VITA

NAME OF AUTHOR: Mark Joseph Schneider

PLACE OF BIRTH: Minneapolis, Minnesota

DATE OF BIRTH: November 22, 1942

UNDERGRADUATE AND GRADUATE SCHOOLS ATTENDED:

Saint Mary's College
University of Minnesota
University of Oregon

DEGREES AWARDED:

Bachelor of Arts, 1964, University of Minnesota
Master of Arts, 1966, University of Oregon

AREAS OF SPECIAL INTEREST:

Comparative Physiology
Biochemistry

PROFESSIONAL EXPERIENCE:

Teaching Assistant, Department of Biology, University of Oregon
Eugene, 1965-1966

AWARDS AND HONORS:

Public Health Service Physiology Traineeship, 1966-1969

PUBLICATIONS:

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EFFECTS OF SIZE AND THERMAL HISTORY ON CENTRAL NERVOUS SYSTEM LIPIDS IN THE FISH, *AEQUIDENS PORTALEGRENSIS*

by

MARK JOSEPH SCHNEIDER

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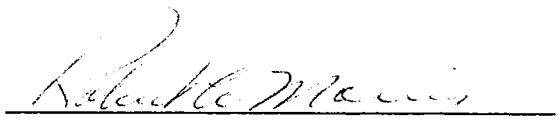
APPROVED: 

(Robert W. Morris)

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DEDICATION

I dedicate this thesis to my wife, Marilynne, for her patience and understanding during the last four years, and to my parents, Joseph and Lillian, for their persistent encouragement.

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INTRODUCTION

Strict adherence to the Van't Hoff relationship would be disadvantageous for poikilothermic organisms. The fact that poikilotherms are able to acclimate metabolically under thermal stress has been well documented. The compensatory ability of several species of fishes has been shown to be related to the body size of the animal. For example, small individuals of the cichlid, Aequidens portalegrensis, make metabolic adjustments to low environmental temperatures; only with increasing size is the ability to compensate for higher temperatures developed (Morris, 1962).

Several investigators have shown that thermal compensation in poikilotherms results in qualitative biochemical alterations. A characteristic pattern of thermally induced fatty acid changes has been revealed in many tissues. It is the aim of the present study to describe these lipid alterations of the central nervous system in Aequidens portalegrensis. Additionally, it is hoped to show that such changes are related to the size of the fish in a manner similar to that of metabolic acclimation.

The growth depensation characteristic of fish populations presents a problem in the study of a size related physiological process; fish of the same size may vary greatly in age. It is necessary at the outset of this study, therefore, to determine the effect age has upon metabolic rate.

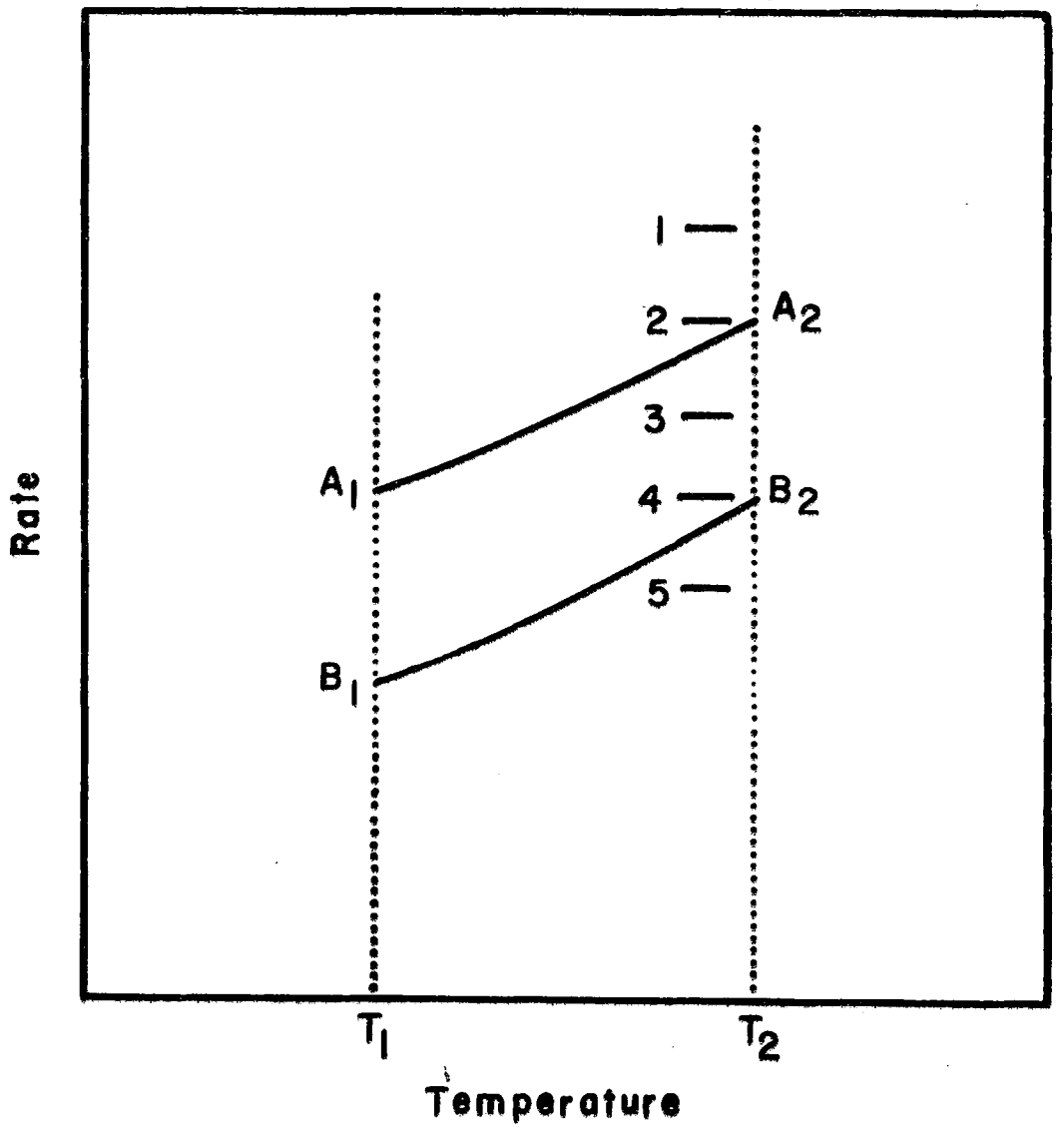
I. Thermal Acclimation

The body temperature of most aquatic poikilotherms is in thermal equilibrium with the environment. If the physiological functions of such an organism were faithful to the Van't Hoff relationship, its activities would be dictated by the ambient temperature fluctuations. This is generally not the case. Many fishes living in waters that freeze over in the winter remain nearly as active during that portion of the year as they are in the summer. Obviously, these poikilotherms have freed themselves from strict adherence to the accelerating affect of temperature on reaction rates.

Figure 1 is a graphic representation of the mode of compensation a poikilotherm produces when faced with a thermal stress. The line connecting points A_1 and A_2 represents the change in a physiological rate as predicted by the Van't Hoff law if an animal is presented with temperature T_2 after having resided at temperature T_1. If the animal now remains in this new thermal environment for an extended period of time, one of four changes may occur in the rate function considered. The numbers 1 through 5 along the line of temperature T_2 represent the five types of acclimation according to Precht (1955). In some cases there will be no compensation as is indicated by type 2, but most organisms show compensation ranging from partial (type 3) to perfect (type 4). In a few cases an over compensation (type 5) and an under compensation (type 1) have been noted (Prosser and Brown, 1962).

A comparison of the rate-temperature curve for a group of poikilotherms of T_1 thermal history with the curve generated by a group of T_2

Figure 1. Effect of temperature change on the rate of a physiological process. (1) Under compensation, (2) No compensation, (3) Partial compensation, (4) Perfect compensation, (5) Over compensation. Modified from Precht, 1955.


Rate
1
2
A2
3
A1
4
B2
5
B1
T1
T2
Temperature

history reveals something of the nature of thermal compensation. If the animals are incapable of compensating, the result would be type 2, the rate-temperature curves for the two groups would coincide. More frequently two curves will be derived like those in Figure 1, curves A_1A_2 and B_1B_2. The upper curve is representative of an animal that has successfully made compensation to cold while the lower curve is that of warm compensation. In classical terminology this represents a "translation of the rate-temperature curve". In addition to such lateral movement it is frequently found that the curve has undergone rotation, i.e., the Q_{10}'s of warm and cold compensation differ (Prosser and Brown, 1962).

The translation of rate-temperature curves has been interpreted to be the result of alteration in the nature of enzyme systems; e.g., a change in the enzyme concentration, ionic strength, or pH (Kanungo and Prosser, 1960; Hochachka and Hayes, 1962). Rotation of the curve suggests a change of Arrhenius' value A. Currently, it is believed that there is a shift in the relative concentrations of the various isozymes comprising an enzyme system (Hochachka, personal communication).

The term that has been given to the phenomenon of compensation to thermal stress is "thermal acclimation". The exact application of this term is to a laboratory induced compensation produced by maintaining animals under controlled temperature regimes. A similar phenomenon has been noted in "summer" and "winter" animals, where temperature changes are the result of the change of season. The term applied here is "acclimatization". There is a third term used in the discussion of compensatory phenomena, "adaptation". This refers to the genetically controlled

ability of the organism to deal with environmental stress such as temperature change. Each of these terms expresses a rather different relationship of the animal to its environment; however, the actual physiological mechanism underlying them may be similar. In most studies of thermal acclimation, groups of animals are maintained at defined temperatures. After an appropriate period the physiological aspect of interest is studied. Those animals kept at the lower temperature are referred to as "cold acclimated"; while those maintained at higher temperature are spoken of as "warm acclimated". These terms will be employed throughout this study.

II. Vital Functions Demonstrating Thermal Acclimation

Of the physiological functions that could serve as indices of thermal acclimation, metabolic rate has frequently been used. Many investigators have constructed rate-temperature curves using the rate of oxygen consumption as the measured parameter of metabolism. Other parameters that have been used are the rates of heart and opercular beat (Krog, 1954; Tsukuda, 1961). A representative study based on oxygen consumption employed two groups of Carassius auratus (Cyprinidae) which were maintained at 5° and 26°C for five weeks (Suhrman, 1955). At the end of this time the oxygen consumption of goldfish from both temperature groups was measured at all temperatures from 10° to 30°C. The derived rate-temperature curves displayed a highly significant difference in oxygen consumption. The curve of the warm acclimated fish was 30% lower than the curve of cold

acclimated fish. This is an example of a translation of the rate-temperature curve to the left on cold acclimation. Metabolic thermal acclimation has been found to be a phenomenon common to many poikilotherms: fish (Suhrman, op. cit.), reptiles (Dawson and Bartholomew, 1956), and crustaceans (Krog, op. cit.). In addition to whole animal metabolism, oxygen consumption at the tissue level demonstrates thermal acclimation (Freeman, 1950).

The mechanism of thermal acclimation in these organisms functions at the cellular and biochemical level (Das and Prosser, 1967; Das, 1967). For this reason, much research has been devoted to elucidation of biochemical changes occurring as a result of thermal acclimation. The alterations of lipids seem to have been of singular general interest possibly because of their complexity as a family of biochemicals and also due to the fact that they demonstrate changes in physical state within the range of physiological temperatures. At the same time that Arrhenius and Van't Hoff were studying the effects of temperature on reaction rates, the investigation of its effects on lipid composition of animals was already in progress. Henriques and Hansen (1901) found that pigs dressed in warm clothing for a period of months had body fat with a higher melting point than that of controls. This is a reflection of the degree of unsaturation of the fatty acids comprising the fats (West and Todd, 1964). With the advent of gas chromatography this problem could be studied with great precision and from such research a characteristic pattern has developed. Generally, when fatty acids have been obtained from a plant or animal which has been maintained at a low temperature the fatty acids exhibit

greater unsaturation and the percentage of long chain acids is much higher than a similar sample of acids obtained from a warm acclimated individual (Fraenkel and Hopf, 1940; Hoar and Cottle, 1952; Kayama et al., 1963; Farkas and Herodek, 1964; Knipprath and Mead, 1965, 1966; and Jagaard et al., 1967). This characteristic development of a greater amount of long chain unsaturates occurs from the level of the whole animal down to the subcellular fractions such as the mitochondrial membranes obtained from the gills of cold acclimated fish (Caldwell, 1967).

III. The Size Dependence of Acclimation Ability

One of the features of the acclimation ability in fishes is that it is often related to the size of the animal. Working with Aequidens portalegrensis, Morris (1962) observed that only the small animals demonstrated metabolic compensatory ability when subjected to cold temperatures. Only as the size of the fish increased beyond approximately 6.0 gm was the ability to acclimate to warm water gained. With the acquisition of this new ability there was a partial loss of the capacity to cold-acclimate. In an eleotrid fish, Carassiops compressus, the size relationship to acclimation ability was different (Morris, 1965a). Here again the smaller individuals were observed to acclimate to cold. In this species, however, fish of any size demonstrated complete lack of acclimation ability when subjected to warm water. The author interpreted this observation as a case of neoteny at the physiological level. In a continuing survey of acclimation in fish, Morris (1965b) found that the small individuals of Forsterygion varium and F. robustum (Clinidae) showed a significantly

greater ability to cold-acclimate than did the larger fish; that is, the smaller animals could adjust their metabolic rate upward to a greater degree than could the larger fish. Although the larger fish demonstrated a capacity to warm-acclimate, no relationship of this ability to size was found. In contrast to these four examples, Ictalurus natalis (Ictaluridae), demonstrates no size relationship in its ability to acclimate (Morris, 1965c). The fact that thermal acclimation is size related in some fish but not in others has been interpreted as an indication of at least two different mechanisms for acclimation (Morris, 1965c).

