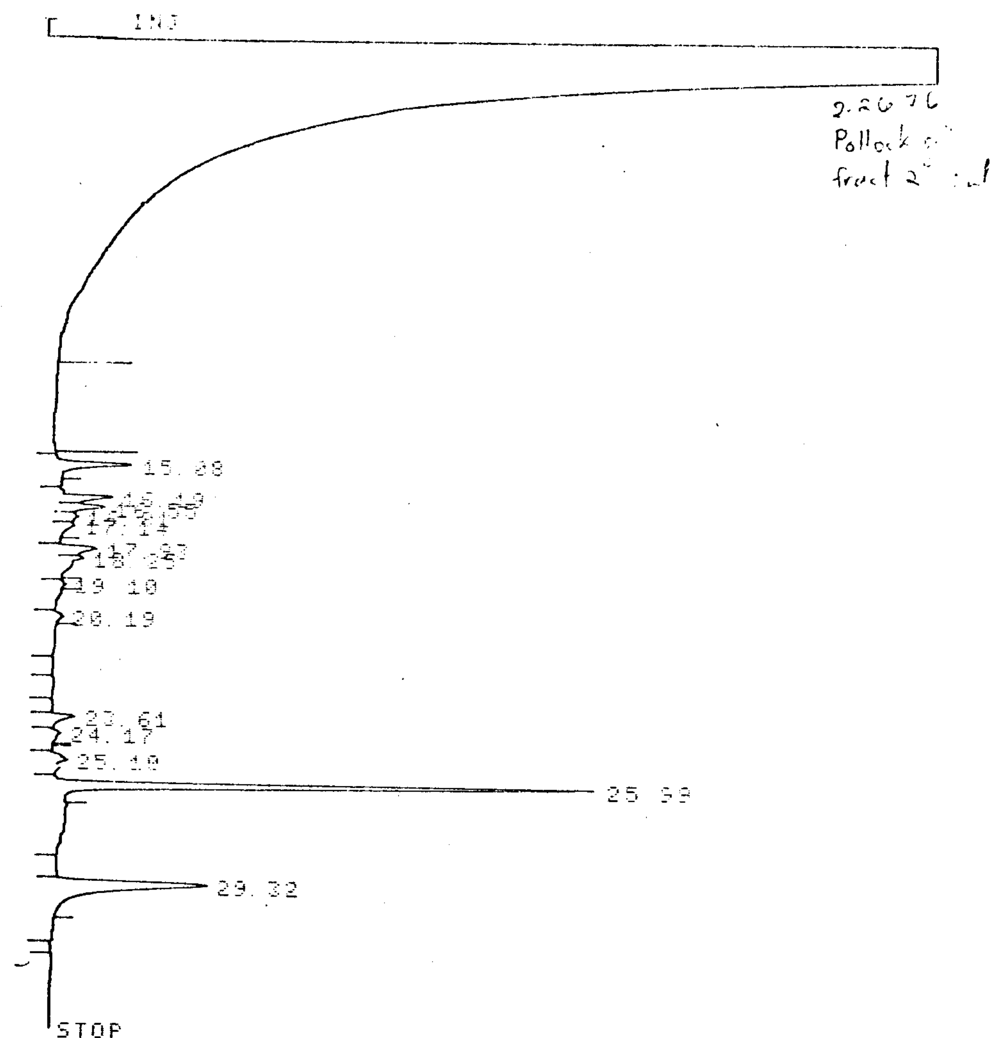
AREA %

	RT	TYPE	AREA	
cont.	[illegible]		[illegible]	[illegible]
cont.	[illegible]		[illegible]	[illegible]
cont.	[illegible]		[illegible]	[illegible]
a.i.s.	[illegible]		[illegible]	[illegible]
	14.82		19950	76.79
septum	[illegible]		[illegible]	[illegible]
	16.13		254	1.028
septum	[illegible]		[illegible]	[illegible]
septum	[illegible]		[illegible]	[illegible]
septum	[illegible]		[illegible]	[illegible]
septum	[illegible]		[illegible]	[illegible]
i.s.	[illegible]		[illegible]	
			119337	

Blank 2-[illegible]-76

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M 10 ATTN 4

286



AREA %

	RT	TYPE	AREA	
septum	~~15.88~~		~~[illegible]~~	~~[illegible]~~
	16.19		2823	4.911
septum	~~16.55~~		~~[illegible]~~	~~[illegible]~~
	16.81	M	1389	2.416
	17.14	M	1175	2.044
	17.93		2341	4.073
septum	~~18.25~~		~~[illegible]~~	~~[illegible]~~
	19.10		248	.431 4
	20.19		560	.974 2
cont.	~~23.61~~		~~[illegible]~~	~~[illegible]~~
	24.17	T	597	1.039
	25.10		887	.1.543
	25.99		23127	40.23
	29.32		14053	24.45
			49413	

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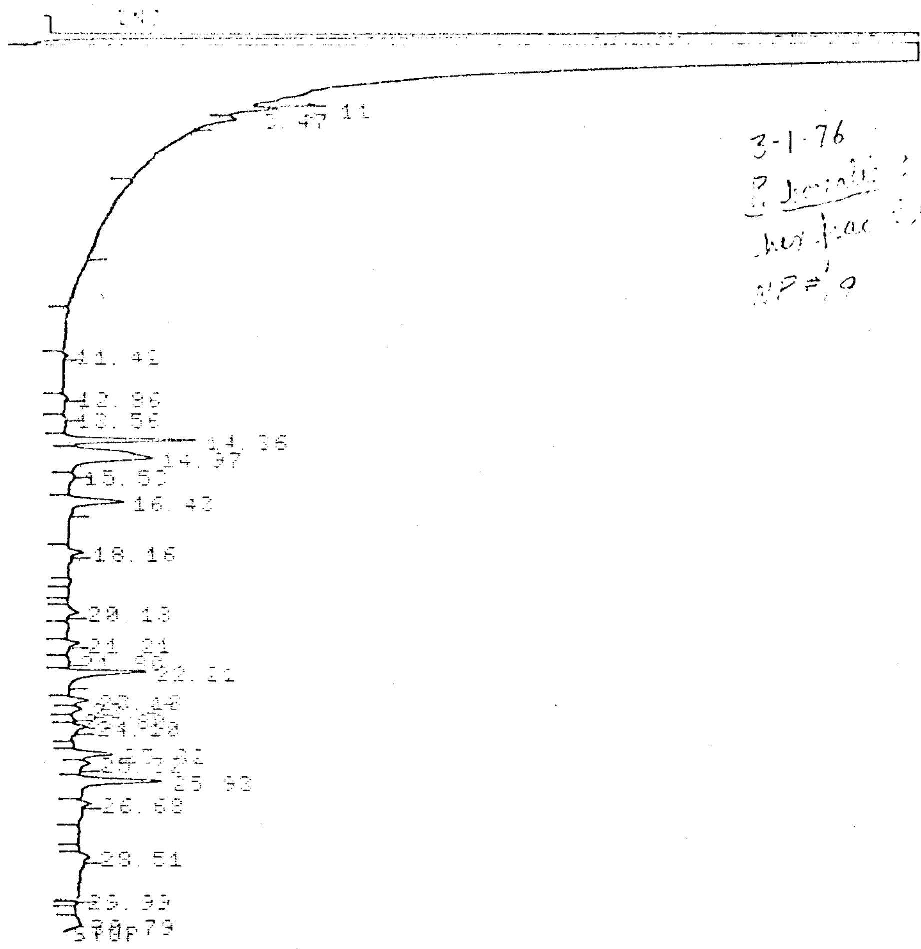
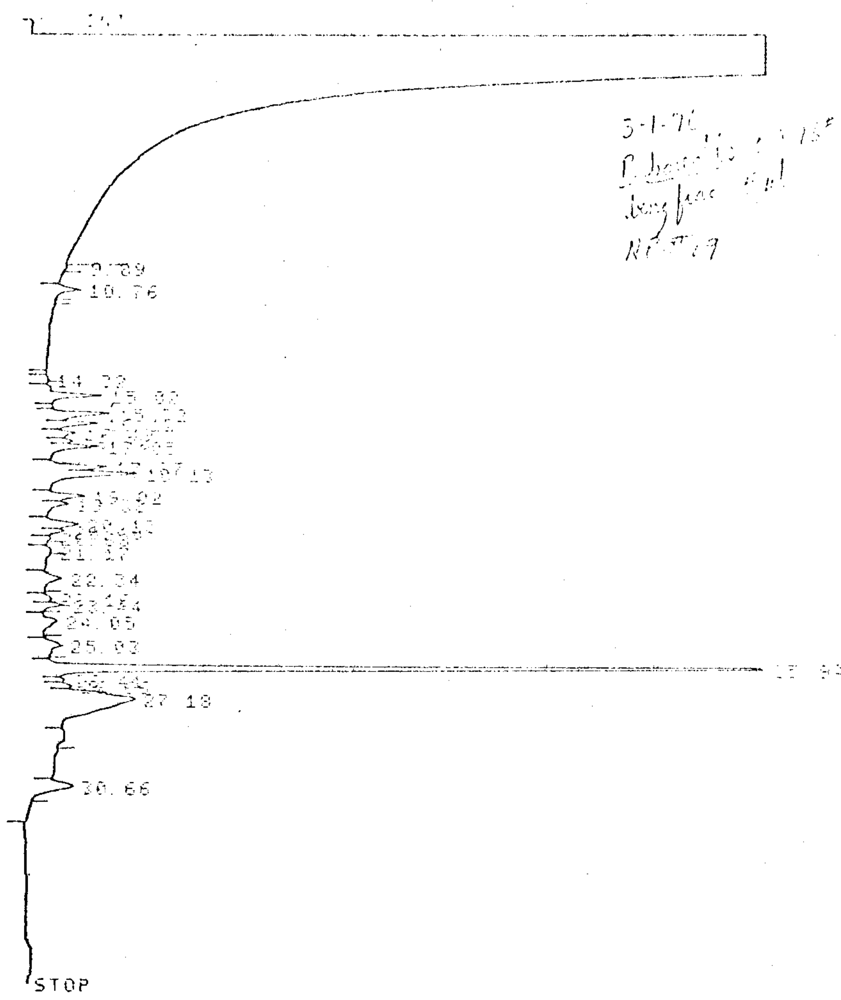
HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

287



Mark	RT	TYPE	AREA	AREA %	Note
	[illegible]		[illegible]	[illegible]	
	[illegible]		[illegible]	[illegible]	
	11.42		237	.662	4
B	[illegible]		[illegible]	[illegible]	
	13.56		116	.324	2
	14.36		5256	14.69	
S	[illegible]		[illegible]	[illegible]	
B	[illegible]		[illegible]	[illegible]	
S	[illegible]		[illegible]	[illegible]	
S	[illegible]		[illegible]	[illegible]	
B	[illegible]		[illegible]	[illegible]	
B	[illegible]		[illegible]	[illegible]	
	21.80		240	.670	7
	22.21		3824	10.69	
B	[illegible]		[illegible]	[illegible]	
	23.47	M	538	1.504	
	23.80	M	113	.315	8
B	[illegible]		[illegible]	[illegible]	
S	[illegible]		[illegible]	[illegible]	
B	[illegible]		[illegible]	[illegible]	
	25.93		4217	11.79	
B	[illegible]		[illegible]	[illegible]	
	28.51		416	1.163	
	[illegible]		[illegible]		
	[illegible]		[illegible]	[illegible]	
			14957		

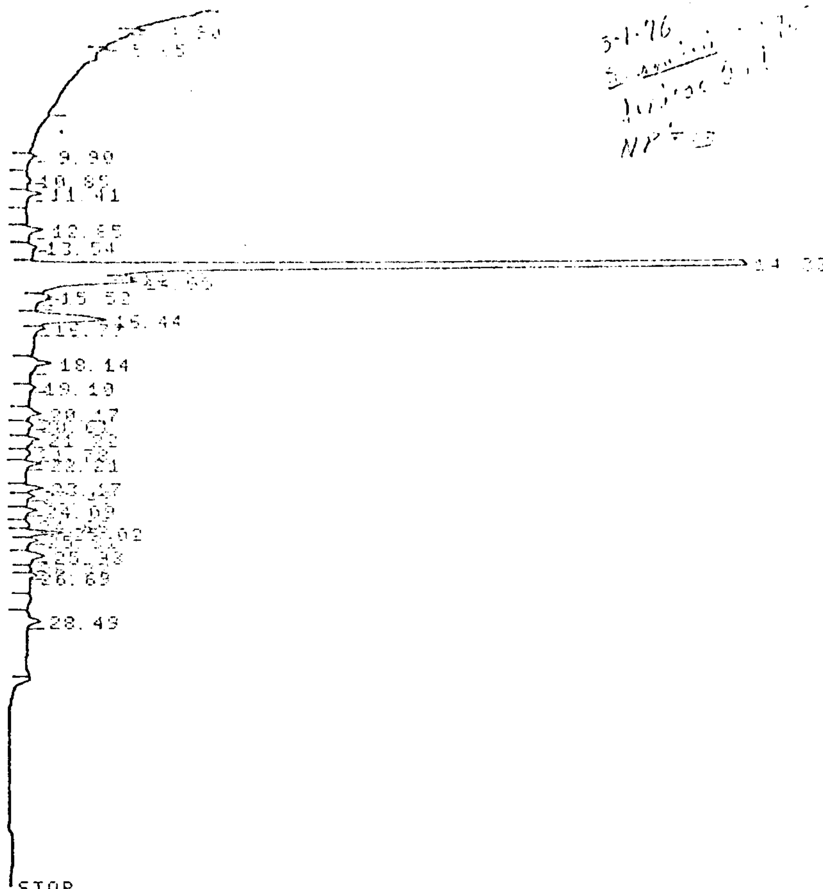
HP 3280A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



RT	TYPE	AREA	AREA %
~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
10.76	T	1484	1.324
14.32		134	.119 5
S ~~15.[illegible]~~		~~[illegible]~~	~~[illegible]~~
15.72		3350	2.989
16.12	M	2365	2.11
S ~~16.[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
16.80	TM	1011	.901 9
17.05	M	2874	2.564
17.87	M	3663	3.268
18.13	M	6750	6.022
19.02	M	2600	2.32
S ~~19.[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
20.13	M	2502	2.232
20.40	M	1043	.930 5
20.68	M	252	.224 8
21.17		311	.277 5
22.34		1783	1.591
23.16		222	.198 1
I ~~23.44~~		~~[illegible]~~	~~[illegible]~~
24.05		1473	1.314
S ~~25.[illegible]~~		~~[illegible]~~	~~[illegible]~~
25.94		44292	39.51
26.41	M	748	.667 3
26.66	M	1345	1.2
27.18	M	22461	20.04
30.66		[illegible]	2.874
		103884	

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

289

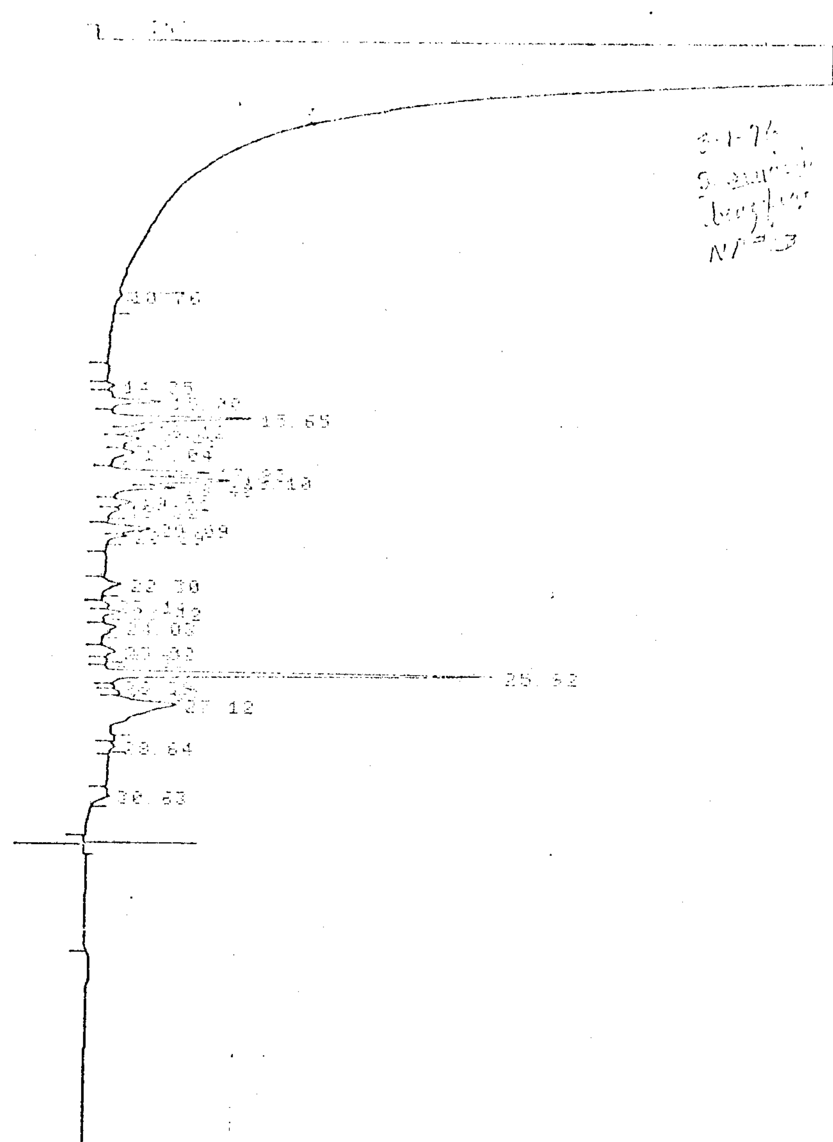


AREA %

RT	TYPE	AREA	
~~2.16~~	~~TA~~	~~[illegible]~~	~~[illegible]~~
~~2.31~~	~~TA~~	~~[illegible]~~	~~[illegible]~~
~~2.42~~	~~T~~	~~[illegible]~~	~~[illegible]~~
~~[illegible]~~	~~TA~~	~~[illegible]~~	~~[illegible]~~
~~[illegible]~~	~~TA~~	~~[illegible]~~	~~[illegible]~~
~~3.25~~	~~TA~~	~~[illegible]~~	~~4.[illegible]~~
~~3.32~~	~~TA~~	~~[illegible]~~	~~[illegible]~~
~~4.90~~	~~T~~	~~[illegible]~~	~~[illegible]~~
~~5.65~~	~~T~~	~~[illegible]~~	~~[illegible]~~
9.90		308	.111 4
10.85		373	.134 9
11.41		784	.283 6
12.85		1049	.379 5
13.54		370	.133 8
14.33		208368	75.37
14.77	M	5381	1.946
s ~~14.[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
15.52	T	876	.316 9
s ~~16.44~~		~~[illegible]~~	~~[illegible]~~
16.77	M	746	.269 9
18.14		1729	.625 4
s ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
20.61		347	.125 5
~~21.[illegible]~~		~~[illegible]~~	~~[illegible]~~
~~21.[illegible]~~		~~[illegible]~~	~~[illegible]~~
22.21	M	811	.293 4
23.17		877	.317 2
s ~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
24 09		553	.2
24 66		310	.112 1
25.02	M	2066	747 [illegible]
s ~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
25 93		1108	400 3
s/s ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
26 69		344	.124 4
28 49		[illegible]	.478 9
		277729	

HP [illegible]80A
OLY 2. STOP 45 REJECT OFF

230

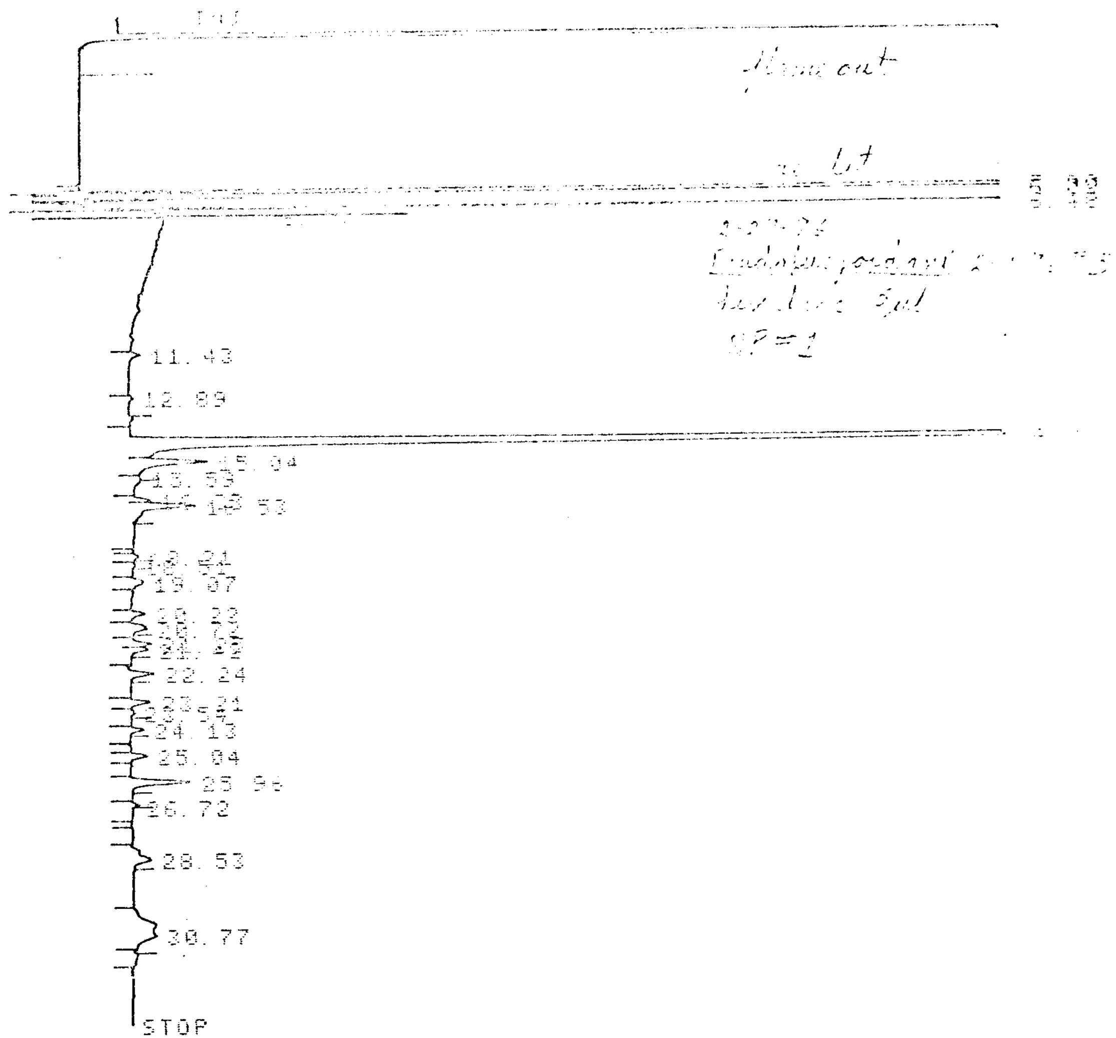


STOP

AREA %

RT	TYPE	AREA	
10.76	T	283	.327 3
14.35		366	.423 2
[illegible]	[illegible]	[illegible]	[illegible]
15.65	M	15496	17.92
15.12	T	847	.979 5
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
17.83		6244	7.221
18.10	M	8529	9.863
18.40	M	4438	5.132
19.01	M	1950	2.255
[illegible]	[illegible]	[illegible]	[illegible]
20.09		3527	4.079
[illegible]	[illegible]	[illegible]	[illegible]
22.30		1656	1.915
23.14		452	.522 7
[illegible]	[illegible]	[illegible]	[illegible]
24.07		1250	1.446
[illegible]	[illegible]	[illegible]	[illegible]
25.31	M	187	.216 2
25.92		18911	21.87
[illegible]	[illegible]	[illegible]	[illegible]
26.67	M	179	.207
27.12		10059	11.63
28.64		253	.292 6
30.63		[illegible]	1.737
		76129	

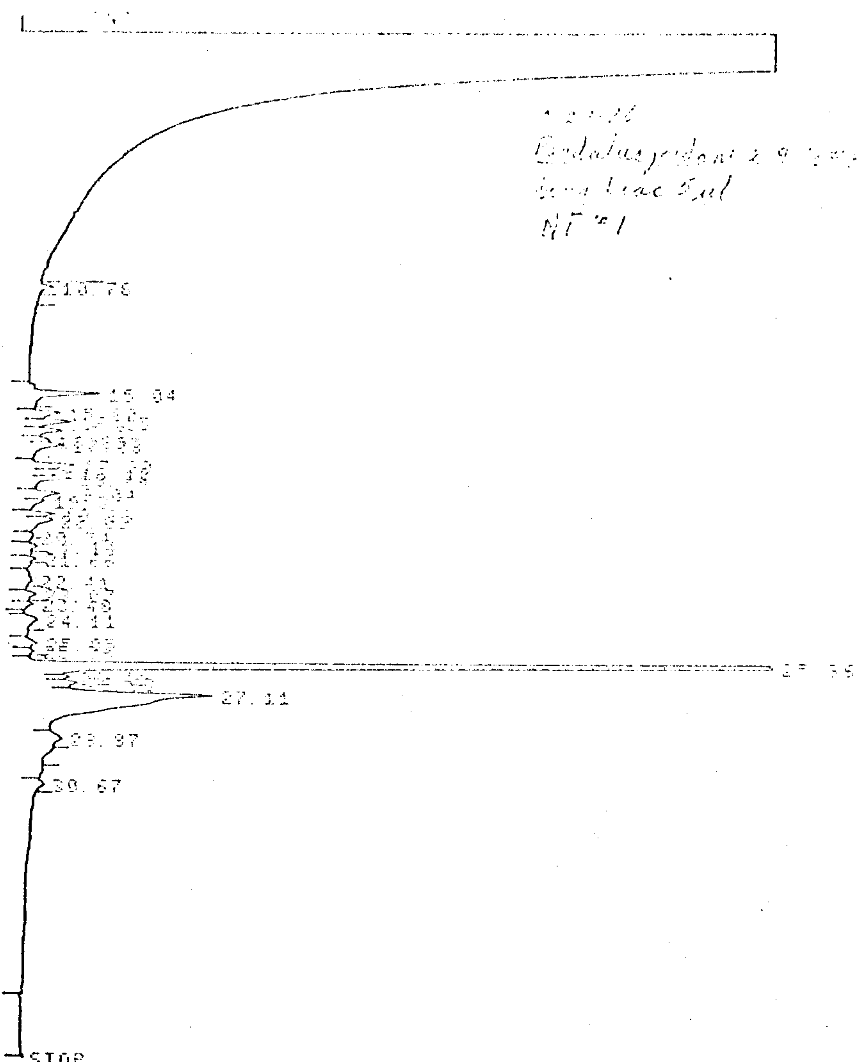
HP 3380A



AREA %

	RT	TYPE	AREA	AREA %
	~~5.90~~		~~[illegible]~~	~~[illegible]~~
	~~6.02~~	~~M~~	~~[illegible]~~	~~[illegible]~~
	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	~~[illegible]~~		~~[illegible]~~	
	~~[illegible]~~	~~T~~	~~[illegible]~~	~~[illegible]~~
	~~[illegible]~~	~~TM~~	~~[illegible]~~	~~[illegible]~~
	~~[illegible]~~	~~TM~~	~~[illegible]~~	~~[illegible]~~
	14.37	PRISTANE	56457	19.39
S	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
	16.33		537	.120 5
S	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	18.51		123	.027 59
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	21.42	M	811	.181 9
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	23.54	M	234	.052 49
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	25.96	small in blank	2719	.608 9
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	28.53		1843	.412 4
	30.77		[illegible]	.938 5
			97131	

HP 3380A
DLY 2   STOP 45   REJECT OFF
MV/M 10   ATTN 4



AREA %

	RT	TYPE	AREA		
	10.78	T	796	.433	1
S	~~15.04~~		~~[illegible]~~	~~[illegible]~~	
	15.80		1703	.926	5
	16.15	M	2156	1.173	
S	~~16.51~~	~~M~~	~~[illegible]~~	~~[illegible]~~	
	16.89	M	999	.543	
S	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~	
	17.90		2654	1.444	
B	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~	
	18.44	M	2450	1.333	
	19.04	M	2107	1.146	
B	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~	
	19.88	M	1132	.615	9
B	~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~	
	20.74	M	228	.124	
	21.14		422	.229	6
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	22.41		734	.399	3
	23.07		478	.260	1
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	24.11		1023	.556	6
B	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~	
	25.36	M	252	.137	1
	25.96		101454	55.2	
	26.41	M	2086	1.135	
	26.67	M	3492	1.9	
	27.11	M	42641	23.2	
	28.87	T	1142	.621	3
	30.67		541	.350	4
			168573		

HP 3380A
DLY 2 STOP 45 REJECT OFF

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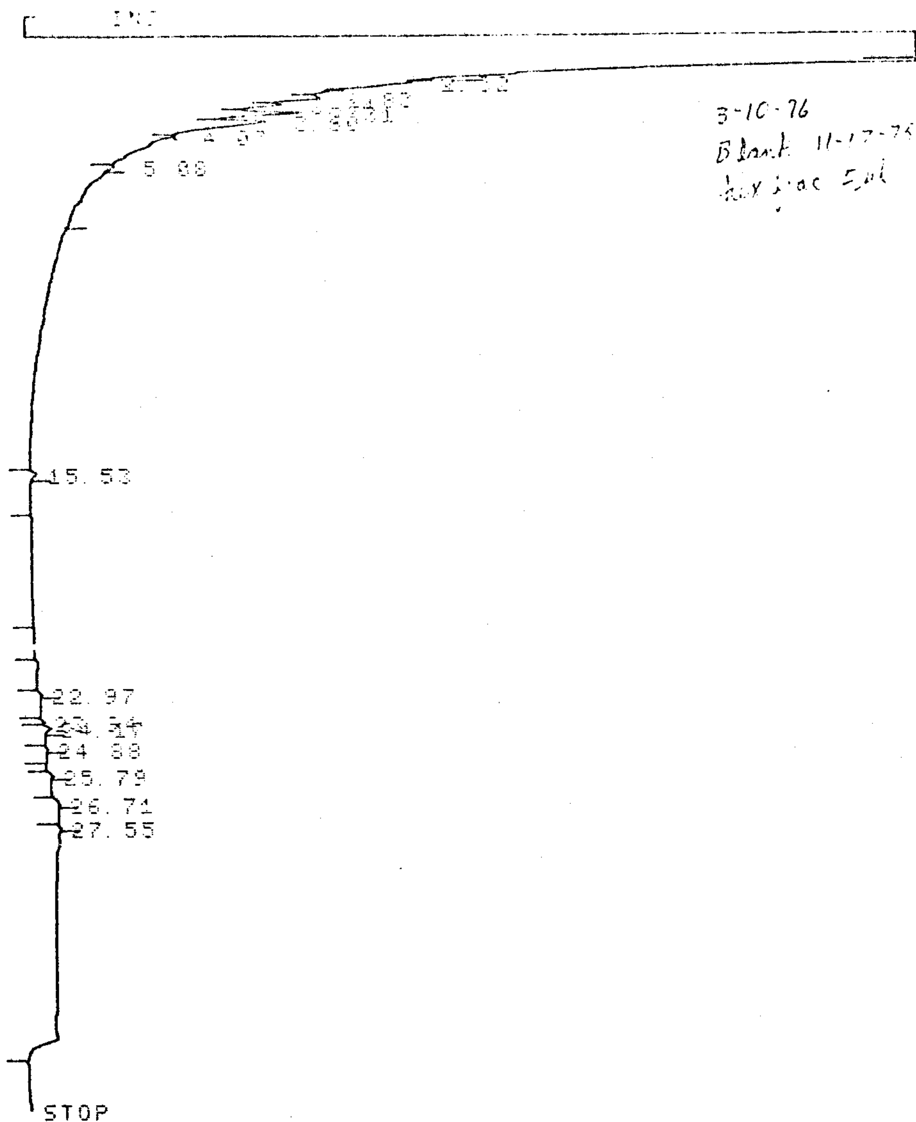
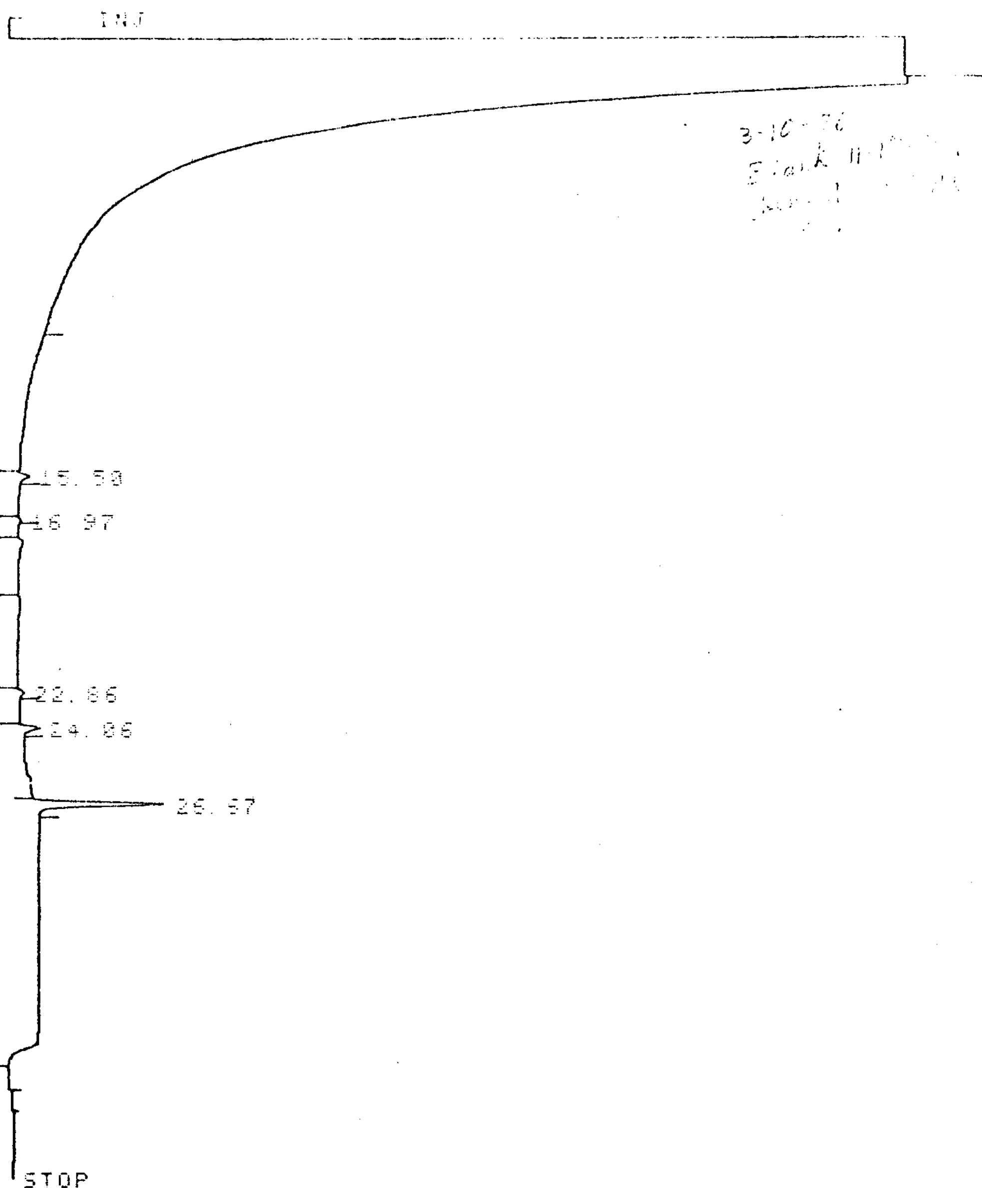


AREA %

RT	TYPE	AREA	
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
~~[illegible]~~		~~1125~~	~~[illegible]~~
~~3.04~~		~~[illegible]~~	~~5.152~~
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
~~3.60~~		~~3787~~	~~34.53~~
~~4.07~~		~~193~~	~~1.759~~
~~5.08~~		~~174~~	~~1.595~~
~~15.53~~		~~[illegible]~~	~~[illegible]~~
22.97		173	1.586
23.34		129	1.183
24.17	M	405	3.713
24.88		145	1.329
25.79		166	1.522
26.71		251	2.301
27.55		113	1.036
		1826	

MeOH

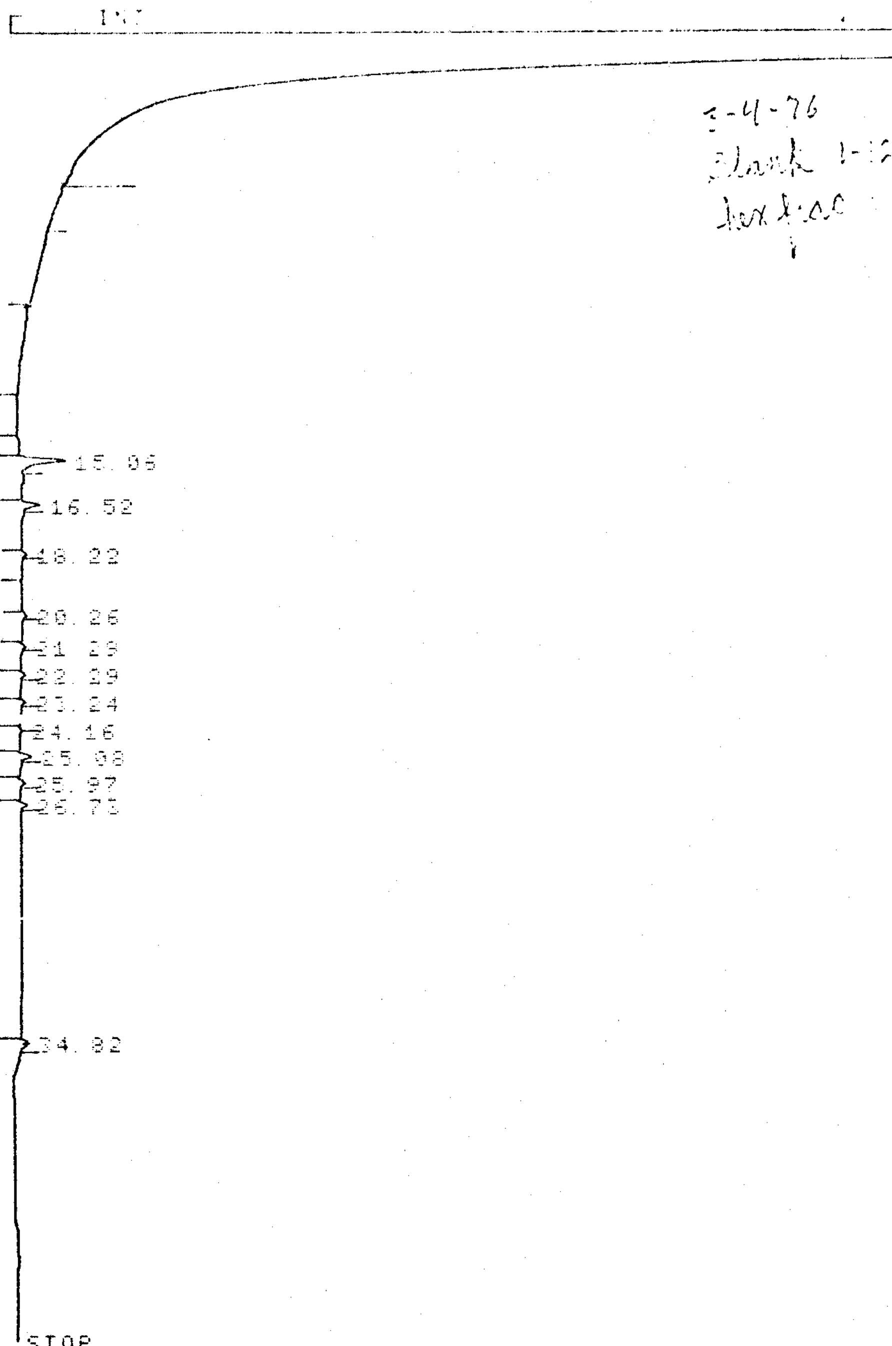
HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



AREA %

	RT	TYPE	AREA	
S	~~15.50~~		~~525~~	~~7.143~~
S	~~16.97~~		~~[illegible]~~	~~1.7[illegible]~~
	22.86		256	3.485
	24.06		801	10.91
	25.67		5631	76.66
			6688	

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M 10 ATTN 4

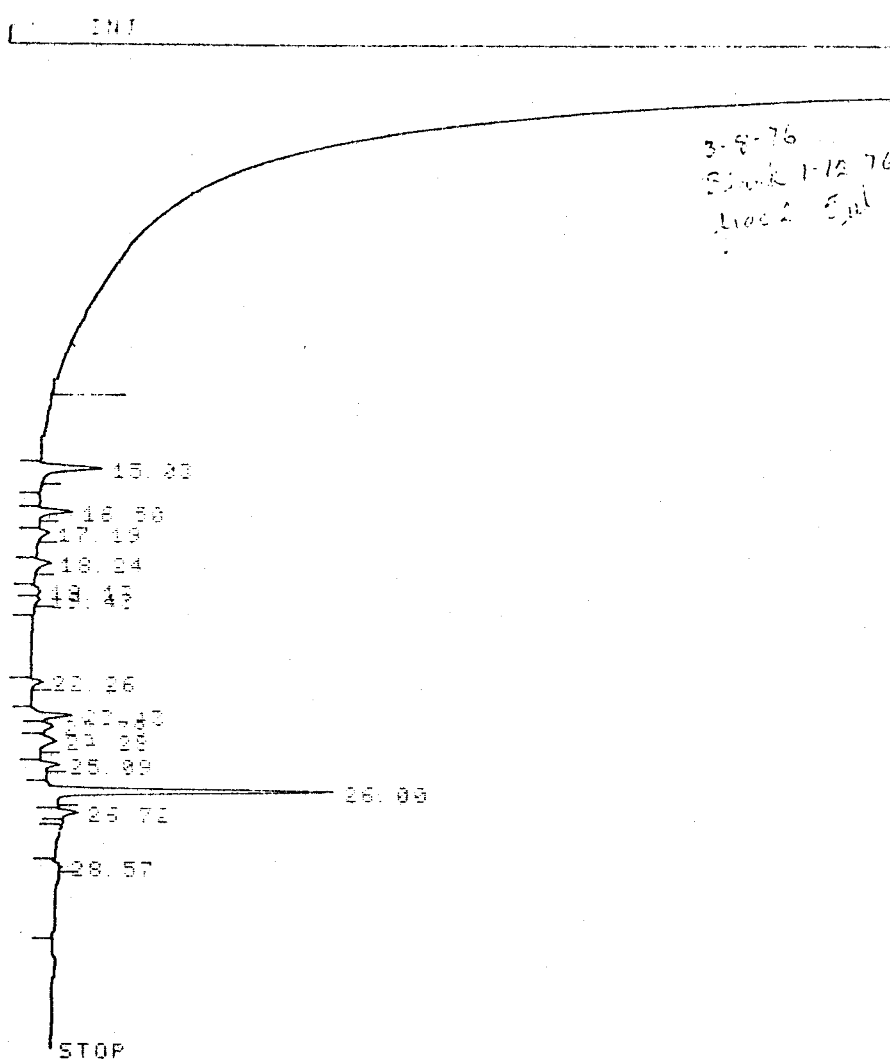


AREA %

	RT	TYPE	AREA	
S	~~15.[illegible]~~		~~[illegible]~~	~~[illegible]~~
S	~~16.[illegible]~~		~~[illegible]~~	~~[illegible]~~
S	~~18.[illegible]~~		~~[illegible]~~	~~[illegible]~~
	20.26		173	2.814
	21.29		196	3.189
	22.29		240	3.904
	23.24		229	3.725
	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	25.08		506	8.232
	25.97		294	4.783
	26.73		318	5.173
	34.82		537	9.712
			2553	

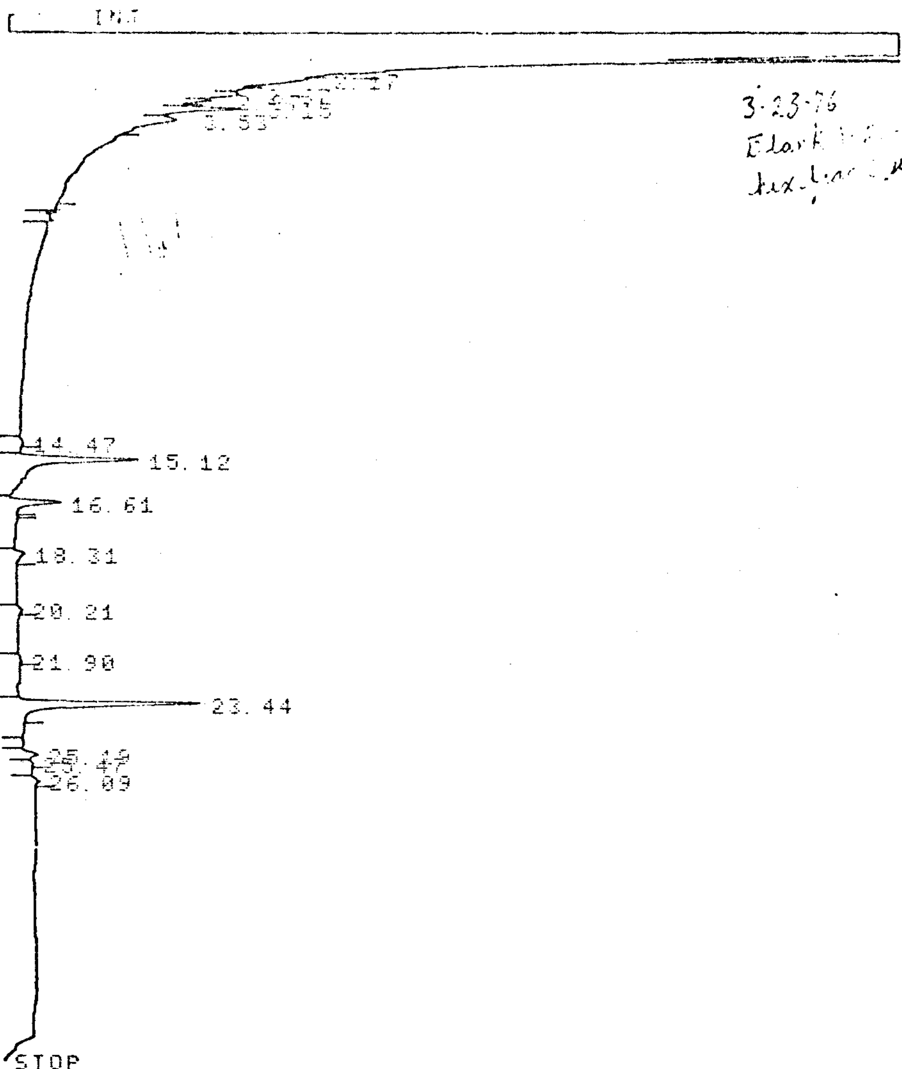
HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

296



RT	TYPE	AREA	AREA %
S [illegible]		[illegible]	[illegible]
S [illegible]		[illegible]	[illegible]
17.19		572	2.124
S [illegible]		[illegible]	[illegible]
19.13		451	1.675
19.43	M	434	1.612
22.26		607	2.254
23.43		1965	7.298
23.78	M	1339	4.97
24.28	M	1477	5.486
25.09		702	2.607
26.00		12377	45.97
26.72		712	2.644
28.57		234	.869 1
		20869	

HP 3380A
DLY 2 STOP 60 REJECT OFF
MV/M .10 ATTN 4

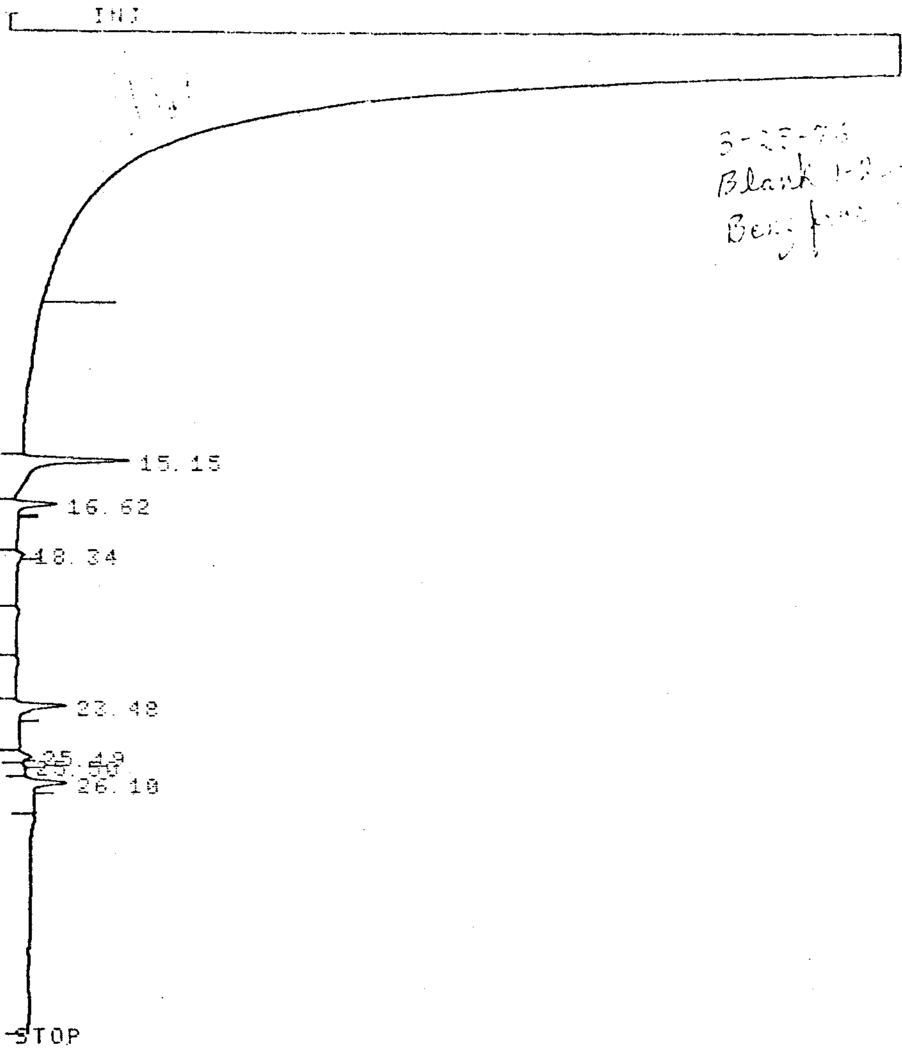


AREA %

	RT	TYPE	AREA		
MeOH	~~2.17~~	~~T~~	~~517~~	~~1.939~~	
	~~2.67~~	~~T~~	~~770~~	~~2.888~~	
	~~2.87~~	~~TM~~	~~264~~	~~.990~~	-1
	~~3.15~~	~~T~~	~~2295~~	~~8.607~~	
	~~3.53~~	~~TM~~	~~1645~~	~~6.17~~	
	14.47		357	1.339	
S	~~15.12~~		~~6971~~	~~26.14~~	
	~~16.61~~	~~T~~	~~2522~~	~~9.459~~	
	~~18.31~~		~~695~~	~~2.607~~	
	20.21		252	.945	1
S	~~21.90~~		~~287~~	~~1.076~~	
	23.44		8919	33.45	
S	~~25.19~~		~~611~~	~~2.292~~	
	25.47	M	230	.862	6
S	~~26.09~~		~~328~~	~~1.23~~	
			9758		

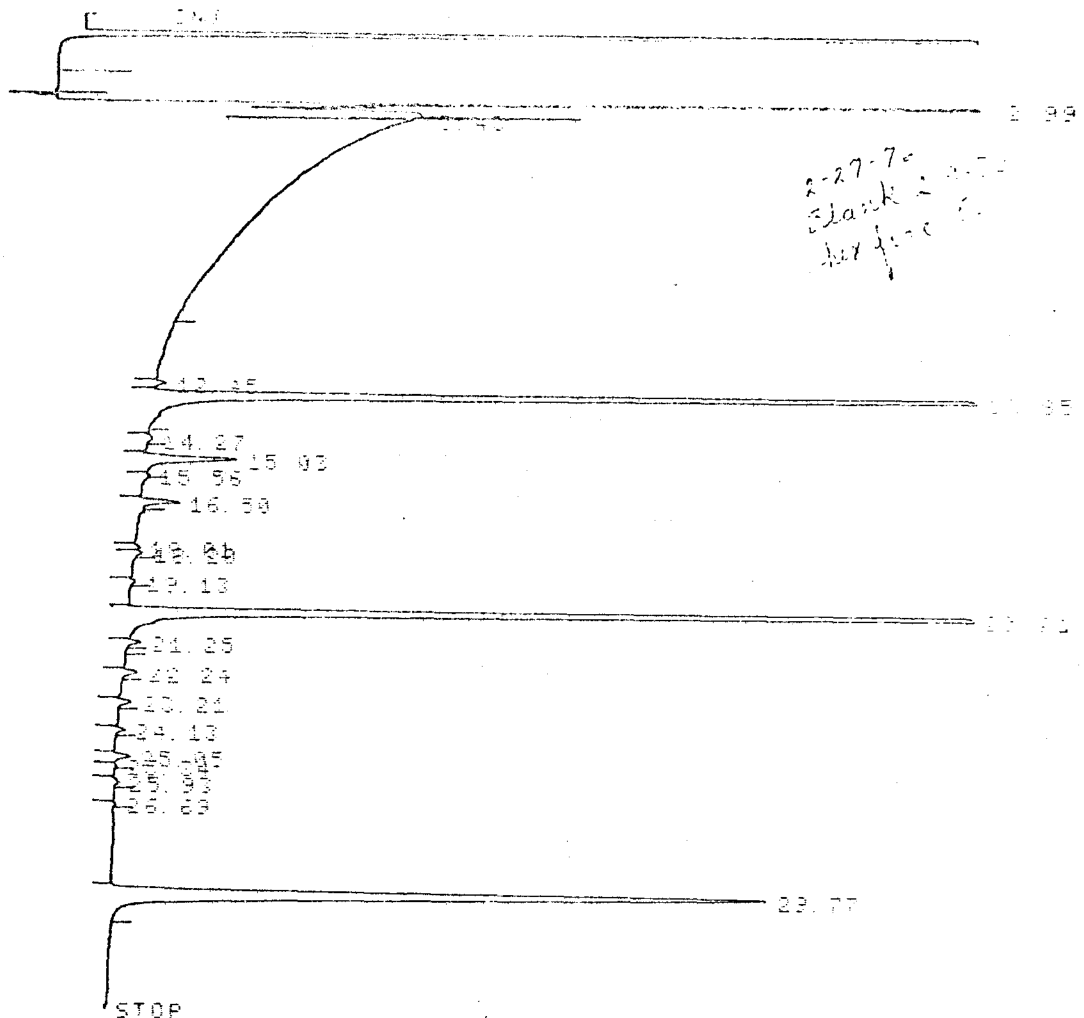
HP 3380A

DLY	2.	STOP	45	REJECT	100
MV/M	.10	ATTN	4		



	RT	TYPE	AREA	AREA %
S	~~15.15~~		~~6710~~	~~47.19~~
S	~~16.62~~	~~T~~	~~2029~~	~~14.27~~
S	~~18.34~~		~~281~~	~~1.976~~
	23.48		2667	18.76
S	~~25.49~~		~~675~~	~~4.747~~
	26.10		1857	13.06
			4524	

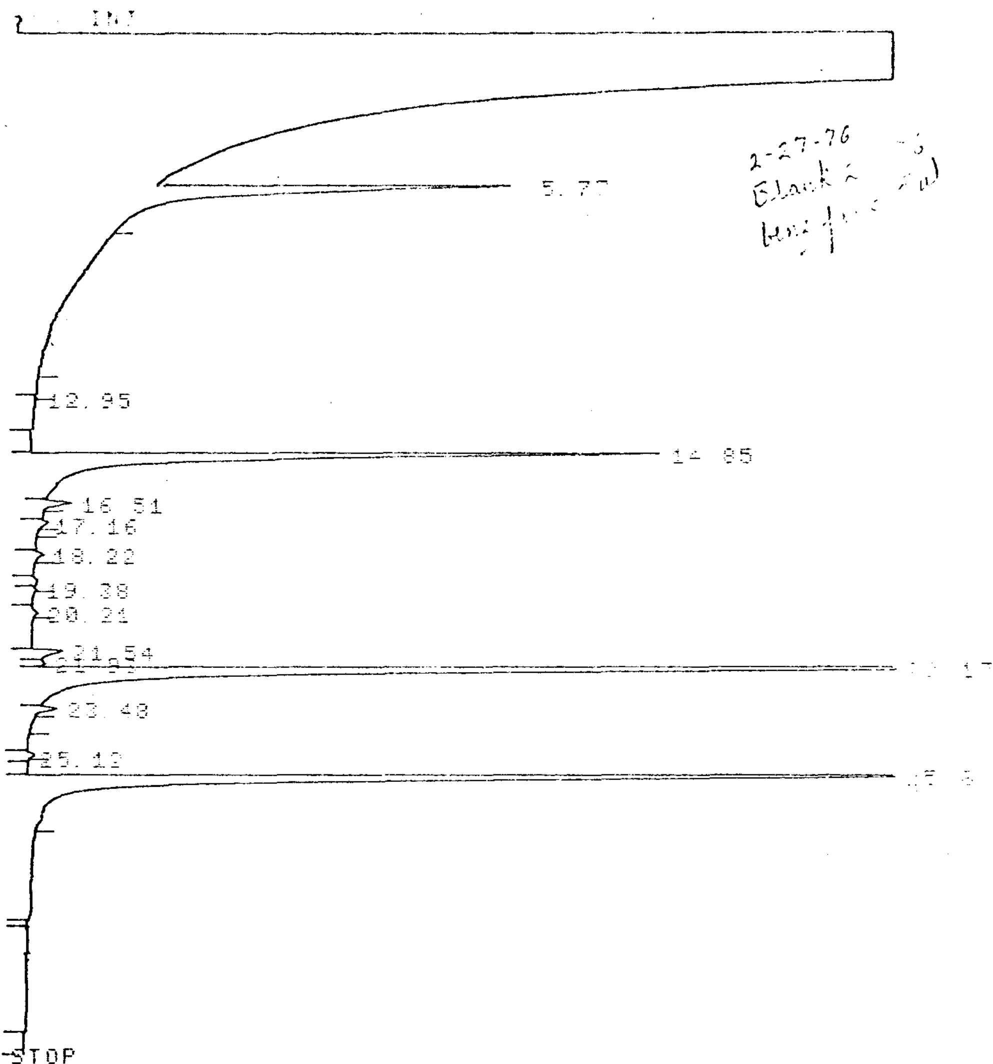
HP 3380A
DLY 2. STOP 45 REJECT 100
MV/M .10 ATTN 4



AREA %

RT	TYPE	AREA	
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
~~[illegible]~~	~~T~~	~~[illegible]~~	~~[illegible]~~
12.45		469	.083 35
12.85	C_{16} M Spike	65610	11.66
14.27		337	.059 9
S ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
15.56	M	118	.020 97
S ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
18.01		209	.037 15
S ~~[illegible]~~	~~M~~	~~[illegible]~~	~~[illegible]~~
19.13		257	.045 68
20.21	C_{22} Spike	74457	13.23
21.25	T	498	.088 51
22.24		651	.115 7
23.21		561	.099 71
24.13		380	.067 54
S ~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
25.34	M	124	.022 04
25.93		351	.062 39
26.69		139	.024 71
29.77	C_{32} Spike	47244	8.397

HP 3380A
DLV 2.   STOP 45   REJECT OFF
MV/M 10   ATTN 4



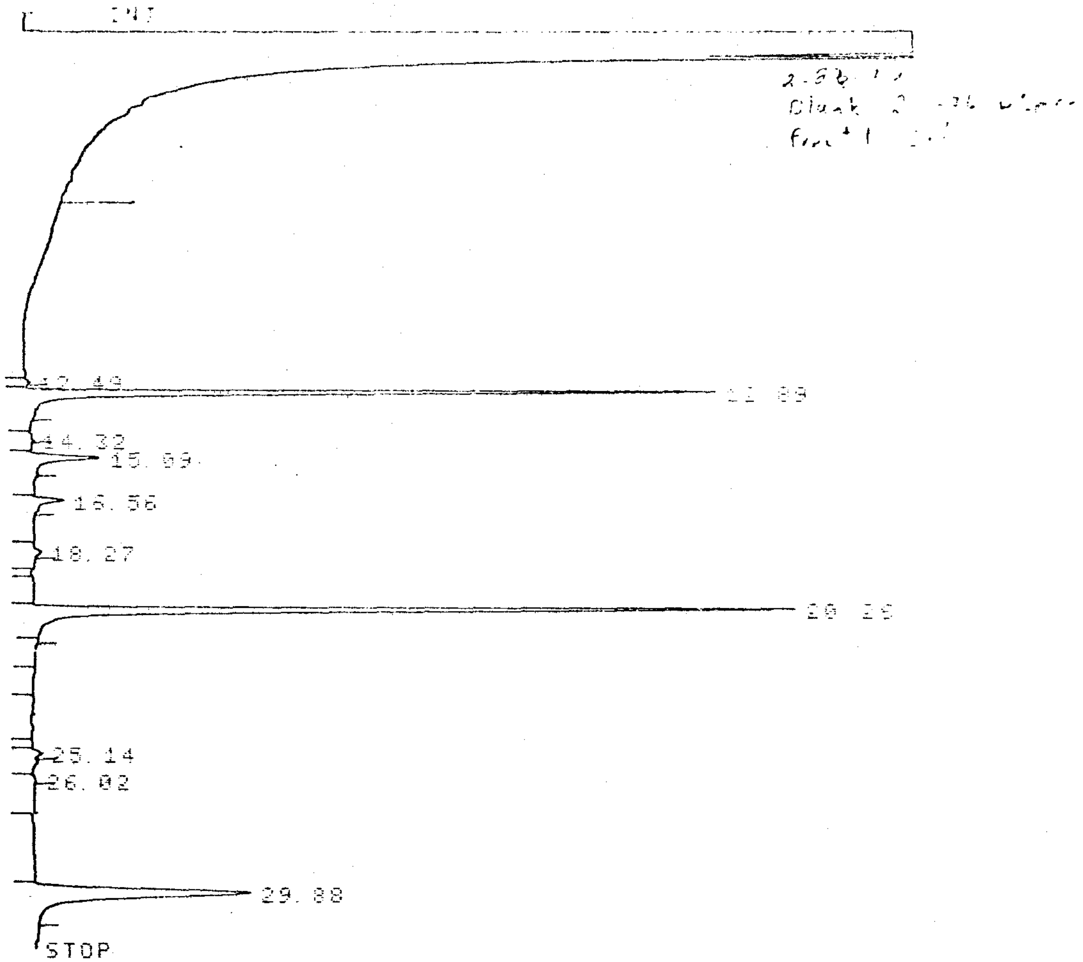
AREA %

	RT	TYPE	AREA	
–	5.77	Naphthalene	25460	12.59
5	~~12.95~~		~~118~~	~~[illegible]~~
–	14.85	ANTHRACENE	45863	22.69
5	~~16.51~~	~~T~~	~~1156~~	~~.571 7~~
	17.16	T	378	.186 9
5	~~18.22~~		~~475~~	~~.235 4~~
	19.38		82	.040 55
	20.21		316	.156 3
	21.54		1676	.828 8
	21.93	T	163	.080 61
–	22.17	CHRYSENE	65934	32.61
	23.48	T	704	.349 1
5	~~25.12~~		~~354~~	~~.175 1~~
–	25.85	PERYLENE	59538	29.44

HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M 10 ATTN 4

301

MV/M .03 ATTN 4

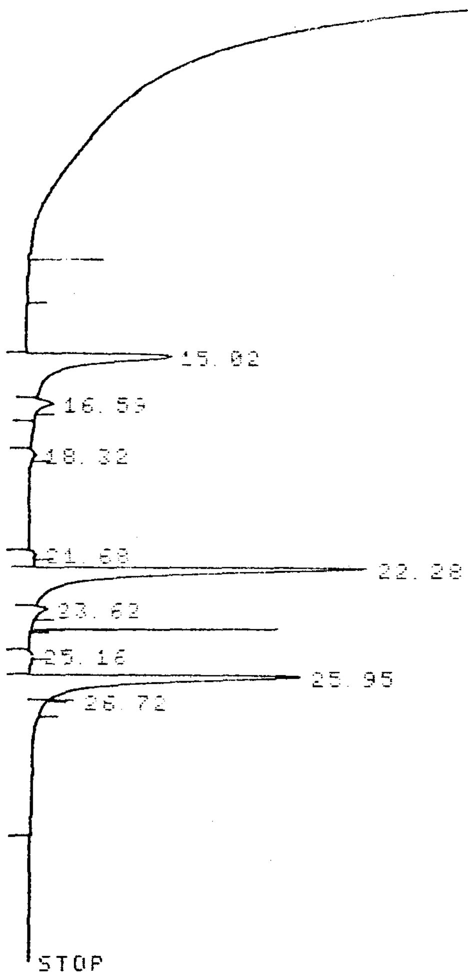


AREA %

	RT	TYPE	AREA	
in std	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
C_{16}	12.99		27727	30.24
	14.32		281	.306 5
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
C_{22}	20.26		35346	38.56
septum	~~25.14~~		~~[illegible]~~	~~[illegible]~~
	26.02		225	.245 4
C_{32}	29.88		21299	23.23

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

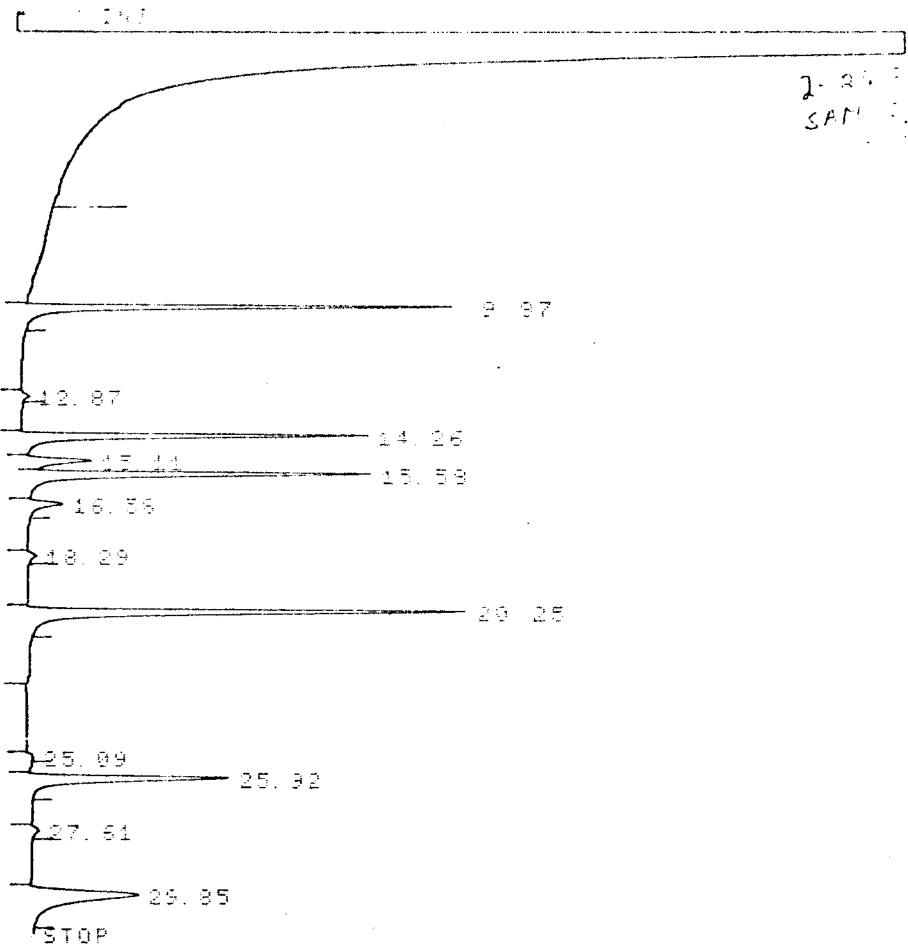
302



AREA %

	RT	TYPE	AREA	
	15.02		17299	24.04
septum	~~[illegible]~~	~~T~~	~~[illegible]~~	~~[illegible]~~
septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
in std	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	22.28		28200	39.19
	23.62	T	687	.954 8
in std	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	25.95		23431	32.57
	26.72	T	281	.390 5

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

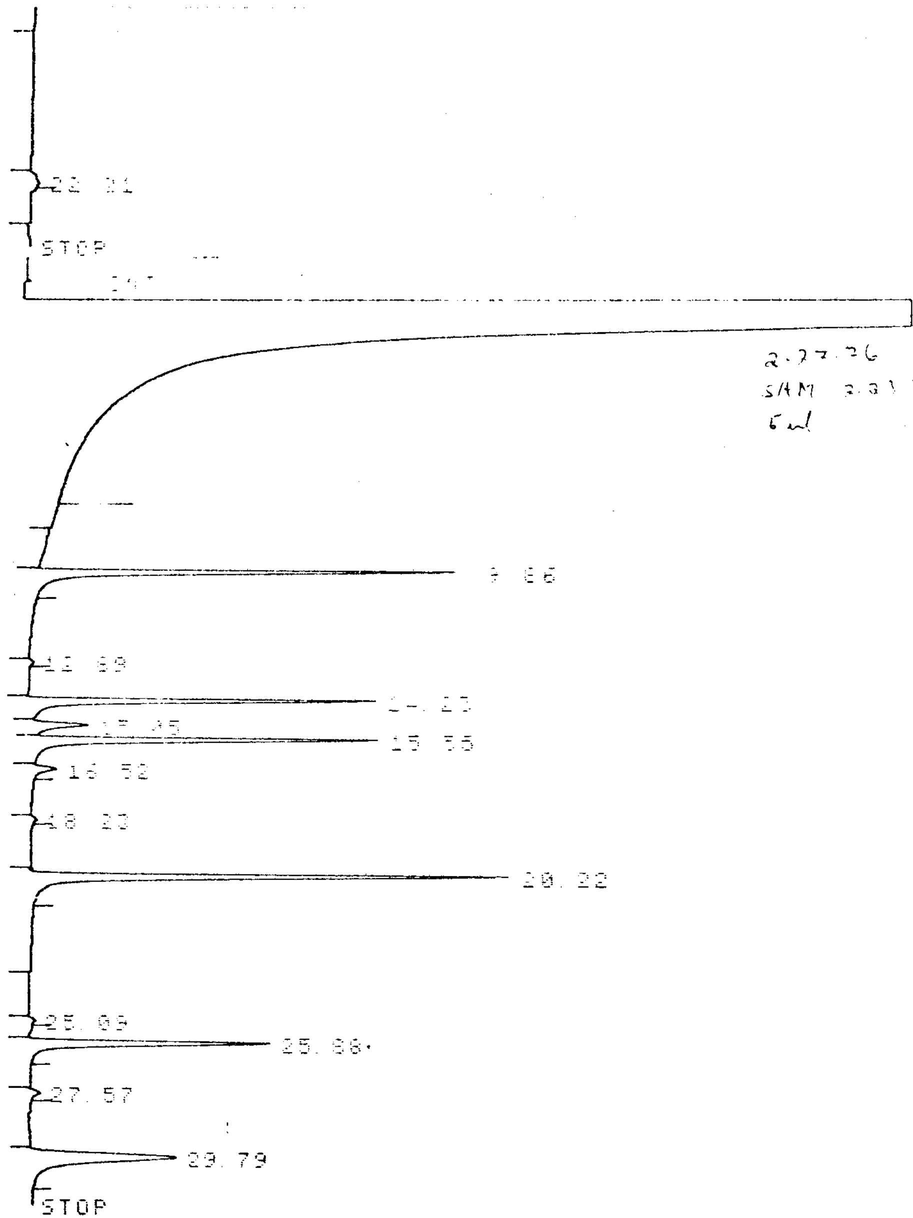


AREA %

	RT	TYPE	AREA	
C_{14}	9.87		17746	17.1
~~cont.~~	[illegible]		[illegible]	[illegible]
C_{17}	14.26		15472	14.91
~~septum~~	[illegible]	[illegible]	[illegible]	[illegible]
C_{18}	15.58	M	16456	15.85
~~septum~~	[illegible]	[illegible]	[illegible]	[illegible]
~~septum~~	[illegible]		[illegible]	[illegible]
C_{22}	20.25		22561	21.74
~~septum~~	[illegible]		[illegible]	[illegible]
C_{28}	25.92		11731	11.3
~~cont.~~	[illegible]		[illegible]	[illegible]
C_{32}	29.85		12339	11.89

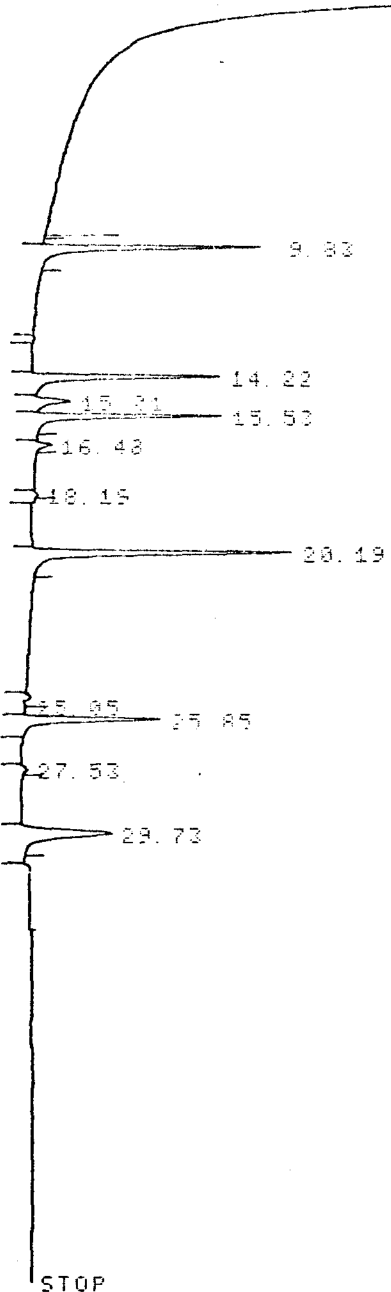
HP 3380A
DLY 2 STOP 45 REJECT OFF
MV/M .10 ATTN 4

304



	RT	TYPE	AREA	AREA %
	9.86		17517	17.14
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	14.23		15186	14.86
S	~~[illegible]~~	~~I~~	~~[illegible]~~	~~[illegible]~~
	15.55	M	15395	15.06
S	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	20.22		22536	22.05
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	25.88		12173	11.91
S	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
	29.79		13741	13.45