IV. Size and Age

Among a brood of young fish, shortly after the onset of active feeding, a very striking disparity in the size of individuals can be noticed. As the fish increase in age, the variance in a size frequency distribution increases. This growth depensation in fish has been documented in many species (Hubbs and Cooper, 1935; Brown, 1946, 1951, and 1957). Explanations for this size disparity have been many. Probably the most obvious explanation would be based on simple genetic variation. This probably plays a part in the phenonenon and cannot be disregarded (Allee et al., 1948). Many investigators have shown, however, that the intensity of growth depensation is affected by many other factors as well: disparity in egg size (Brown, op. cit.), social order (Greenberg, 1947), aggressive behavior (Magnuson, 1962), and production of self-inhibiting growth factors (Rose, 1959).

Whatever the cause of growth depensation may be, the resultant

size disparity in fish of the same age presents a problem in investigations of any physiological function that is size related. It is necessary to know which factor, size or age, is of greater importance.

V. The Central Nervous System and Thermal Acclimation

In the search for an explanation of the process through which the effects of acclimation are produced many investigators have suggested involvement of the central nervous system, since it is the most sensitive to temperature stress (Freeman, 1950; Brett, 1956; and Tsukuda, 1961). They reasoned that the thermal limit of an organism is set by the resistance of the most sensitive essential tissue and this appeared to be the central nervous system. The early studies of Battle (1926) indicated that, on gradual heating, the order of tissue failure was as follows: myoneural junctions, nerve, somatic muscle, smooth muscle, and finally cardiac muscle. More recently a similar work dealing with the nerve-muscle preparation from Rana pipiens displayed a corresponding result (Orr, 1955).

Evidence gathered from behavioral studies also suggests that the central nervous system is involved in thermal acclimation. If a fish is placed in water where there is a gradation of temperature the animal will seek out a "preferred temperature". Further, this selected temperature is determined by thermal history (Fry and Hart, 1949; and Pitt et al., 1956). For Carassius auratus acclimated to temperatures ranging from 10° to 35°C, in increments of 5°C, the selected temperatures were: 17°, 25°, 27°, 31°, 31°, and 32°C (Fry and Hart, op. cit.).

The first direct evidence for the involvement of the central nervous system in thermal acclimation is found in the work of Roots and Prosser (1962). These investigators determined the temperature of cold blockage of nervous activity for simple and conditioned reflexes in Carassius auratus, Lepomis macrochirus (Centrarchidae), and Amieurus melas (Ictaluridae) that had been acclimated to 5°, 15°, 25°, 30° (and 35°C for the goldfish only). Lower acclimation temperature decreased the temperature at which the simple reflex response to an electrical stimulus was blocked. A typical result for the goldfish was blockage at 10°, 5°, and 1°C for fish acclimated to 35°, 25°, and 15°C respectively. Section of the spinal cord just posterior to the medulla had no effect on the results; therefore, the site of cold blockage of the reflex was the spinal cord. Similar results were obtained for the blockage of conditioned reflexes. The two conditioned reflexes were: inhibition of respiration and a light avoidance response. The temperatures of cold blockage were considerably higher for each acclimation class than in the simple reflexes. These authors interpreted the site of blockage to be the brain. It is apparent that the cold blocking temperature is related to both the acclimation temperature and the nerve pathway involved and that the site of cold blockage was the central nervous system.

Another work dealing directly with the effects of temperature acclimation on the function of the central nervous system measured an evoked midbrain potential in Salmo gairdnerii (Salmonidae) acclimated to 4°, 10°, and 16°C (Konishi and Hickman, 1964). Subjection to 4°C after acclimation to 10°C caused a lengthening of the spike latency and the

duration of the post-synaptic potential. Maintenance of fish at 4°C produced a compensatory shortening of the response to a level comparable to that found in 10° fish measured at 10°C. Abrupt transferal of 10° fish to 16°C did not result in a significant shortening of the latent period as might have been expected, but the preparations quickly lost their responsiveness. With acclimation to 16°C the survival time of the preparation was increased.

The results of the studies of Roots and Prosser, and of Konishi and Hickman denote that thermal acclimation in fish entails alterations of the central nervous system which allow normal functioning at higher or lower temperature than before acclimation. Just what constitutes these alterations was of interest to Johnston and Roots (1964). In their research they found that changes in lipids reported in the earlier literature could be found at the level of the nervous system. The amount of lipid material in the brain of the goldfish (Carassius auratus) increased with cold acclimation as well as an increase in the long chain fatty acids of high degree of unsaturation. In particular, they found the greatest difference in the relative amounts of arachidonic (20:4), docosapentaenoic (22:5), and docosahexaenoic (22:6). (The number before the colon represents the number of carbon atoms in the fatty acid chain; the number after the colon, the number of double bonds in the chain). Johnston and Roots (op. cit.) suggest that nervous system acclimation is due to the ability to control the chain lengths and unsaturation of fatty acids comprising the neuronal lipids. They propose this as a mechanism for maintenance of a specific state of the cellular membranes allowing compensatory

changes in the biophysical properties of the central nervous system.

VI. Statement of the Problem

As in other species of fish, growth depensation is striking in Aequidens portalegrensis. The resultant size disparity often reaches such an extreme that the largest fish is ten times the size of the smallest fish in the same brood. Where such a great size range exists for animals of the same age it may be important to know how age may affect physiology. It is essential to determine which factor, size or age, is of greater influence on physiological processes. The first portion of the present work deals with this problem. Groups of fish of comparable size ranges but of different ages were studied respirometrically to ascertain the effect of age on weight-specific metabolic rate. And in other experiments on similar age groups of fish, again of comparable size ranges, sensitivity to temperature change was determined, i.e., Q_{10} values for the age groups were determined.

The investigations of Morris (1962; 1965a, b, and c) showed that the ability of several species of fish to acclimate was related to body size. Of particular interest was Aequidens portalegrensis. Only the smaller individual A. portalegrensis could acclimate to low temperatures and only with increasing size did the capacity to acclimate to higher temperature become established. The involvement of the central nervous system in acclimation was documented earlier and a possible role of fatty acid alteration in the central nervous system was considered. A second phase of the present study was aimed at describing the qualitative

nature of thermally induced changes in the lipids of the central nervous system (brain and spinal cord) of A. portalegrensis. The resulting data were also analyzed to determine whether or not such changes were related to body size. Positive results would be evidence that thermally induced changes in the central nervous system, arising as a result of thermal history, might themselves come to be causative of the subsequent metabolic changes (Prosser et al., 1965).

Since the brain and spinal cord were excised and treated separately in all stages of this study a comparison of the lipids obtained from the two tissues was made. It was hoped that such a comparison might indicate whether the lipids were associated with the neurones or the myelination.

The term "lipids" encompasses a rather large group of biochemicals which can be placed in the following classes: waxes, hydrocarbons, free fatty acids, phospholipids, glyolipids, glycerides, and sterols. The section of this work which deals with the lipids of the central nervous system and their relationship to the size of the organism, considered the total fatty acids derived from all saponifiable classes. Some additional information was gathered by investigating the distribution of fatty acids among separate classes of lipids. In this portion of the investigation the total extractable lipids were separated into three fractions: (1) sterols, glycerides, sterol esters, and wax-hydrocarbons, (2) free fatty acids, (3) phospholipids. From each of these three fractions the fatty acids were collected and analyzed.

Thermally induced alterations of the fatty acid pattern of central

nervous system lipids are the result of changes in fatty acid metabolism. It is hypothesized that the fish is capable of lengthening or shortening, saturating or unsaturating fatty acid chains according to requirements. Many of the fatty acids derived from the diet act as precursors for such metabolism. In addition, dietary fatty acids often have a direct influence on the fatty acid pattern of the consumer. It was hoped that knowledge of the fatty acid composition of the food might be revealing with regard to fatty acid metabolism and dietary influence. Total fatty acid analyses were, therefore, made from several samples of the food.

MATERIALS AND METHODS

I. Aequidens portalegrensis

The goal of this research was information about the process of thermal acclimation in poikilotherms, particularly in fish. Obviously the choice of the experimental organism was to be made on the basis of acclimation performance. Morris (1962) had shown that Aequidens portalegrensis displayed near perfect (type 3 of Precht) acclimation. Additionally, the ability of this fish to thermal-acclimate displayed a well defined relationship with size. A. portalegrensis was chosen as the experimental animal because it had these two acclimation characteristics.

Little is known about the ecology or natural history of this member of the family Cichlidae. It has proven to be a useful laboratory fish, requiring relatively little care and is easily reared in the large numbers needed for statistically meaningful results. Upon reaching sexual maturity the fish can be induced to spawn regularly, the eggs being laid on pottery placed on the bottom of the aquarium. In raising the young fish the practice was to scrape the eggs off the pottery several hours after they had been laid and fertilized. They were then transferred to beakers where they were artifically incubated. The original stock of fish had been obtained from tropical fish suppliers and raised in Dr. Morris's laboratory for 10 to 12 generations at the outset of this study.

Previous experience had shown that A. portalegrensis was most easily maintained on a diet of Lumbricus terrestris which could be obtained from a local supplier. The preparation of this diet differed for

the two portions of the research. The first section has been referred to in the INTRODUCTION as Size and Age; the second section deals with the lipids of the central nervous system.

The three age classes of fish used in the respirometry were fed worms that had been chopped in a Waring blender and then poured into porcelain pans and frozen. Twice a day chunks of this frozen worm suspension were placed in the aquaria and as the ice melted, the fish fed on the settling bits of worm.

It is known that the fatty acid composition of fish tissues can be markedly influenced by the dietary fatty acids (Kelly, Reiser, and Hood, 1958). While observing the fish feeding, it was noticed that recognizable bits of worm could be seen falling from the frozen food chunks. Most commonly identified were whole gonads. It was, therefore, considered necessary to provide a more homogeneous diet with regard to the lipid content during the portion of the research dealing with lipids. This was accomplished by blending the earthworms until a thin slurry was obtained and then suspending this in warm agar which, after cooling, could be cut into cakes which the fish readily consumed. Here again the animals were fed twice daily and at the end of each day the uneaten food was removed from the aquaria. The fish were not able to digest the agar but passed it into the water apparently stripped of the nutritional components. Throughout the acclimation period the fish continued to grow and at all times appeared to be in excellent condition.

With one exception, the fish in all experiments were maintained in fifteen gallon glass tanks which were routinely cleaned every fourteen

days. The one exception was the group of fish from which the lipid extracts were fractionated into the lipid classes. These fish were kept in outdoor cement tanks having inside dimensions of 4' x 4' x 4'. The water in these tanks was changed every fourteen days. As mentioned above, the indoor aquaria were fitted with continuously operating bottom sand filters. The outdoor tanks contained filters consisting of glass wool, charcoal, and sand. In the fifteen gallon tanks the sand was littered with bits of pottery which offered cover for the animals. All of the fish maintained indoors were under constant illumination from room lights and during the day additional light from the four windows in the room. The two outdoor tanks were only illuminated by natural daylight. The fish occupied these tanks during the months of June, July, and August.

Throughout the research, the temperatures of the aquaria were maintained with immersion heaters or heating tapes (in the case of the cement tanks) controlled by Fenwall thermostats. This system maintained the desired temperature within one half of a degree centigrade. Three acclimation temperatures were used: 22°, 27°, and 30°C. The animals used in the respirometric study of size and age were all maintained at 27°C from the time of spawning. The age of the youngest group of these fish was five months. Many of these same fish were employed in the subsequent lipid studies for which they were acclimated to 22° and 30°C. The period of time spent by these fish at the two temperatures was three months. Although Doudoroff (1942) had shown that as little as 48 hours was needed for a fish to compensate for a rise in temperature, diet was another factor to be considered here. With the change in the preparation of the

worm food there was a potential change in the dietary fatty acids. It was felt necessary, therefore, to allow ample time for the dietary effect to become stabilized.

II. Size and Age

The rate of oxygen consumption was the index used in the calculation of weight-specific metabolic rate and Q_{10}. These measurements were used to reveal any differences of metabolic performance that might be attributed to age. A number of fish from different age classes but similar size ranges were used. The instrument used for these measurements was a multiple version of the modified Barcroft respirometer described by Morris (1963). This apparatus consists of a rack on which are mounted five round bottomed flasks which serve as respirometer vessels; each of the flasks is connected by a manometer to a common compensation vessel. The compensation vessel eliminates fluctuations due to variations in atmospheric pressure. The rack can be lowered into a 35 gallon water bath. The temperature of this bath can be controlled to within $\pm$ 0.1°C by a "push-pull" system of heater and refrigeration coils actuated by a mercury thermostat. Inside each of the respirometer flasks is a small bucket which holds a potassium hydroxide saturated wick which absorbs any carbon dioxide as it is produced by the experimental animal. On the bottom of each vessel is a magnetic stirring bar separated from the fish by a perforated lucite plate. This stirring bar maintains an equilibrium between the gas and liquid phases. As the animals respire, oxygen is removed from the water and is replaced by oxygen in the gas phase resulting in

a reduction of pressure in the system which is registered on the manometer. A direct reading micrometer syringe built into each respirometer vessel allows restoration of the manometer level by measured amounts. The volume decrease required to re-establish the initial manometer level equals the volume of oxygen consummed.