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M 10 ATTN 4



3-1-76
SAM 3-1

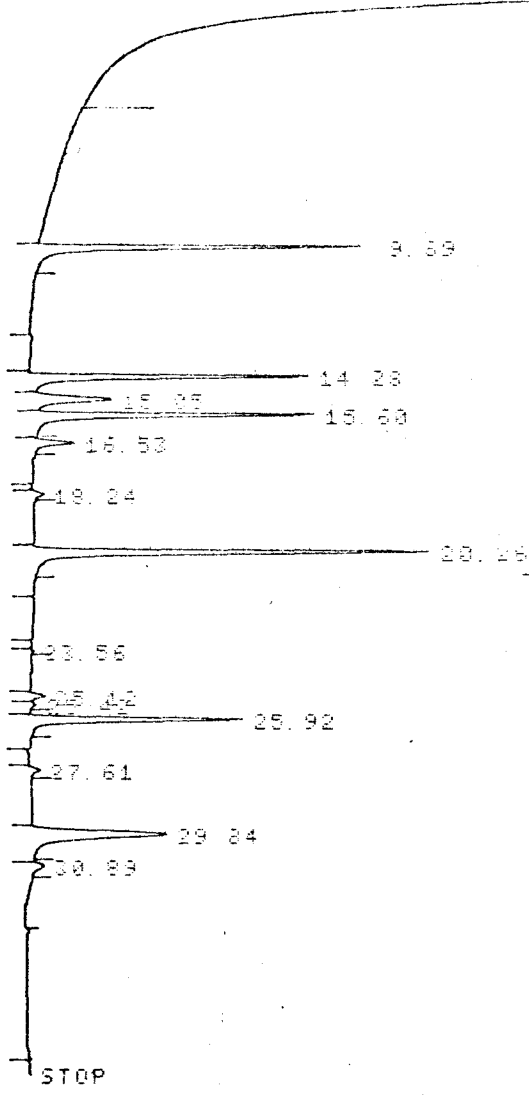
STOP

AREA %

RT	TYPE	AREA	
9.83		9447	16.81
14.22		8356	14.87
~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
15.53	M	8238	14.66
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
20.19		12421	22.11
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
25.85		6700	11.93
~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
29.73		7565	13.5
		52750	

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

306

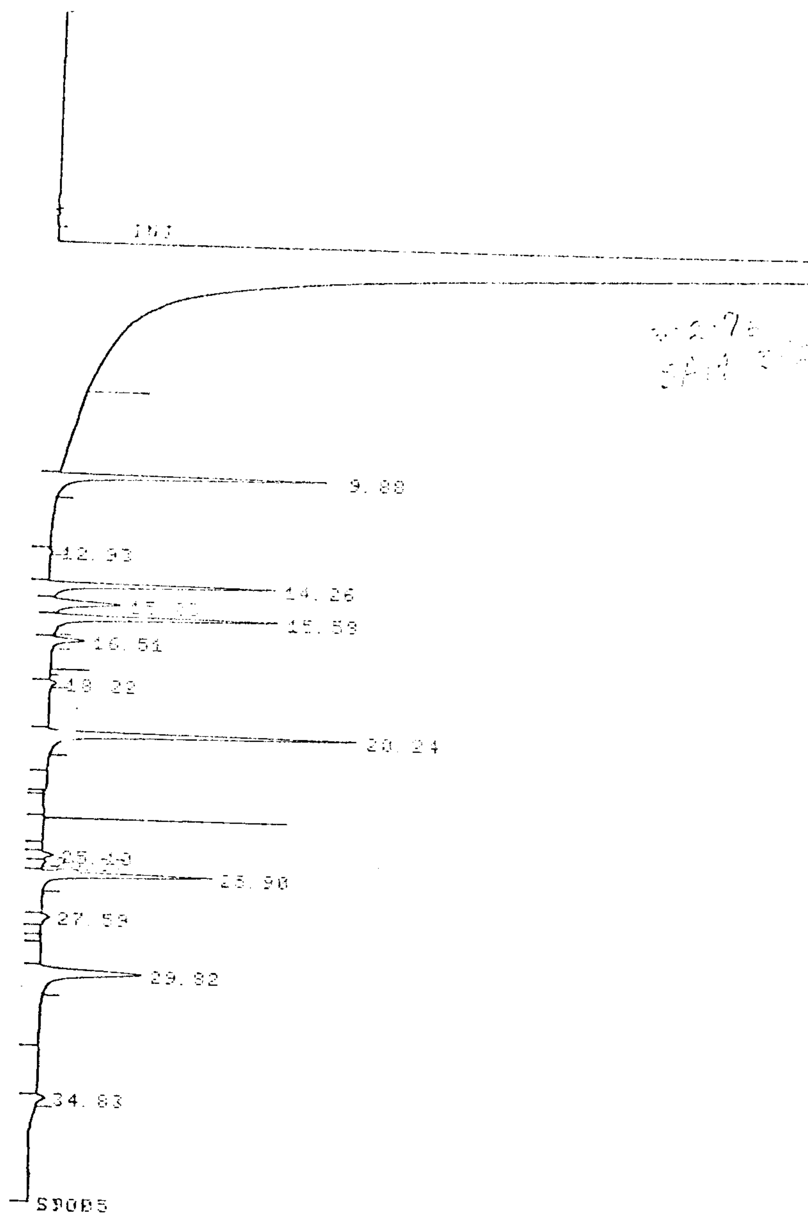


3-2-76
SRM 3-1

AREA %

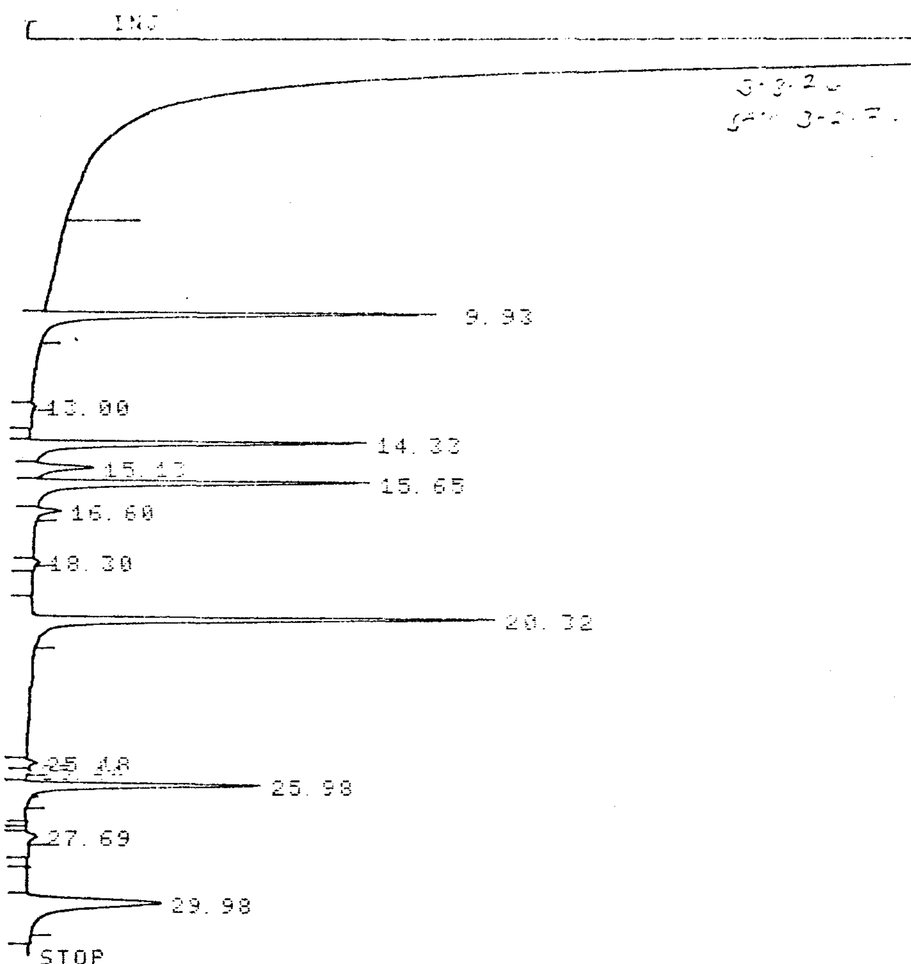
	RT	TYPE	AREA	
C_{14}	9.89		13891	15.75
C_{17}	14.28		12031	13.64
Septum	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
C_{18}	15.60	M	12456	14.12
Septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
Septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
C_{22}	20.26		18148	20.58
	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
Septum	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
Septum	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~	~~[illegible]~~
C_{28}	25.92		10015	11.36
cont.	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~
C_{32}	29.84		11505	13.05
cont.	~~[illegible]~~		~~[illegible]~~	~~[illegible]~~

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



	RT	TYPE	AREA	AREA %
C_{14}	9.88		13587	15.93
cmt.	[illegible]		[illegible]	[illegible]
C_{17}	14.26		11866	13.91
septum	[illegible]		[illegible]	[illegible]
C_{18}	15.58	M	11750	13.79
septum	[illegible]		[illegible]	[illegible]
septum	[illegible]		[illegible]	[illegible]
C_{22}	20.24		17725	20.79
septum	[illegible]		[illegible]	[illegible]
septum	[illegible]		[illegible]	[illegible]
C_{28}	25.90		9724	1[illegible].4
cont	[illegible]		[illegible]	[illegible]
C_{32}	29.82		11109	1[illegible].02
cont.	[illegible]		[illegible]	[illegible]
	[illegible]		[illegible]	[illegible]

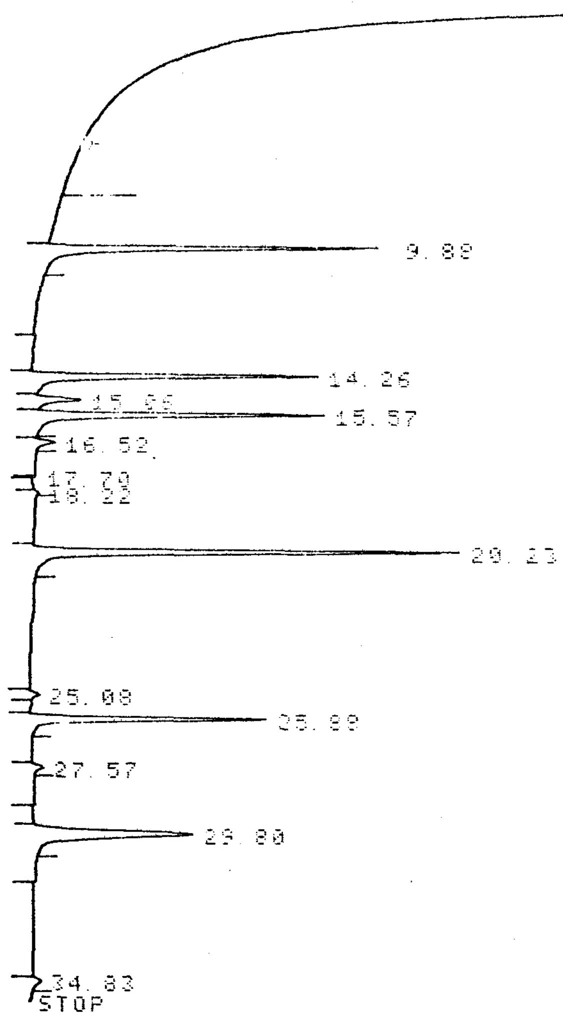
HP 3380A
DLY 2.
MV/M 10
STOP 45
ATTN 4
REJECT OFF



AREA %

	RT	TYPE	AREA	
C_{14}	9.93		17025	17.09
cont.	~~13.00~~		~~169~~	~~.169 7~~
C_{17}	14.33		14689	14.75
septum	~~15.43~~	~~T~~	~~[illegible]~~	~~3.[illegible]~~
C_{18}	15.65	M	14934	15.
septum	~~16.60~~	~~M~~	~~1207~~	~~1.212~~
septum	~~18.30~~		~~224~~	~~.224 9~~
C_{22}	20.32		22070	22.16
septum	~~25.[illegible]~~		~~[illegible]~~	~~[illegible]~~
septum	~~25.44~~	~~M~~	~~229~~	~~[illegible]~~
C_{28}	25.98		11786	11.83
cont.	~~27.69~~		~~[illegible]~~	~~[illegible]~~
C_{32}	29.98		13115	13 17

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

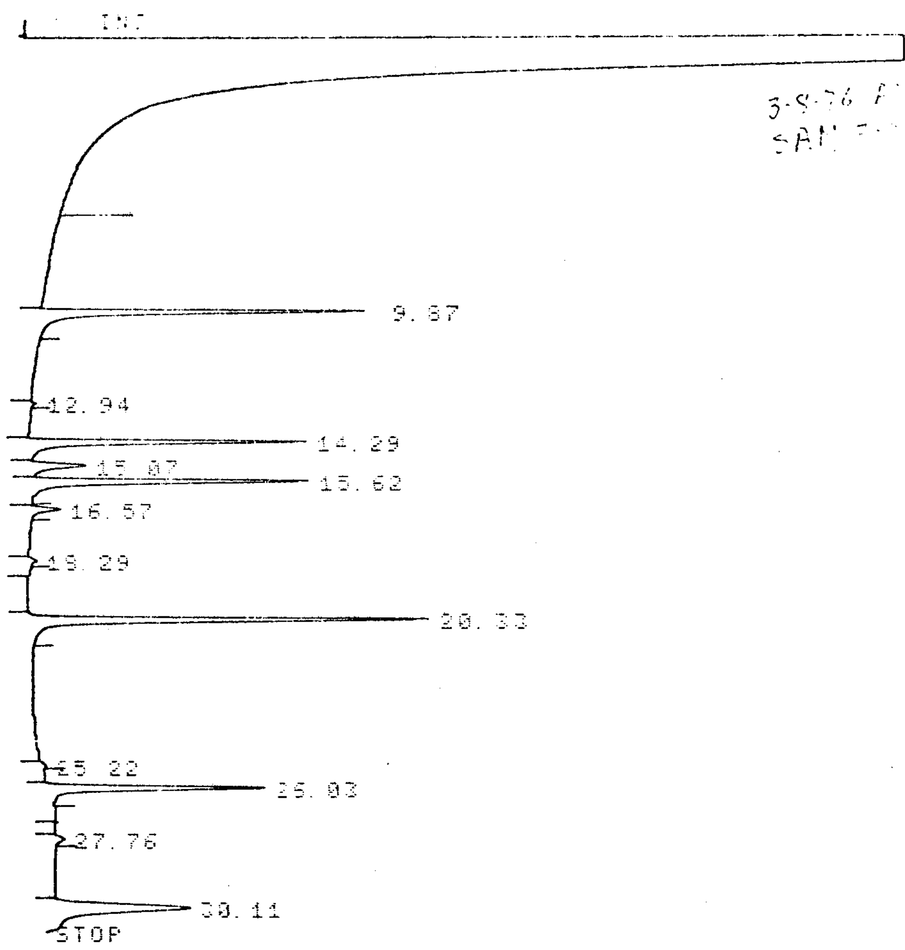


3-4-76
SAM 3-4-76

AREA %

RT	TYPE	AREA	
9.88		14474	16.52
14.26		12564	14.34
~~15.05~~	~~T~~	~~[illegible]~~	~~[illegible]~~
15.57	M	12681	14.47
~~16.52~~		~~[illegible]~~	~~[illegible]~~
~~17.70~~		~~[illegible]~~	~~[illegible]~~
~~18.22~~		~~[illegible]~~	~~[illegible]~~
20.23		19255	21.97
~~25.08~~		~~[illegible]~~	~~[illegible]~~
25.88		10928	12.47
~~27.57~~		~~[illegible]~~	~~[illegible]~~
29.80		12818	14.63
~~34.83~~		~~[illegible]~~	~~[illegible]~~

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

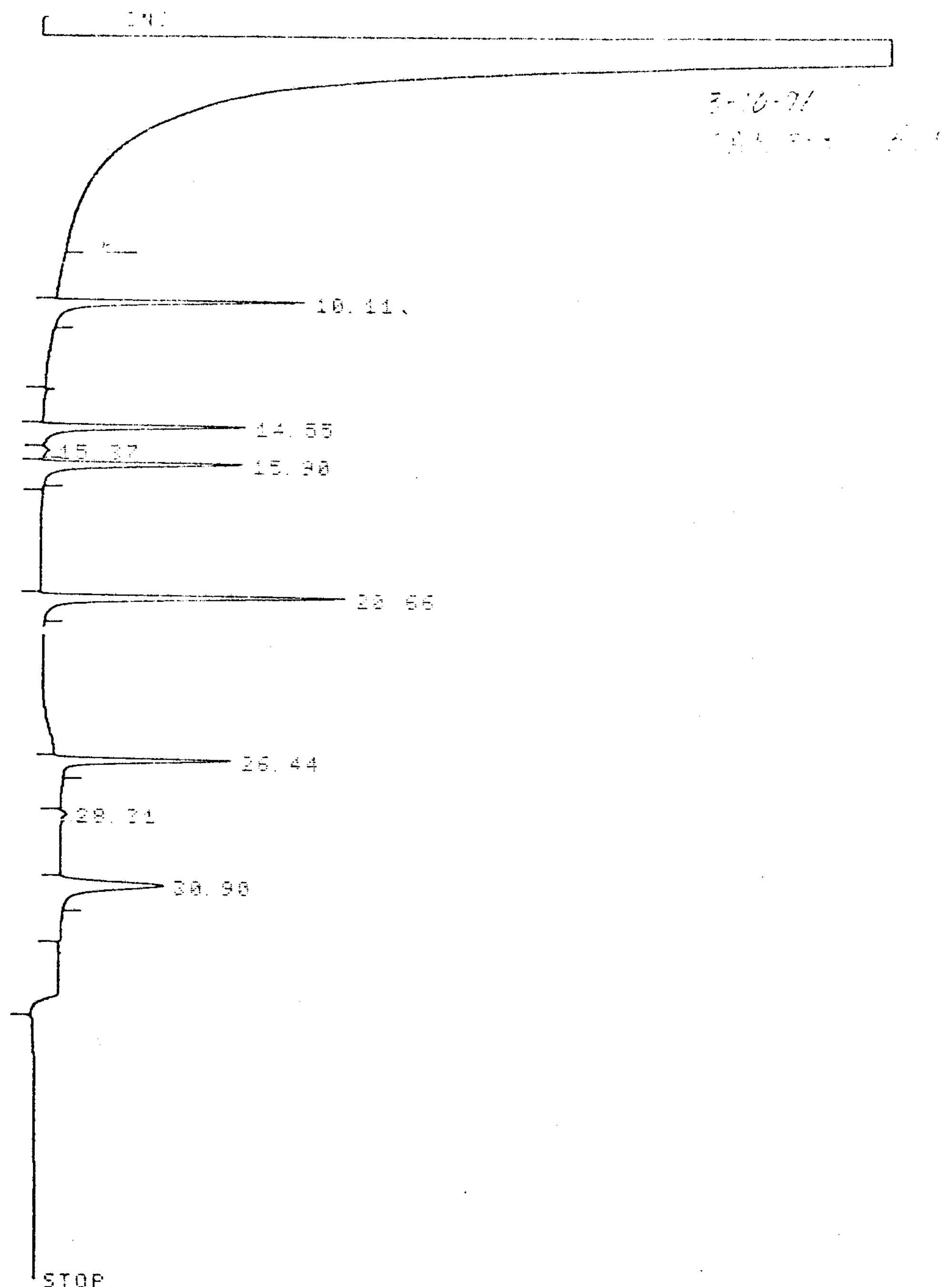


RT	TYPE	AREA	AREA %
9.87		14429	16.42
~~12.94~~		~~245~~	~~.279~~
14.29		12342	14.05
~~15.07~~	~~I~~	~~[illegible]~~	~~[illegible]~~
15.62	M	12529	14.26
~~16.57~~		~~[illegible]~~	~~1.62~~
~~18.29~~		~~422~~	~~[illegible]~~
20.33		18782	21.38
~~25.22~~		~~[illegible]~~	~~[illegible]~~
26.03		10060	11.45
~~27.76~~		~~[illegible]~~	~~[illegible]~~
30.11	I	13935	15.85

3/8/76
SAM 5µl

HP 3380A
DLY 2. STOP 60 REJECT OFF
MV/M .10 ATTN 4

311

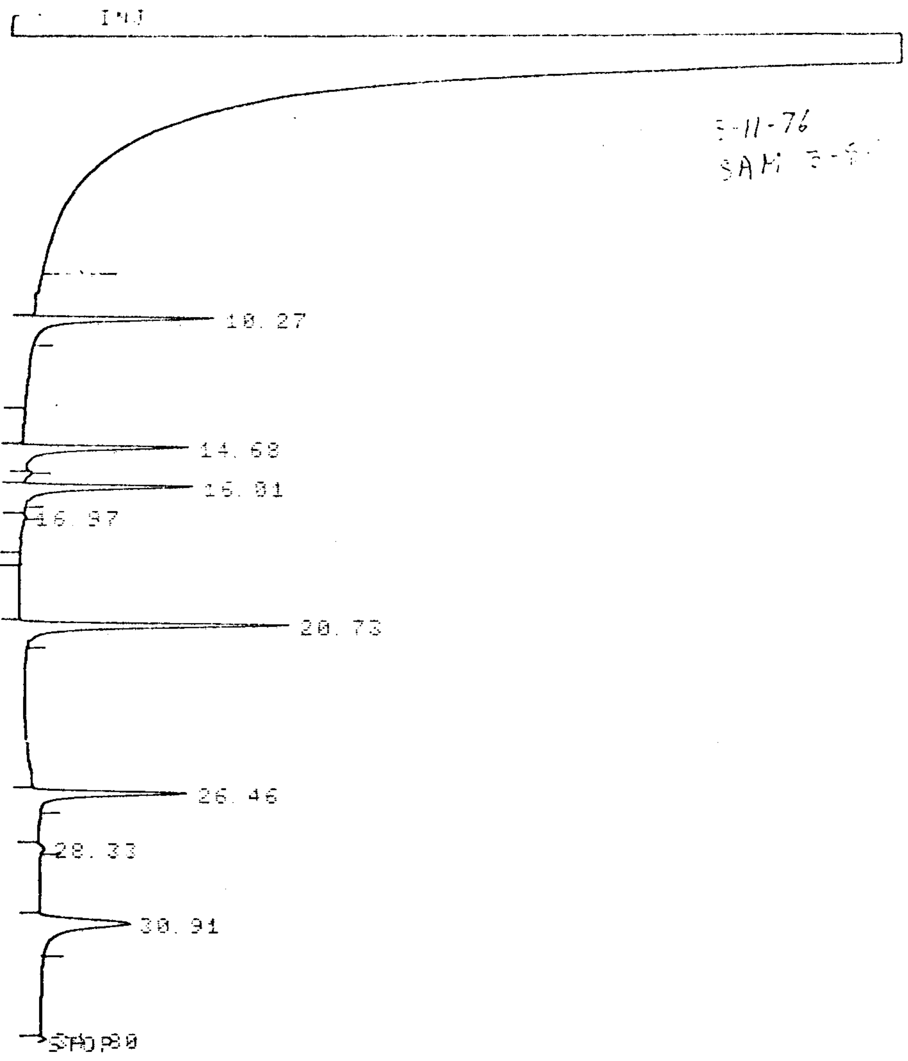


AREA %

RT	TYPE	AREA	
10.11		12302	17.51
14.55		10567	15.04
~~15.37~~		~~[illegible]~~	~~[illegible]~~
15.90		10587	15.07
20.66		16200	23.06
26.44		9060	12.9
~~28.71~~		~~[illegible]~~	~~[illegible]~~
30.90		13555	15.17
		69371	

RF = [illegible]

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4



AREA %

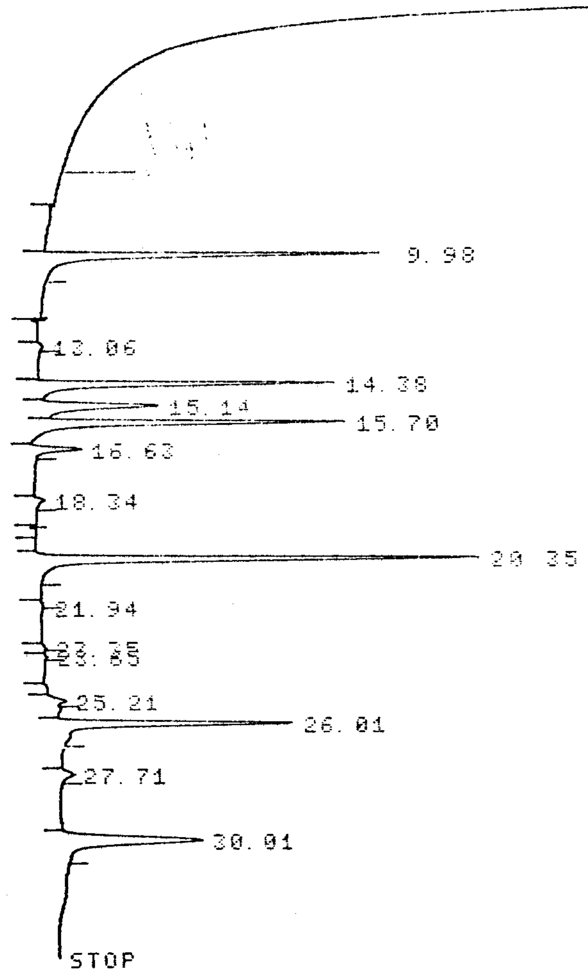
RT	TYPE	AREA	
10.27		9033	15.32
14.68		7903	13.4
15.01		8716	14.78
S ~~16.97~~		~~[illegible]~~	~~[illegible]~~
20.73		14311	24.26
26.46		8231	13.95
S ~~28.33~~		~~357~~	~~[illegible]~~
30.91		9955	16.88
~~34.00~~	~~I~~	~~342~~	~~[illegible]~~
		58149	

$RF = \frac{371}{58149} = 6.38 \times 10^{-3}$

HP 3380A
DLY 2. STOP 45 REJECT OFF
MV/M .10 ATTN 4

313

3-23-76
SAM 3-23 5μl



AREA %

	RT	TYPE	AREA	
	9.98		15392	15.35
S	~~13.06~~		~~252~~	~~.251 3~~
	14.38		14027	13.99
S	~~15.14~~	~~T~~	~~7374~~	~~7.352~~
	15.70	M	14685	14.64
S	~~16.63~~	~~M~~	~~2323~~	~~2.316~~
S	~~18.34~~		~~682~~	~~.68~~
	20.35		20596	20.54
S	~~21.94~~		~~204~~	~~.203 4~~
S	~~23.35~~		~~116~~	~~.115 7~~
S	~~23.65~~		~~138~~	~~.137 6~~
S	~~25.21~~		~~534~~	~~.532 4~~
	26.01		11605	11.57
S	~~27.71~~		~~707~~	~~.704 9~~
	30.01		11662	11.63
			87967	

$RF = 4.217 \times 10^{-3}$

HP 3380A
DLY 2. STOP 45 REJECT 100
MV/M .10 ATTN 4

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 5 R.U. NUMBER: 275/276/294

PRINCIPAL INVESTIGATOR: Dr. D. G. Shaw

Submission dates are estimated only and will be updated, if necessary, each quarter. Data batches refer to date as identified in the data management plan.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]		
	From	To	Batch 1	2	3
Silas Bent Leg I #811	8/31/75	9/14/75	None	4/30/76	4/30/76
Discoverer Leg III #810	9/12/75	10/3/75	None	None	4/30/76
Discoverer Leg IV #812	10/3/75	10/16/75	6/30/76	None	4/30/76
Surveyor #814	10/28/75	11/17/75	None	6/30/76	None
North Pacific	4/25/75	8/7/75	6/30/76	None	None
Contract 03-5-022-34	Last	Year	4/30/76	4/30/76	4/30/76
Moana Wave MW 001	2/21/76	3/5/76			

Note: [1] Data Management plan has been approved and made contractual.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 5

PRINCIPAL INVESTIGATOR: Dr. David G. Shaw

Period April 1, 1975 - March 31, 1976* (12 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	105,577.00	39,542.46	66,034.54
Staff Benefits	17,948.00	6,690.40	11,257.60
Equipment	135,300.00	125,094.91	10,205.09
Travel	6,004.00	1,702.03	4,301.97
Other	106,100.00	98,577.39	7,522.61
Total Direct	370,929.00	271,607.19	99,321.81
Indirect	60,391.00	22,618.29	37,772.71
Task Order Total	431,320.00	294,225.48	137,094.52

* Preliminary cost data, not yet fully processed.

RU 275
(subcontract)

ANNUAL REPORT

TO

UNIVERSITY OF ALASKA

Period from July 1, 1975 to March 31, 1976

FROM

Institute of Geophysics and

Planetary Physics

University of California

Los Angeles, California 90024

for

"Characterization of Organic Matter in Sediments from Gulf of Alaska, Bering and Beaufort Seas"

Subcontract No. F-01770, PO# F01770

I. SUMMARY

The objectives of the investigation were aimed at assessing the organic content of the sediments of the eastern Bering Sea and Gulf of Alaska, and in particular, the identification and quantification of paraffinic hydrocarbons.

The results show that the sediments sampled generally contain less than 1% organic carbon and less than 0.1% nitrogen and sulfur. A search for natural gases which may result from either biogenic production or from natural seeps, showed that the gas content was methane and was in concentration approximately equal to that of dissolved gas in sea water.

Seven sediment samples from Eastern Gulf of Alaska have been analyzed for organic solvent-extractable components. The total extracts amounted to about 140-200 ppm extractable component. The paraffin contained in this fraction varied from 2-8 ppm. The distribution pattern of the saturated hydrocarbons showed no recognizable isoprenoids (pristane or phytane) and no n-alkanes lighter than C_{19}. Because of an odd/even ratio >1 and the presence of hydrocarbons $> C_{27}$, the distribution pattern is interpreted as resulting from a mixture of planktonic and terrestrial plant-derived material with no evidence for petroleum-derived hydrocarbons.

II. INTRODUCTION

This study has been undertaken to primarily determine the baseline level of hydrocarbons in the Gulf of Alaska and the Bering Sea. When exploration, production and transportation of submarine petroleum begins, accidental loss of petroleum may result in substantial environmental damage. In order to determine whether such petroleum release has occurred on the ocean floor, it is necessary to first establish the background levels of hydrocarbons in recent marine sediments and to determine with some precision their distribution pattern. It is now known that hydrocarbons originating from organisms in young sediments differ in certain specific characteristics from petroleum-derived hydrocarbons. THe analyses undertaken at UCLA were therefore designed to survey the distribution of hydrocarbons in the sediment and evaluate their nature and concentration.

As an adjunct to these studies, known weights of sediment were placed in cans, distilled water was added and the cans sealed. In our laboratory, hydrocarbon gases trapped in the sediment were released by heating the cans to 80°C and removing gases in the head space with a syringe and injecting them into the gas chromatograph. Using this approach, we wanted to determine both the type of light hydrocarbon gases present and their distribution. Biogenic gases generally only contain methane, whereas wet petroleum gases include a variety of volatile hydrocarbons from methane to higher molecular weights.

To assess the biological richness of the sediment, we analyzed for organic carbon, nitrogen and sulfur. Changes in these are all indicative of diagenesis and hence fermentative processes occurring within the sediment column which will affect the hydrocarbon distribution. Results on these are given in this Report.

In progress are studies on the humic acids and kerogen contents of the sediment. However, because of the lengthy procedures required to extract and purify these components, results are presently not available from this study.

III. CURRENT STATE OF KNOWLEDGE

With the exception of measurement of total carbon contents from a variety of environments in the Gulf of Alaska and Arctic Ocean, little is known about the nature of organic substances in the sediment. A preliminary report by Shaw, Cheek and Paul (University of Alaska, 1976) on petroleum uptake in intertidal sediments at Port Valdez gives a single analysis for an undisturbed sediment. None of the stations we report here coincide with the station sampled by Shaw *et al*. The distribution pattern for the paraffinic hydrocarbons found by Shaw shows a high concentration of pristane, C_{21} and C_{28}. This is a most unusual distribution and may indicate a hydrocarbon mixture from both polluted and non-polluted sources.

IV. STUDY AREA

A total of 34 sediment samples for high molecular weight hydrocarbons and 27 samples for volatile hydrocarbons were collected in summer 1975 from the western Gulf of Alaska and from the Bering Sea. A description of the cruise report is included in the Appendix I, and the stations occupied for sampling are shown in Fig. 1. Many of the stations occupied for sampling had rocky or gravel bottoms, and it was not possible to collect a meaningful sample for chemical analysis.

In order to obtain additional samples for analysis, ten sediment

samples adjacent to Prince William Sound were supplied to us by Dr. David Shaw. The locations of these samples are shown on the map in Fig. 2. Because these latter samples were not hermetically sealed on collection, they cannot be used for volatile hydrocarbon analysis, but are suitable for high molecular weight hydrocarbon studies.

V. METHODS

The methods of this report involve three separate types of analytical procedures:

(1a) Elemental analysis for sulfur was carried out on dry samples by use of an induction furnace following the Leco Corporation method.

(1b) Total carbon and organic carbon (carbon remaining after treatment with 3N HCl) were determined by a Leco acid-base semi-automatic carbon determinator and then calcium carbonate is calculated as follows: (% total C - % C after acidification) x 8.33 = % $CaCO_3$.

(1c) Total nitrogen was measured as NH_4^+ extracted by sulfuric acid oxidation of the organic matter in evacuated, closed tubes at 340°C for 2½ hours. The ammonia was purified by microkjeldahl distillation NaOH added, and then measured colorimetrically.

(2) Volatile hydrocarbon gases were determined on sediment samples which had been placed in a cleaned can and covered with distilled water to leave a headspace of about 200 ml. The cans were sealed and analyzed on shore. There, the cans were placed in a bath at 80°C and

energized ultrasonically for one hour. At that time, the lid was punctured with a device which allowed a known volume of gas to be removed and introduced into a gas chromatograph. The column, 0.035" I.D. x 10 ft. was packed with n-Octane/ Porasil C Durapak, 100/120 mesh, and programmed from -10°C to 100°C.

The gases were identified by coinjection with known standards, and the amount of gas measured was calibrated against a standard gas mixture, and the results calculated as ppb of dry sediment.

(3) The analysis of high molecular weight hydrocarbons ($> C_{15}$) essentially follows the procedure described by BLM. In order to maintain uniformity in analysis for the purpose of realistic comparisons of results on OCS samples, it was decided to use the preferred method. A detailed description of the method was given in Appendix II. All samples were maintained in a frozen condition from the time of collection to the time of analysis.

VI. RESULTS

1. Carbonate, Organic Carbon, Nitrogen and Sulfur

With the exception of four samples in the western Gulf of Alaska, where the local calcium carbonate content reached 40%, the calcium carbonate content is generally < 5% (see Table 1).

Organic carbon content is 1% or lower. Only two samples exceed 1%. The samples from the Bering Sea and the mouth of Cook Inlet are particularly low in carbon, nitrogen and sulfur.

The nitrogen content varied, but many samples displayed a C:N ratio of $\sim$10. Surprisingly, the sulfur content is very low, probably indicating oxygenated conditions at the sediment surface. It might

be of interest to determine whether this sulfur is biologically produced syngenetically with the sediment, or whether it has been introduced by glacial and river erosion.

2. High Molecular Weight Hydrocarbons

Seven sediment samples from the Western Gulf of Alaska have been analyzed for extractable hydrocarbons. The results are presented in Tables 2 and 3 and in Figs. 3 - 9.

The total extractable fraction after sulfur removal by copper and saponification to remove fatty acids, yielded values in the range of 141 to 196 ppm hydrocarbon (Table 2). The total extractable component was broken down into paraffinic and cyclic (saturate), aromatic and polar components. As seen from Table 2, the saturate fraction usually contained the lowest amount of extractable material, whereas the polar component contained the greatest extractable amount. The nature of the aromatic and polar components is presently not known.

The distribution pattern for the recognizable saturated hydrocarbons is shown in Table 3 and in Figs. 3 - 9. Some very notable features are observed. First, there are no detectable n-alkanes lighter than C_{19}. Second, there are no detectable isoprenoids, in particular pristane or phytane. Third, the odd/even ratio for n-alkanes over a range C_{21}-C_{31} is >1.0, but does not reach the higher values (~ 2) often observed in young sediments. Fourth, there appears to be an absence of C_{17} or C_{22} olefinic hydrocarbons often associated with young sediment, where the organic matter has largely originated from planktonic sources. Fifth, the higher molecular weight hydrocarbons

in the range $C_{27} - C_{32}$ are present, but generally in amounts lower than the $C_{20} - C_{26}$ components.

To demonstrate that the identification of the chromatogram peaks is correct, a series of four known standard compounds, nC_{16}, nC_{17}, nC_{24} and nC_{28} alkanes were coinjected with GASS 43 extract. It can be seen from Fig. 10 that nC_{24} and nC_{28} superimposed on the peaks so identified from the hexane fraction of sediment extract in Figs. 3 - 9.

A possible explanation for the lack of n-alkanes below C_{19} might be the lack of sensitivity in detection. However, high detectable signals can be obtained with nanogram quantities of hydrocarbons as seen in Fig. 11, where $\sim$ 100 ng standards were injected. Since signal strengths of 10^{-2} to 10^{-1} could be detected, it must be surmised that indeed, n-alkanes $< C_{19}$ are missing from the sample.

3. Volatile Hydrocarbons

The method employed for analysis is capable of measuring $C_1 - C_6$ alkanes in the concentration range of $\leq$ 1 ng. The resolution on the column is excellent, as can be seen from a series of standard gases shown in Fig. 12. The results, however, showed that only traces of gases other than methane were present. Their concentration was too low to detect. The only measurable gas was methane, in the amounts shown in Table 4.

With the exception of EBBS 28, all the CH_4 concentrations are 10 ng/g dry sediment. These values fall in a concentration range similar to those of average seawater in the amounts of seawater contained in the pore water of the canned sediments. There is no evidence for

petroleum gas seeps.