Black, Fry, and Scott (1939) reported that the handling required to place a fish into a respiration chamber was sufficient stimulus to cause it to consume oxygen at their maximum rate for some time afterward. For each experiment in which this instrument was employed the procedure was as follows: one fish was placed in each respirometer vessel the evening before the day the measurements were to be made. After all five chambers were loaded, the rack was lowered into the water bath and an air pump attached to one end of the system. This allowed a small stream of air to flow through all five respirometer flasks and the compensation chamber during the night. The following morning the air source was disconnected, the micrometer syringes zeroed, and the vessels sealed. Then, the measurements were begun. Readings were made at fifteen minute intervals for one hour. In the measurements for the calculation of the weight-specific metabolic rate, three separate hour long determinations were made for each fish. Between each one hour run the instrument was opened, the syringes returned to zero and after a pause of one half hour, the next run initiated. The average of the three determinations was used in the statistical analysis. The measurements for the Q_{10} calculations required a different protocol. The first run in the morning was made at the cool temperature (24°C). A single, one hour determination was made.

Then the instrument was opened again, the air source reconnected and the temperature of the water bath raised to the higher temperature (30°C). The system was allowed to stabilize until afternoon when it was resealed and the second determination was made. At the end of each day's recordings the fish were removed from the vessels, dried by blotting with paper towels, and weighed.

In both experiments food was withheld from the fish. The fish were selected alternately from the age classes and an attempt was made to use a range of sizes in any given experiment.

III. Lipids

The fish used in the work dealing with lipids were denied food for two days prior to their sacrifice in order that a more accurate body weight could be obtained. The animals were taken from cold and warm acclimation tanks on alternate days, blotted on paper toweling and weighed. Preparations of the brain and spinal cord were made from each fish. First the brain was excised by severing the spinal cord immediately posterior to the tenth cranial nerve, removing the top of the skull with a scalpel and then the brain was freed of its nerve connections and lifted out. The brain was then rinsed in distilled water to remove cerebro-spinal fluid and blood. It was then placed in a glass and Teflon tissue homogenizer and the lipids extracted in several volumes of chloroform:methanol (2:1, v/v) (Folch, 1957). The brain extract was filtered and dried by passing it through a glass wool and Na_2SO_4 filter bed. At this point the samples were stored at -15°C in sealed vials until all fish had been

sacrificed. Immediately after the brain of a fish had been removed, a scalpel was passed down the back, cutting the neural arches and exposing the spinal cord. It was then lifted out, homogenized and extracted; the extract was dried and stored. The entire operation from the severing of the spinal cord to the storage of the spinal cord extract took five minutes.

In the first experiment, where the total fatty acid samples were obtained and the acclimation-size relationship was the subject of the study, there was some pooling of extracts. Proportionately less tissue was obtainable from fish of decreasing size. As size decreased the extent of extract pooling became greater, especially in the case of the spinal cords due to the relatively small amount of tissue obtained from an individual fish. There was no pooling of material for specimens greater than four grams. A total of 110 fish were used in this experiment.

In the second experiment where the total extract was first fractionated into the three lipid classes it was necessary to pool material from greater numbers of fish. In both cold and warm acclimated animals the brain and spinal cord material was pooled into two groups; that extracted from fish weighing less than and those weighing more than 5.0 gm. Thus there were eight aggregate samples obtained: warm brain, less than 5.0 gm; warm brain, more than 5.0 gm; etc. The choice of 5.0 gm as the weight for pooling separation was based on the size distribution of the available fish. At the time the brains and spinal cords were removed from this group of fish, the tissue was weighed. After homogenization and pooling, the sample was dried in a rotary evaporator and the

evaporation flask containing the dried extract weighed so that a determination of lipid content could be calculated. Due to the extensive pooling necessary prior to the fractionation, any statistical treatment of the data was ruled out but the data served as important indicators of the class from which the total fatty acid composition was contributed. A total of 115 fish were utilized for this experiment.

The samples of earthworm fatty acids were obtained from whole animals which were homogenized in chloroform:methanol (2:1, v/v) in a Virtis "23" tissue homogenizer and treated as were those from the fish. No lipid class fractionation was performed on the worm samples.

The total lipid extract and the individual classes after fractionation were next saponified and methylated according to the procedure described by Böttcher (1959). The resultant methyl esters were analyzed for percent of composition on a Beckman GC-5 gas chromatograph equipped with a hydrogen flame ionization detector. The instrument was fitted with two matching 12' x 1/8" stainless steel columns. The reason for the unusually long columns will be apparent later. The column packing was Chromosorb W-HP of 100/120 mesh with 15% liquid phase of diethylene glycol succinate (DEGS). The carrier gas was nitrogen and the flow rate was 30 cc/min. The model GC-5 is equipped with a linear temperature programmer thus allowing much shorter analysis times than under isothermal conditions. The result is better resolution and uniform-shaped peaks. In the first experiment the column temperature at the time of sample introduction was 180°C. It was then raised over a period of 32 minutes to 215°C. A better protocol was worked out for later samples including those

of the three lipid classes and those of the worm extracts. Here, the initial temperature was 160°C, and then increased to 225°C during 16 minutes. This change in protocol decreased the analysis time by nearly twenty minutes and the fatty acids displaying the longest retention times now appeared as sharp peaks. The signal produced by the Hydrogen flame ionization detector was recorded on a Speedomax G strip chart recorder equipped with a Disc integrator.

In all experiments identification of the fatty acid components of the samples was determined by considering combined evidence gained through several methods: fatty acid standards; peak subtraction; plots of the log of retention time versus the number of carbon atoms; and, in the case of branched acids, urea complexing.

The technique of gas chromatography is only a means of separating components of a sample and therefore one employs several methods for the identification of the separated components. The most direct method is the comparison of retention time of the individual sample peaks with that of standards. Standards for the following fatty acids were used in this study: 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:4, 22:0, 22:1, 22:6, 24:0, 24:1. In addition to these more common fatty acids, standards were obtained for branched acids and fatty acids of uneven carbon chain length. These were: Iso 14:0, Anteiso 15:0, 15:0, Iso 16:0, Anteiso 17:0, Iso 18:0, Anteiso 19:0, Iso 20:0, and Anteiso 21:0. All the standard fatty acids were purchased from Applied Science Laboratories, State College, Pennsylvania. Finally, a sample of the multi-branched fatty acid, phytanic, was obtained from the laboratory

of Dr. A. A. Benson, Scripps Institute of Oceanography, La Jolla, California.

Many unsaturated fatty acids can be identified by the method of peak subtraction resulting from the catalytic hydrogenation of the fatty acid sample. This hydrogenation is performed by suspending a sample of fatty acids in methanol in the presence of Adam's platinum oxide catalyst in a hydrogen atmosphere (Hammarstrand, 1966). The reaction is carried out at room temperature in a round bottomed flask equipped with a magnetic stirrer. This system is set up, the flask purged with hydrogen, and the stirrer motor turned on. After twelve hours the hydrogenated sample is recovered from the methanol. The gas chromatogram of the sample after hydrogenation reveals an apparent increase in the amount of the saturated components equal to the total of the contributing unsaturated components.

The identification of some saturated and unsaturated fatty acids was accomplished by plotting the log of the retention time versus the number of carbon atoms in the fatty acid chain (Evans, Cooney, and Panek, 1962). For fatty acids with the same number of double bonds, the graph of log retention time against the number of carbon atoms yields a straight line for isothermal gas chromatography. In the case of temperature programmed gas chromatography, as was used in the present study, the graph yields a curve. A separate curve is obtained for each homologous series; saturates, monoenes, dienes, etc.

When branched and straight chain saturated fatty acids are in a urea saturated methanol solution and crystallization is precipitated,

the straight chains are selectively included in the forming crystal lattice. Several repetitions of this process remove the saturated straight components, leaving the branched acids in solution (Schlenk, 1954; Nicolaides and Ray, 1965). This method was employed to concentrate the components of the sample believed to be branched fatty acids.

The aggregate lipid extracts from the second group of acclimated A. portalegrensis were fractionated into three lipid classes by short column chromatography. The column was a 60 ml. sintered glass funnel containing one inch of basic Silica Gel-G (Keith, 1966). The lipid extract was transferred to the head of this column in diethyl ether. The first elution was with diethyl ether which removed those components not bound to the column packing: sterols, glycerides, sterol esters, and wax-hydrocarbons. The free fatty acids were next eluted from the column with diethyl ether:formate (50:1, v/v). The phospholipids, which were still on the column, were not eluted from the silica gel as were these first two fractions. The polar phospholipids, which are more strongly bound to the silica gel, were removed from the column when it was flushed with methanol:formate (100:1, v/v). The phospholipids are replaced by the methanol. During all three elutions a slight and constant pressure of nitrogen was maintained on the column. The fractionation process was carried out at 15°C. Upon the completion of the fractionation process, all three portions were subjected to saponification and methylation. The resultant fatty acid methyl esters were analyzed by gas chromatography.

About a third of the phospholipid fraction was saved for thin layer chromatography. The thin layer plates were glass, 20 x 20 cm and

coated with 75 micra of Silica Gel-G. The samples were applied as spots and the plates developed in chloroform:methanol:water (65:25:4, v/v/v). The plates were developed to 10 cm. at room temperature. To avoid any temperature fluctuations, the developing chambers were placed in styrofoam insulated boxes. The separated phospholipid components were visualized by spraying the plates with 50% H_2SO_4 and then charring at 100°C. The percent of composition of each component in the samples was determined by scanning the plate with a Photovolt electronic spot photometer, Model 501 A. The output from this was recorded on a Varicord variable recorder 42 B and quantitated with an Integraph integrator, Model 49. The separated components were identified by comparison of the distance travelled from the point of origin by the sample spot, with that distance for the standard phospholipids. The standards used were: sphingomyelin, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, and cardiolipin. These standards were obtained from Nutritional Biochemical Corporation, Cleveland, Ohio.

Chemicals used in all parts of the research were analytical reagent grade. All solvents used in the preparation of lipid samples were examined for impurities which might interfere with their gas chromatographic analysis. This was done by evaporating 150 ml. of the particular solvent in a rotary evaporator and then subjecting any residue to gas chromatography. If any peaks were observed in the chromatography record, the solvent was discarded. Also the agar used in the food preparation was examined for any interfering components.

IV. Statistics

The regression coefficients for the weight-specific metabolic rate were calculated for each age class and tested for significant differences by Student's "t". The means of the Q_{10} values obtained for the different age classes were similarly tested.

The alterations of brain and spinal cord lipid patterns, resulting from thermal acclimation, were examined for size relationships. Percents of composition of individual fatty acids were subjected to linear regression analysis on body size. The generated regression coefficients were examined for significance. The regression coefficient from the acclimation groups were compared by Student's "t". The mean percentage composition between different acclimation groups and between brain and spinal cord groups of similar acclimation history were similarly tested. All of these calculations were performed by computer (see Appendix I). In all stages of statistical analysis the 0.99 level of confidence was accepted as statistically significant.

RESULTS

Weight data refer to wet weight of the fish or of the tissue excised. The rates of metabolism are reported as milliliters oxygen per gram per hour. Fatty acid compositions are stated as percents of the total. The statistical procedures are from Mather (1951) and probabilities are from the tables of that textbook.

I. Effects of Size - Age

The data for determination of the weight-specific metabolic rate come from two separate groups of fish. These groups differ in size range and each includes two age classes; five months and ten months. The results of this study appear in Tables 1 and 2, and Figures 2 and 3.

In group 1 the five month old fish range in size from 2.0 to 5.3 gm with a mean weight of 2.9 gm. The fish ten months of age range in size from 3.0 to 7.8 gm with a mean weight of 4.2 gm. In order to minimize handling the fish before respirometry, the body weights were obtained immediately after the respiration rates had been measured. At the time the fish were removed from the holding tanks and transferred to the respirometer and attempt was made to use fish of comparable size from each age class. In this first experiment I was unsuccessful in this effort for group 1. In group 2 the five month old fish range in body size from 1.2 to 3.6 gm with a mean weight of 1.6 gm. The body size of ten month fish ranges from 0.72 to 2.78 gm with a mean of 1.4 gm.

The average of three, one-hour determinations of oxygen consumption is used in the calculation of the linear regression of metabolic

rate as a function of body weight. The fish used in this study were maintained at 27° ± 0.5°C from the time they were spawned and the determinations of metabolic rate were made at that temperature. There is no indication that oxygen consumption is depressed by a reduction in the oxygen available in the resprometer vessels. There are also no significant differences in oxygen consumption between any of the three, one-hour determinations.

The regression of the log of metabolic rate on the log of body weight is calculated by the method of least squares. For group 1 the regression coefficients for age classes five and ten months are -0.5846 and -0.6835, respectively (Figure 2). A t test indicates that these are not significantly different ($P > 0.70$). The regression coefficients for group 2 are: five month old fish, -0.7619; ten month old fish, -0.7254 (Figure 3). As with the first group, there is no significant difference between the two age classes ($P > 0.80$).

Neither group 1 nor group 2 show any difference between age classes on the basis of weight-specific metabolic rate. The regression lines shown in Figures 2 and 3 are not coincident in the area of overlapping body weight. This is most obvious in group 1 (Figure 2). The mean log body weights and mean log oxygen consumptions were examined to determine if any significant difference exists between the means. There is no significant difference between oxygen consumption rates of the two age classes in either group 1 ($P > 0.50$) or group 2 ($P > 0.20$). Also, there is no difference between the means of the log body weight for group 2 ($P > 0.30$). In group 1, the ten month old fish were significantly larger than the five month old fish ($P < 0.001$).

TABLE 1. Comparison of weight-specific oxygen consumption rates. Group 1. Age classes: 5 months and 10 months. Fish acclimated to 27°C and rates of oxygen consumption measured at that temperature.