VII. DISCUSSION

The methods used in this study are compatable with other similar studies being conducted by BLM elsewhere on the OCS. The data demonstrate that the sediments from the Bering Sea and Gulf of Alaska are poor in organic matter. This may either be due to lack of a source of organic carbon or due to rapid degradation of organic matter at the sediment-water interface, because of the presence of highly oxygenated bottom waters. The low abundance of sediment sulfur and dissolved methane, both attest to the lack of reducing conditions. Hence, hydrocarbons are either not forming in the sediment column, or else they are being rapidly degraded. We cannot tell from the present data which process dominates.

It is apparent that petroleum-derived hydrocarbons are either absent or else they have been sufficiently altered so that the lighter hydrocarbons have already been oxidized away. Without knowing the hydrocarbon content and characteristics of the regional plankton, it is difficult to predict what percentage of the HMWHC are contributed from primary producers in the water column and what fraction may originate from land.

VIII. CONCLUSIONS

A series of analyses for carbon, sulfur, nitrogen, high molecular weight hydrocarbons and volatile hydrocarbons indicate that sediment from the Bering Sea and Gulf of Alaska are poor in organic matter and contain only minor or trace amounts of methane. The pattern for high

molecular weight hydrocarbons is different from the distribution of these compounds in temperate climate marine OCS sediments. More analyses need to be undertaken to determine if the distribution is characteristic of the entire Gulf of Alaska and Bering Sea. There is presently no evidence for a contribution of petroleum hydrocarbons to the young sediments.

IX. NEEDS FOR FURTHER STUDY

A. Continuation of extraction and analysis of hydrocarbons in sediments from western Gulf of Alaska and eastern Bering Sea to characterize the hydrocarbons in sediments from these areas, and to compare with the results obtained from eastern Gulf of Alaska.

B. Develop procedure for the analysis of light hydrocarbons in canned sediment samples from EBBS and GASS stations that will concentrate the headspace gases and provide a much greater sensitivity level. This will enable us to determine if the methane concentrations given in this report are the result of entrapped pore water or are derived from the sediments. Furthermore, if enough gas can be concentrated, isotopic analysis, using vacuum lines and mass spectrometer in this lab will distinguish methane derived from biogenic processes from that derived from thermochemical processes.

C. Collection of sediment samples from known gas seep areas to ensure that sample collection procedures effectively maintain ambient light hydrocarbon concentrations.

D. Determine extraction efficiency by injecting analytical standards

in wet sediments as outlined in BLM analytical procedure (Appendix II).

E. Collection of plankton from the water column over the areas in which sediment samples were collected and the extraction and characterization of the plankton hydrocarbons. This is necessary to define the input of hydrocarbons derived from plankton to the sediments and to enable better interpretation of the hydrocarbon distribution in the sediments.

X. SUMMARY OF FOURTH QUARTER OPERATIONS

A. Ship Activities

1a. Dates:

May 20 to June 15, 1976 - Eastern Gulf of Alaska
June 15 to August 1, 1976 - Western Gulf of Alaska and Cork Inlet
September 15 to October 15, 1976 - Northern Bering Sea

1b. Vessel: R.V. SEA SOUNDER, chartered by U.S.G.S.

2. Scientific Party:

Edward Ruth: Staff Research Associate, U.C.L.A.
Mark Sandstrom: Post-Graduate Geochemist, U.C.L.A.
Daniel Stuermer: Post-Doctoral Research Fellow, U.C.L.A.

3. Methods:

a. Collection of undisturbed surface sediments, free of hydrocarbon contamination, in frame-supported Van Veem grab sampler.

TABLE 1. ELEMENTAL ANALYSIS

Station	Latitude	Longitude	Water Depth (m)	Carbon (%)			Nitrogen (%)	Sulfur (%)
				Total	Organic	$CaCO_3$		
EASTERN BERING SEA								
EBBS 8	58°17.9'N	159°31.6'W	26	0.27	0.23	0.33	0.026	0.051
EBBS 12	56°09.5'	162°09.5'	84	0.45	0.14	2.58	0.018	0.019
EBBS 17	55°26.4'	165°49.1'	119	0.92	0.76	1.33	0.089	0.090
EBBS 19	56°40.5'	163°56.6'	75	0.76	0.39	3.25	0.064	0.066
EBBS 24	58°46.4'	162°29.4'	47	0.37	0.33	0.33	0.032	0.057
EBBS 28	57°10.4'	165°04.4'	70	0.76	0.59	1.33	0.140	0.079
EBBS 35	56°12.4'	168°20.4'	160	0.54	0.41	1.08	0.038	0.014
EBBS 37	57°05.3'	167°00.6'	75	0.53	0.41	1.00	0.045	0.051
EBBS 38	57°40.1'	166°05.8'	66	0.84	0.66	1.50	0.074	0.045
EBBS 40	58°07.3'	165°15.6'	46	0.31	0.32	0.00	0.033	0.044
EBBS 41	58°46.9'	164°14.2'	36	0.61	0.37	2.00	0.008	0.027
EBBS 43	58°42.5'	166°16.0'	37	0.49	0.30	1.58	0.029	0.038
EBBS 46	57°34.5'	168°04.5'	70	0.95	0.42	4.40	0.043	0.045
EBBS 54	56°56.4'	170°54.8'	105	1.01	0.68	2.75	0.104	0.096
EBBS 56	58°06.5'	169°05.4'	71	0.61	0.47	1.67	0.083	0.099
EBBS 58	58°43.5'	167°21.0'	44	0.61	0.31	2.50	0.033	0.054
EBBS 59	59°11.9'	167°17.1'	38	0.53	0.27	2.17	0.029	.058
EBBS 64	58°01.5'	171°17.0'	90	1.07	0.77	2.50	0.094	0.127
EBBS 65	57°24.9'	172°04.6'	109	1.08	0.67	3.42	0.074	0.077
PMEL 45B	55°39.8'	164°04.1'	95	1.22	0.76	3.83	0.035	0.042
WESTERN GULF OF ALASKA								
GASS101	59°18.6'N	152°18.6'W	170	0.53	0.18	2.92	0.009	0.010
GASS102	59°10.1'	152°04.4'	101	2.44	0.18	18.83	0.019	0.016
GASS103	59°00.6'	151°48.2'	96	5.41	0.53	40.65	0.092	0.043
GASS104	58°50.0'	151°26.4'	100	2.65	0.39	18.83	0.046	0.020
GASS105	58°40.1'	151°07.3'	160	1.37	0.76	5.08	0.058	0.037
GASS119	57°06.0'	156°00.6'	250	1.25	0.74	4.25	0.083	0.063
GASS120	56°55.0'	155°44.1'	294	1.51	1.08	3.58	0.132	0.075
GASS121	56°43.6'	155°28.0'	238	1.81	1.13	5.66	0.150	0.010
GASS122	56°31.1'	155°11.9'	42	5.02	0.18	40.32	0.023	0.042
GASS123	56°19.1	154°55.1'	12	1.69	0.78	7.58	0.083	0.066
GASS124	56°07.0'	154°39.0'	107	1.41	0.92	4.08	0.105	0.071
GASS133	55°44.8'	158°49.3'	68	0.83	0.35	4.00	0.029	0.037
GASS134	55°33.6'	158°40.0'	152	1.59	1.09	4.17	0.101	0.150
GASS135	55°20.0'	158°25.4'	145	0.97	0.42	4.58	0.052	0.039
GASS137	54°55.0'	157°58.5'	102	2.15	0.34	15.08	0.019	0.020
GASS160	53°44.2'	164°26.3'	195	0.60	0.31	2.42	0.030	0.169
EASTERN GULF OF ALASKA								
GASS 01	59°50.2'N	149°30.5'W	254	0.98	0.77	1.75	0.036	0.034
PWS 13	60∘35.0	146°55.0'	439	1.29	0.71	4.83	0.051	0.041
GASS 41	59°55.1'	142°39.5'	118	0.99	0.84	1.25	0.038	0.042
GASS 43	59°45.0'	143°52.8'	123	0.98	0.66	2.67	0.046	0.058
GASS 50	59°47.7'	145°09.0'	170	0.95	0.78	1.42	0.048	0.061
GASS 51	59°57.6'	145°07.8'	147	1.09	0.73	3.00	0.081	0.052
GASS 52	60°07.6'	145°06.5'	85	1.12	0.81	2.58	0.044	0.042
GASS 54	60°13.9'	146°48.6'	208	1.05	0.51	4.50	0.033	0.028
GASS 55	60°04.5'	146°42.6'	117	1.39	0.92	3.92	0.081	0.050
PWS 107	61°00.3'	146°45.9'	368	1.01	0.92	0.75	0.087	0.050
PRECISION				± 0.08	± 0.08	± 0.08	± 0.003	±0.006

TABLE 2. Characterization of Sediment Organic Fractions Determined By Gravimetric Analysis of Extracts

Sample	Organic Carbon (%)	Solvent Extractable (μg/g dry sediment)	Liquid Chromatography (μg/g dry sediment)		
			Saturate	Aromatic	Polar
GASS 41	0.84	143.11	7.07	31.80	70.67
GASS 43	0.66	141.10	18.71	18.71	76.92
GASS 50	0.78	170.36	29.77	47.97	82.70
GASS 52	0.81	146.41	15.52	28.83	88.73
GASS 55	0.92	162.32	20.55	26.71	100.68
PWS 107	0.92	159.55	12.27	39.27	98.18
GASS 51	0.73	195.82	26.11	11.19	143.60

TABLE 3. GAS CHROMATOGRAPHIC DATA - High Molecular Weight Hydrocarbons

COMPOUND	CONCENTRATION (µg/g dry sediment)						
	GASS 41	GASS 43	GASS 50	GASS 51	GASS 52	GASS 55	PWS 107
nC_{15}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nC_{16}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nC_{17}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pristane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nC_{18}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phytane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nC_{19}	0.0	0.0	0.043	0.067	0.0	0.0	0.425
nC_{20}	0.0	0.060	0.199	0.327	0.329	0.462	0.713
nC_{21}	0.110	0.163	0.574	0.394	0.966	0.996	0.406
nC_{22}	0.438	0.322	0.737	0.628	1.269	1.393	0.765
nC_{23}	0.531	0.432	0.724	0.641	1.362	1.438	1.083
nC_{24}	0.523	0.207	0.557	0.505	0.365	1.119	0.117
nC_{25}	0.702	0.201	0.727	0.463	0.368	1.338	0.149
nC_{26}	0.671	0.152	0.582	0.376	0.230	0.759	0.088
nC_{27}	0.790	0.189	0.499	0.449	0.412	0.245	0.233
nC_{28}	0.715	0.134	0.460	0.423	0.247	0.213	0.069
nC_{29}	0.984	0.187	0.758	0.415	0.138	0.248	0.194
nC_{30}	0.573	0.077	0.541	0.166	0.095	0.082	0.045
nC_{31}	0.499	0.083	0.394	0.160	0.171	0.094	0.134
nC_{32}	0.052	0.0	0.0	0.0	0.0	0.0	0.0
Total n-Alkanes	6.588	2.206	6.795	5.014	5.952	8.387	4.421
Odd/Even Ratio	1.217	1.230	1.195	1.923	1.348	1.082	1.223
Total Isoprenoid	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Major Peak	nC_{29}	nC_{23}	nC_{29}	nC_{23}	nC_{23}	nC_{23}	nC_{23}
Pristane/ Phytane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nC_{17}/Pristane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
nC_{18}/Phytane	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unresolved background	0.70%	88.6%	77.19%	80.8%	46.5%	45.9%	64.3%

TABLE 4. GAS CHROMATOGRAPHIC DATA - Low Molecular Weight Hydrocarbon

STATION	CH_4 Concentration (ng/g dry sediment)	STATION	CH_4 Concentration (ng/g dry sediment)
EBBS 17	1.1	GASS 101	3.4
EBBS 19	3.3	GASS 103	0.0
EBBS 28	20.9	GASS 105	2.1
EBBS 35	4.3	GASS 119	3.1
EBBS 37	0.4	GASS 122	0.7
EBBS 38	3.8	GASS 124	0.7
EBBS 40	3.0	GASS 133	4.2
EBBS 41	1.4	GASS 134	5.8
EBBS 43	0.0	GASS 135	1.1
EBBS 46	6.4		
EBBS 54	2.8		
EBBS 56	3.2		
EBBS 58	8.8		
EBBS 65	12.2		

FIGURE 1.

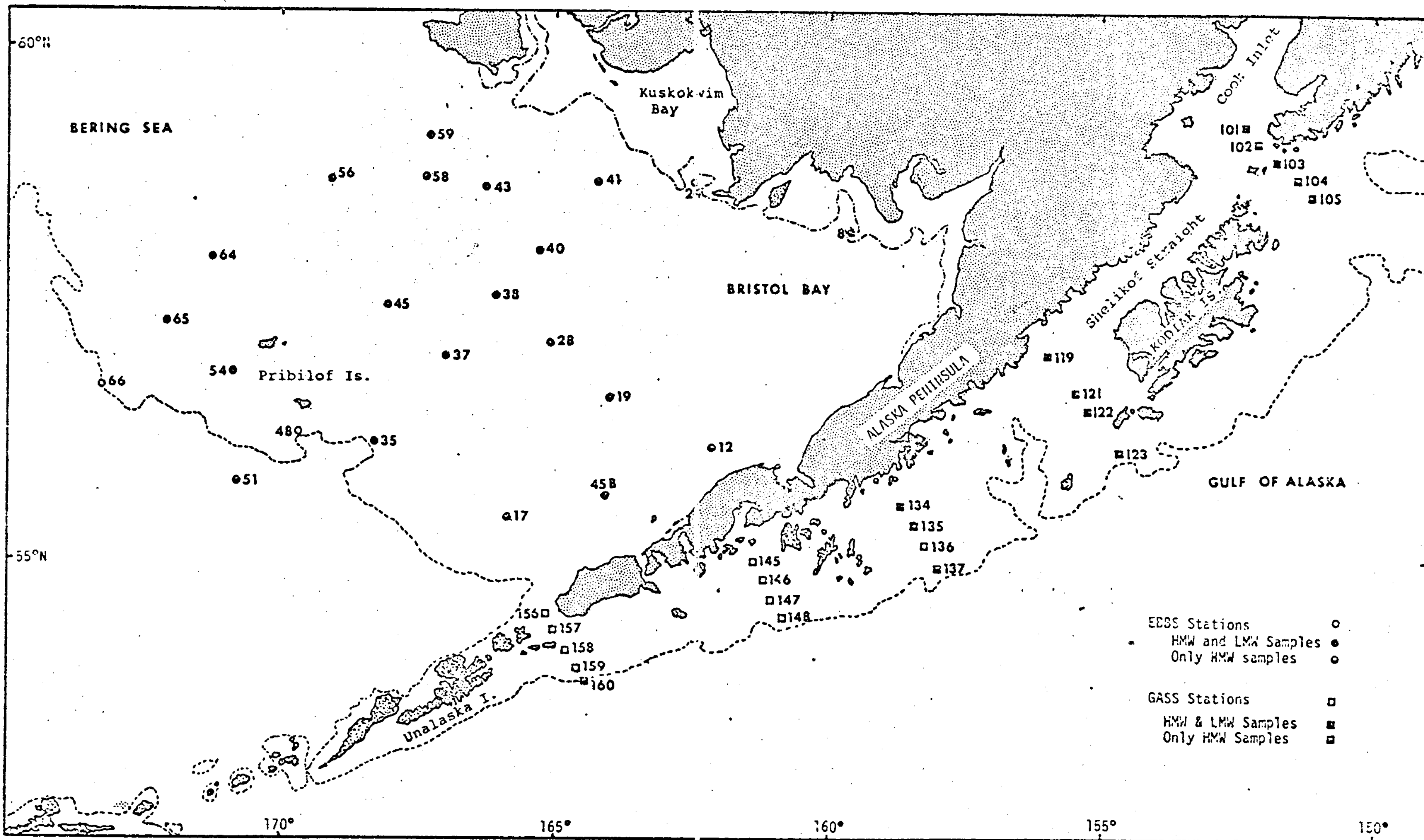
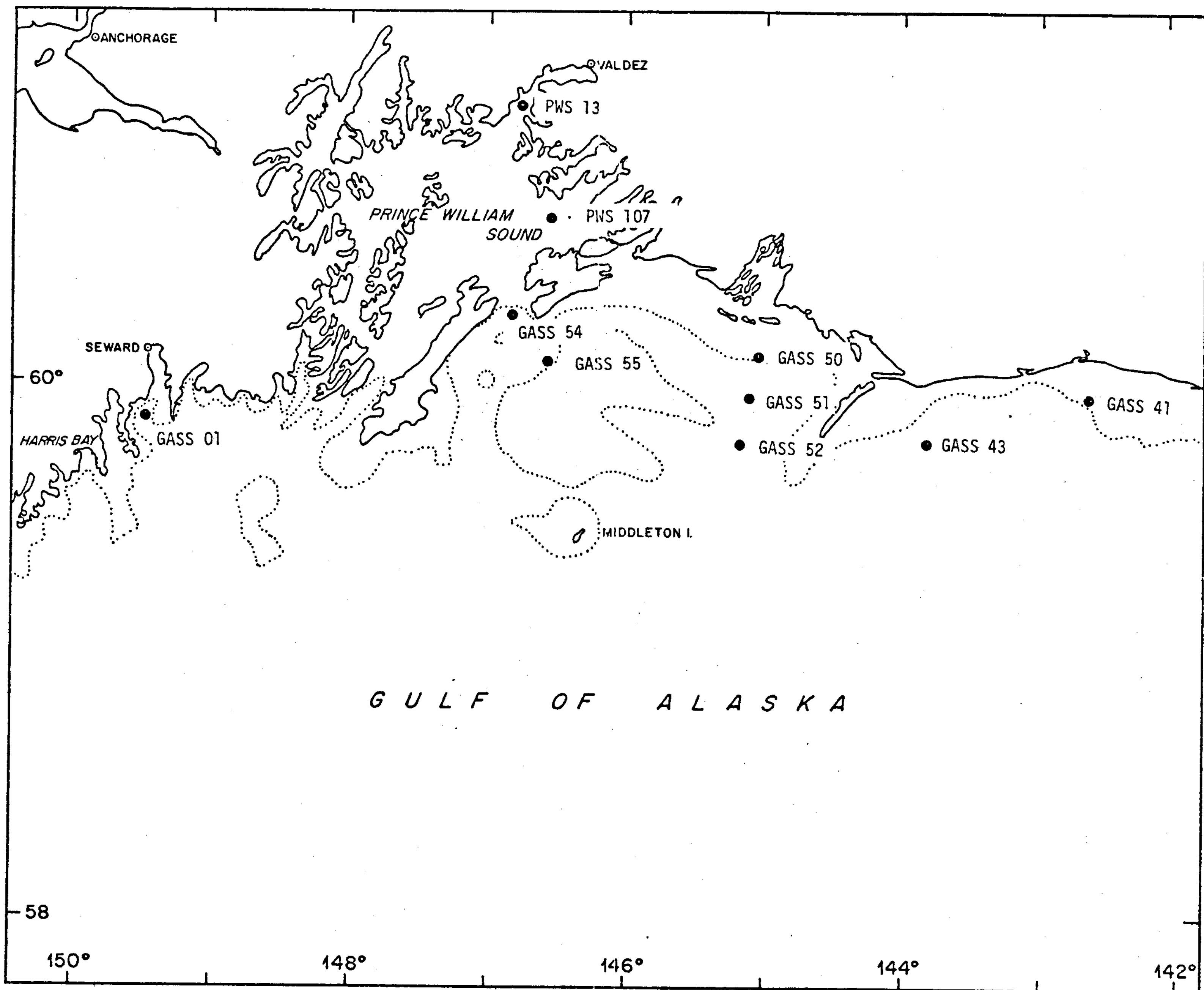


Fig. 2



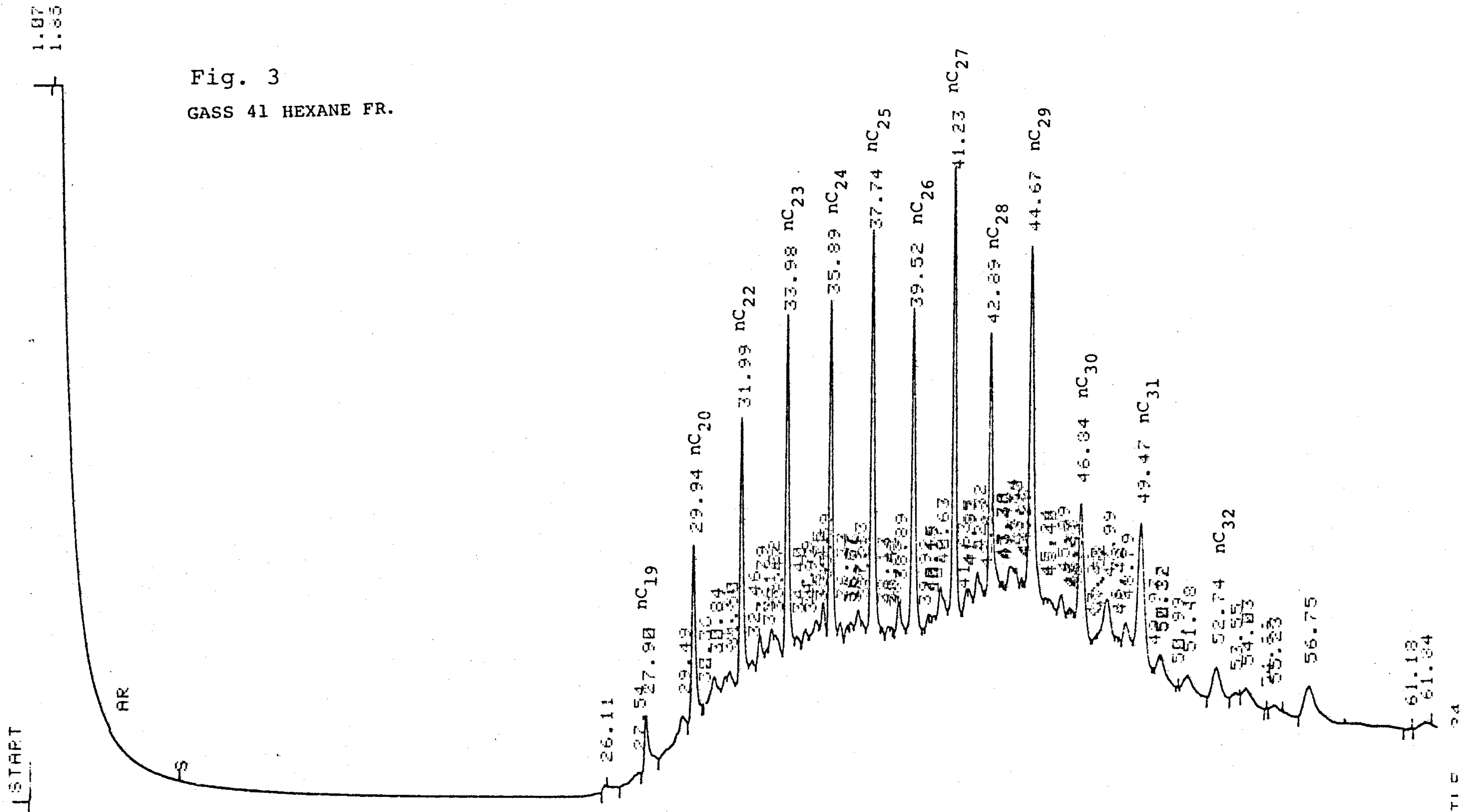
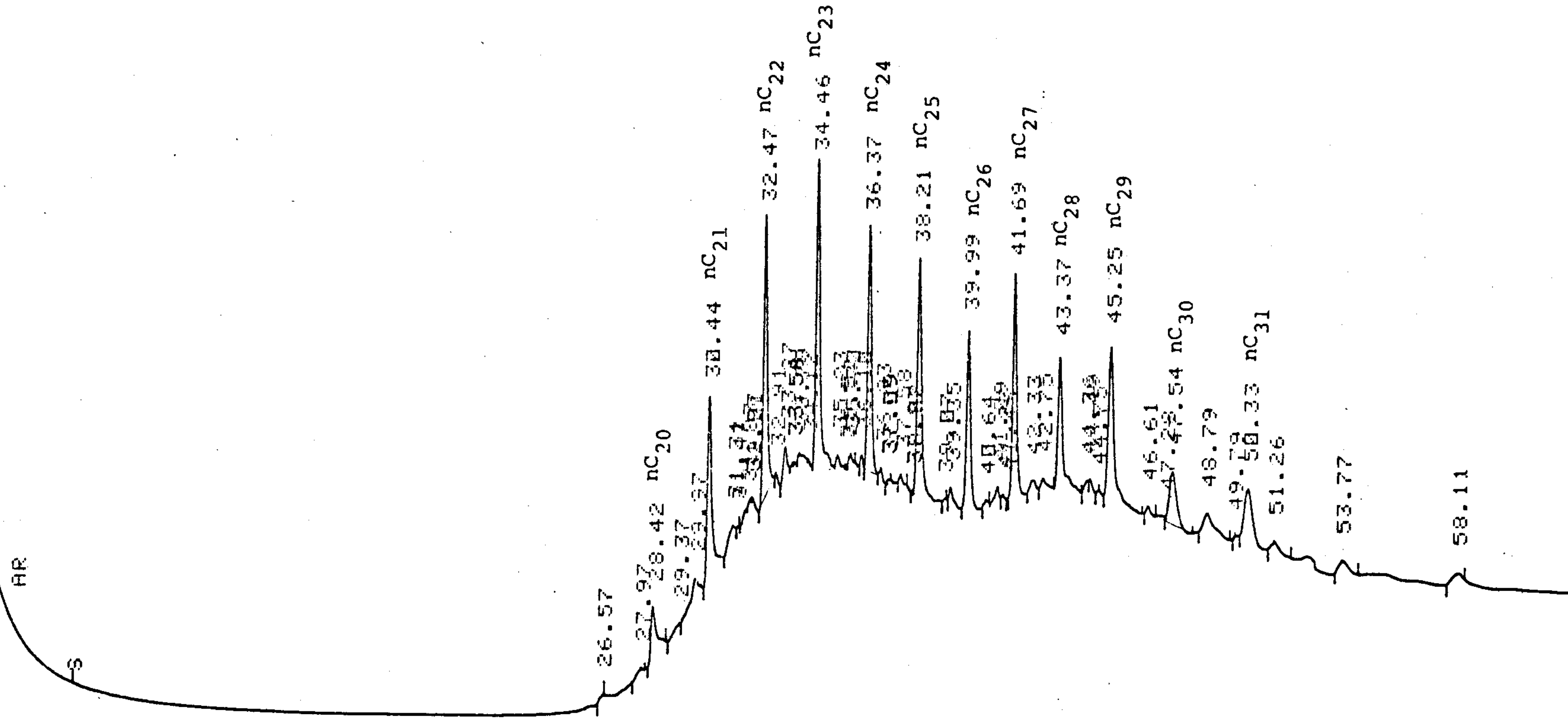


Fig. 3
GASS 41 HEXANE FR.

Fig. 4
GASS 43 HEXANE FR.



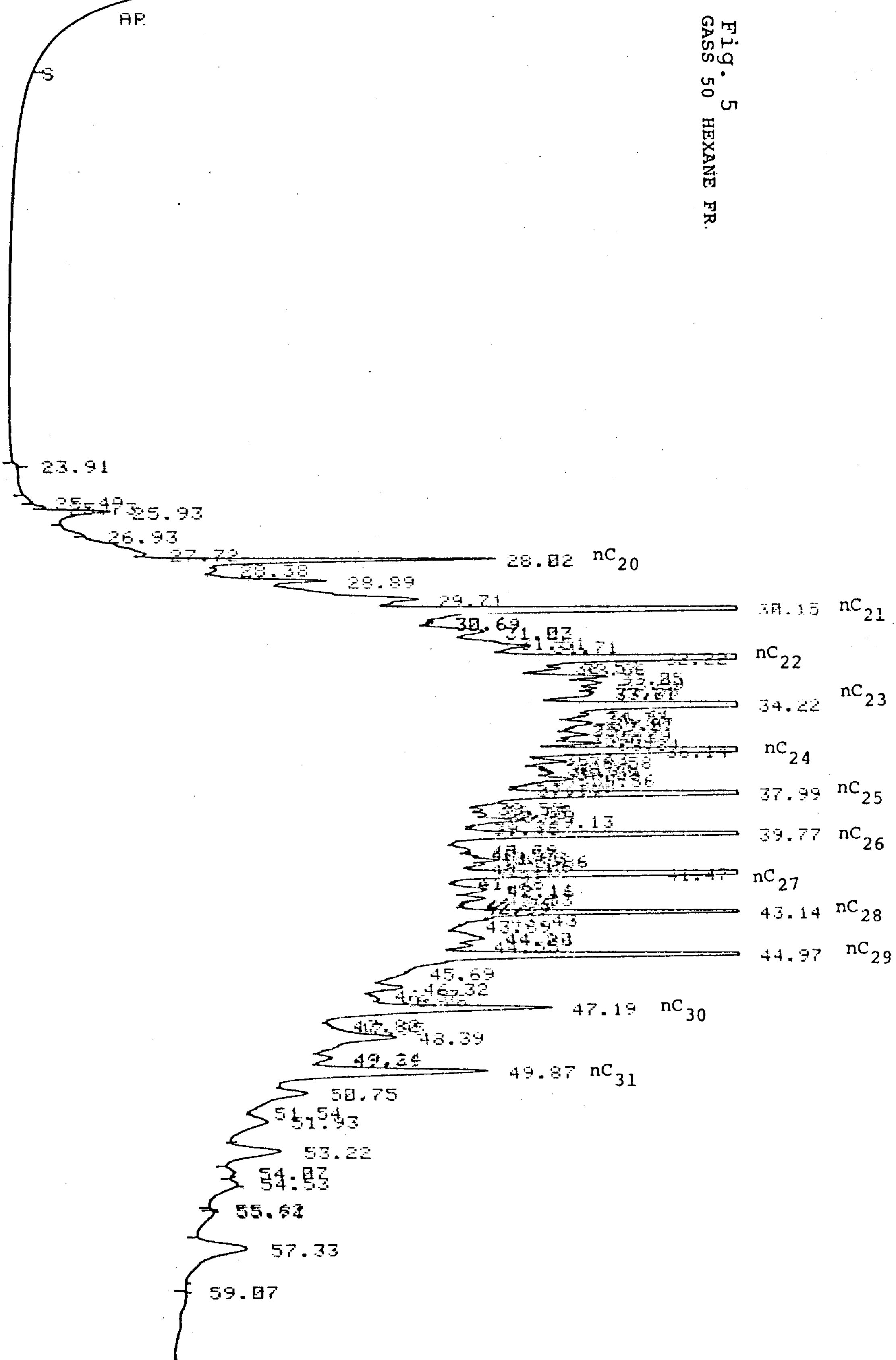


Fig. 5
GASS 50 HEXANE FR.

Fig. 6

GASS 51 HEXANE FR.

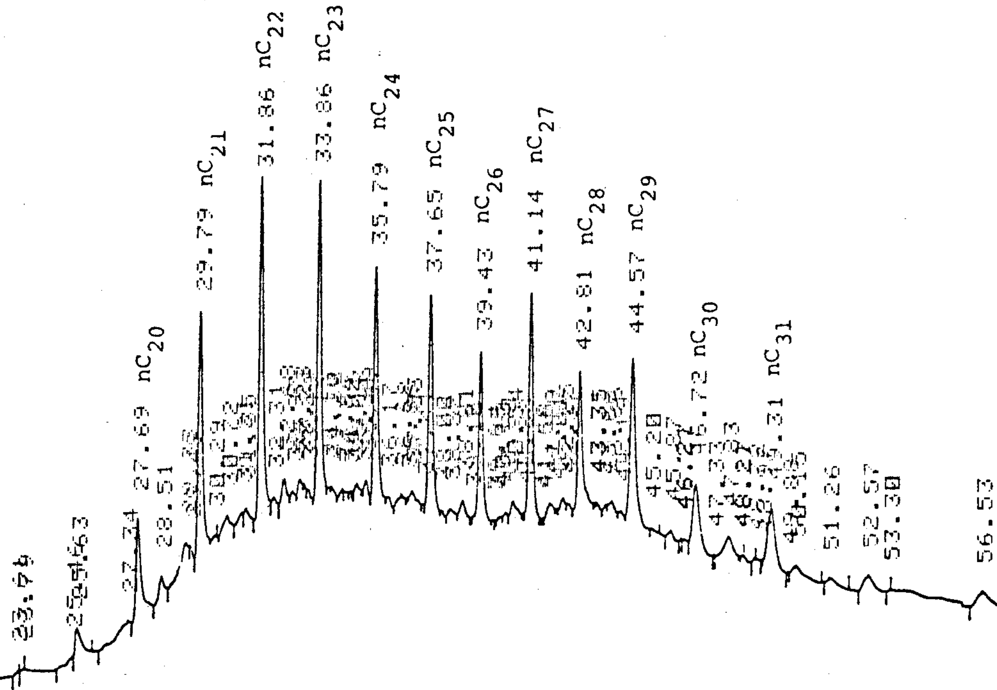
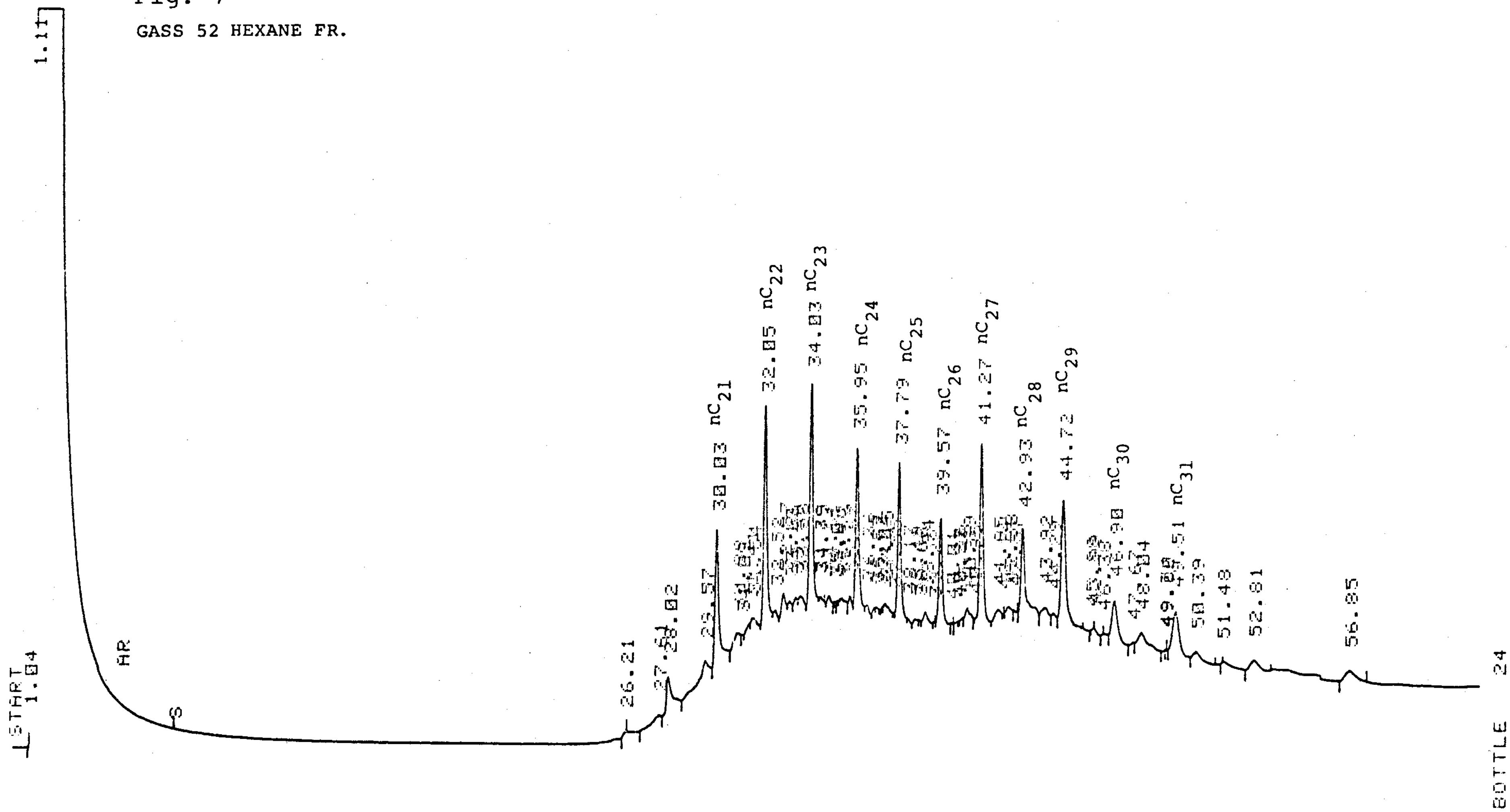


Fig. 7

GASS 52 HEXANE FR.