Age	Number of Fish	Mean Log Weight, gm	Standard Error of Mean Log Weight	Mean Log O_2 Consumption ml O_2/gm/hr	Standard Error of Mean Log O_2 Consumption
5 Months	20	0.4720	0.0293	$\bar{1}$.3263	0.0336
10 Months	20	0.6226	0.0239	$\bar{1}$.2998	0.0230

t of Mean Log Weight	t of Mean Log O_2 Consumption	Degrees of Freedom	Probability for Weight	Probability for O_2 Consumption
3.9841	0.6506	38	<0.001	>0.50

Regression Coefficient	Standard Error of Regression Coefficient	t of Regression Coefficient	Degrees of Freedom	Probability
-0.5846	0.2308	0.3515	36	>0.70
-0.6835	0.1596			

Figure 2. Comparison of weight-specific metabolic rate as a function of body weight. Group 1. Age classes: (A) 10 months; mean weight, 4.2 gm. (B) 5 months; mean weight, 2.9 gm.

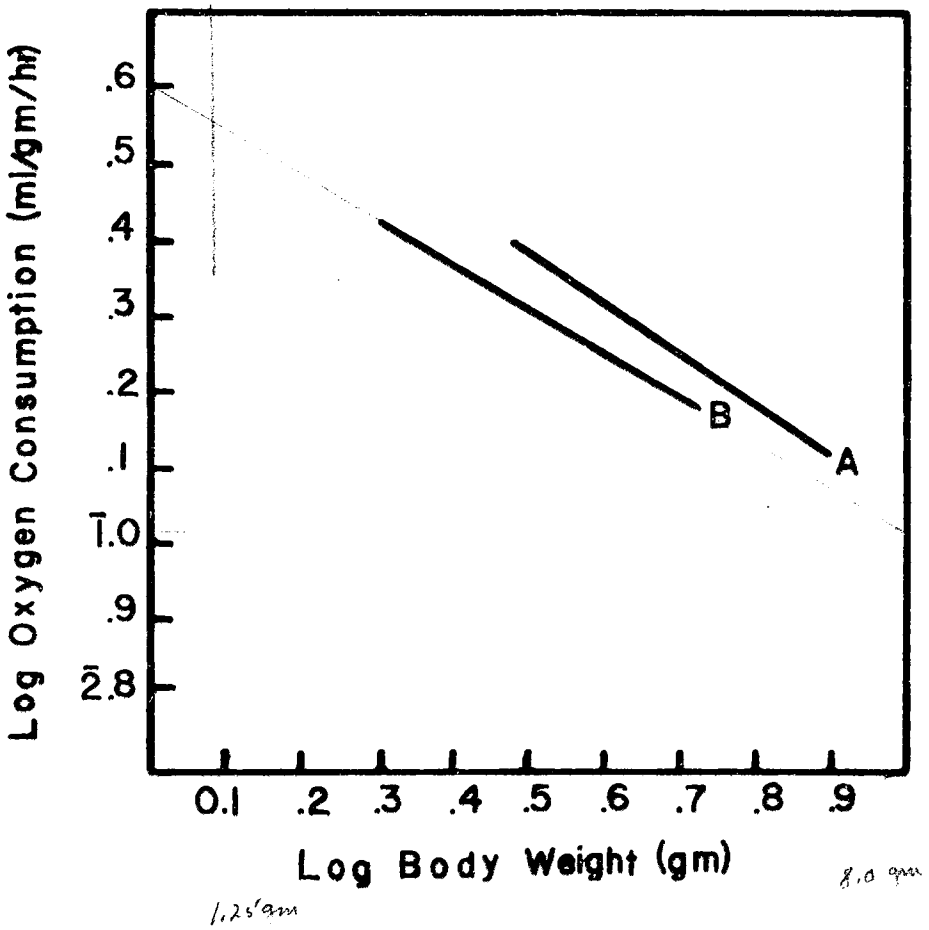

Log Oxygen Consumption (ml/gm/hr)
.6
.5
.4
.3
.2
.1
1.0
.9
2.8
B
A
0.1
.2
.3
.4
.5
.6
.7
.8
.9
Log Body Weight (gm)

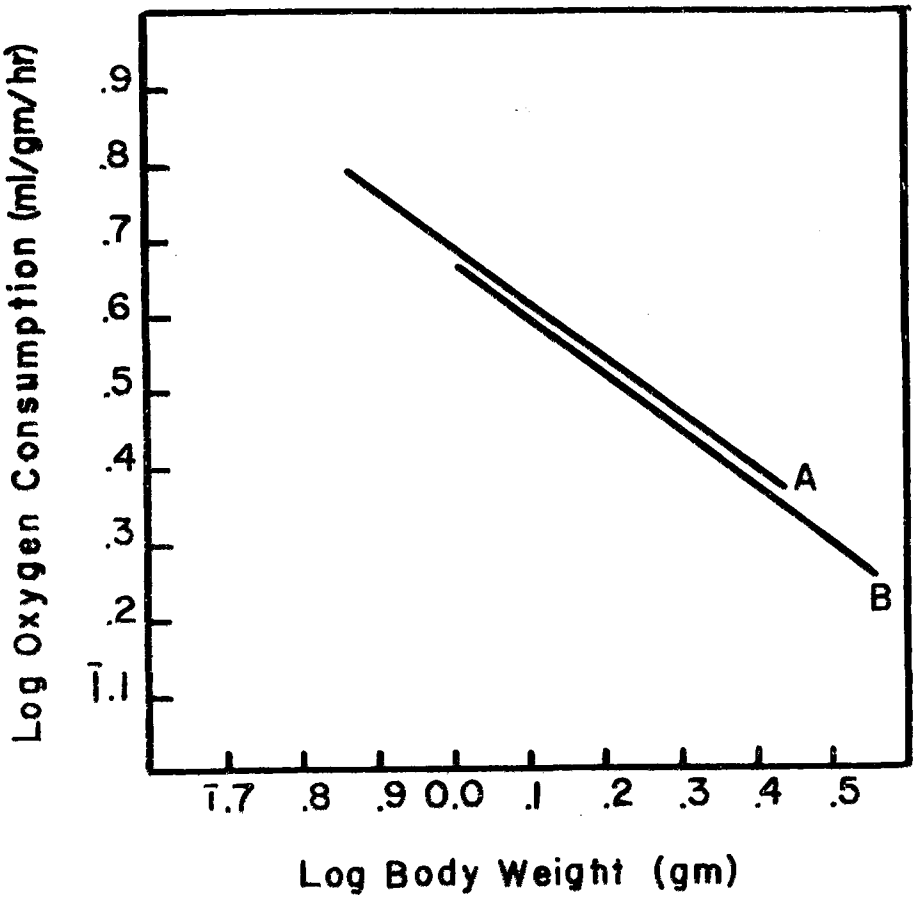
TABLE 2. Comparison of weight-specific oxygen consumption rates. Group 2. Age classes: 5 months and 10 months. Fish acclimated to 27°C and rates of oxygen consumption measured at that temperature.

Age	Number of Fish	Mean Log Weight, gm	Standard Error of Mean Log Weight	Mean Log O_2 Consumption ml O_2/gm/hr	Standard Error of Mean Log O_2 Consumption
5 months	12	0.2129	0.0417	$\bar{1}$.5134	0.0400
10 months	13	0.1513	0.0154	$\bar{1}$.5792	0.0406

t of Mean Log Weight	t of Mean Log O_2 Consumption	Degrees of Freedom	Probability for Weight	Probability for O_2 Consumption
0.9620	1.1542	23	>0.30	>0.20

Regression Coefficient	Standard Error of Regression Coefficient	t of Regression Coefficient	Degrees of Freedom	Probability
-0.7619	0.1853	0.1493	21	>0.80
-0.7254	0.1255			

Figure 3. Comparison of weight-specific metabolic rate as a function of body weight. Group 2. Age classes: (A) 10 months; mean weight, 1.4 gm. (B) 5 months; mean weight, 1.6 gm.


Log Oxygen Consumption (ml/gm/hr)
.9
.8
.7
.6
.5
.4
.3
.2
1.1
A
B
1.7
.8
.9
0.0
.1
.2
.3
.4
.5
Log Body Weight (gm)

The data appearing in Table 3 are the results of Q_{10} determinations. The fish used for this experiment are of three age classes and two body size ranges. These fish were acclimated to 27°C and the oxygen consumption rates measured at 22° and 30°C. The animals of the first weight group were five and ten months old. The mean weights for these age classes are 1.40 and 1.29 gm respectively. The second group of animals consists of age classes ten and fifteen months. The ten month age class has a mean body weight of 6.71 gm while the fifteen month age class has a mean of 6.92 gm. A test for differences of the mean weights of age classes is negative for both groups. The mean Q_{10} of metabolism of the five month old fish of group 1 is 1.81; for the ten month old fish this value is 1.80. The t test for significant difference is negative ($P > 0.70$). In group 2 the mean Q_{10} of the ten month old animals is 1.96. The fifteen month old fish have a mean Q_{10} of 2.20. There is no significant difference in Q_{10} between the age classes in group 2 ($P > 0.30$). The results of the Q_{10} experiments are shown in Table 3.

II. Lipids

The methods used for identification of the gas chromatographic peaks representing the separated components of fatty acid samples were described earlier. Results from using these different methods are shown in Figures 4, 5, 6, and 7. Figure 4 is a gas chromatogram of the total fatty acids of an A. portalegrensis brain. The peaks have been numbered and the identity of a numbered peak is indicated in the legend of that figure. The numbering system is consistent for Figures 4, 5, and 6.

TABLE 3. Comparison of the Q_{10} of oxygen consumption for fish of ages: 5 months, 10 months, and 15 months. Fish acclimated to 27°C.

Age	Number of Fish	Mean Weight, gm	Standard Error of Mean Weight	Mean Q_{10} 24° - 30°C	Standard Error of Mean Q_{10}
5 Months	15	1.40	0.187	1.81	0.237
10 Months	19	1.29	0.114	1.80	0.346
10 Months	10	6.71	0.789	1.96	0.190
15 Months	14	6.92	0.579	2.20	0.126

Age	t of Mean Weight	t of Mean Q_{10}	Degrees of Freedom	Probability for Weight	Probability for Q_{10}
5 Months 10 Months	0.49	0.31	32	>0.60	>0.90
10 Months 15 Months	0.26	1.04	22	>0.70	>0.30

The standard fatty acids samples mentioned in MATERIALS AND METHODS were used to identify the majority of the peaks. Additional information was gained by catalytic hydrogenation. A chromatogram illustrating peak subtraction after hydrogenation is shown in Figure 5. The unsaturated fatty acid peaks are subtracted from the chromatogram and appear as additions to saturates according to chain length. In some instances certain unsaturates have not been totally hydrogenated; e.g.; peaks 9 (16:1), 18 (18:3), 21 (20:4), and 22 (22:2).

Urea adduction of hydrogenated samples removes the saturated straight chain fatty acids permitting concentration of branched chain acids (Figure 6). The relative amounts of peaks 3 (Iso 14:0), 5 (Anteiso 15:0), 6 (Multibranched 16:0), 7 (Iso 16:0), 9 (Anteiso 17:0), and 10 (Phytanic) are increased by urea adduction. It was the presence of these branched acids that necessitated the use of twelve foot chromatography columns. For routine fatty acid analysis six foot columns are sufficient. The initial analyses performed on six foot columns demonstrated that the fatty acid samples from A. portalegrensis were very complex. The section of the chromatogram representing components having fewer than eighteen carbon atoms consisted of many components differing only slightly in boiling points. In order to resolve these components the length of the chromatography columns had to be doubled.

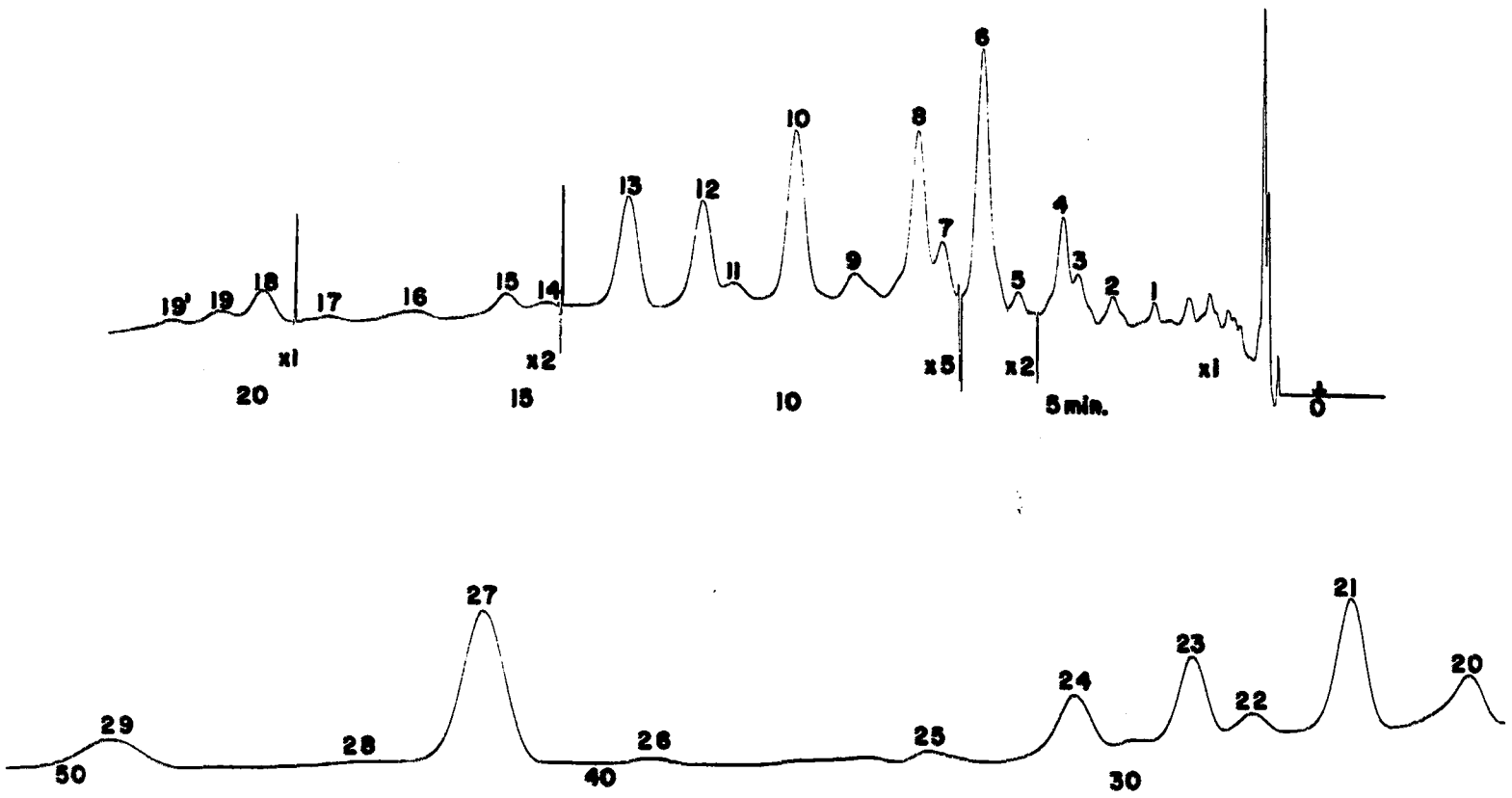
The fourth technique utilized in the identification of the components of the samples was the graphic method, plotting the log of retention time against the number of carbon atoms (Figure 7). Each curve on the graph represents a homologous series, e.g., saturates, monoenes, dienes, etc.

Figure 4. Fatty acid composition of Aequidens portalegrensis brain. The identification of the numbered peaks of the gas chromatogram.

Peak #	Peak Identity	Peak #	Peak Identity
1	12:0*	14	16:3
2	13:0	15	18:2
3	Iso 14:0	16	20:0
4	14:0	17	20:1
5	Anteiso 15:0 and 14:1	18	18:3
		19	20:2 (2 peaks)
6	Multibranch 16:0	20	22:0
		21	20:4
7	Iso 16:0	22	22:2
8	16:0	23	22:2
9	Anteiso 17:0 and 16:1	24	24:0
		25	24:1
10	Phytanic	26	22:5
11	16:2	27	22:6
12	18:0	28	24:5
13	18:1	29	24:6

*Number before the colon refers to chain length and number after the colon to the number of double bonds.

Note: the numbers below the recorder trace represent the retension time in minutes and the electrometer attenuation factor.


19'
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
x1
x2
x5
x2
x1
20
15
10
5 min.
0

29
28
27
26
25
24
23
22
21
20
50
40
30

Figure 5. Gas chromatogram of brain fatty acid sample after hydrogenation. Numbering system for identification of peaks like that in Figure 4.

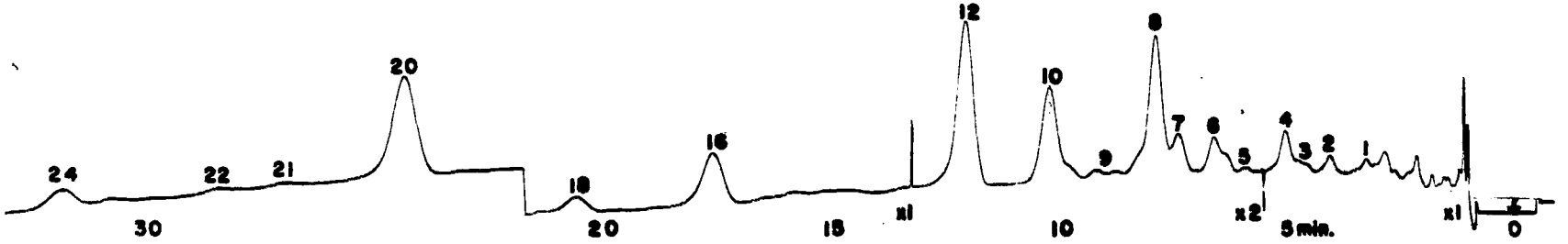

24
22
21
20
18
16
12
10
9
8
7
6
5
4
3
2
1
30
20
15
x1
10
x2
5min.
x1
0

Figure 6. Gas chromatogram of brain fatty acid sample after urea adduction. Process results in the concentration of branched fatty acids present in the sample. Numbering system for identification of peaks like that in Figure 4.

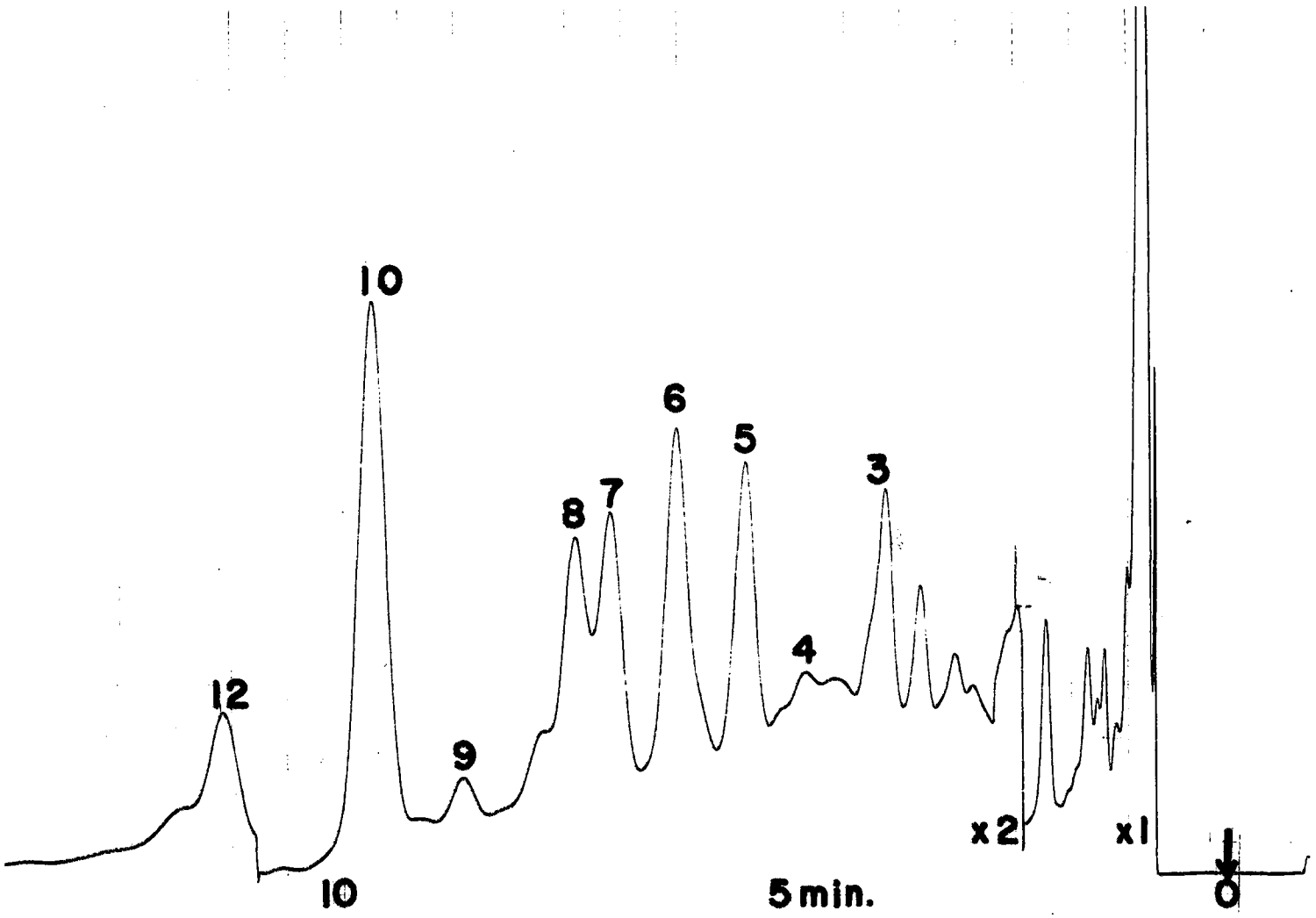
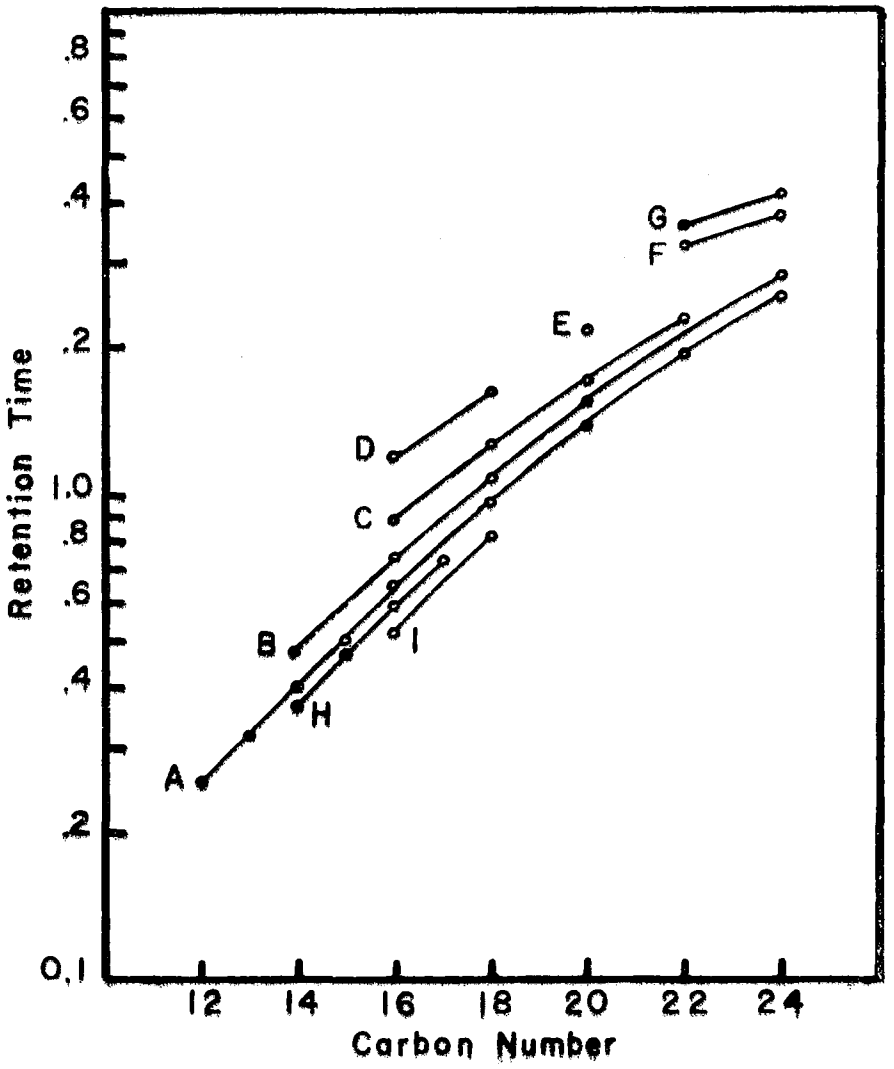

10
6
5
8
7
3
12
4
9
x2
x1
10
5 min.
0

Figure 7. Plot of log retention time against the number of carbon atoms in fatty acid chain. (A) saturates, (B) monoenes, (C) dienes, (D) trienes, (E) tetraene, (F) pentaenes, (G) hexaenes, (H) branched components, (I) multibranched components.


Retention Time
.8
.6
.4
.2
1.0
.8
.6
.4
.2
0.1
12
14
16
18
20
22
24
Carbon Number
A
B
C
D
E
F
G
H
I

The first experiment dealing with lipids was designed to determine whether the relationship between body size and thermal acclimation of metabolism is reflected in changes of the fatty acid patterns of the central nervous system lipids. The percent composition of individual fatty acids were plotted against the body weights of the fish from which the extracts were collected. The resulting calculations are found in Tables 4, 5, 6, and 7. Tables 4 and 5 present the data for spinal cord extracts from cold and warm acclimated animals. Tables 6 and 7 contain the results of studies of brain extracts of cold and warm acclimated fish. In only two instances was significant relationship apparent between body size and percent of fatty acid composition; 22:6 of the spinal cord extract from cold acclimated fish, and 24:6 of the brain extract from warm acclimated fish. Whether or not this apparent relationship is meaningful would have to be determined by further studies.

The regression coefficients for corresponding fatty acids of extracts from cold and warm acclimated animals were tested by Student's t for differences between acclimation groups. Only one component revealed such a difference: again 22:6. The regression coefficient for docosahexaenoic acid (22:6) from the spinal cord of cold acclimated fish is 0.1598. The value of this coefficient for the same component from the spinal cord of warm acclimated fish is 0.0064. The difference is significant ($P < 0.01$).

Extracts of brains and of spinal cords were studied to learn whether the percent composition could be related to thermal history. The result of these studies is seen in Table 8. Examination of the differences of

TABLE 4. A. portalegrensis spinal cord total fatty acids from cold acclimated fish. Regression of percent composition on fish body weight.