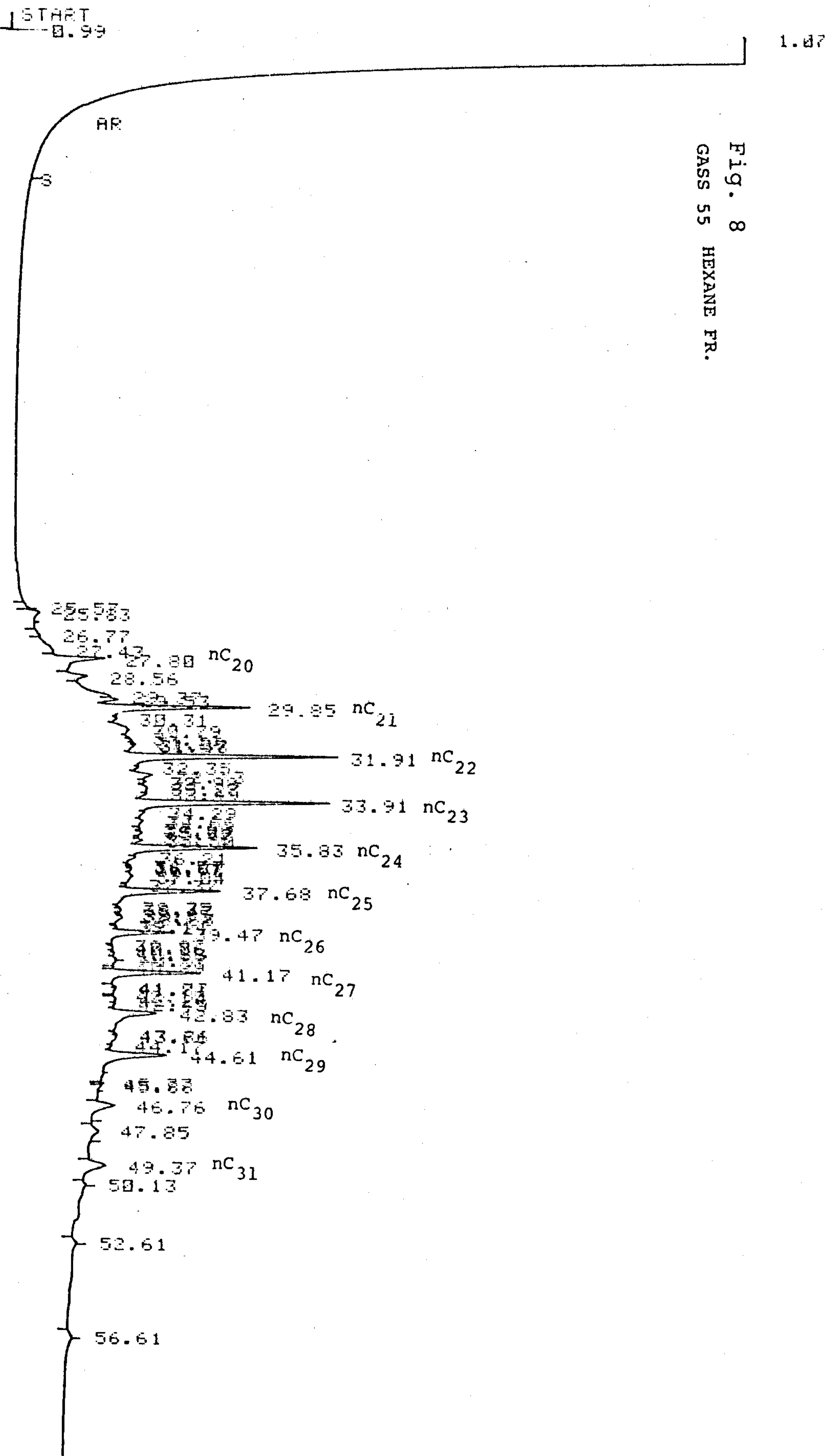


Fig. 8

GASS 55 HEXANE FR.

Fig. 9

PWS 107 HEXANE FR.

1.10

START

AR

23.79

27.93 nC_{20}

30.03 nC_{21}

32.10 nC_{22}

34.09 nC_{23}

36.01 nC_{24}

37.86 nC_{25}

39.63 nC_{26}

41.33 nC_{27}

nC_{28}

44.77 nC_{29}

45.61

46.42

46.99 nC_{30}

47.75

48.14

49.05

49.59 nC_{31}

50.45

51.54

52.89

53.65

56.93

BOTTLE 24

Fig. 11 n-Alkane Standard

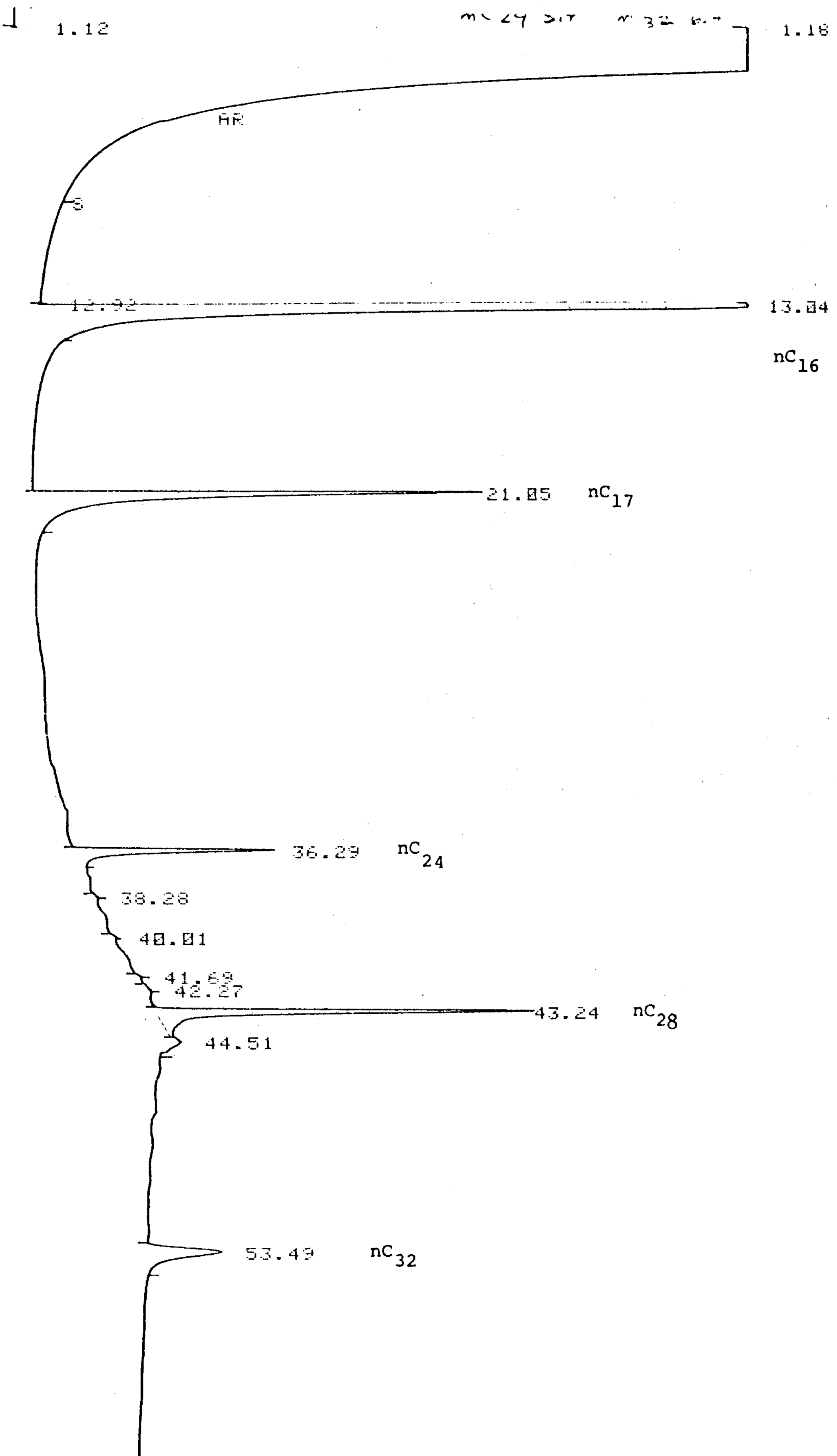


Fig. 10 GASS 43 HEXANE FRACTION COINJECTED WITH n-ALKANE STANDARD.

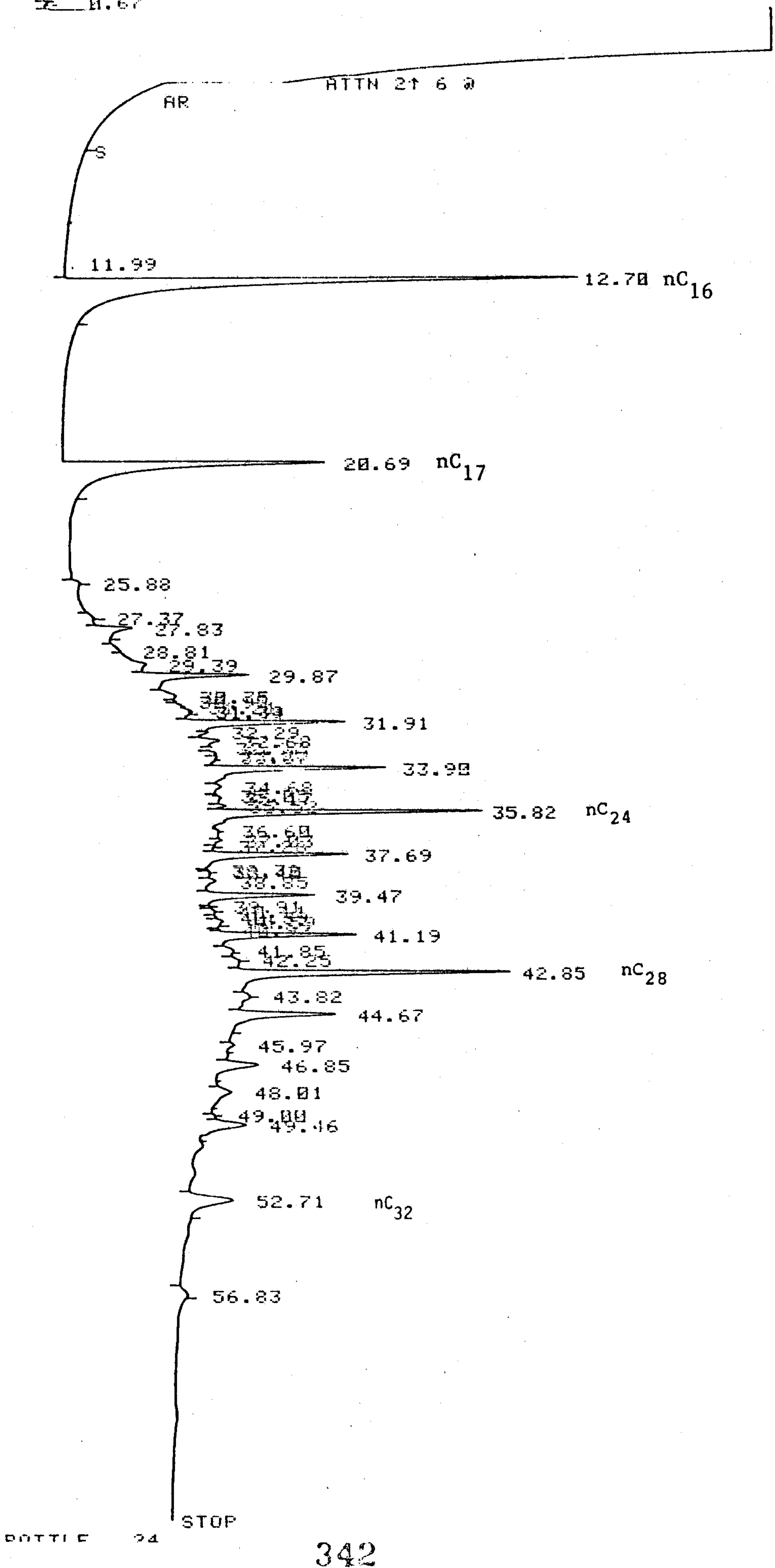


Fig. 12 LOW MOLECULAR WEIGHT HYDROCARBON STANDARD

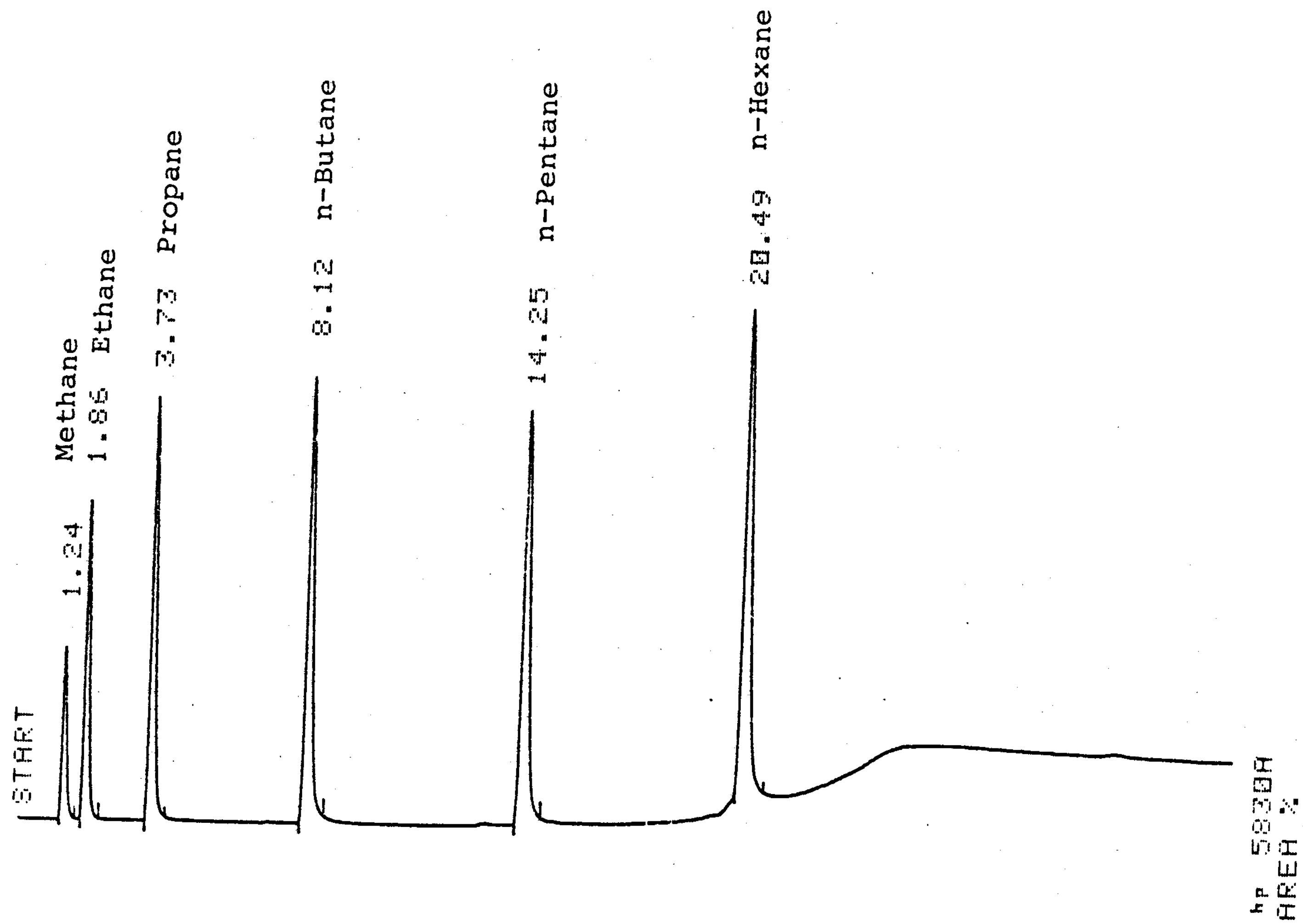


Fig. 10 GASS 43 HEXANE FRACTION COINJECTED WITH n-ALKANE STANDARD.

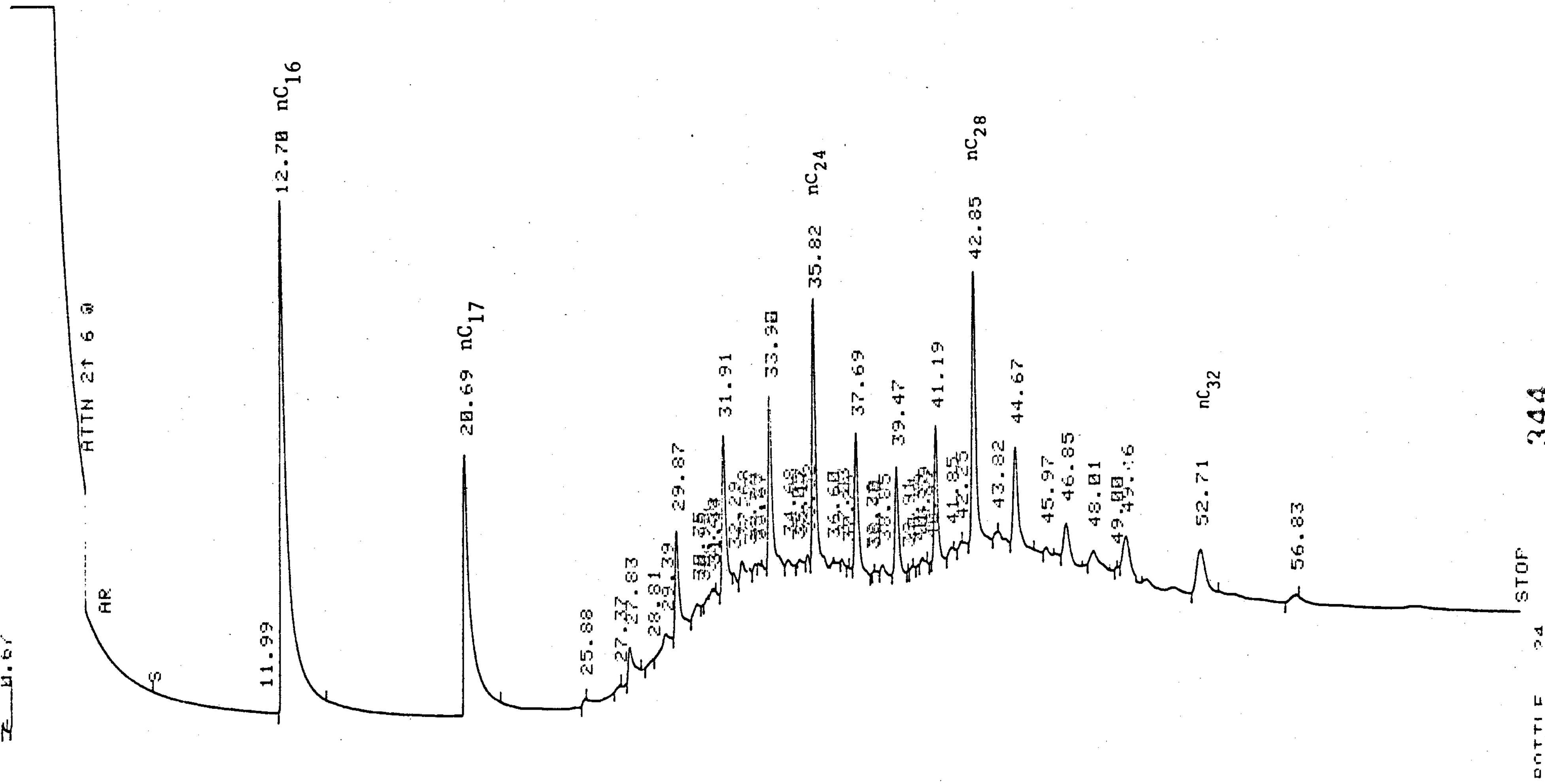


Fig. 11 n-Alkane Standard

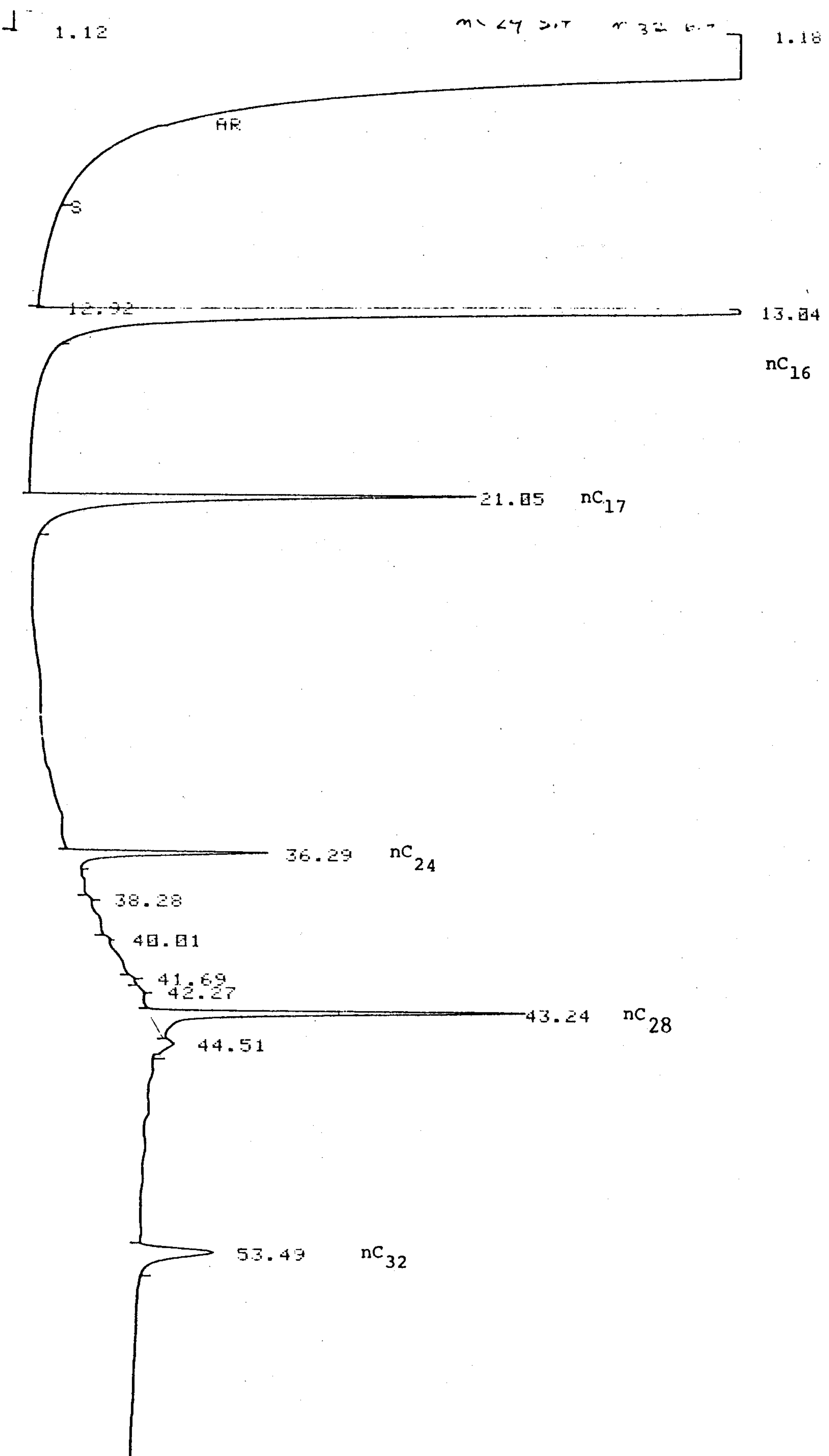
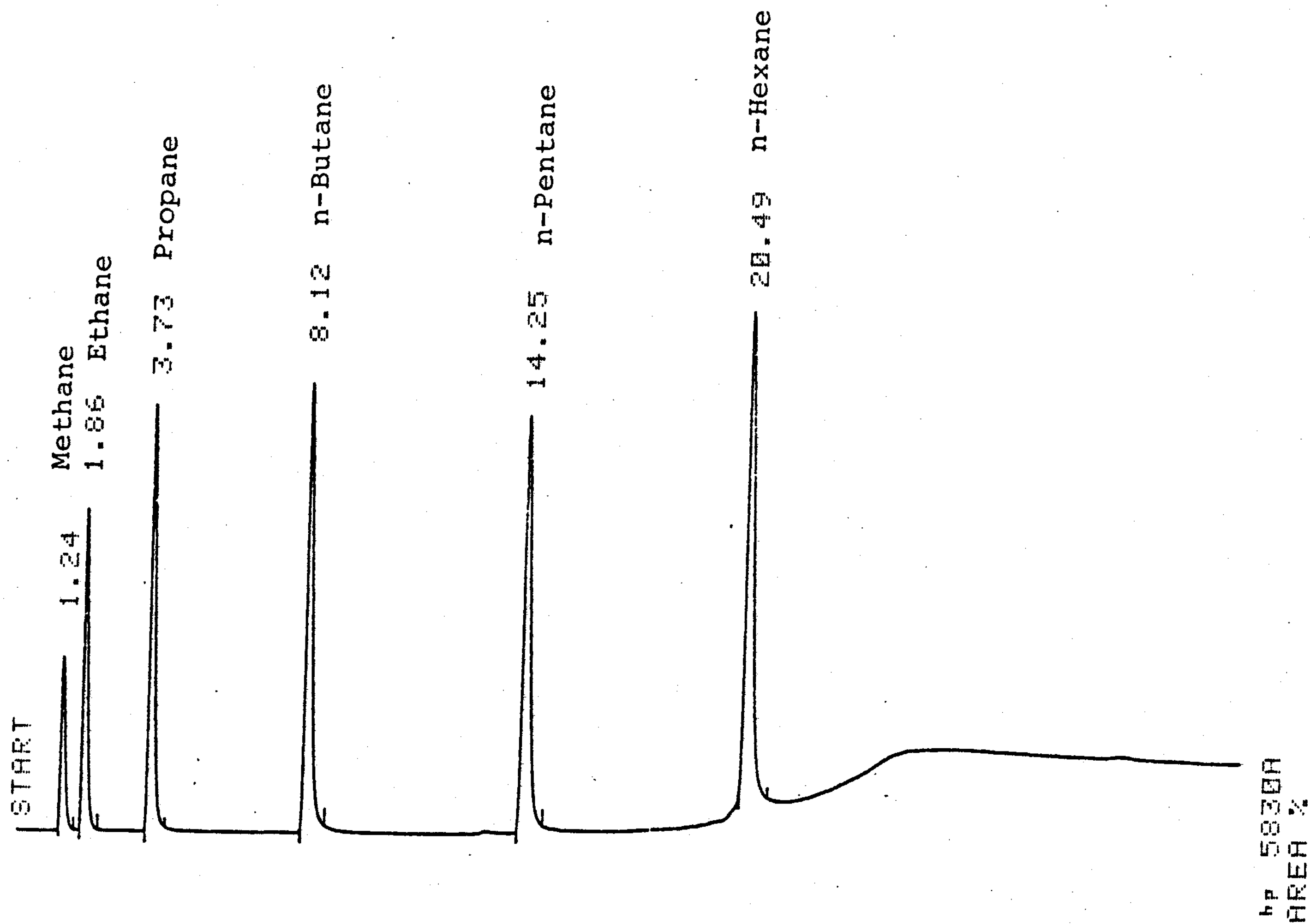


Fig. 12 LOW MOLECULAR WEIGHT HYDROCARBON STANDARD



APPENDIX I

CRUISE REPORT: DISCOVERER - LEGS III AND IV

TO

UNIVERSITY OF ALASKA

from

Institute of Geophysics & Planetary Physics

University of California

Los Angeles, California 90024

for

"Characterization of Organic Matter in Sediments from

Gulf of Alaska, Bering and Beaufort Seas"

Subcontract No. F-91770, PO #F01770

CRUISE REPORT: DISCOVERER - LEGS III AND IV

The initial phase of our efforts to characterize hydrocarbons in marine sediments from eastern Bering Sea and western Gulf of Alaska was completed with the collection of surface sediments on Legs III and IV on the NOAA ship DISCOVERER. Leg III began September 11, 1975 in Dutch Harbor, Alaska; 23 stations were occupied and 22 high molecular weight (HMW) and 18 low molecular weight (LMW) hydrocarbon samples were collected. Leg IV terminated October 10, 1975, in Kodiak, Alaska, after 22 stations were occupied and 12 HMW hydrocarbon and 9 LMW hydrocarbon samples had been collected. Hydrocarbon samples could not be obtained at all stations occupied because of insufficient sediment cover.

The suite of samples collected in the Bering Sea appear sufficient to define and characterize background levels of hydrocarbons in this area, while further sampling in the western Gulf of Alaska area is necessary. Additional recommendations for sampling in these areas may be made after initial extraction and analysis of the sediment is completed. Specific problems and recommendations concerning the sampling program are detailed below.

One of the objectives of this study includes the collection of uncontaminated, undisturbed surface sediments. Approximately 350 gm wet sediment are needed for HMW and LMW hydrocarbon analysis, so a sampling device that samples a large surface area is needed. Accordingly, an aluminum Van Veem grab sampler with teflon hinges (greaseless) and large top opening doors that allow easy sampling of the surface of the recovered sample was obtained for use on Legs III and IV of the DISCOVERER.

Lack of time prohibited testing this device before its use on the DISCOVERER and it proved to be unsuccessful in repeated sampling attempts because of problems with the weights and aluminum latch. (A similar Van Veem grab has since been modified and operated successfully).

Consequently, a steel Van Veem grab on board DISCOVERER was used to collect samples during Legs III and IV. Although generally reliable in recovering samples, it was less than ideal in sampling for hydrocarbon analysis because of possible contamination from oil or grease on hinges, and the inability to remove sediment samples without dumping and homogenizing the entire sample into a pan or tray.

One problem encountered during the sampling operation was the inability to accurately determine when the grab sampler was at the sediment surface. On many occasions during Legs III and IV, the Van Veem grab was retrieved untriggered, or triggered and empty, and it was unclear whether or not the sampler had been on the sediment surface. Consequently, it is recommended that a tensionometer on the winch cable A-frame be installed so that it is easier to determine when the sampler penetrates the sediment surface. Furthermore, a pinger placed on the winch cable could more accurately determine when the sampler is close to the sediment surface, although this involves more time and personnel to monitor recorders on the bridge.

Initial plans for subsequent sampling programs are to modify and test and aluminum Van Veem grab so that it successfully recovers sediment, and to determine the most efficient rate of descent and method of bottom penetration for this type of Van Veem grab.

Alternatively, we recommend the construction and use of a Soutar Van Veem sampling device for subsequent cruises because we feel that such a

device is not only more efficient and reliable, but it is best for our sampling requirements. This type of sampler, constructed of aluminum and teflon, is mounted on a frame which will ensure vertical penetration of the sediment and sample recovery. Additional advantages include ease of setting the trigger, the ability to store the grab within the frame protected from ship contamination, and also the potential of mounting a camera or pinger on the A-frame. Moreover, the large top-opening doors should allow the collection of the fine surface layer of sediment because there is no hydrostatic head formed by the descent of the sampler and, upon retrieval the sediment surface is undisturbed.

Another problem related to the low sediment recovery was the apparent lack of sufficient sediment cover for grab sampling. It is assumed that rocky, erosional bottom environments are common in many of the western Gulf of Alaska stations; and in the Bering Sea at stations EBBS 12 and 24.

It is recommended that future stations be chosen in depressions, troughs, and deeps where there is greater probability for sediment accumulation. Pre-cruise planning should involve the use of available sub-bottom profiles and existing bottom sampling data to define areas of sufficient sediment cover. Furthermore, the use of a 3.5 kHz depth recorder while approaching a potential station would ensure that the station finally chosen had sufficient sediment cover. This would allow some preliminary surveying to find suitable areas, if necessary. On one station during Leg IV, GASS 146, three grab samples of silty sand and gravel were collected for the benthic organisms study, but the ship

apparently drifted into a rocky area before the hydrocarbon grab sample could be collected. Use of a 3.5 kHz depth recorder might have helped maintain position over the apparently patchy sediment cover.

Finally, samples were not collected at some of the proposed stations because of lack of available time for sampling. Engine repairs in Kodiak delayed departure and consequently shortened Leg IV by 2 days; rough weather forced termination of Leg IV 1.5 days earlier than planned. The main effect of this shortened time was the failure to occupy proposed stations 106 through 110 on the continental shelf west of Kodiak Island.

During Leg III in the Bering Sea, the large number of stations for other studies and the desire to complete the entire sampling grid resulted in a small amount of time alloted to the hydrocarbon sampling program on some stations. It is recommended that future cruises allow sufficient time for collection of sediment samples, taking into account the occasional necessity of repeated attempts.

TABLE I: LEG III DISCOVERER, EASTERN BERING SEA

STATION	Latitude	Longitude	Hydrocarbon Samples HMW	Hydrocarbon Samples LMW	Comments
EBBS 8	58°17.9' N	159°31.6' W	X	X	Sandy silt
EBBS 12	56°09.5'	162°07.6'	X	0	Lt. green sand
EBBS 17	55°26.4'	165°49.1'	X	X	Dk. green silty clay
EBBS 19	56°40.5'	163°56.6'	X	X	Dk. green silty clay
EBBS 24	58°46.4'	162°29.4'	X	0	Sand, gravel
EBBS 28	57°10.4'	165°04.4'	X	X	Lt. green silty clay
EBBS 35	56°12.4'	168°20.4'	X	X	Dk. green sandy silt
EBBS 37	57°05.3'	167°00.6'	X	X	Dk. green silty clay
EBBS 38	57°40.1'	166°05.8'	X	X	Lt. Green silty clay
EBBS 40	58°07.3'	165°15.6'	X	X	Silty sand
EBBS 41	58°46.9'	164°14.2'	X	X	Lt. green sand
EBBS 43	58°42.5'	166°16.0'	X	X	Dk. green sandy silt
EBBS 46	57°34.5'	168°04.5'	X	X	Dk. green silty clay
EBBS 48	56°19.1'	169°41.9'	0	0	Rocky bottom (?)
EBBS 51	55°48.6'	170°48.0'	X	0	Shipeck sampler--(may be contaminated)
EBBS 54	56°56.4'	170°54.8'	X	X	Dk. green silty clay
EBBS 56	58°06.5'	169°05.4'	X	X	Dk. green silty clay
EBBS 58	58°43.5'	167°21.0'	X	X	Green silty sand
EBBS 59	59°11.9'	167°17.1'	X	X	
EBBS 64	58°01.5'	171°17.0'	X	X	Dk. green silty clay
EBBS 65	57°24.9'	172°04.6'	X	X	
EBBS 66	56°45.6'	173°13.7'	0	0	Rocky bottom (?)
PMEL 45B	55°39.8'	164°04.1'	X	X	Green sandy silt
TOTAL: 23			21	18	

TABLE II: LEG IV DISCOVERER - WESTERN GULF OF ALASKA

Station	Latitude	Longitude	Hydrocarbon Samples		Comments
			HMW	LMW	
GASS 101	59°18.6'N	152°23.5'W	X	X	Light green sand
GASS 102	59°10.1'	152°04.4'	X	0	
GASS 103	59°00.6'	151°48.2'	X	X	Light green silty sand
GASS 105	58°40.1'	151°07.3'	X	X	Light green silty clay
GASS 119	57°06.0'	156°00.6'	X	X	Light green silty clay
GASS 121	56°43.6'	155°28.0'	X	X	Light green clay
GASS 122	56°31.1'	155°11.9'	X	X	Sand, shell fragments
GASS 124	56°07.0'	154°39.9'	X	X	Lt. grn. silty clay; pebbles
GASS 133	55°44.8'	158°49.3'	0	0	Rocky bottom (?)
GASS 134	55°33.6'	158°40.0'	X	X	Lt. green clay
GASS 135	55°20.0'	158°25.4'	X	X	Lt. green sandy silt
GASS 136	55°07.5'	158°13.3'	0	0	Rocky bottom (?)
GASS 137	54°55.0'	157°58.5'	X	0	1/2 Jar sand, gravel
GASS 147	54°36.3'	161°1.62'	0	0	Rocky bottom (?)
GASS 146	54°49.5'	161°11.8'	0	0	Rocky bottom (?)
GASS 145	55°01.0'	161°19.8'	0	0	Rocky bottom (?)
GASS 148	54°23.7'	160°49.2'	0	0	Rocky bottom (?)
GASS 156	54°29.0'	165°11.4'	0	0	Only recovered mussell shell
GASS 157	54°16.7'	165°00.3'	0	0	Sponge, shells
GASS 158	54°4.2'	164°46.9'	0	0	Rocky bottom (?)
GASS 159	53°52.0'	164°33.7'	0	0	Rocky bottom (?)
GASS 160	53°44.2'	164°26.3'	X	0	Sandy silt
TOTALS 22 STATIONS			12	9	

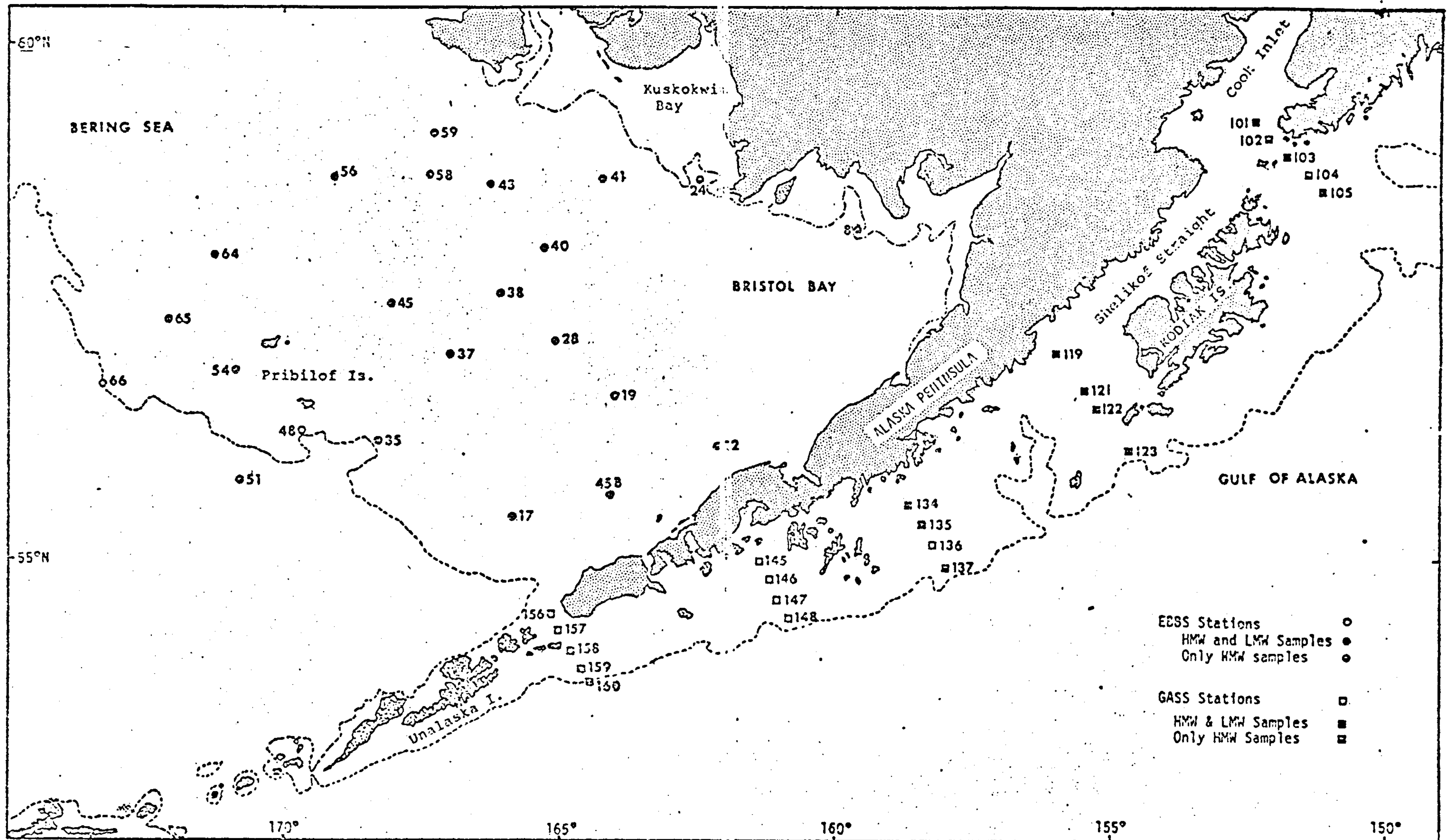


Figure 1: Locations of stations occupied during Legs III (EBBS) and IV (GASS) of NOAA ship, DISCOVERER.

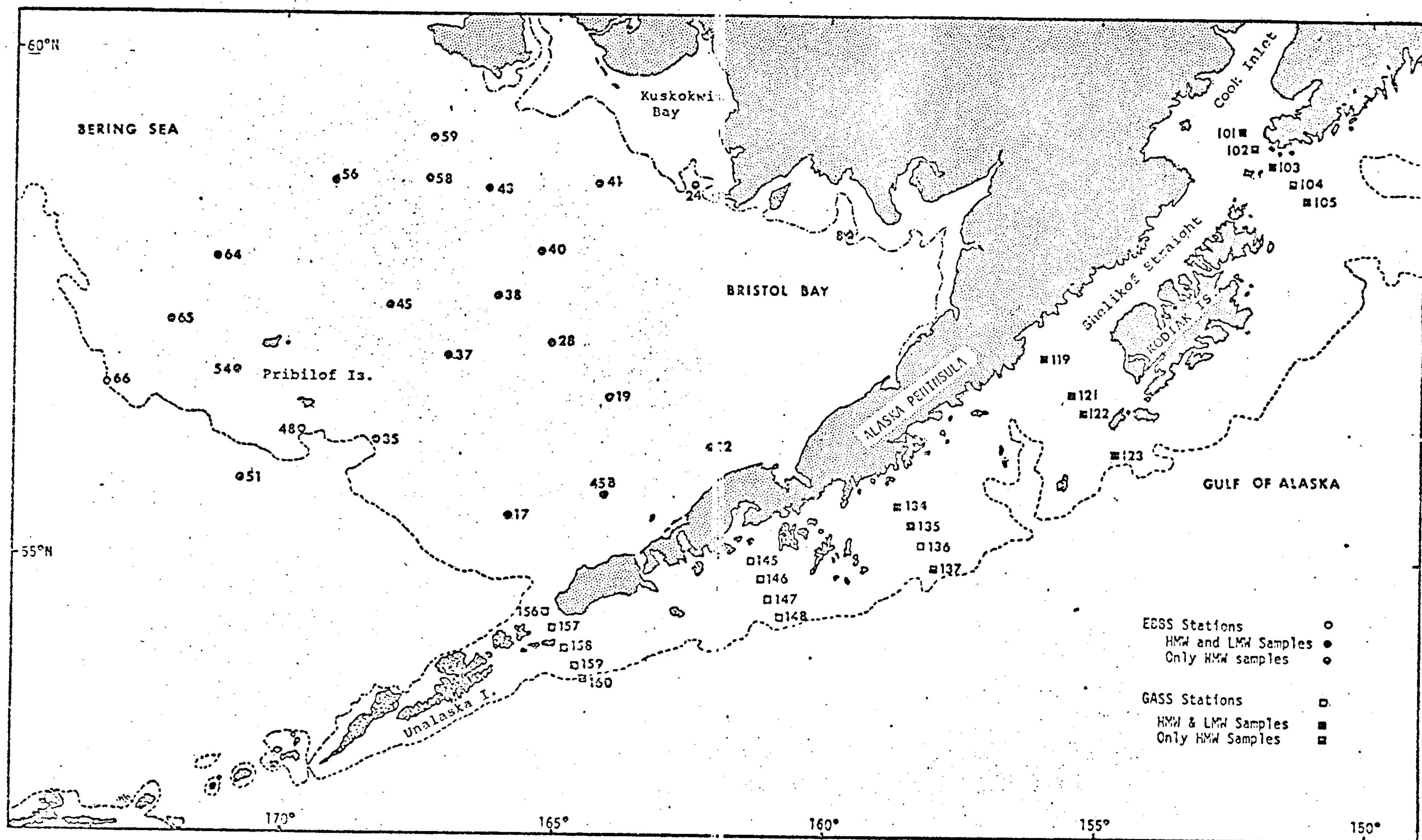


Figure 1: Locations of stations occupied during Legs III (EBBS) and IV (GASS) of NOAA ship, DISCOVERER.

APPENDIX II

HIGH MOLECULAR WEIGHT HYDROCARBON EXTRACTION PROCEDURE FOR SEDIMENT

Recommended by BLM February, 1976

Extraction of Sediment Samples

The recommended minimum sample weight is 100 g. This amount can be increased for sediments found to be very low in extractable organics. Every fifth sample will be spiked with a 0.1 μg hydrocarbon standard/g sediment using an appropriate n-alkane or iso-alkane standard and a polycyclic aromatic standard which falls outside of the spectra of the compounds being measured. The frozen sample is weighed into a pre-extracted sochlet thimble and rinsed with distilled water to remove organic salts. The water is double-distilled and percolated through Chromosorb-102 resin to remove trace organics. It should be stored in either glass or teflon containers. Excess water will be removed under vacuum. The filtrate is then extracted three (3) times with 25 ml of n-hexane. The extracts are then combined and saved for later addition to the sediment extract.

The sediment sample is then freeze-dried and removed when dry. This procedure serves to minimize sample manipulation. The freeze-dried sediment is then extracted by the soxhlet technique.

1. The Soxhlet Extraction

The freeze-dried sediment in soxhlet thimble is placed in the extractor and extraction is allowed to proceed for 100 hours, or 300 turnovers, with one solvent change after twenty-four (24) hours. The solvent system used for sediment extraction and pre-extraction of the soxhlet thimbles is a toluene:methanol (3:7) azeotrope. The extracts

obtained from the sediment extraction and water washing are then combined and reduced in volume using a rotary evaporator. The dry weight of the extracted sediments is determined while in the thimble.

2. Sulfur Determination

The presence of elemental sulfur is determined on at least one sample per suite by dipping activated copper wire into the extract. If the wire becomes immediately coated, all samples should be tested for sulfur. The sulfur should be removed by charging the extract onto an activated copper column. It is then eluted with three column volumes of toluene. The solvent is again reduced in volume with a rotary evaporator. It is then saponified in accordance with the procedure described in paragraph E. The sample is transferred to a tared vial, the remaining solvent removed with pre-purified N_2 and weighed on an analytical balance sensitive to 0.1 mg. The sample is then dissolved in a small volume of n-heptane for column chromatographic analysis.

Saponification

All samples requiring saponification will be handled as described below. Saponification will be carried out by refluxing the sample with a 1:1:1 mixture of 0.5 N KOH in methanol:toluene:water. This mixture will be refluxed either under pre-purified nitrogen or with a filter of molecular sieve or silica gel to prevent contamination from external hydrocarbons in the laboratory.

Upon completion of the saponification, the mixture shall be diluted with an equal volume of distilled water or a saturated NaCl solution. If no emulsion exists, the toluene layer should be decanted, followed by

three extractions of the aqueous mixture with n-heptane. The volume of n-heptane used for each extraction should be equivalent to the volume of toluene initially used in the saponification. The toluene and n-heptane fractions are then combined and reduced in volume with a rotary evaporator.

If an emulsion exists, the entire mixture should be extracted three times with the n-heptane. The extracts obtained should be placed in glass centrifuge tubes with teflon-lined cap and then spun down so that the phases can be easily separated. A refrigerated centrifuge may aid separation. THe organic phases will then be combined and back-extracted with an equal volume of saturated sodium chloride solution. The saturated sodium chloride solution will then be re-extracted once with n-heptane and all the organic phases will be combined. The organic solvents will then be reduced in volume on a rotary evaporator.

Column Chromatography (L.C.)

All sample types will be chromatographed in the manner described below. A weight ratio of one-hundred (100) parts alumina to one (1) part lipid sample and two-hundred (200) parts silica gel to one (1) part lipid sample will be used. The column should have a length-to-i.d.-ratio of 20:1. Both the silica gel and the neutral alumina will be Activity I. The columns will be prepared by first suspending the absorbents in n-hexane and then pouring a slurry of silica in n-hexane into a standing column of n-hexane and allowing it to settle. This will be followed by pouring the alumina slurry into the column. The column should then be rinsed with two column volumes of n-hexane. At no time should the column be allowed to run dry. The extract will then be

applied to the column in a small volume of n-hexane and the aliphatic fraction eluted with two column volumes of n-hexane. This will be followed by elution of aromatics with two column volumes of benzene. The eluates from the two fractions will then be taken to near dryness on a rotary evaporator. They will then be transferred to screw-cap vials with either aluminum- or teflon-lined caps, and the remainder of the solvent removed with a light stream of pre-purified nitrogen.

Add a small measured volume of a suitable solvent to the residue, and, using a one (1) μℓ syringe, place a one (1) μℓ aliquot on the weighing pan of a micro-balance. An alternative method is the use of tared vials and determination of weight difference using an analytical balance. After the solvent evaporates and the balance has come to equilibrium, usually one to two minutes, the residue can be weighed. The weight of the total residue can then be determined by extrapolation. This method helps in avoiding problems associated with the presence of salts, and sulfur in the vial which may not have been completely removed. Additionally. an appropriate sample volume for injection into the gas chromatograph can be determined in this manner. Appropriateness of sample volume is a function of gas chromatographic operating conditions and the composition of the sample itself.

Gas Chromatography (G.C.)

Each eluted fraction obtained from the column chromatographic separation will be re-dissolved in a small volume of n-hexane and aliquots will be withdrawn and weighed on a microbalance to determine appropriate volumes for injection on the gas chromatograph. Stainless

steel capillary columns coated with OV-101, should be used for the anlalysis. The columns should be high resolution with at least 50,000 theoretical plates. The gas chromatograph will be capable of linear temperature programming and will be operated with a hydrogen flame detector with a sensitivity of at least 5×10^{-11} gms/sec for n-decane at a signal-to-noise ratio of 5:1. Retention indices will be computed based on known standards.

The gas chromatographic analysis should allow for isolation and characterization of the following: normal alkanes from C_{14} to at least C_{32}; condensed and non-condensed cycloalkanes (in a cursory way, if present) and homologous series of alkyl benzenes and alkyl-substituted polycyclic aromatics such as chrysene with retention times up to n-C_{32}.

Following is part 2 of the quarterly report R.U.# 275/276/294 for the period ending December 31, 1975. This was received after the printing of the Quarterly Reports, July - September 1975, therefore is included here.

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OCS COORDINATION OFFICE

University of Alaska

Quarterly Report for Quarter Ending December 31, 1975

Project Title: Hydrocarbons: Natural Distribution and Dynamics on the Alaskan Outer Continental Shelf

Contract Number: 03-5-022-56

Task Order Number: 5

Principal Investigator: Dr. David G. Shaw

I. Task Objectives

The primary objective of this project is to produce data on the kinds and amounts of hydrocarbons in waters, sediments and biota of the Alaskan OCS.

II. Field and Laboratory Activities

A. Field

1. Suspended sediments from the vicinity of the mouth of the Copper River were collected during a cruise of the R/V Acona 6 October - 11 October.

2. Surface water samples were taken in the Gulf of Alaska on a cruise of the NOAA ship Surveyor 28 October - 17 November. During this cruise, a joint sampling exercise was carried out with the National Bureau of Standards. Surface water was collected in the Lynn Canal.

B. Laboratory

1. The GC-MS-data system has been delivered to Fairbanks and Dr. Douglas McIntosh has been hired to operate the system. Installation and start-up are expected in early 1976.

2. The check of methods for biota analysis has been completed and analysis begun.

3. Analysis of water samples collected in August, 1975 were completed. Data for these analysis will be submitted when a Data Management Plan is in place. Analysis of water collected in October is currently in progress. A contamination problem was encountered in this work but is now thought to have been overcome. It is believed not to have affected the intercalibration samples.

4. Mr. Gregory Malinky has been hired to carry out our investigation of the interaction of hydrocarbons with Gulf of Alaska suspended sediments. An outline of the experimental plan for this work is included as Appendix A.

III. Results

None are available at this time.

IV. Problems

A. No progress has been made in the area of Beaufort Sea logistics.

B. Other problems described in my September, 1975 report have now been resolved.

V. Subcontractor Activities

A report describing I. R. Kaplan's progress in sediment collection and analysis will be forwarded in approximately two weeks.

APPENDIX A

The aim of this project is to study the absorption of hydrocarbons onto suspended sediments. We have limited the choice of hydrocarbons to constituents of crude oil. The hydrocarbons to be studied are hexane, cyclohexane, bengene, and tetrahydronapthalene, a napthend-aromatic. In this way the behavior of the six carbon chain can be followed from an aliphatic to a cycloalkane, trans-aromatic. The tetrahydronapthalene is a fused cycloalkane-aromatic ring system containing one ring of each.

Having some idea of the type of hydrocarbons absorbed and the avenues of suspended sediment transport, one can then make an educated guess as to where the hydrocarbons carried by suspended sediments will be deposited and what type of hydrocarbons they will be. Thus, the harmful hydrocarbons of an oil spill may be carried and deposited miles from the accident adversely affecting the biota there.

A synopsis of the experimental variables are shown below:

1. Temperature - probably two will be considered; one possibly being the average yearly temperature of the Gulf of Alaska.

2. Salinity - three or four solutions will be considered here starting with distilled water and increasing salinity to that of sea water.

3. Sediment size - possibly two size fractions will be considered; if the nature of the sediment permits, the two most common sizes of suspended sediments will be studied.

4. Hydrocarbons - one from each of the four classes of hydrocarbons of petroleum-alkane, cycloalkane, aromatic, naptheno-aromatic.

5. If time permits, the affect of a surfactant on absorption will also be studied as it may hinder or enhance absorption.

6. Mixing turbulence - hydrocarbon absorption may be a function of mixing turbulence.

7. Mineralogy of sediment - absorption may be a function of mineralogy of the size fraction.

8. Contact time - must be long enough for steady state.

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 5 R.U. NUMBER: 275/276/294

PRINCIPAL INVESTIGATOR: Dr. D. G. Shaw

Submission dates are estimated only and will be updated, if necessary, each quarter. Data batches refer to date as identified in the data management plan.

Cruise/Field Operation	Collection Dates		Estimated Submission Dates[1]			
	From	To	Batch 1	2	3	
Silas Bent Leg I #811	8/31/75	9/14/75	None	3/31/76	3/31/76	
Discoverer Leg III #810	9/12/75	10/3/75	None	None	3/31/76	
Discoverer Leg IV #812	10/3/75	10/16/75	6/30/76	None	3/31/76	
Surveyor #814	10/28/75	11/17/75	None	6/30/76	None	
North Pacific	4/25/75	8/7/75	6/30/76	None	None	
Contract 03-5-022-34	Last	Year	4/30/76	4/30/76	4/30/76	

Note: [1] Estimated submission dates are contingent upon final approval of data management plan submitted in draft form Oct. 9, 1975 and University of Alaska approved form Nov. 20, 1975 to NOAA. No format for data submission is necessary as per data management plan.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 5

PRINCIPAL INVESTIGATOR: Dr. David G. Shaw

Period April 1 - December 31, 1975* (9 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	105,577.00	26,452.64	79,124.36
Staff Benefits	17,948.00	2,104.66	15,843.34
Equipment	135,300.00	119,544.91	15,755.09
Travel	6,004.00	1,432.26	4,571.74
Other	106,100.00	93,641.65	12,458.35
Total Direct	370,929.00	243,176.12	127,752.88
Indirect	60,391.00	15,130.91	45,260.09
Task Order Total	431,320.00	258,307.03	173,012.97

* Preliminary cost data, not yet fully processed.

ANNUAL REPORT

MICROBIAL RELEASE OF SOLUBLE TRACE METALS FROM OIL IMPACTED SEDIMENTS

Contract # 03-5-022-56
Research Unit # 278
Reporting Period 4/1/75-3/31/76
Number of Pages 7

DR. ROBERT BARSDATE
Institute of Marine Science

UNIVERSITY OF ALASKA

March 31, 1976

MICROBIAL RELEASE OF SOLUBLE TRACE METALS FROM OIL IMPACTED SEDIMENTS

I. Summary

This project is an investigation of the possible remobilization of trace metals from crude oil impacted sediments. The initial results of laboratory experiments suggest that the copper concentration of sediment pore water may increase following the addition of oil, and tentatively the effect is ascribed to the occlusion of trace metal binding or exchange sites by components of the oil.

II. Introduction

This work has been undertaken to determine the potential for release of soluble trace metals following the oil impaction of marine sediments. The object of the work is to determine by the use of experimental laboratory techniques whether or not changes in the concentration of dissolved trace metals in the interstitial waters of sediments are likely to occur as a result of introduction of crude oil. A further objective is to relate such metal changes to variations in microbial activity or other biological or chemical perturbations taking place within the sediments. If dissolved metal concentrations increase in sediment pore waters following oil spills, benthic organisms may be affected adversely. In addition in continental shelf waters and other shallow water regimes, the possibility exists that increased trace metal levels in sediment pore waters will be reflected as increases in trace metals in the open waters above, potentially disturbing the species composition and productivity of plankton algae and inhibiting the development of sensitive growth stages of certain zooplankters.

III. Current State of Knowledge

The dissolved trace metal level in sediment pore water is set largely by inorganic chemical reaction with solid phases, prehaps the most important of which are relatively insoluble metal compounds and sorption/ion exchange sites on a variety of surfaces including clay minerals, hydrous metal oxides, and organic detritus. Displacement of metal from these binding sites or masking of these binding sites by direct or indirect effects of oil would tend to increase the concentration of metals in solution. In addition soluble metal-complexing compounds may react with trace metals and further increase their concentration in interstitial waters. These complexes may be relevant to oil spill impact since microorganisms are involved in the production and decomposition of organic complexes and since both the intensity and the nature of microbial activity is perturbed by oil.

IV. Study Area

Sediments used in this work were collected on a 1975 *Discoverer* cruise in the Bering Sea, and additional detrital material was collected at Izembek Lagoon, an embayment of the Bering Sea. However, in large part this is a process study which may have relevance to impact in many marine locations.

V. Sources and Methods

The primary activity of this study has been to follow the distribution and/or abundance of metals with time in small experimental containers (with defined conditions of temperature, pH, and other environmental parameters) to which oil has been added. Two principal analytical methodologies have been employed. The first, a radioisotope tracer technique, is being used to determine the distribution of exchangable trace metals between sediments and water. The second

analytical technique is anodic stripping voltammetry, which is a sensitive method for the determination of the abundance of cadmium, copper, lead, and zinc. In this work it has been used with chemical pretreatment for the determination of total dissolved metals, and it also is suitable for the study of organic complexing agents. In some experiments microbial activity will be estimated by changes in dissolved oxygen (in oxidized systems) or production of sulfide from radioactively-labeled sulfate (in reduced systems).

VI, VII Results and Discussion

Since a great deal of effort has been required for the development and implementation of the techniques, relatively little output is yet available from this study. The results of the first "production" experiments using anodic stripping voltammetry for total dissolved copper are presented in Table 1. In this experiment copper increased from 0.4 to 3.5 ug Cu/l one day after the addition of sediment and decreased slightly to 3.4 ug Cu/l after seven days. In a parallel set of experiments to which a small amount of oil had been added, the copper concentrations increased to 3.9 ug Cu/l. Although the increase is small (13% over controls), the difference is statistically significant. Since the oil-induced increase in copper concentration appeared within the first day of the experiment and remained nearly constant after seven days, it appears likely that abiotic chemical processes rather than microbial processes are involved.

VIII. Conclusions

The initial results of this project indicate a distinct but small increase in total dissolved copper following the addition of oil to water-sediment systems. Since the increase appears within twenty-four

Table 1. Effects of Prudhoe Bay crude on the copper concentrations in water-sediment experimental systems containing 1 g (wet weight) Bering Sea sediment and 100 ml sea water.

Flask	Oil Added (cc/g sed)	Dissolved Copper (μg Cu/l) at various times Initial*	1 day	7 days
1	0	0.4, 0.4	3.5, 3.5	3.5, 3.3
2	0	0.4, 0.3	–	3.6, 3.2
3	0	0.3, 0.5	–	3.3, 3.4
4	0.1	0.3, 0.4	3.8, 4.1	3.8, 3.8
5	0.1	0.5, 0.4	–	3.6, 3.9
6	0.1	0.4, 0.4	–	4.1, 4.0

*Prior to addition of sediments or oil.

hours, the most likely but highly tentative conclusion is that the oil has occluded trace metal sorption/ion exchange sites on the sediment particles. The work is in too early a stage to permit any meaningful conclusions concerning the generality of the results or the potential for environmental perturbation.

IX. Needs for Further Study

A substantial amount of further work already is covered in the context of this project, and hopefully by its termination considerably more insight will exist as to the nature and extent of trace metal perturbations induced by oil. However, it should be noted that the project originally emphasized the investigation of trace metal effects induced by microbial activity, and the initial results strongly suggest that other, probably physiochemical, processes are active and also must be considered. It is quite possible that definitive results will not be achieved within the context of this present small-scale study.

X. Summary of 4th Quarter Operations

The major objectives for this quarter were to carry out the first production runs for the measurement of dissolved trace metals in water-sediment systems with and without oil and to initiate radiotracer studies of similar systems. This first aspect was done with anodic stripping voltammetry, and the results for copper have been presented above. Cadimum and lead determinations also were done for the same set of experiments, but this information has not yet been processed. In all approximately 100 individual analyses were made. Preliminary experiments to determine the distribution of trace metals between water and sediments have been carried out using cadimum ^{109} as a radiotracer. No ship or field activities were scheduled for this quarter.

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 7 R.U. NUMBER: 178

PRINCIPAL INVESTIGATOR: Dr. Robert J. Barsdate

No environmental data are to be taken by this task order as indicated in the Data Management Plan. A schedule of submission is therefore not applicable[1].

NOTE: [1] Data Management Plan has been approved and made contractural.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: March 31, 1976

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 7

PRINCIPAL INVESTIGATOR: Dr. R. J. Barsdate

Period April 1, 1975 - March 31, 1976* (12 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	8,233.00	749.50	7,483.50
Staff Benefits	1,383.00	127.42	1,255.58
Equipment	2,500.00	1,064.96	1,435.04
Travel	1,300.00	-0-	1,300.00
Other	3,300.00	526.90	2,773.10
Total Direct	16,716.00	2,468.78	14,247.22
Indirect	4,709.00	428.71	4,280.29
Task Order Total	21,425.00	2,897.49	18,527.51

* Preliminary cost data, not yet fully processed.

Following is part 2 of the quarterly report R.U.# 278 for the period ending December 31, 1975. This was received after the printing of the Quarterly Reports, July - September 1975, therefore is included here.

RECEIVED
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UNIVERSITY OF ALASKA
FAIRBANKS, ALASKA 99701

January 20, 1976

Dr. Herbert E. Bruce
OCSEAP Project Office
P. O. Box 1808
Juneau, Alaska 99802

Reference: Contract Number 03-5-022-56

Dear Herb:

Enclosed is the quarterly report for the period ending December 31, 1975 for Task Order #7.

Sincerely yours,

Donald H. Rosenberg
OCS Coordination Office

DHR/brm

Enc.

cc: Dr. J. Robinson - w/enc
Dr. G. Weller - w/enc

PLEASE REPLY BY AIRMAIL

OCS COORDINATION OFFICE

University of Alaska

Quarterly Report for Quarter Ending December 31, 1975

Project Title: Microbial Release of Soluble Trace Metals from Oil-Impacted Sediments

Contract Number: 03-5-022-56

Task Order Number: 7

Principal Investigator: Robert J. Barsdate

I Task Objectives

The principal goals for this quarter have been to assemble the equipment and apparatus for the major experimental work and to initiate pilot runs to verify the feasibility of experimental design, checking such things as adequate sensitivity and freedom from artifacts such as oxygen leakage and trace metal contamination.

II. Field Activities

As stated in the previous quarterly report, the present sample inventory appears adequate, and no additional field activities appear justified.

III Results

A. Trace Metal Determinations

In a set of preliminary experiments without oil, trace metal release from Bering Sea sediments was investigated using various water/sediment ratios. As had been anticipated, the experimental work, as far as trace metals are concerned, can be carried out over a wide range of water/sediment ratios; therefore, the water/sediment ratios to be employed in the final work can be set to optimize the results of the sediment respiration measurements, which will be carried out parallel to the trace metal work. Obviously the potential for contamination always is present in low level metal analyses, but no specific problems were detected in this work.

B. Sediment Respiration

The oxygen probes ordered over three months ago have not arrived as yet, and, since it will be necessary to start

the oxygen work soon in order to stay on schedule, several alternatives have been examined. They are: (1) the use of a different style probe, which currently is available within IMS and can be borrowed for this work; (2) the use of laboratory-fabricated microprobes with the polarographic instrumentation now available in this laboratory; or (3) restriction of the work to Winkler titrations for oxygen. Any of these alternatives would be adequate for the work at hand, but all do involve some minor negative factors. If the originally ordered probes do not arrive shortly, one of the alternatives will be chosen and implemented.

IV. Problems Encountered

The oxygen probe problem has been identified and provisions have been made for alternative action, as noted above. A possible problem related to the loss of facilities with the programmed laboratory move has not yet been resolved.

OCS COORDINATION OFFICE

University of Alaska

ENVIRONMENTAL DATA SUBMISSION SCHEDULE

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56 T/O NUMBER: 7 R.U. NUMBER: 178

PRINCIPAL INVESTIGATOR: Dr. Robert J. Barsdate

No environmental data are to be taken by this task order as indicated in the Data Management Plan. A schedule of submission is therefore not applicable[1]

NOTE: [1]Data management plan was submitted to NOAA in draft form on October 9, 1975 and University of Alaska approval given on November 20, 1975. We await formal approval from NOAA.

OCS COORDINATION OFFICE

University of Alaska

ESTIMATE OF FUNDS EXPENDED

DATE: December 31, 1975

CONTRACT NUMBER: 03-5-022-56

TASK ORDER NUMBER: 7

PRINCIPAL INVESTIGATOR: Dr. R. J. Barsdate

Period April 1 - December 31, 1975* (9 mos)

	Total Budget	Expended	Remaining
Salaries & Wages	8,233.00	751.03	7,481.97
Staff Benefits	1,383.00	127.68	1,255.32
Equipment	2,500.00	511.96	1,988.04
Travel	1,300.00	-0-	1,300.00
Other	3,300.00	472.01	2,827.99
Total Direct	16,716.00	1,862.68	14,853.32
Indirect	4,709.00	429.59	4,279.41
Task Order Total	21,425.00	2,292.27	19,132.73

* Preliminary cost data, not yet fully processed.

ANNUAL REPORT

TITLE: Research Unit 332. Determine the incidence and pathology of marine fish diseases in the Gulf of Alaska, Bering Sea, and Beaufort Sea

PRINCIPAL INVESTIGATORS:

Dr. Bruce B. McCain, University of California (Davis), CA, and National Marine Fisheries Service, Northwest Fisheries Center, 2725 Montlake Boulevard East, Seattle, Washington

Dr. Sefton R. Wellings, University of California (Davis), CA

April 1976

ANNUAL REPORT FOR RESEARCH UNIT 332

April 1, 1976

I. SUMMARY OF OBJECTIVES, CONCLUSIONS AND IMPLICATIONS WITH RESPECT TO OCS OIL AND GAS DEVELOPMENT

The main purpose of this investigation is to obtain baseline data on the present health status of demersal fishes in waters near Alaska's Outer Continental Shelf so that future environmental effects of oil exploration and development in these areas can be evaluated. The principal criterion of fish health being employed is the frequency of externally visible pathological conditions. In addition, the histopathological and microbiological properties of each major disease are being characterized. The geographical areas included in this work are the Bering and Beaufort Seas, and the Gulf of Alaska.

During September and October, 1975, about 30,000 bottom-dwelling fish from the Bering Sea were examined for diseases. The three most commonly observed diseases and their frequency of occurrence were (1) epidermal papillomas of rock sole (*Lepidopsetta bilineata*), 1.0%; (2) lymphocystis of yellowfin sole (*Limanda aspera*), 2.1%; and (3) epidermal tumors of the pseudobranch of adult Pacific cod (*Gadus macrocephalus*), 7.4%. Both the appearance and the histological characteristics of epidermal papillomas on rock sole resembled those found on several species of flatfish in Puget Sound, Washington. The frequency of tumor-bearing rock sole ranged from 0-23%, with the highest occurrence at sampling stations around the periphery of the Bering Sea in water depths of 20-30 fathoms. The virus-caused lymphocystis lesions were always on the "blind" side fins and skin surfaces of yellowfin sole, the largest lesions being found on the operculum, and the most common site of

infection was the pectoral fin. The frequency of lymphocystis was 0-15%, with a gradual increase of incidence from the northern to southern sampling stations.

The Pacific cod tumors were always bilateral, and contained remnants of normal-appearing pseudobranch tissue on the surface or deep inside the tumors. Portions of some tumors were necrotic and liquefied. Tumor-bearing adult cod were most commonly found in the south and southeastern Bering Sea.

A major implication of these data is the need to monitor pathological abnormalities of demersal fishes in near-shore areas of the Bering Sea. The 1.0% frequency of tumors in Bering Sea rock sole appears to be a value expected in older fish near an area where the prevalence of tumors would be quite high (25-50%) among younger fish of that species. In the case of rock sole, young fish are found in the shallow, near-shore waters.

Lymphocystis has never before been reported to occur in marine fish on the northern Pacific coast of North America. Although the causative agent is known to be a virus, and can be transmitted in the laboratory to both marine and freshwater fish, the mechanism of natural transmission is not known. Environmental factors, such as low salinity, higher temperature, suspended sediments, and other factors which affect natural disease resistance, are thought to influence the prevalence of the disease. All of these conditions are more common in near-shore areas and estuaries.

II. INTRODUCTION

A. General nature and scope of study

Accomplishment of the goals of this study depends upon close cooperation with personnel performing resource assessment investigations of demersal fishes in Alaskan waters. As fish are captured and processed by these investigators, members of our unit examine the samples or subsamples of fish for pathological abnormalities. In addition to recording biological data and the types and frequencies of abnormalities, affected fish and normal-appearing fish of the same species and age are subjected to techniques designed to better characterize each disease and to provide evidence for their cause(s). These procedures include the isolation and study of disease-associated microorganisms (e.g., bacteria, fungi, and viruses), and the determination of the gross and histopathological properties of the fish and affected tissues.

B. Specific objectives

The specific objectives of this investigation include the following: (1) Determine the frequency of each major type of pathological condition in demersal fishes in the Bering and Beaufort Seas and Gulf of Alaska; (2) Establish the distribution of each disease in Alaskan waters sampled; (3) Define the histological features of each disease by examining tissues from lesions, associated major internal organs and blood, using procedures designed for light and/or electron microscopy; (4) Isolate disease-associated microorganisms from lesions and internal tissues, use taxonomic tests to identify them, and determine if any microorganism is disease specific; and (5) Compare the length, weight, age, and sex frequencies of diseased fish with those of normal-appearing fish of the same species.

C. Relevance to problems of petroleum development

The research to be described in this report is relevant in two main ways to understanding the effects of petroleum development on the marine animals in the waters of Alaska's Outer Continental Shelf regions.

The most important contribution is to provide baseline data on the health of demersal fish prior to the time when the environmental impacts of oil drilling occur so that future effects of oil on fish can be assessed. Also, knowledge of the possible causes of pathological abnormalities in demersal fishes will provide a clearer understanding of the ways in which exposure of a fish to oil could affect the frequency of a particular disease. For example, petroleum may cause pathological abnormalities through the action of toxins or carcinogens (Kuhnhold, 1972; Morrow, 1974) or indirectly by lowering the resistance of fish to disease agents.

III. CURRENT STATE OF KNOWLEDGE

Very limited knowledge is presently available concerning the pathology of fish species in or associated with the Gulf of Alaska and Bering and Beaufort Seas. Because our investigation is concerned primarily with diseases which produce externally visible pathological conditions, this literature review will emphasize diseases of marine fishes in the Northern Hemisphere with obvious symptoms and/or in which the disease-causing organisms is readily detected. At least eight different agents or factors are known to be responsible for diseases of these types. They are as follows: (1) bacteria, (2) fungi, (3) protozoa, (4) helminths,

(5) copepods, (6) viruses, (7) environmental factors, and (8) genetic anomalies. In addition, neoplasia or hyperplasia may be caused by one or more of the above.

The bacterium, *Vibrio anguillarum*, is known to cause red, hemorrhagic skin lesions and in many cases death in several groundfishes, including cod, eels, and pleuronectides (Ljungberg, 1963; Buckman, 1952; Hodgkiss and Shewan, 1950). Other groups of bacteria, such as the pseudomonads and mycobacteria, are reported to cause ulcerated skin, fin erosions, and various other lesions (Zobell and Wells, 1934; Hodgkiss and Shewan, 1950; Mahoney *et al*., 1973).

Fungi are not known to be a widespread cause of marine fish diseases. They have been shown to cause epizootics among herring in the Northwest Atlantic. The disease results in extensive hyphal growth in the internal organs and muscles. The genus *Ichthyophonus* is most commonly isolated from fungal disease (Sindermann, 1966).

Protozoan parasites infest a great many bottom-dwelling fish along the Pacific coast of North America (Margolis, 1970). The presence of these organisms does not always result in pathological damage to the host. Myxosporidia, however, are known to cause a condition in fish muscles referred to as "milkiness." Several marine fish species are affected including halibut (*Hippoglossus stenolepis*), starry flounder (*Platichthys stellatus*), and Hake (*Merluccius productus*) (Margolis, 1953; Patashnik and Groninger, 1964). In most cases the disease is not obvious in freshly captured fish, but the condition can be readily detected after the affected fish have been refrigerated for 4-24 hrs. During this period, proteolytic enzymes present in the spores, when released into

muscle tissue, are thought to cause foci of softened and liquefied muscle in which cysts containing spores can be macroscopically observed.

Another group of protozoans, the microsporidia, form easily detected cysts or "tumors" in the somatic muscles of many groundfishes. Members of the families Gasterosteidae, Zoarcidae, and Gadidae have been reported with this disease (Weissenberg, 1921; Nigrelli, 1946; Polzanski, 1955).

The helminths, including trematodes, cestodes, and nematodes, produce disease symptoms in marine fish in greatly varying degrees. Trematode species have metacercariae which form cysts under the skin, but are thought not to cause serious disease (Wolfgang, 1954). Cestodes have been reported in several fishes in Canadian Pacific waters (Arai, 1967). The larval cestodes may be found in the musculature, but few gross pathological problems have been observed. Larval nematodes are present in the muscles of a large number of marine species (Scott and Martin, 1957; Templeman _et al_., 1957). In some cases, pathological damage results from the movement of these larvae to other parts of the fish; for example, the liver may become heavily infested and dysfunctional.

Many copepod species attach to the gills and body surfaces of a wide variety of marine fishes in the northeastern Pacific Ocean (Arai, 1969). Some of these parasites are able to invade internal organs, such as the heart (Mann, 1954), and some cause skin ulcerations.

Another type of disease found in Alaskan waters is characterized by the presence of tumors. The two most frequently reported types of neoplasia are epidermal papillomas of pleuronectid fishes, and pseudobranchial tumors of cod. The causes of these tumors are not yet known. Papillomas

were identified on large numbers of starry flounder and Arctic flounder (Liopsetta glacialis) in waters of the Aleutian Islands in 1886 (Turner, 1886). Frequency of papillomas of about 32% was found in sand sole (Psettichthys melanostictus) in the Northern Hecate Strait of Canada in 1965 (Nigrelli et al., 1965). Recently, several rock sole with papillomas were captured in the Bering Sea by a National Marine Fisheries Service vessel (Johnson, 1974).

Nine species of Pleuronectidae have been reported to have papillomas in Puget Sound, Washington (Miller and Wellings, 1971; McArn and Wellings, 1971; Wellings et al., 1969). The life history and histopathology of the disease have been characterized (Brooks et al., 1969; Wellings et al., 1967). The cause of the disease has been investigated, but no causative agent or factor has yet been determined (McCain, 1974; Wellings, McCain and Miller, 1976).