Peak Identity	Mean % Composition	Standard Deviation	Regression Coefficient	Standard Error of Regression Coefficient
12:0	3.07	0.69	-0.0400	0.0841
13:0	3.85	0.86	-0.0498	0.1051
Iso 14:0	1.73	0.45	-0.0352	0.0549
14:0	5.90	1.30	-0.1201	0.1584
Ante 15:0 and 14:1	2.43	0.56	-0.0582	0.0677
Multibranch 16:0	9.43	2.12	-0.1752	0.2584
Iso 16:0	5.87	1.35	-0.0989	0.1652
16:0	15.46	3.44	-0.1838	0.4214
Ante 17:0 and 16:1	6.21	1.34	-0.0599	0.1650
Phytanic	11.60	2.59	0.1228	0.3181
16:2	2.55	0.62	-0.0447	0.0758
18:0	7.46	1.92	-0.0106	0.2363
18:1	7.21	1.75	0.0747	0.2150
16:3	1.22	0.31	0.0017	0.0375
18:2	0.82	0.20	0.0227	0.0247
20:0	0.86	0.19	0.0074	0.0240
20:1	0.47	0.15	0.0173	0.0180
18:3	0.99	0.24	0.0521	0.0267
20:2	0.34	0.12	0.0129	0.0142
22:0	0.93	0.27	0.0320	0.0326
20:4	1.39	0.38	0.0664	0.0444
22:2	0.95	0.28	-0.0015	0.0346
22:2	3.63	1.02	0.1829	0.1186
24:0	1.63	0.41	0.0409	0.0500
24:1	0.30	0.12	0.0459	0.0108
22:5	0.21	0.07	0.0068	0.0090
22:6	0.99	0.46	0.1598	0.0447
24:5	0.23	0.07	0.0054	0.0085
24:6	2.27	0.57	0.0257	0.0695

Body size range: 39.98 to 0.92, mean 9.03, standard deviation 0.017
Number of samples: 23

TABLE 5. *A. portalegrensis* spinal cord total fatty acids from warm acclimated fish. Regression of percent composition on fish body weight.

Peak Identity	Mean % Composition	Standard Deviation	Regression Coefficient	Standard Error of Regression Coefficient
12:0	3.58	0.80	-0.1259	0.1166
13:0	4.14	0.93	-0.1478	0.1367
Iso 14:0	2.35	0.57	-0.0477	0.0855
14:0	7.02	1.50	-0.1700	0.2228
Ante 15:0 and 14:1	2.60	0.61	-0.0891	0.0903
Multibranch 16:0	10.29	2.22	-0.0264	0.3334
Iso 16:0	5.77	1.26	-0.0068	0.1884
16:0	14.90	3.07	-0.0661	0.4612
Ante 17:0 and 16:1	5.20	1.15	-0.0119	0.1723
Phytanic	11.61	2.59	0.4348	0.3786
16:2	2.49	0.64	0.1075	0.0929
18:0	7.01	1.69	-0.0163	0.2531
18:1	5.99	1.38	-0.0560	0.2067
16:3	1.51	0.38	-0.0111	0.0570
18:2	0.63	0.17	0.0159	0.0249
20:0	1.07	0.27	0.0835	0.0371
20:1	0.42	0.13	0.0072	0.0188
18:3	1.09	0.24	0.0312	0.0356
20:2	0.64	0.18	-0.0176	0.0262
22:0	0.81	0.20	0.0291	0.0291
20:4	0.78	0.20	-0.0010	0.0303
22:2	0.95	0.22	-0.0106	0.0332
22:2	4:26	1.01	0.0342	0.1512
24:0	1.97	0.61	0.0310	0.0911
24:1	0.32	0.11	0.0247	0.0154
22:5	0.15	0.06	0.0172	0.0079
22:6	0.32	0.09	0.0064	0.0139
24:5	0.51	0.21	-0.0272	0.0317
24:6	1.63	0.40	0.0087	0.0595

Body size range: 35.71 to 0.87 gm, mean 6.70, standard deviation 0.014
Number of samples: 25

TABLE 6. A. portalegrensis brain total fatty acids from cold acclimated fish. Regression of percent composition of fish body weight.

Peak Identity	Mean % Composition	Standard Deviation	Regression Coefficient	Standard Error of Regression Coefficient
12:0	1.71	0.42	-0.0177	0.0529
13:0	2.39	0.54	-0.0290	0.0674
Iso 14:0	1.47	0.30	0.0036	0.0374
14:0	3.47	0.78	-0.0394	0.0968
Ante 15:0 and 14:1	2.47	0.51	-0.0282	0.0636
Multibranch 16:0	9.85	1.95	0.1655	0.2398
Iso 16:0	6.86	1.33	-0.0214	0.1659
16:0	15.81	3.04	-0.1248	0.3782
Ante 17:0 and 16:1	6.17	1.19	-0.0191	0.1481
Phytanic	15.71	3.09	0.2219	0.3827
16:2	3.61	0.66	-0.0216	0.0813
18:0	7.59	1.55	-0.0845	0.1922
18:1	7.16	1.39	0.0493	0.1731
16:3	0.63	0.17	0.0016	0.0212
18:2	0.40	0.11	0.0093	0.0135
20:0	0.46	0.11	-0.0003	0.0140
20:1	0.31	0.08	0.0074	0.0105
18:3	0.61	0.13	0.0117	0.0169
20:2	0.19	0.05	0.0009	0.0062
22:0	1.25	0.72	-0.0248	0.0897
20:4	2.28	0.48	0.0228	0.0597
22:2	0.48	0.14	0.0046	0.0185
22:2	2.44	0.60	0.0233	0.0742
24:0	1.23	0.26	0.0041	0.0325
24:1	0.17	0.05	0.0087	0.0063
22:5	0.17	0.08	-0.0025	0.0099
22:6	4.06	0.93	-0.1034	0.1139
24:5	0.09	0.05	-0.0037	0.0064
24:6	2.04	0.81	-0.0509	0.0004

Body size range: 39.98 to 0.92 gm, mean 8.55, standard deviation 0.016
Number of samples: 29

TABLE 7. A. portalegrensis brain total fatty acids from warm acclimated fish. Regression of percent composition on fish body weight.

Peak Identity	Mean % Composition	Standard Deviation	Regression Coefficient	Standard Error of Regression Coefficient
12:0	1.88	0.48	-0.0740	0.0693
13:0	2.97	0.73	-0.0775	0.1068
Iso 14:0	1.64	0.38	-0.0092	0.0565
14:0	4.20	0.94	-0.0608	0.1394
Ante 15:0 and 14:1	2.65	0.58	-0.0341	0.0865
Multibranch 16:0	9.68	2.08	-0.0327	0.3086
Iso 16:0	6.00	1.31	-0.0956	0.1932
16:0	14.77	3.19	0.0457	0.4750
Ante 17:0 and 16:1	5.91	1.31	-0.0591	0.1937
Phytanic	14.67	3.21	0.1503	0.4770
16:2	2.57	0.63	-0.0645	0.0924
18:0	7.26	1.55	0.0272	0.2298
18:1	6.76	1.46	0.0889	0.2165
16:3	0.75	0.20	-0.0227	0.0300
18:2	0.72	0.16	-0.0032	0.0239
20:0	0.79	0.17	0.0029	0.0258
20:1	0.44	0.12	-0.0005	0.0187
18:3	1.11	0.24	0.0019	0.0365
20:2	0.31	0.08	-0.0138	0.0112
22:0	0.98	0.30	0.0283	0.0445
20:4	2.19	0.49	0.0179	0.0729
22:2	0.48	0.14	-0.0169	0.0209
22:2	2.65	0.76	0.0337	0.1133
24:0	2.06	0.49	-0.0241	0.0720
24:1	0.54	0.17	0.0200	0.0252
22:5	0.59	0.21	-0.0073	0.0309
22:6	3.12	0.81	0.0012	0.1210
24:5	0.62	0.26	-0.0313	0.0386
24:6	1.73	0.52	0.2050	0.0642

Body size range: 35.71 to 0.87 gm, mean 6.85, standard deviation 0.014
Number of samples: 24

TABLE 8. Results of t tests of the difference between means of percent composition of fatty acids from cold and warm acclimated fish. Only those peaks are presented in which a significant difference was found ($P \leq 0.01$).

Comparison of extracts from spinal cords of warm- and cold acclimated fish. Degrees of Freedom, 46.

Peak Identity	t	Change in % Composition with Cold Acclimation
18:2	3.62	increase
20:2	8.85	decrease
20:4	4.11	increase
22:5	10.85	increase
22:6	3.15	increase
24:5	6.12	increase

Comparison of extracts from brains of warm- and cold acclimated fish. Degrees of Freedom, 51.

Peak Identity	t	Change in % Composition with Cold Acclimation
18:2	10.99	decrease
20:0	10.09	decrease
20:1	7.47	decrease
18:3	7.98	increase
20:2	17.39	decrease
24:0	3.40	decrease
24:1	12.60	decrease
22.5	9.49	decrease
24:5	7.63	decrease

the means of spinal cord extracts from cold and warm acclimated fish shows significant difference in six fatty acids. Five acids display an increase with cold acclimation: 18:2, 20:4, 22:5, 22:6, and 24:5. One acid, 20:2, decreases with cold acclimation. A similar comparison of brain fatty acids from cold and warm acclimated fish displays a significant decrease in eight acids with cold acclimation: 18:2, 20:0, 20:1, 20:2, 24:0, 24:1, 22:5, and 24:5. Only in 18:3 did cold acclimation result in an increase in the mean percent composition.

The differences in percent composition of fatty acids extracted from brains and spinal cords of the same acclimation temperature are presented in Table 9. This comparison was made in order to determine whether the extractable material is involved in the myelination or the neurones. For cold acclimated fish the fatty acids: 12:0, 16:3, 18:2, 20:0, 20:1, 18:3, 20:2, 22:2, 24:1, 22:5, and 24:5 are on a percentage composition basis, significantly greater in the spinal cord than in the brain. The fatty acids 20:4, and 22:6 are greater in the brain extracts. Following warm acclimation I found that individual percentage of composition of acids: 16:3, 20:0, 20:2, and 22:2 were significantly greater in the spinal cord than in the brain. Warm acclimation resulted in a greater percent of composition in the brain for acids: 20:4, 24:1, 22:5, and 22:6.

All analyses of fatty acid patterns discussed thus far deal with the total fatty acids from all saponifiable classes of lipids. Further analyses were made on separate lipid classes. Lipid classes were separated by short column chromatography and fatty acids of the separate classes were analyzed. The results of these analyses appear in Table 10.

TABLE 9. Results of t tests of the difference between means of percent composition of fatty acids from brain and spinal cord extracts. Comparisons made for those groups of the same acclimation temperature. Only those peaks are presented in which a significant difference was found ($P \leq 0.01$)

Comparison of extracts from brains and spinal cords of cold acclimated fish. Degrees of freedom, 50

Peak Identity	t	Tissue with Higher % Composition
12:0	2.69	spinal cord
16:3	6.07	spinal cord
18:2	9.47	spinal cord
20:0	9.89	spinal cord
20:1	6.92	spinal cord
18:3	6.52	spinal cord
20:2	10.66	spinal cord
20:4	3.25	brain
22:2	5.64	spinal cord
24:1	8.99	spinal cord
22:5	4.27	spinal cord
22:6	3.46	brain
24:5	24.42	spinal cord

Comparison of extracts from brains and spinal cords of warm acclimated fish. Degrees of freedom, 52

Peak Identity	t	Tissue with Higher % Composition
16:3	5.01	spinal cord
20:0	3.42	spinal cord
20:2	10.65	spinal cord
20:4	5.67	brain
22:2	8.88	spinal cord
24:1	6.64	brain
22:5	10.35	brain
22:6	4.22	brain

TABLE 10. A. portalegrensis fatty acids obtained from lipid fractions: Fraction I: glycerides, sterols, sterol esters, and wax-hydrocarbons. Fraction II: free fatty acids. Fraction III: phospholipids. (A) Brain extract from 22°C acclimated fish. (B) Brain extract from 30°C acclimated fish. (C) Spinal cord extract from 22°C acclimated fish. (D) Spinal cord extract from 30°C acclimated fish. Numbers in columns A - D are % composition.