Pacific cod with adenomas and/or adenocarcinomas associated with the pseudobranch have been captured in the Bering Sea (Weber, 1975), and in the coastal waters of British Columbia (Levings, 1968; Wellings, 1969). In a recently completed study of commercially caught Pacific cod from various fishing grounds off British Columbia, Forrester (1976) reported finding an average frequency of pseudobranchial tumors of 3.6% in about 110,000 fish examined. These tumors can become quite large (6 x 3 cm) and are readily recognized. Similar tumors have been found in walleye pollack (Theragra chalcogrammas) (Takahashi, 1929), a fish common in Alaskan waters, and in Gadus morhua near Norway (Lange, 1973).

Other types of neoplastic diseases are likely to be found in Alaskan groundfishes. For example, lipo-osteomas, fibro-osteomas, and fibromas have been described in Pacific halibut from Alaska (Wellings, 1969).

Few virus-caused diseases have been reported in Alaskan marine fishes. A disease which is present in other marine waters of the Northern Hemisphere is lymphocystis (Weissenberg, 1965). This disease takes the form of numerous nodules on the body surface. The nodules are composed of giant connective tissue cells. Species affected include many members of the Pleuronectidae family.

Another virus with approximately the same geographical distribution and physical properties as lymphocystis virus has been recently found near British Columbia (Evelyn, 1976). The virus is called piscine erythroyctic necrosis virus and was found in Pacific herring (*Clupea harengus pallasi*) and chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*). Diseased fish were lethargic, had low hematocrits, and a high percentage of the red blood cells had cytoplasmic inclusion bodies.

IV. STUDY AREA

Proposed Study Area. The originally contracted study areas were the Bering and Beaufort Seas and the Gulf of Alaska.

Areas Studied. During 1975, the Bering Sea was studied. Efforts were made to examine fishes from the Beaufort Sea, but due to severe ice conditions in this area few fish samples were available.

V. SOURCES, METHODS AND RATIONALE OF DATA COLLECTION

A. Field Procedures

Demersal fishes captured by otter trawl were distributed according to species into baskets. The fish in these baskets were then examined by members of R.U. 332 for the presence of externally

visible pathological abnormalities. In addition, subsamples of fish were routinely autopsied for internal pathology. Diseased fish were speciated, measured, weighed, sexed, photographed, autopsied, aged by examination of otoliths, and the abnormalities recorded. Pertinent catch data were also noted; including haul number, location, and bottom type. For each haul, the prevalence of each type of disease was recorded.

Autopsy procedures included the taking of specimens for histopathological, bacteriological, virological, and hematological examinations. Tissues to be subjected to histological procedures were preserved in 10% buffered formalin and/or 2% glutaraldehyde for light and electron microscopy, respectively. Bacteria were isolated by inoculating Petri dishes, containing either trypticase soy agar (TSA) or Ordal's seawater cytophaga agar (OSCA), with fluid or tissue obtained with sterile probes, swabs, syringe and needles, or forceps. The resulting bacterial colonies were purified and stored in tubes containing OCSA. For virological tests, fresh tissue was cut into small pieces, placed in glass ampules, and frozen in liquid nitrogen. These samples will then be inoculated onto fish cell cultures in order to detect viruses. Blood smears were prepared from each autopsied fish, and will be examined for the presence of abnormal cell numbers and types, and parasitic organisms of the circulatory system.

In addition, diseased fish captured by the chartered vessel, R.V. Oregon, were frozen and returned to the NWFC, Seattle. The fish were thawed, photographed, measured, sex determined, and the disease signs were noted. Due to the fact that these fish were frozen, histological and microbiological tests could not be performed.

B. Laboratory Procedures

Tissues to be examined by light microscopy for histopathology were embedded in paraffin, sectioned with a microtome, and the sections were stained by a variety of methods, including hematoxylin and eosin, Oil-Red-O, Sudan black and Masson's trichrome. Microscopic examination of sections from diseased tissue and major organs allows for determination of abnormalities of tissue structure, the types of cells involved, and the presence or absence of intra- or extracellular microorganisms.

Tissue to be examined by the electron microscope which had been previously preserved in 2% glutaraldehyde was treated with osmium tetroxide, dehydrated in absolute ethanol, embedded in Spurr's Low Viscosity Epoxy Resin and sectioned on a MT2B Dupont-Sorvall microtome. Sections were examined with either a Ziess EM95 or an AEI-EM801 electron microscope. Examination of tissue in this manner allows detection of intracellular damage, identification of disease-specific cells, and observation of virus particles.

Microbiology. Bacterial and fungal isolates collected in the field were returned to the laboratory for further testing. Tests were performed to define the major taxonomic properties of each isolate with the intention of eventually conducting pathogenicity tests on organisms routinely isolated from a particular disease. Pathogenicity tests will be conducted by injecting microorganisms into test fish of the same or closely related species as the original host.

Tests used to classify the bacterial isolates included: Gram stain, oxidase test, determination of motility by hanging drop and motility methods, colony characteristics (shape, color), and methyl red and Voges-Proskauer tests. Tests using special growth media were also used, including Triple Sugar Iron medium and sugar utilization media.

Virus isolation procedures were initiated by freezing fresh tissue samples in liquid nitrogen (-196°C). The frozen samples were delivered to the NWFC laboratory at Seattle where they will be homogenized and inoculated onto fish cell cultures. Fish cell cultures derived from English sole and chinook salmon tissue are presently being maintained in the laboratory. Additional cultures will be initiated from rock sole, yellowfin sole, and Pacific cod from the Bering Sea during the 1976 sampling period. Efforts to obtain cell cultures from the species in which the major diseases were found are motivated by the apparently strict species-specificity of these abnormalities. For example, lymphocystis was found only in yellowfin sole, and the virus, which is the etiological agent of this disease, will most probably infect only cells from yellowfin sole. Other strains of the lymphocystis virus have been shown to be highly species-specific. Serum neutralization tests will be performed with the Bering Sea lymphocystis virus to determine the strain type involved.

Biological Data Analysis. For each major pathological condition, the available biological data, including species, length, weight, sex, and age, were compiled and compared to data for the total populations. Length frequencies, sex ratios, and length/weight and

age/length relationships of diseased and normal-appearing fish captured in 1975 were plotted in graphic form, and trends and differences were noted. After completion of the 1976 sampling, all data will be put on computer cards and the above mentioned comparisons will be analyzed using appropriate standard statistical methods, such as the Chi-square test, analysis of variance, regression analysis, and covariance tests.

By comparing the biological characteristics of normal fish and fish with pathological abnormalities, the growth and survival rates of affected individuals will be better understood. In addition, such comparisons may better define some of the behavior patterns of affected fish, especially with respect to interactions with normal fish of the same age, and could provide insights into possible modes of disease transmission.

VI. RESULTS

A. Field Activities

Members of our research unit were aboard the R.V. Miller Freeman from September 8 to October 24, 1975 during its sampling cruise in the Bering Sea. Catches of demersal fishes were examined for pathological abnormalities. In addition, samples of diseased fish captured in the Bering Sea were received from the R.V. Oregon.

R.V. Miller Freeman. Approximately 30,000 demersal fish, representing about 25 different species, were examined. Rock and yellowfin sole, and Pacific cod were each found to have a significant pathological condition (Table I) while the remaining 22 species were found to be essentially free of external evidence of disease (Table II).

Rock sole had skin tumors, known as epidermal papillomas, in 1.0% of the rock sole examined. The disease frequency for each haul ranged from 0 to 23%, with the highest occurrence at sampling stations around the periphery of the Bering Sea in water depths of 20 to 30 fathoms (Fig. 1).

The tumors were usually partially elevated, pigmented (gray to brown), with a cauliflower-like surface (Fig. 2). They varied in size from 2.5 x 3.1 x 0.8 cm to 9.1 x 6.0 x 1.0 cm. Tumor-bearing fish ranged in length from 120 mm to 470 mm.

The ages of papilloma-bearing fish ranged from 3 to 11 years. The age distribution of tumor-bearing males and females was bimodal, with peaks at 9 to 10 years and 5 to 6 years. This distribution mimicked the age pattern of normal female rock sole, but was significantly different from the age distribution of the normal males. Five-year old normal males were the most numerous, with the frequency of males of ages 6 to 10 years decreasing gradually. However, there were no tumor-bearing 7 and 8 year old male rock sole.

In hauls where sex and length of both tumor-bearing and normal rock sole were compared, 64% of the tumor-bearing fish were males, compared with 50% males for all the rock sole. In addition, the lengths of tumor-bearing males and females did not correspond with that of the total sample population (Fig. 3). Relatively fewer fish of intermediate length (23-29 cm) had tumors, while the smaller (16-22 cm) or larger (30-36 cm) rock sole and most of the largest males had tumors. Preliminary examination of length/age data suggests that both male and female tumor-bearing rock sole were 2 to 4 cm shorter than normal rock sole of the same age.

Lymphocystis was detected only in yellowfin sole at a frequency of 2.1%. A range of 0 to 15% was observed for each haul, with a gradual increase in prevalence from the northern to southern sampling stations (Fig. 4).

The principal signs of this disease were growths which were pink to red, ovoid, from 1 to 25 mm in maximum dimensions, and composed of abnormally large hypertrophied cells up to 2 mm in diameter (Fig. 5). Lesions were always on the blind side fins and skin surfaces, the largest growths being found on the operculum, and the most common site of infection was the pectoral fin.

The length frequency of yellowfin sole from the total sample (both normal and diseased fish) showed a bimodal distribution with maxima at 16 and 24 cm, indicating the presence of two dominant age groups. Yellowfin sole with lymphocystis had a length frequency which corresponded to the large length group.

Approximately 7.4% of the adult Pacific cod captured by the R.V. Miller Freeman had tumors of the pseudobranch. The tumor frequency per haul varied from 0 to 100%, and tumor-bearing cod were most commonly found in the south and southeastern Bering Sea (Fig. 6).

The cod tumors were lobulated, smooth, pale yellow to yellow-pink, and measured 1.5 x 1.0 x 0.8 cm for the smallest tumors to 4.5 x 3.5 x 2.0 cm for the largest (Fig. 7). Portions of some tumors were necrotic and liquefied. The tumors were always bilateral, and usually contained remnants of normal-appearing, dark-red pseudo-branchial tissue on the surface.

Other pathological abnormalities which were observed to occur in two or more fish included "green liver disease." found in Pacific pollock (Theragra chalcogramma); and "ulcers and boils" disease, seen in Pacific cod. The former abnormality may or may not be pathological and is being investigated in the same manner as the above mentioned diseases. The "ulcers and boils" disease was characterized as a type of epidermal hyperplasia with focal necrosis.

R.V. Oregon. As a part of resource assessment studies by the R.V. Oregon in the Bering Sea during the summer of 1975, demersal fish with obvious pathological conditions were frozen and returned to the NWFC, Seattle. Because large numbers of fish were processed daily by the crew of the R.V. Oregon, little emphasis could be placed on careful examination of every fish for disease signs. Therefore, no disease frequency data could be obtained from their collections.

Thirty-four rock sole with epidermal papillomas captured near Amak Island in August were delivered to the NWFC. Nine of the 34 had two tumors each, and the remainder had one. The tumors ranged in size from 2.0 x 2.0 x 0.5 cm to 5.5 x 7.5 x 1.0 cm, with an average of about 3.0 x 3.0 x 0.7 cm. They ranged in length from 110 to 250 mm, which corresponded to otolith-determined ages of three to six years, respectively. Of the 43 tumors examined, 31 had spread to both sides of the fish.

Two yellowfin sole with lymphocystis were also received. Both were captured near Amak Island. The lesions were located on the blind pectoral fins.

Photographs were taken of all the above mentioned fish. Due to the fact that the fish had been frozen, no histological, microbiological or hematological specimens were taken.

B. Laboratory Activities

The two main types of laboratory analyses performed on specimens from fish with pathological abnormalities and from normal fish involved histopathology and microbiology. Most of the histopathological procedures were performed at the Department of Pathology, University of California, Davis; while the microbiological work was done at the NWFC, Seattle.

Histopathology. Examination of sections of epidermal papillomas demonstrated the typical papillary structure of the thickened layer of epidermal cells supported by a branching fibrovascular stroma (Fig. 8). A tumor-specific cell, known as an X-cell, was observed in both the stromal and epidermal areas. Typical X-cells are characterized as being larger than normal epidermal cells, and having a pale nucleus, a large intense nucleolus, and granular cytoplasm.

Electron microscopic examination of X-cells showed that the cytoplasm contained numerous vesicular bodies (Fig. 9). The nucleus contained a large nucleolus, and the chromatin-like material was evenly dispersed around the nucleus. This was in contrast to the normal-appearing cells in the tumors which had generally even-staining cytoplasm, and a nucleus with chromatin condensed around the periphery of the nuclear membrane.

Lymphocystis growths were packed with typical appearing hypertrophied lymphocystis cells ("Giant" cells) (Russell, 1974; Templeman, 1965). The diameter of the cells ranged from about 0.10 mm to 1.0 mm.

A variety of cytoplasmic inclusion bodies were observed. One type was usually centrally located and contained very dense, basophilic material which, as will be described below, contained aggregates of virus particles (Fig. 10). Another basic type of inclusion had a fibrillar structure and also contained virus particles (Fig. 11).

The virus particles observed in lymphocystis cells were hexagonal in shape and approximately 200 nm in diameter (Fig. 12). The virus resembled lymphocystis virus reported in other species of pleuronectids (Walker and Weissenberg, 1965; Russell, 1974). Frequently, the virus particles were in aggregates which contained a variety of maturation forms ranging from irregularly-shaped capsids to hexagonally-shaped empty capsids to mature virions. Interspersed between the aggregates were striated structures of, at present, unknown identity. Possible identities include viral precursors, myofilaments, or collagen.

Although the tumors of the pseudobranch of Pacific cod were clearly pseudobranch-associated, the pseudobranchs were not the specific sites of tumorigenesis. The tumors appeared to be derived from the epidermal cells overlying pseudobranchial tissue and were separated from the normal-appearing glandular tissue by a thickened connective tissue capsule (Fig. 13). Thin bands of this stroma infiltrated the tumor tissue and contained numerous small blood vessels.

Another important feature of the cod tumor was the presence of X-cell-like cells (Fig. 14). As was mentioned above, X-cells are tumor-specific cells heretofore found only in epidermal papillomas of flatfish. No X-cells were observed in tumor-associated pseudobranch glandular tissue.

Microbiology. Attempts were made to isolate bacteria and fungi from lesions or tumors and major organs of fish with pathological abnormalities. Normal fish of the same species were also sampled in order to gain knowledge of the normal flora.

A total of 108 bacterial isolates were obtained. Using previously mentioned taxonomic tests, the isolates were placed into 59 taxonomic groups. All groups except one were Gram stain negative. Of the Gram negative bacteria, 53 were rod-shaped and 5 were cocci. No *Vibrio* or *Aeromonas* species were found. Most of the Gram negative rods were probably pseudomonads.

No one taxonomic group was routinely isolated from fish having one of the three main fish diseases; papillomas, lymphocystis or pseudobranchial tumors. However, seven bacterial isolates from the same general taxonomic group were taken from five different Pacific pollock with the previously mentioned "green liver disease." These seven isolates are pseudomonads and are currently being subjected to additional taxonomic tests. They will be compared to isolates obtained from pollock captured in the 1976 cruises.

No fungi were successfully isolated from diseased or normal fishes. This fact does not imply non-existence of marine fungal diseases in the sampling area, however, as suspected fungal growths from sampled fish failed to grow in the growth media used on board. Modified fungal isolation techniques will be conducted on future cruises.

Tissues to be used in virus isolation procedures were frozen in the field in liquid nitrogen, returned to the NWFC, Seattle, and transferred to a -70°C freezer. Due to breakage of ampules while

adding additional liquid nitrogen to the storage container at the Naval Station, Adak, Alaska, only a few ampules for each of the three main diseases were received at the NWFC, Seattle.

Because of the apparently strict species specificity of lymphocystis and cod pseudobranchial tumors, transmission of these diseases may depend upon using fish or cell cultures of the affected species or experimental hosts. Because of this possibility and the few frozen samples, attempts to transmit these diseases *in vitro* were postponed until after the 1976 sampling cruises. During these cruises, attempts will be made to initiate cell cultures from various tissues (i.e., fin, gonad, etc.) of affected fish species.

Cell cultures presently being maintained and those initiated during the cruises will then be used to detect potentially biologically active virus in lymphocystic growths, pseudobranchial tumors, and epidermal papillomas by methods described above.

VII. DISCUSSION

Each of the three major pathological conditions found in demersal fishes captured in the Bering Sea appeared to be very species specific. Nevertheless, each of these conditions, epidermal papillomas, cod pseudobranchial tumors, and lymphocystis, have been reported in other species, either in Alaskan waters or in other areas of the Northern Hemisphere. This and other aspects of each of these diseases will be discussed below.

A. Epidermal Papillomas of Rock Sole

The gross pathology and histopathology of rock sole epidermal papillomas were similar to tumors found on pleuronectid fishes in Puget Sound, Washington and other areas along the West Coast of the United States (McArn *et al.*, 1968; Wellings *et al.*, 1976; Mearns and

Sherwood, 1974). In Puget Sound, the tumor disease affects mostly the early juvenile (0's) and one-year old fish (1's). The disease is seasonal, with frequencies reaching as high as 50% in the fall and winter among young fish along the beaches, and decreasing to less than 5% by late spring and early summer. This decrease in near-shore areas is due in part to movement of one-year old fish to deeper waters. As the flounder grow older, the tumor frequency continues to decline; in fact, tumor-bearing flounder over four-year olds are extremely rare (Miller and Wellings, 1971; Angell *et al.*, 1975).

Using the extensive epizootiological data from Puget Sound as a model system, the 1.0% prevalence of papillomas on Bering Sea rock sole, usually five years in age or older, suggests that younger rock sole in shallow coastal waters of the Bering Sea may have quite high tumor frequencies (25 to 50%) at certain times of the year.

An unusual aspect of the age group distribution of tumored rock sole was the large number of fish 9 to 11 years old bearing tumors. Approximately half of all tumor-bearing rock sole were in this age range. Also, the older age of the tumor-bearing fish may explain why over 70% of the tumors had spread to both sides of the fish, and why, in some of the oldest fish, the tumors covered between one-quarter and one-third of the total body surface area.

That the tumors had dramatic effects on the life histories of affected rock sole is suggested by two types of evidence. Preliminary data suggest that tumor-bearing flatfish were shorter. Also, in hauls where both tumor-bearing and non-tumor-bearing rock sole were present,

most of the larger male rock sole had papillomas, suggesting that large, normal rock sole may move away from these sampling stations, leaving the diseased, less competitive fish behind.

Besides the rock sole, two other species of pleuronectids are known to have epidermal papillomas in Alaskan waters, the starry and Arctic f-ounders (Turner, 1886). Because very few starry flounder and no Arctic flounder were captured by the R.V. Miller Freeman, tumors in these species were not detected.

The cause of flounder papillomas is not known. Papillomas in mammals can be induced by viruses (Shope, 1935) and chemical carcinogens (Lappe, 1968). So far, exhaustive efforts to isolate a virus from papillomas of flounders from Puget Sound, or to transmit the disease have failed (Wellings *et al.*, 1976). Therefore, the possibility of these tumors being caused directly or indirectly by chemical agents or carcinogens has become more plausible. Petroleum oil (Zobell, 1971) and metabolic products of some marine organisms (Nelson-Smith, 1972) are known to contain potent carcinogens.

B. Lymphocystis of Yellowfin Sole

Lymphocystis has never before been reported to occur in marine fish on the northern Pacific coast of North America. The disease has been reported in other areas of the Northern Hemisphere including the Eastern Grand Bank of North America, where 1% of captured American plaice (*Hippoglossoides platessoides*) were found with the disease (Templeman, 1965); and the Northeast Irish Sea, where between 2.4 and 33.3% of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) were infected (Russell, 1974; Shelton and Wilson, 1973).

Lymphocystis is known to be caused by a virus (Weissenberg, 1965) which is a member of the group of viruses called icosahedral cytoplasmic deoxyribonucleic acid viruses. The virus particles observed by us in electron micrographs of cells from yellowfin lymphocystis resemble very closely the members of this group.

The mode of transmission of this virus is not known. The freshwater form of lymphocystis has been transmitted by injecting fish subdermally with filtered tumor homogenates (Wolf, 1962). In the marine environment, theories of disease transmission include: (1) lymphocystis is endemic, and fish are made susceptible by surface abrasion (i.e., due to net damage, abrasion from sharp objects such as rocks or animal spines), or by decreased mucous secretion; (2) the virus is not normally present, but is associated with fishing nets, suspended sediments originating in estuaries, or transmitted by vectors, such as fish or invertebrates (Templeman, 1965; Shelton and Wilson, 1973).

Because the lymphocystis lesions were always on the "blind" side of yellowfin sole and usually located in the opercular region, the method of viral transmission may involve invasion of virus normally present on the skin surface through abrasions caused by contact with sharp objects on the ocean bottom. If this interpretation is correct, then environmental conditions, which could cause weakening of the physical barriers associated with the skin of yellowfin sole, could also allow invasion by lymphocystis virus.

Preliminary examination of length frequency data showed that yellowfin sole with lymphocystis had a length frequency which corresponded to the average length of larger fish in the bimodally

distributed normal yellowfin sole populations. This observation suggests that either age dependent changes in behavior may make this species more susceptable to the virus, or that the older year class may have been exposed to more virus or environmental stress.

C. Pseudobranchial Tumors of Cod

Until our recent careful histological examination of well-preserved pseudobranchial tumors in Pacific cod from the Bering Sea, they had been considered to be adenomas. However, our observations demonstrated that the tumors originated from the epidermal covering of the pseudobranchs. Therefore, the pseudobranchial tumors are a type of epidermal tumor with some similarities to the epidermal papillomas of pleuronectids.

Although the pseudobranchial tumors do not have a papillary structure of epidermal papillomas, they do contain cells which are identical in appearance to X-cells, the tumor-specific cells in pleuronectid papillomas. The validity of this observation has not yet been proven; but, if it is true, then a great deal more may be eventually learned about the cause and/or origin of both pseudobranchial tumors and epidermal papillomas. For example, both types of tumors may be caused by X-cells, which may be single-celled parasites, or a virus may cause both conditions and X-cells are a stage of virally transformed cells.

Another clue to the cause of pseudobranchial tumors is that all tumor-bearing fish examined in the Bering Sea had bilateral tumors, each approximately the same size. The tumors are probably not the result of a random cell mutation which leads to cell transformation

and eventually to a tumor. To have this event happen simultaneously in both glands would be very unlikely. More likely, the tumors are caused by an infectious agent, environmental factors, or a systemic physiological or pathologic disorder in the affected fish.

Contrary to the above mentioned bilateral nature of Pacific cod tumors, Lange (1973) reported a unilateral pseudobranchial tumors in a cod (*Gadus morhua*) near Norway. The gross pathology of the tumors in the two species appear to be very similar. If the two tumor diseases are the same, the following questions must be considered: (1) Do Pacific cod also have unilateral tumors which have so far not been detected?, (2) Are there only bilateral tumors in Pacific cod, suggesting the disease manifestations are the same, but the causes are different?, and (3) Are there other species of cod in Alaskan waters and elsewhere that have pseudobranchial tumors?

D. Reliability of Data

The types of pathological abnormalities so far detected in the demersal fish populations of Alaska are chronic conditions. Chronic disease is the main type of disease one would expect to find in fish captured by the methods used aboard the R.V. *Miller Freeman*. This is because fish with chronic disorders live longer than fish affected with acute diseases. Acutely diseased fish, such as those infected with virulent bacteria or viruses, would be removed much more rapidly from the population; either from the direct affects of the disease, or by predators. Therefore, the fish diseases described in this report very probably represent only a portion of the diseases by which demersal fishes are affected.

An index of the reproducibility of the disease frequency data resulted from both the R.V. Miller Freeman and R.V. Oregon sampling the same sampling station near Amak Island. The crew of the latter vessel did not record disease frequency data, but they did notice a very large number of rock sole with epidermal papillomas (34 tumor-bearing rock sole were collected at this site and returned frozen to the NWFC, Seattle). At this same site, members of R.U. 332 found a much higher than average frequency of tumor-bearing rock sole (about 5%). This result indicates that if diseased fish are present at a sampling site, and the disease can be visually recognized, then these fish can be detected using current methodology.

VIII. CONCLUSIONS

At least three important diseases of demersal marine fishes are present in the Bering Sea. These diseases are epidermal papillomas of rock sole, lymphocystis of yellowfin sole, and pseudobranchial tumors of Pacific cod. Several conclusions can be drawn from the data concerned with these diseases.

The frequency of 1.0% for papillomas of rock sole ranging in age from 3 to 11 years suggests that juvenile rock sole located in near-shore waters have a much higher frequency. Preliminary analysis of biological data for tumor-bearing and normal rock sole also suggests that tumor-bearing fish are shorter in length than normal fish and that, of normal and tumor-bearing male fish over 30 mm in length, tumor-bearing fish were more numerous. These observations indicate that the papillomas have a significant affect on the life histories of rock sole.

Lymphocystis growths from yellowfin sole contained typical-appearing lymphocystis virus, which suggests that, if this virus is similar to other lymphocystis virus isolates, this disease is infectious. If this is true, then host defense mechanisms probably play an important role in disease transmission. Therefore, environmental stress (i.e., high temperature, pollution) which affect the disease defenses could increase the frequency of lymphocystis in yellowfin sole.

The tumors associated with the pseudobranchs of Pacific cod are not adenomas, as they were once thought to be. They were found to be epidermal tumors surrounding normal-appearing pseudobranchial tissue. One other species of cod besides Pacific cod has been reported to have these tumors; thus, other species of cod in Alaskan waters (i.e., saffron cod) may have this condition.

The sampling techniques employed during the capture and examination of demersal fishes in the Bering Sea appeared to be reliable in terms of detecting externally visible fish diseases.

IX. NEEDS FOR FURTHER STUDY

A. Additional Field Activities

So far our field work has been mainly concerned with demersal fishes captured in the deeper waters of the Bering Sea. As was mentioned above, our data and the reports of others suggest that the shallow coastal waters of the Bering Sea and Gulf of Alaska may have fishes with higher frequencies of epidermal papillomas and lymphocystis, and more species may be affected. Also, because of additional environmental stresses in coastal waters such as higher temperatures, more suspended sediments, and lower salinity, fish in these areas may have other pathological conditions.

Therefore, we feel it would be very important to the objectives of our research unit to be allowed to examine demersal and pelagic fishes and epibenthic invertebrates in the coastal waters of the Bering Sea and Gulf of Alaska in much the same manner as we have done in the deeper waters of the Bering Sea. Sampling vessels will have to be smaller, but all of our sampling and examination procedures performed on the R.V. Miller Freeman could also be done on smaller charter vessels.

Because of the large biomass of epibenthic invertebrates in Alaskan waters, and the recent approval by OCSEAP of an amended contract which would permit our hiring of a biologist specializing in invertebrate pathology, we will increase our emphasis on field and laboratory investigations of invertebrate diseases. This person would participate in all baseline marine animal disease studies in Alaska, as well as conducting research in cooperation with other NMFS groups on the effects of petroleum on invertebrates.

B. Additional Laboratory Activities

The relationship between the exposure of marine animals to crude oil and/or petroleum products and the frequency of disease in these animals is poorly understood. We would like to investigate two general types of possible effects that petroleum may have on disease induction, (1) the effects on disease resistance, and (2) the carcinogenic or tumor-inducing effects.

C. Effects of Oil on Disease Resistance

Petroleum could make a marine fish more susceptible to an infectious disease by either disrupting the animals' physical barriers to disease (i.e., mucous) or by depressing its immune response. We

anticipate performing experiments in which fish species known to be in Alaskan waters would be exposed to oil and subsequently challenged with infectious bacteria or viruses. Types of bacteria would include vibrios and pseudomonads. A possible virus could be the lymphocystis virus described above.

An immune defense mechanisms which is important for resistance to certain types of infectious agents and neoplastic diseases (tumors) is cell-mediated immunity. We plan to test the effect of oil on cellular immunity in fishes, adapting methods of assay of cellular immunity used in higher animals.

D. Possible Carcinogenic Effects of Oil

The epidermal papillomas found in Bering Sea rock sole could be induced by chemicals and carcinogens currently present in the environment. Carcinogens present in petroleum could increase the prevalence of this disease if levels of these hydrocarbons in the marine environment are increased. Since other work done by us has shown that this is a disease of young fish, we wish to expose various life stages of flounder (Alaskan species yet to be chosen) to crude oil. Flounder eggs will be fertilized and the larvae reared in the laboratory to maturity, when the papillomas would be expected to appear. At various life stages, the animals will be exposed to oil either in water or absorbed to bottom sediments. Exposed animals will be examined histologically by light, electron and scanning microscopy, analytically for the presence of aromatic hydrocarbons, and visually for the development of papillomas or other diseases, such as fin erosion and bacterial diseases.

In addition, young rock sole and starry flounder (species of flounder found in the Bering Sea) captured in waters near the NWFC, Seattle, will be exposed to crude oil-soaked bottom sediments and sea water containing crude oil. Examination of treated fish will be the same as described above for the early life stages.

X. SUMMARY OF 4th QUARTER OPERATIONS

A. Laboratory Activities

1. Ship or field trip schedule - no trips were made.

2. Scientific party:

Dr. Bruce B. McCain, PhD
Department of Pathology
University of California (Davis): and
NWFC, Seattle, WA
(Principal Investigator; participated in bacteriology and virology, coordinated data analysis, and prepared progress reports and manuscripts.)

Dr. S.R. Wellings, MD, PhD
Department of Pathology
University of California (Davis)
(Co-Principal Investigator; participated in examination and interpretation of histological preparations, and aided in manuscript preparation.)

Mark S. Myers
Department of Pathology
University of California (Davis); and
NWFC, Seattle, WA
(Performed bacteriology and aided in the analysis of biological data.)

Charles Alpers
Department of Pathology
University of California (Davis)
(Participated in the preparation, examination and interpretation of histological specimens.)

William D. Gronlund
NWFC, Seattle, WA
(Mr. Gronlund is an employee of the NMFS, and part of his duties involve work with R.U. 332. He aided in the analysis of the biological data, and helped make preparations for the 1976 sampling trips.)

3. Methods

All methods used during this period are described in the attached annual report.

4. Data collected or analyzed

Our main research efforts involved histological and microbiological procedures in the laboratory; although, some work was done to prepare for the 1976 field activities.

Laboratory Activities. The writing of a manuscript was begun concerning the pseudobranchial tumors of Pacific cod. Upon completion within the next month, it will be submitted for publication to the Journal of the National Cancer Institute. Continuing electron microscopic examination of these tumors has revealed that the structure of the tumor-specific X-cells is identical to that of the X-cells found in the epidermal papillomas of rock sole.

Careful attention is presently being given to the finding of collagen in the lymphocystis cells from lymphocystis growths on yellowfin sole. Generally, collagen is thought to be an extracellular product. Further electron microscopic studies of these cells and the intracellular collagen may offer insight into the origin of the cells and the viral pathogenesis.

Tests were continued on the 20 bacterial isolates from Bering Sea fish considered to be most disease-related. Additional tests included flagella stains, antibiotic resistance tests, indole production tests, and the 20-test Analylab Products Inc. microdiagnostic strips. Preliminary results suggest that the 20 isolates represent five different groups of pseudomonads.

Field Activities. In preparation for our 1976 field work, supplies were purchased and loaded onboard the R.V. Miller Freeman, a NMFS employee was trained to participate in sample collection, and, in cooperation with Dean Dale, PMEL, Seattle, two new record types were prepared for data processing disease data. One record type will be used to record disease frequency per haul, and the other for recording the characteristics of each diseased animal. Also, data sheets containing the same information as the computer record types were prepared and printed.

Other OCSEAP investigators concerned with fishes of the Bering and Beaufort Seas were contacted and our needs for the fish pathology study were explained. The Beaufort Sea investigators are Eugene Reguski of the Alaska Department of Fish and Game, Fairbanks, Alaska, and Dr. Andrew G. Carey of Oregon State University, Corvallis, Oregon. An outline containing descriptions and pictures of each of the marine fish diseases known to be in Alaska and describing specimen handling and preservation was prepared and will be given to these investigators prior to the 1976 sampling season. Three copies of this outline were given to the R.U. 175 for use by scientists aboard charter vessels that will be sampling in the Bering Sea.

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Table 1. Incidence of the three most common diseases of demersal fish captured during the second and third legs of R.V. Miller Freeman cruise M-75-1 in the Bering Sea

Disease	Species Affected	Total Fish Examined	Number of Diseased Fish	Percent Incidence
tumor of the pseudobranch	*Gadus macrocephalus*	1,510	111	7.4
epidermal papilloma	*Lepidopsetta bilineata*	6,937	66	0.95
lymphocystis disease	*Limanda aspera*	13,239	272	2.1

Table II. Species of fish examined and found to be without detected disease during the second and third legs of R.V. Miller Freeman cruise M-75-1 in the Bering Sea

Species	Species
Arrowtooth flounder	Red Irish lord *
Greenland halibut	Great sculpin
Pacific halibut *	Spinyhead sculpin *
Flathead sole	Sandfish *
Bering flounder	Saffron cod *
Rex sole *	Walleye pollock
Longhead dab *	Capelin *
Starry flounder	Rainbow smelt *
Alaska plaice	Snake prickleback *
Sturgeon poacher	Wattled eelpout
Pacific herring	*Sebastes* sp. *

* Limited sampling; 50 or less, individuals examined.

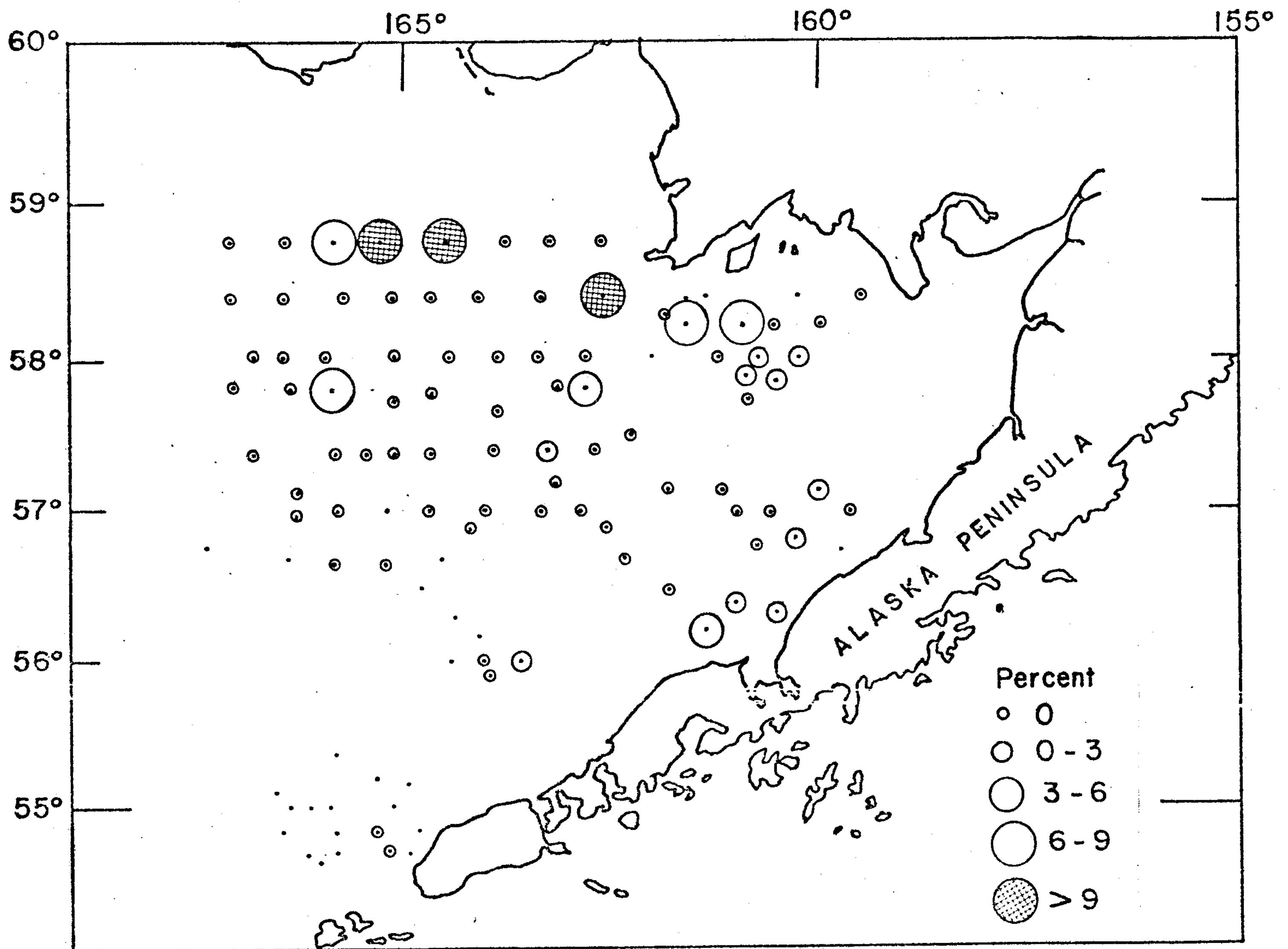


Figure 1. Approximate frequencies of rock sole with papillomas at sampling stations in the Bering Sea where rock sole were captured.