		Fraction I				Fraction II				Fraction III			
Peak #	Peak Identity	A	B	C	D	A	B	C	D	A	B	C	D
1	12:0	1.10	1.62	1.21	1.29	1.50	1.93	1.33	1.12	0.35	0.17	0.85	0.53
2	13:0	1.87	3.79	1.72	2.47	1.70	2.28	1.51	1.56	1.55	0.70	0.85	1.35
3	Iso 14:0	1.64	1.85	1.59	2.23	1.48	2.03	1.04	1.49	2.92	0.91	1.24	1.51
4	14:0	4.82	4.90	3.84	4.65	4.60	4.59	4.36	4.46	2.71	1.41	2.98	3.55
5	14:1	1.43	2.48	4.25	4.45	1.32	1.73	1.42	1.82	7.83	3.73	6.44	12.97
6	Ante 15:0	4.56	5.03	8.31	2.78	3.41	4.59	3.17	3.34	-	-	-	-
7	Multibranch 16:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.99	7.96	6.01	6.44
8	Iso 16:0	3.30	4.96	3.66	3.01	1.54	2.19	1.69	2.35	3.57	3.19	3.98	5.12
9	16:0	16.71	13.36	14.45	14.27	20.23	15.96	15.52	13.34	15.08	12.41	22.70	11.72
10	Ante 17:0 and 16:0	13.35	12.74	10.43	14.21	3.61	4.38	3.90	4.43	12.91	10.10	9.26	10.45
11	Phytanic	0.83	0.00	2.64	0.00	0.00	0.00	0.00	0.00	10.59	12.25	6.89	7.64
12	16:2	3.46	3.49	3.45	2.95	1.73	0.86	1.88	2.60	1.47	0.00	1.88	2.44
13	18:0	6.54	8.61	10.67	10.32	23.81	19.11	18.50	12.50	4.85	6.95	8.44	6.38
14	18:1	15.79	11.22	13.64	14.85	11.37	9.66	15.08	14.15	5.32	7.19	11.89	9.43
15	16:3	1.83	3.26	0.81	0.00	0.00	0.00	0.00	0.00	0.59	0.98	0.00	1.87

(Continued)

TABLE 10 (Continued)

Peak #	Peak Identity	Fraction I				Fraction II				Fraction III			
		A	B	C	D	A	B	C	D	A	B	C	D
16	18:2	5.36	4.41	3.90	3.75	2.19	3.31	5.45	4.43	1.31	0.68	1.46	2.25
17	20:0	1.52	2.36	1.87	4.17	0.79	2.55	3.56	5.05	0.30	0.34	1.24	2.27
18	18:3 (2 peaks)	6.39	5.26	7.96	7.40	2.53	7.02	4.89	5.10	1.29	0.94	1.81	4.92
19	20:2 (2 peaks)	3.98	3.34	4.10	4.36	2.53	5.56	5.86	2.57	0.42	0.92	1.23	3.57
20	22:0	0.96	1.46	1.42	3.41	2.28	1.41	2.36	1.46	0.00	0.97	0.53	3.13
21	22:1	0.13	0.67	0.17	1.18	3.25	0.56	0.84	0.89	0.00	0.00	0.05	0.00
22	20:4	1.50	1.30	1.02	0.30	2.96	1.89	1.29	0.46	0.88	4.81	2.41	2.86
23	22:2	0.13	0.27	0.23	1.12	0.08	0.33	0.08	0.00	0.04	8.03	0.13	0.51
24	22:2	1.32	0.40	1.15	0.59	1.30	0.76	1.26	1.31	0.21	1.03	1.15	2.54
25	24:0	0.00	0.00	0.00	0.25	0.35	0.37	0.00	0.32	0.04	0.20	0.32	0.23
26	24:1	0.00	0.18	0.11	0.00	0.00	0.30	0.00	0.00	0.06	0.15	0.00	0.00
27	22:5	0.50	0.68	0.50	0.00	0.11	0.00	0.54	0.00	0.06	0.25	0.37	0.15
28	22:6	1.23	2.03	0.93	0.00	1.64	2.24	0.86	0.45	3.24	13.70	5.79	2.04

Number of fish: 22°C acclimated: 57
30°C acclimated: 58

Fraction I contains glycerides, sterols, sterol esters, and wax-hydrocarbons. Free fatty acids comprise all of Fraction II. Phospholipids are the only component of Fraction III. There are four fatty acid patterns displayed in Table 10 for each of the three fractions. These four patterns represent: (A) brain extract from cold acclimated fish, (B) brain extract from warm acclimated fish, (C) spinal cord extract from cold acclimated fish, and (D) spinal cord extract from warm acclimated fish. Due to the large amount of material needed for such a comprehensive analysis each column of numbers represents the average of percent composition of only two aggregate samples. These two samples represent either fish equal to or greater than 5.0 gm or fish less than 5.0 gm. Representative gas chromatograms of the fatty acid patterns are shown in Figures 8, 9, and 10. Fraction I is composed largely of 16:0 and 18:1. The most conspicuous components in Fraction II are 16:0 and 18:0. The composite peak of 16:1 and Anteiso 17:0 is relatively small in the second fraction and there is no 16:3. Fraction III is of particular interest because it contains, almost exclusively, all of the multibranched 16:0 and phytanic acids. This fraction also contains greater proportions of Iso 16:0 and 22:6 than do the other two fractions.

At the time the tissue was excised for the preceding analysis, the wet weight of the individual brains and spinal cords was obtained. After the lipids were extracted, the solvent was evaporated to a constant weight. From this weight the percent of total lipid content of the tissue was calculated. The tabulated result of this calculation appears in Table 11. For both brain and spinal cord, from both acclimation groups, there is

Figure 8. Gas chromatogram of fatty acids derived from Fraction I. Fraction I contains glycerides, sterols, sterol esters, and wax-hydrocarbons.

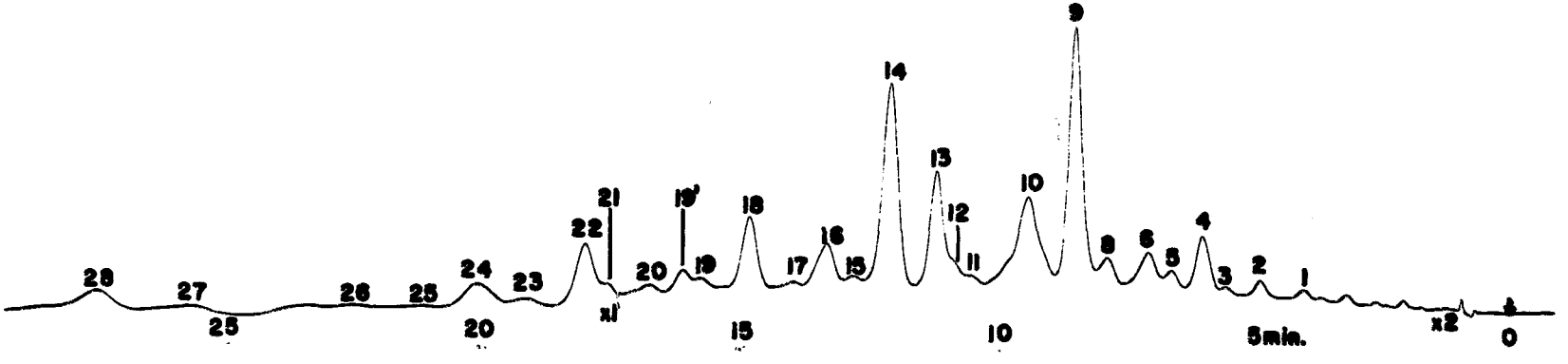

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Figure 9. Gas chromatogram of fatty acids derived from Fraction II. Fraction II contains the free fatty acids.

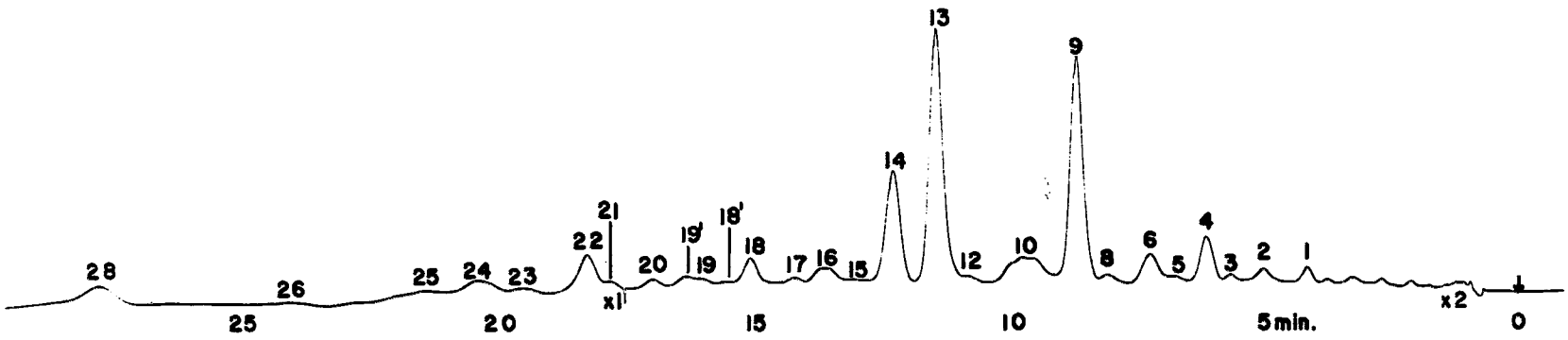
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Figure 10. Gas chromatogram of fatty acids derived from Fraction III. Fraction III contains the phospholipids.

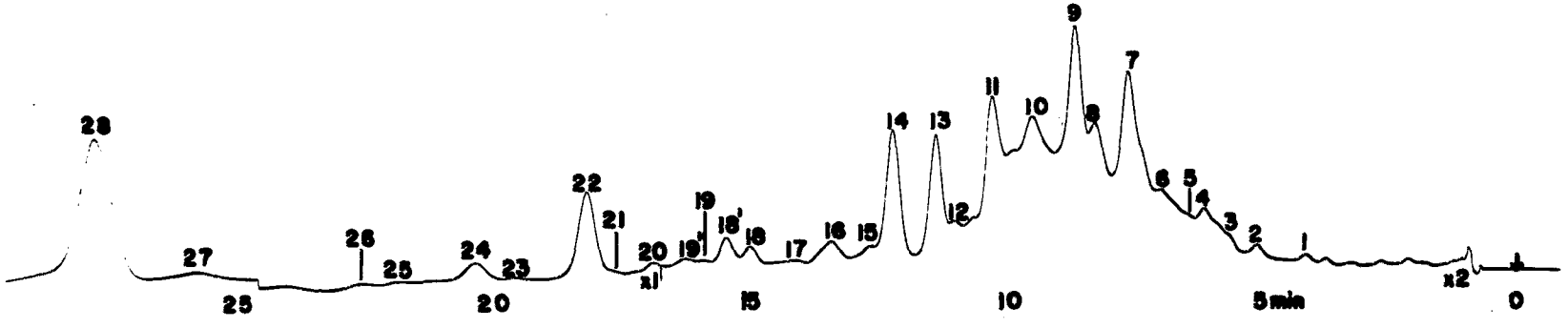
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TABLE 11. A. portalegrensis total lipid content of tissues.

Sample		N	Aggregate Wet Weight of Tissue, gm.	Weight of Lipid Extract, gm.	% Wet Weight
Brain, warm acclimated fish,	< 5.0 gm	43	1.488	0.154	10.35
Brain, warm acclimated fish,	≥ 5.0 gm	15	0.684	0.092	13.45
Brain, cold acclimated fish	< 5.0 gm	47	1.832	0.101	5.51
Brain, cold acclimated fish,	≥ 5.0 gm	10	0.568	0.057	10.04
Spinal cord, warm acclimated fish,	< 5.0 gm	43	0.185	0.010	5.41
Spinal cord, warm acclimated fish,	≥ 5.0 gm	15	0.105	0.016	15.24
Spinal cord, cold acclimated fish,	< 5.0 gm	47	0.238	0.016	6.72
Spinal cord, cold acclimated fish,	≥ 5.0 gm	10	0.091	0.020	21.98

a lower percent of total lipid in those tissues from fish of less than 5.0 gm. than from fish equal to or greater than 5.0 gm. For brain tissue there was a reduction in total lipid content with cold acclimation while the opposite was true with regard to spinal cord material.

A portion of the phospholipid fraction separated on the short column was not subjected to saponification and gas chromatography. These phospholipids were chromatographed on thin layer plates. Table 12 is a presentation of the identified components and their percent composition. Figures 11 and 12 are a collection of the densitometer strip charts for the thin layer chromatography plates. The table and the two figures show that the samples from the spinal cord of warm acclimated fish both larger and smaller than 5.0 gm are missing. This is due to the very small size of the samples. When these samples were chromatographed on thin layer plates the separated components could best be described as shadows rather than distinct spots. As a result, these thin layer plates could not be quantified by densitometry. The successful chromatography reveals that the major phospholipids for both brain and spinal cord are phosphatidyl serine and phosphatidyl choline; followed by somewhat smaller percentages of phosphatidyl ethanolamine, phosphatidyl inositol, and cardiolipin. Sphingomyelin was found in trace amounts.

Considering the well-established fact that fatty acid composition reflects, at least to some degree the diet, an analysis of the food organism was carried out. Certain dietary fatty acids may function as precursors in an organism's fatty acid metabolism. Knowledge of the earthworm fatty acid pattern could aid in revealing the fatty acid metabolism

TABLE 12. A. portalegrensis phospholipids. Identification and percent of composition. (A) Brain, warm acclimated fish, < 5.0 gm. (B) Brain, warm acclimated fish, ≥ 5.0 gm. (C) Brain, cold acclimated fish, < 5.0 gm. (D) Brain, cold acclimated fish, ≥ 5.0 gm. (E) Spinal cord, cold acclimated fish, < 5.0 gm. (F) Spinal cord, cold acclimated fish, ≥ 5.0 gm.

Peak Number	Peak Identity	A	B	C	D	E	F
1	Sphingomyelin	T	T	T	T	T	T
2	Phosphatidyl choline	32.41	6.21	8.70	26.85	61.83	-
3	Phosphatidyl inositol	-	6.29	10.31	-	-	15.42
4	Phosphatidyl serine	55.14	64.57	57.00	56.88	23.71	45.95
5	Phosphatidyl ethanolamine	11.15	18.42	19.85	14.60	14.46	34.19
6	Cardiolipin	1.30	4.52	4.15	1.78	0.00	4.66

Note: The symbol " - " is used to indicate where the peaks for phosphatidyl choline and the phosphatidyl inositol were not resolved and therefore were considered together. The percent of composition resides in the table position corresponding to the component of greater relative quantity. "T" represents trace amounts of sphingomyelin. A trace amount is less than 0.02%.