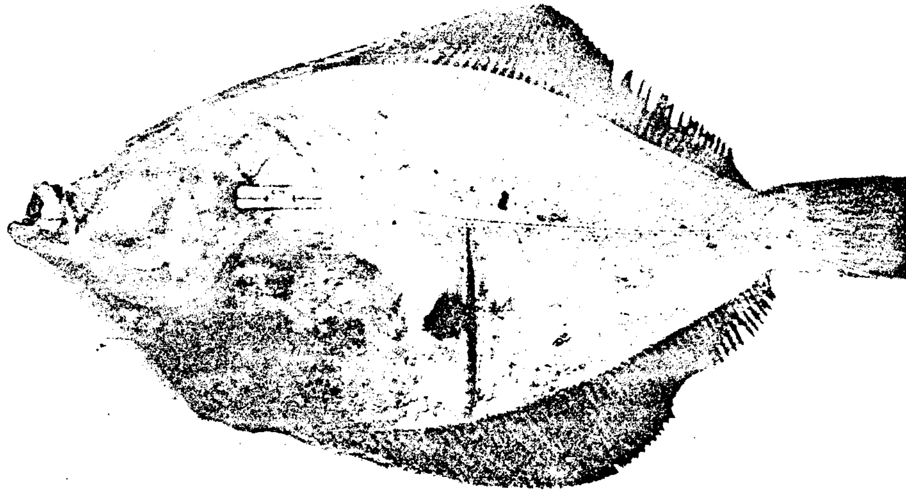


Figure 2. Rock sole with an epidermal papilloma on the abdomen of the "blind" side.

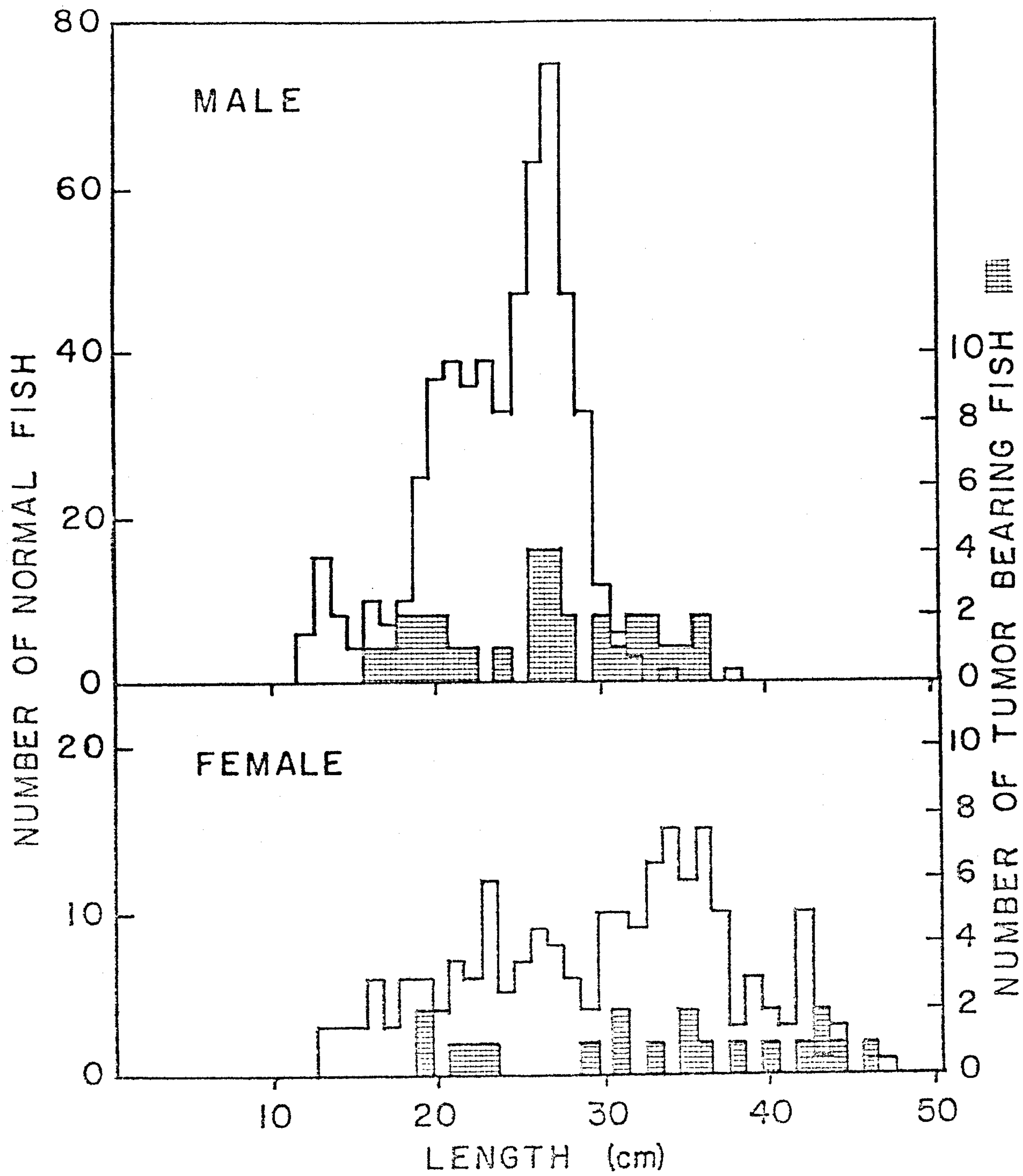


Figure 3. Length frequencies of normal and tumor-bearing male and female rock sole in catches containing fish with epidermal papillomas. Cumulative data is plotted in open bars, and frequencies of tumor-bearing fish are in solid bars.

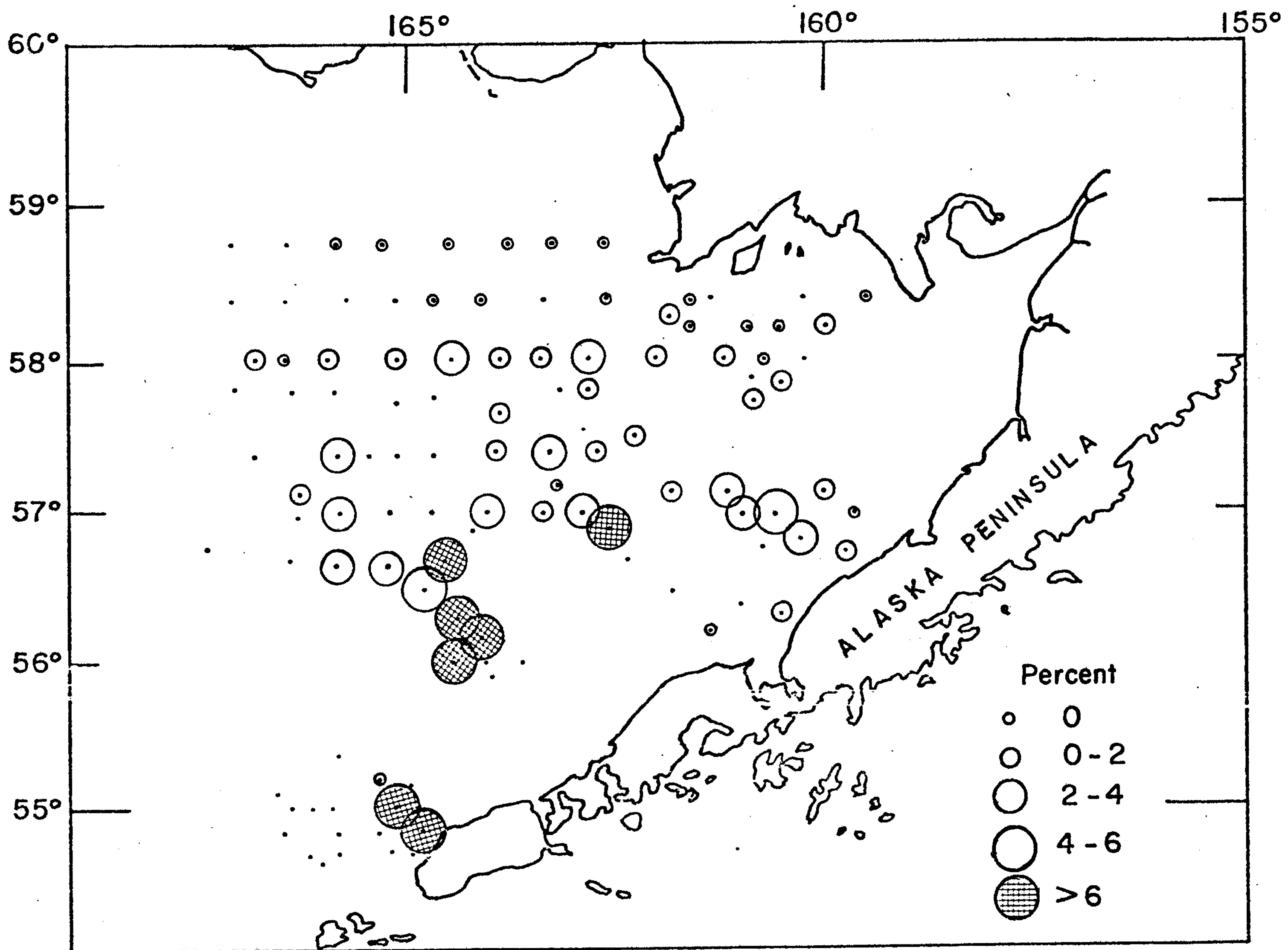


Figure 4. Approximate frequencies of yellowfin sole with lymphocystis at sampling stations in the Bering Sea where yellowfin sole were examined for the disease.

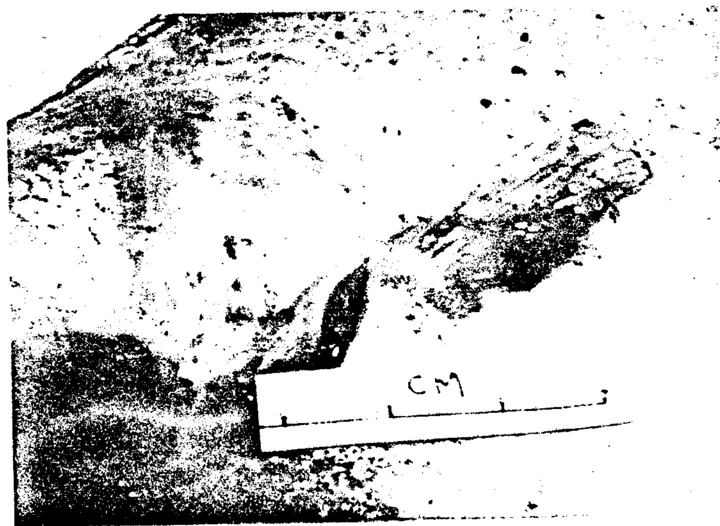


Figure 5. Yellowfin sole with a lymphocystis lesion on the "blind"-side pectoral fin.

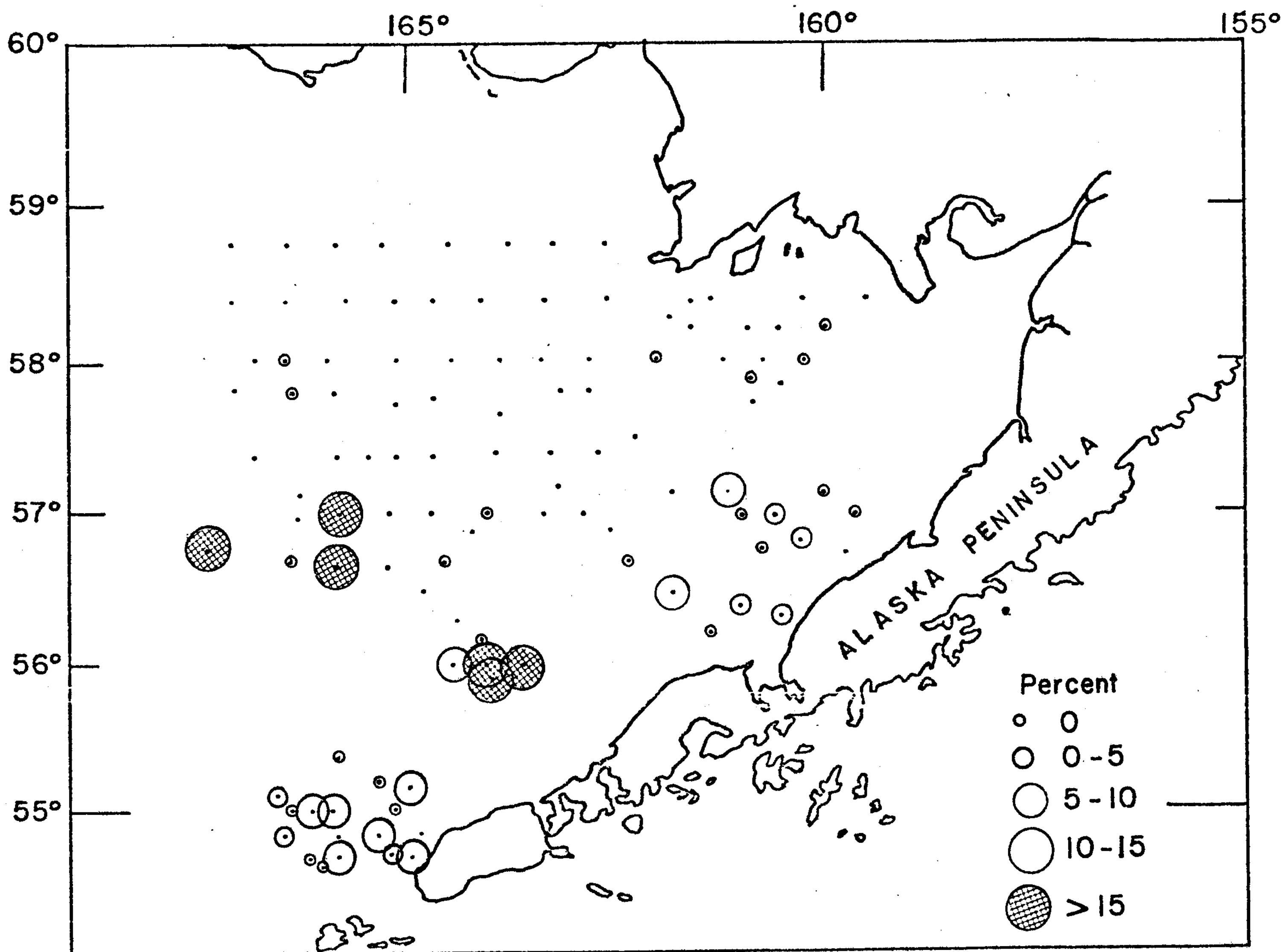


Figure 6. Approximate frequencies of pseudobranchial tumors of Pacific cod at sampling stations in the Bering Sea where this species was captured.

Figure 7. Two, bilateral pseudobranchial tumors in the pharyngeal region of a Pacific cod.

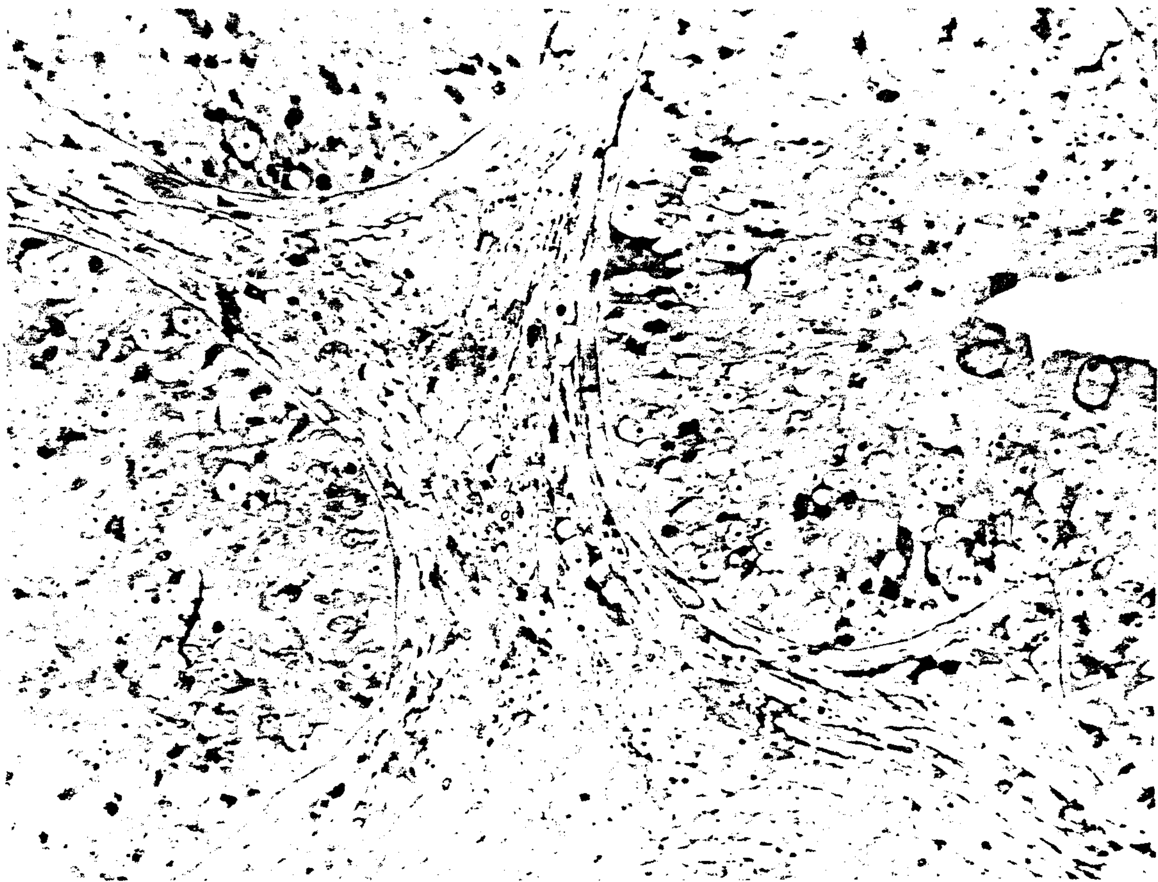
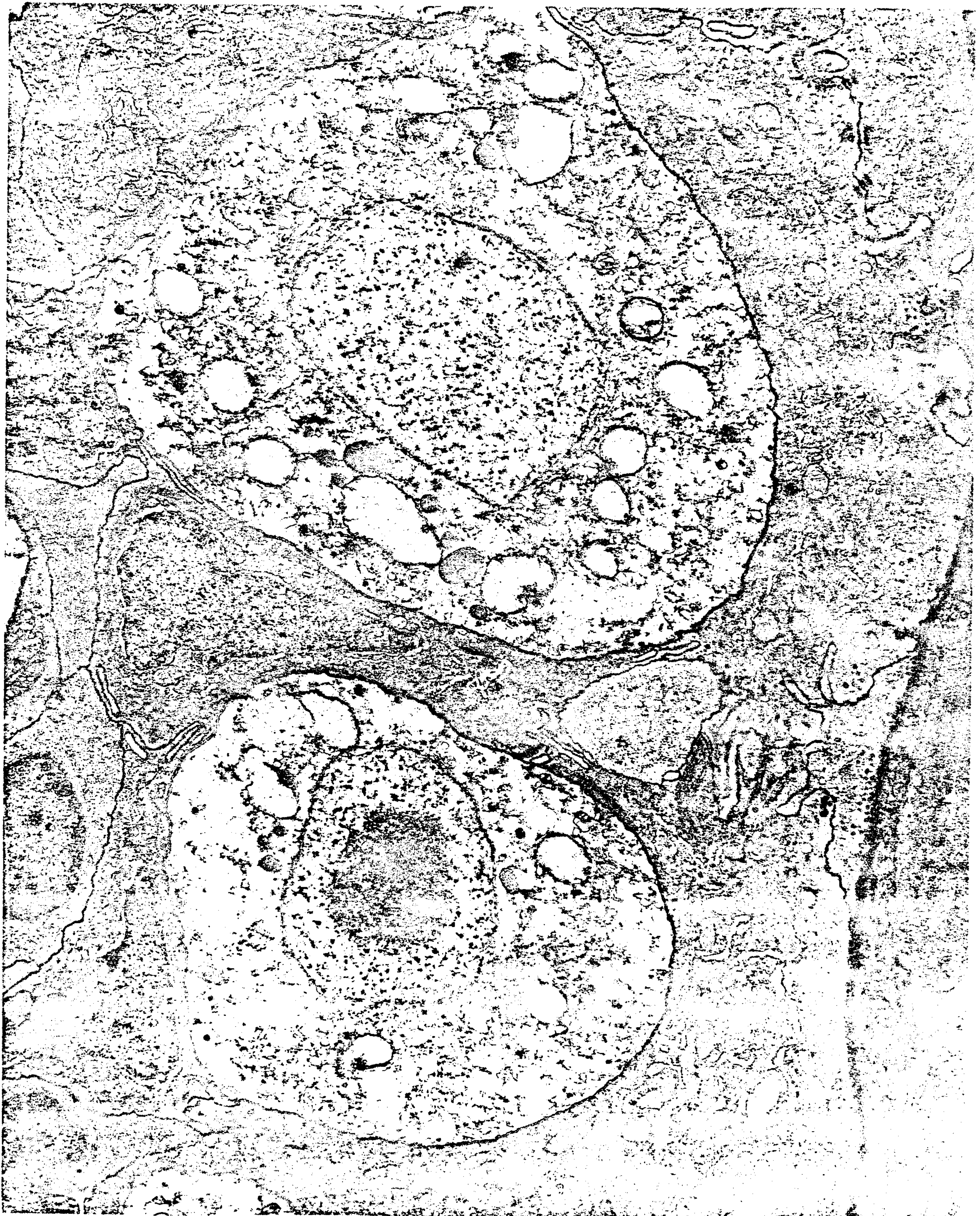


Figure 8. Section of an epidermal papilloma from a rock sole. Fibrovascular stroma extends up through hyperplastic epithelium; both areas contain X-cells characterized by their large size, pale nucleus and large, intensely stained nucleous. (Stained with H and E)

Figure 9. Electron micrograph of two X-cells in a rock sole epidermal papilloma. Upper X-cell has prominent nucleolus, and both cells have vesicular cytoplasma with few mitochondria. A normal-appearing cell can be seen wedged between the X-cells. (mag., 12,000 X).



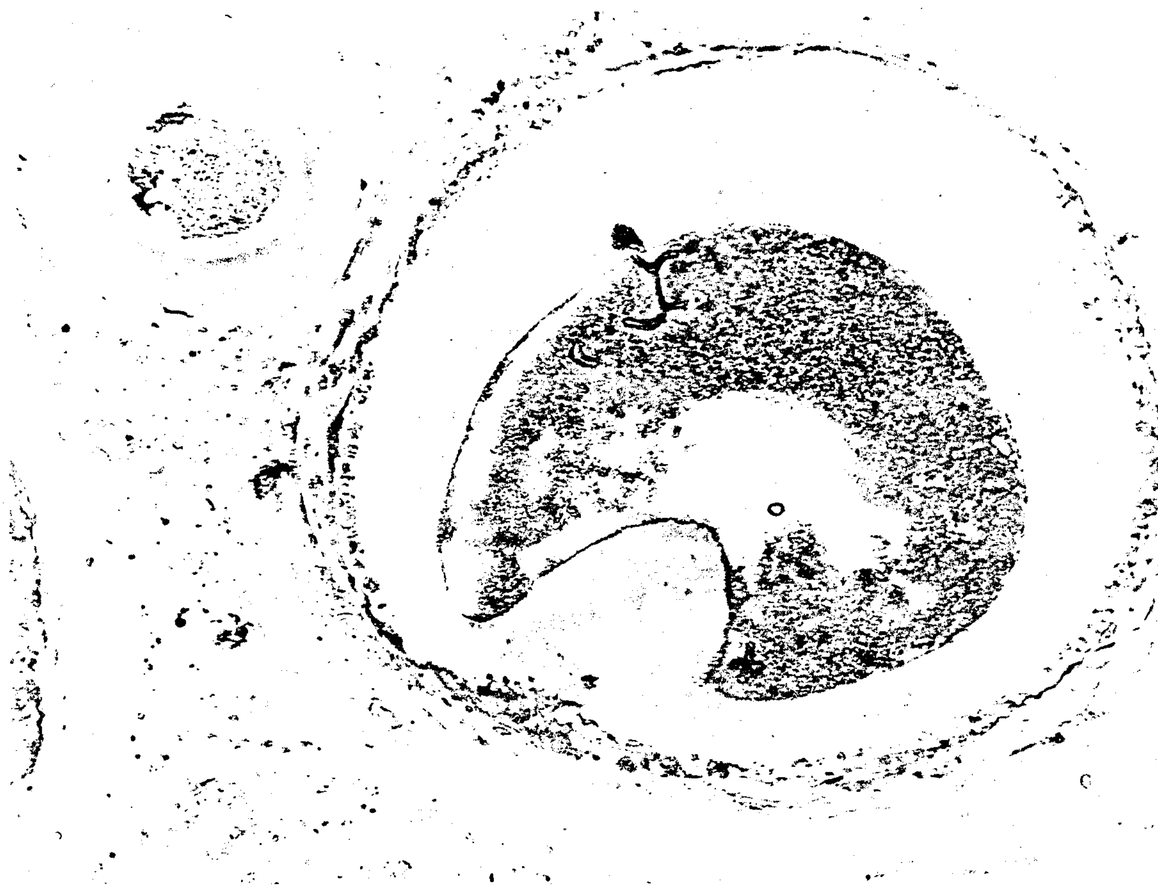


Figure 10. Two lymphocystis cells, approximately 0.3 and 0. 1 mm in diameter, from a yellowfin sole, respectively. The larger cell has a centrally located inclusion body from which the electron micrograph in Figure 12 was taken.

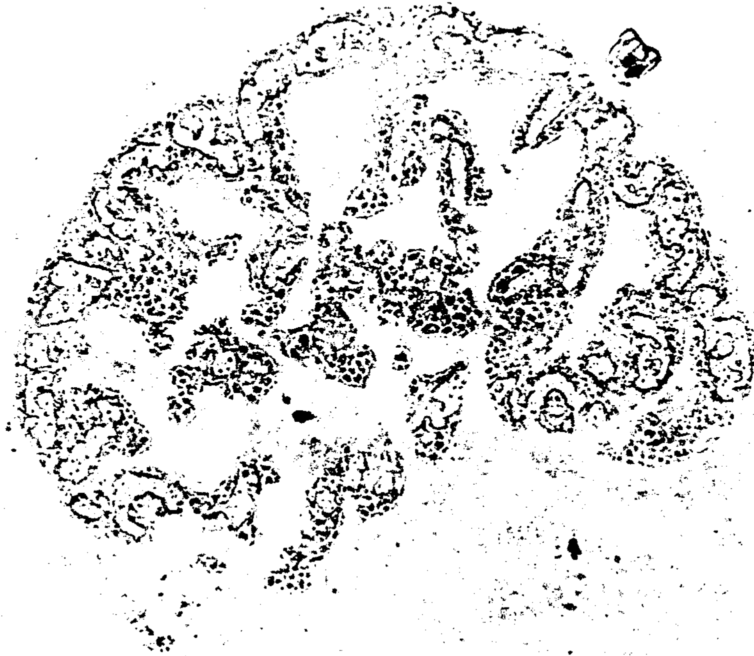
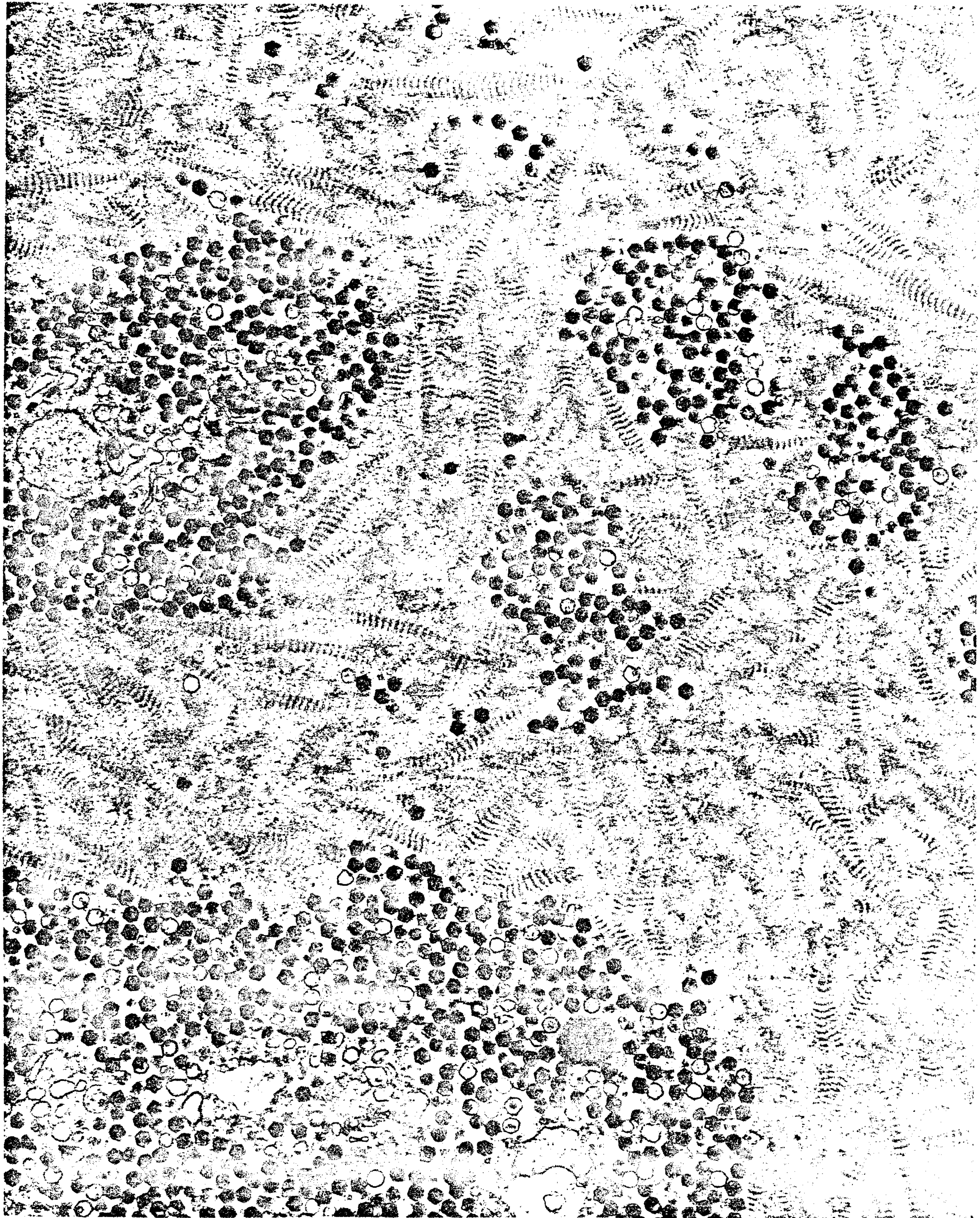


Figure 11. A section of a lymphocystis cell with a fibrillar-type inclusion body. A lightly staining cell nucleus occupies the lower portion of the cell.

Figure 12. Electron micrograph of a portion of the inclusion body seen in Figure 11. Five clumps of lymphocystis virus can be seen. The virions are hexagonal, about 200 nm in diameter (mag., 16,000 X).



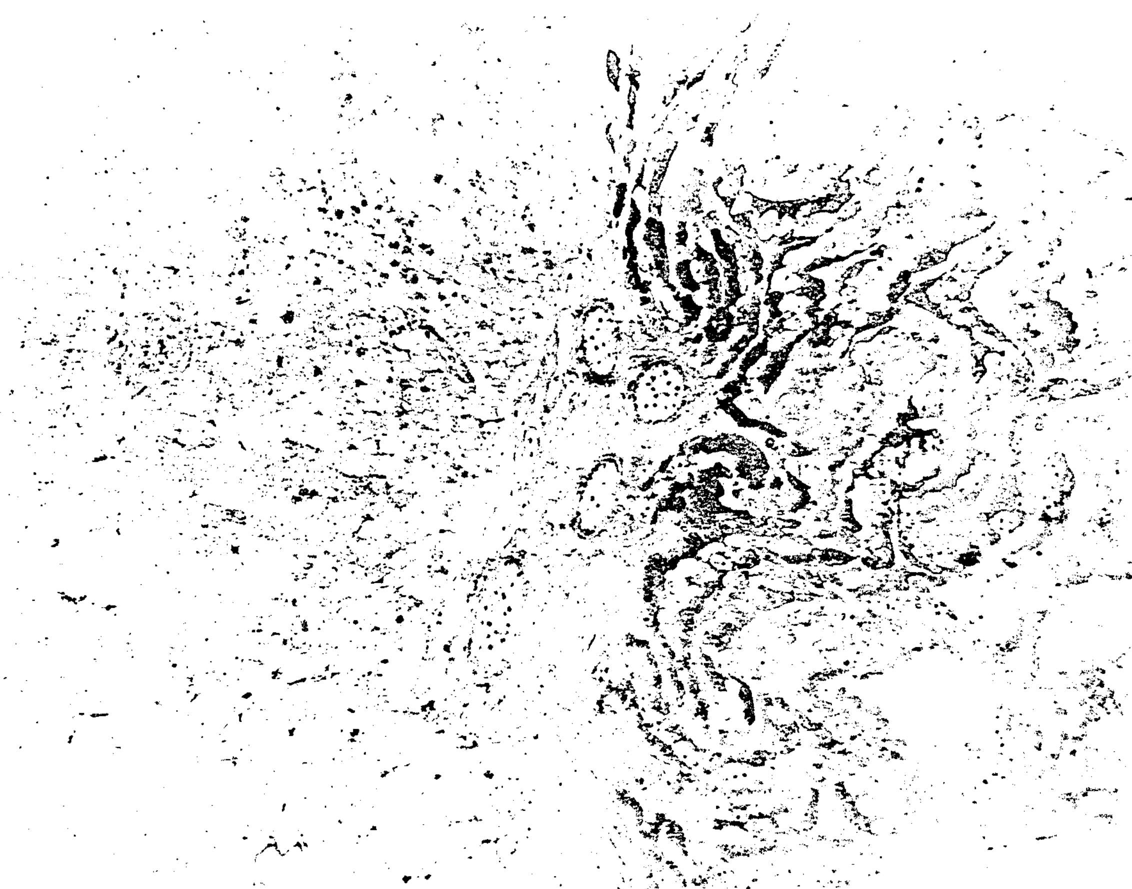


Figure 13. A section of a pseudobranchial tumor from a Pacific cod. Normal-appearing pseudobranch tissue is on the right, a portion of the connective tissue capsule is in the middle, and the epidermal tumor is on the left. (Stained with H and E)

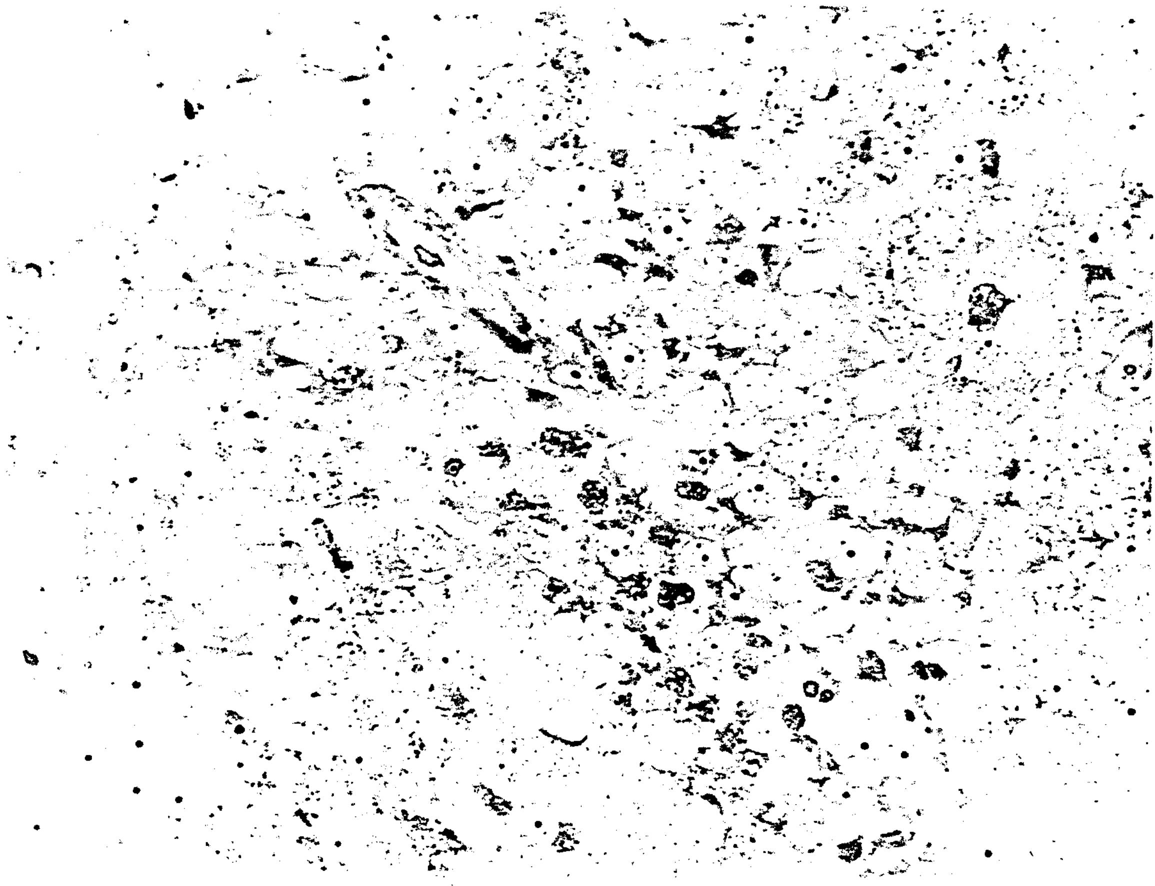


Figure 14. A higher magnification of the epidermal tumor shown in Figure 13. X-cell-like cells can be seen throughout the field. (Stained by H and E)

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