Figure 11. Densitometer strip charts of phospholipid thin layer chromatography plates.

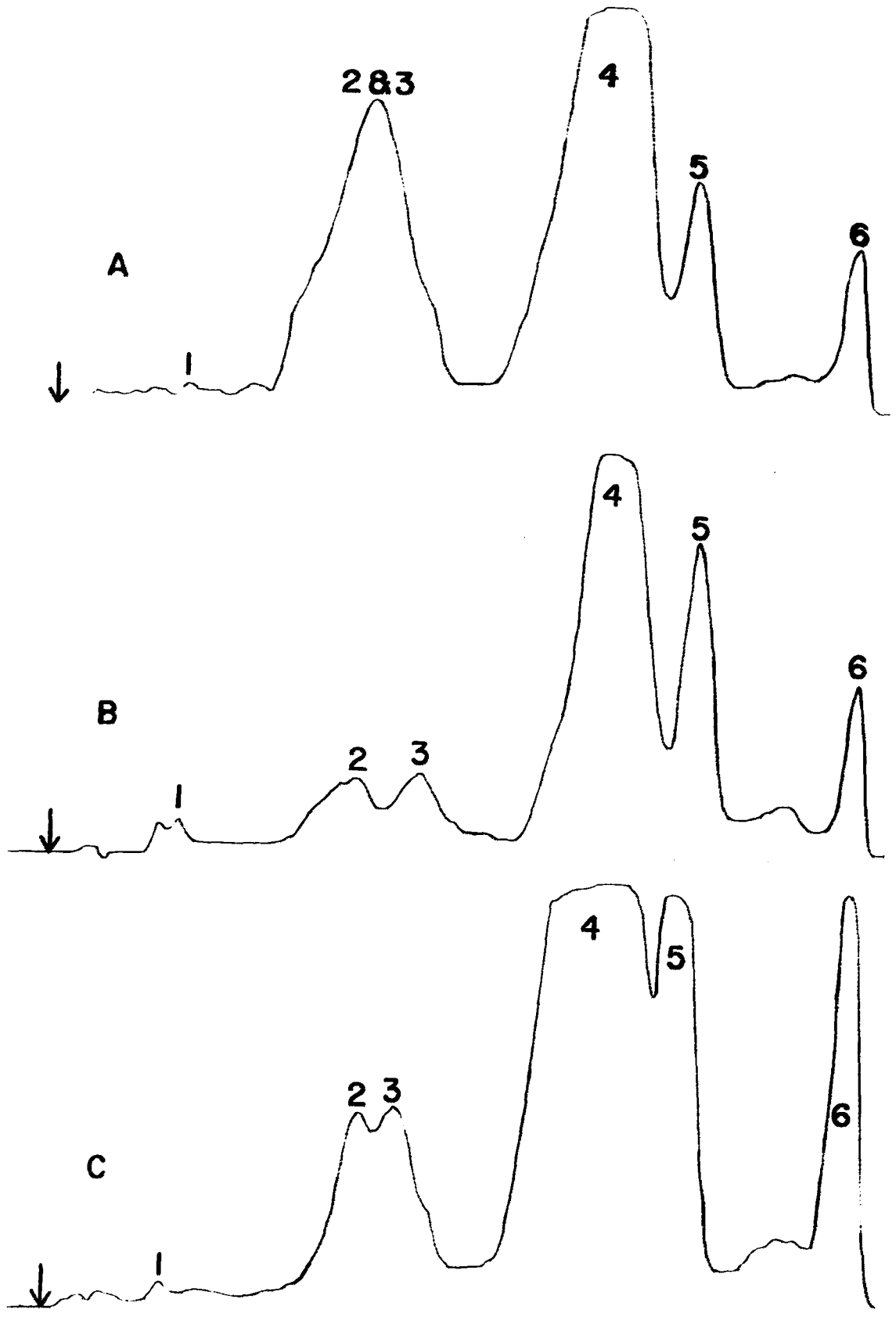
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Figure 12. Denitometer strip charts of phospholipid thin layer chromatography plates.

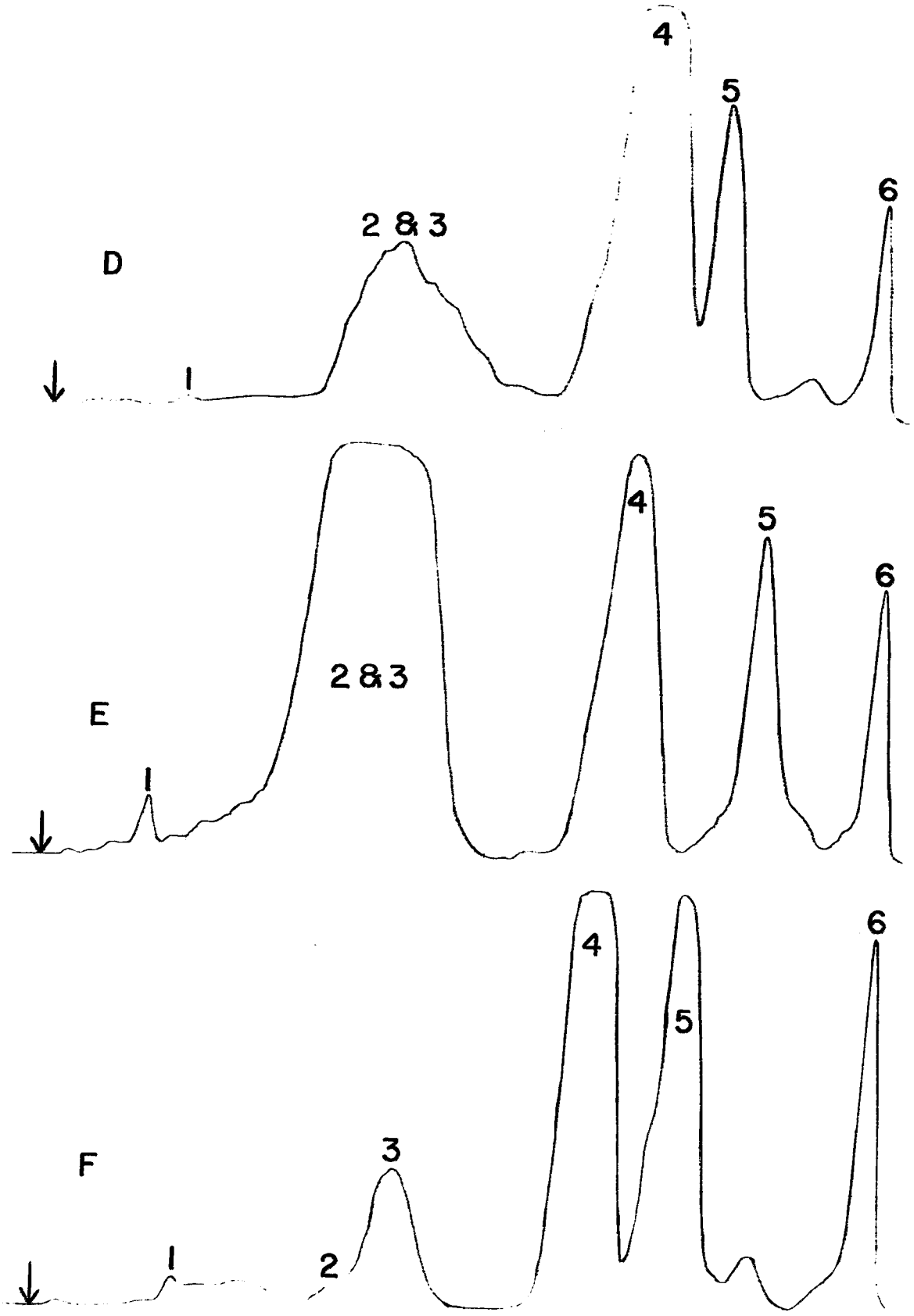
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TABLE 13. Identification and percent of composition of total fatty acids from Lumbricus terrestris. The mean percent composition represents 4 samples.

Peak #	Peak Identity	Mean Composition	Standard Error Mean % Composition
1	12:0	0.24	0.085
2	13:0	0.26	0.043
3	Multibranch 14:0	1.30	0.053
4	Iso 14:0 and 14:0	5.33	0.177
5	Ante 15:0	13.60	0.228
6	Multibranch 16:0	15.75	0.657
7	Iso 16:0 16:0, and unknown	24.41	0.720
8	Ante 17:0	6.60	0.382
9	Phytanic	10.69	0.599
10	18:0	5.31	0.059
11	18:1	3.98	0.118
12	18:1	0.67	0.122
13	18:2	1.71	0.086
14	20:0	0.53	0.121
15	18:3	2.64	0.353
16	20:2	0.21	0.067
17	20:2	0.34	0.046
18	22:0	0.48	0.234
19	20:4	2.41	0.643
20	22:2	0.82	0.451
21	24:0	2.87	0.878

of A. portalegrensis. The results of the analysis performed on earthworm appear in Table 13. The most obvious components of the fatty acids are the branched chain members. Of particular note are: multibranched 16:0, Iso 16:0, and phytanic acid. Conspicuous by their absence are the polyunsaturates: 16:3, 22:5, 22:6, 24:5, and 24:6. These five acids appear in the extracts of both tissues considered in A. portalegrensis.

DISCUSSION

I. Size and Age

The rate at which an animal consumes oxygen can be modified by numerous environmental factors (Fry, 1957; Prosser and Brown, 1962). The relationship between metabolism and any single factor can often be ascertained by appropriately controlled environment. In the early phases of this investigation, respirometric studies were conducted to determine whether or not age, within specified limits, significantly influenced the metabolism of Aequidens portalegrensis. The results summarized in Tables 1 and 2 and in Figures 2 and 3 show that there was no difference in the level of metabolism between the age classes involved. These data also show that the relationship between weight-specific metabolic rate and size is the same for the age classes compared. The lack of coincidence of regression lines in the area of overlapping body weight was noted earlier. This was particularly noticeable in group one. This lack of coincidence is apparently a result of chance and is probably of no biological significance.

The temperature sensitivity of oxygen consumption, expressed as Q_{10}, showed no significant difference between age classes in two weight groups considered. The acutely measured Q_{10} of metabolism of A. portalegrensis is not related to the age of the fish.

The metabolic rate of an organism has often been used as a general index of response to its environment. Investigations of metabolic thermal acclimation in A. portalegrensis indicated that the ability to

acclimate was related to the size of the fish (Morris, 1962). The striking depensation in growth of these fish prompted a study of the possible influence of age on physiological processes. It was concluded from the present results that age is not a factor of any significant influence over the size range, and within the age limits, considered. Hence, age was not considered further in this investigation.

II. Lipids

Many poikilotherms possess the ability to compensate for changes in environmental temperatures. In many species, changes in the fatty acid patterns of the constituent lipids are concomitant with acclimation to temperature changes. This investigation was aimed at determining the extent to which thermally induced changes in the fatty acids of the central nervous system reflected size-related differences in acclimation of metabolism. A size-related change in percent composition was found in only two cases: 22:6 from spinal cord material of cold acclimated fish, and 24:6 from brain extract of warm acclimated fish. Since each of these instances in which there was apparent significance was from a total of twenty-nine similar comparisons, the reality of these two results becomes questionable. Twenty-nine fatty acids were identified and their composition percentages analyzed for their relationship to size. The examination of the regression coefficients for differences between acclimation groups revealed one of significance, again 22:6 in the spinal cord. This result is probably a reflection of the apparent significance of the size related change found in the acid from the cold acclimated

fish and is, therefore, also suspect.

The hypothesis that changes in the fatty acid pattern resulting from thermal history may be related to size can not be totally disregarded. It should be pointed out that the fatty acid samples were derived from the total saponifiable lipids of the brain and spinal cord. The fatty acid patterns observed were, therefore, a composite derived from several lipid classes: phospholipids, glycerides, free fatty acids, glycolipids, and protein-bound lipids. With regard to the lipids of the central nervous system, the phospholipids would be of primary structural importance (Rossiter, 1962). Although the phospholipids are the major contributors to the fatty acid pattern of the brain and spinal cord (Rossiter, op. cit.), fatty acids derived from other lipid classes may have "masked" any size-related changes. Such a result could accrue from a fixed pattern of these other classes thus effectively muting any size-related changes occuring in the phospholipids (Knipprath and Mead, 1968). Another possible explanation is that the changes in the fatty acid compositions of the other classes were so vagrant as to invalidate statistically any size-related changes of the phospholipids. These speculations indicate a necessary direction of future investigations of the relationship between size and lipid change resulting from thermal acclimation.

A comparison of the fatty acid patterns derived from tissues obtained from animals of both acclimation groups revealed significant differences in percent composition of several fatty acids. Table 8 has been constructed by referring to the cold acclimation pattern as an increase

or decrease relative to the pattern for warm acclimation. The increase in long chain polyunsaturates, which has come to be the accepted pattern of cold acclimation, was seen in the spinal cord composition. There were significant increases in 18:2, 20:4, 22:5, 22:6, and 24:5. The decrease in 20:2 can be explained by the function of this fatty acid as an intermediate in the synthesis of 20:4 and possibly the polyunsaturates of C_{22} and C_{24} (Mead, 1960).

In the lipids of the brain, an opposite, unprecedented result occurred. The signficant differences of fatty acid composition were decreases with cold acclimation. The fatty acids 18:2, 22:5, and 24:5, found to increase in the spinal cord, decreased in the lipids of the brain. Also, 20:0, 20:1, 20:2, 24:0, and 24:1 were found to decrease in the brain lipids of animals with a low temperature history. It may be significant that none of these five acids are polyunsaturates. Two are saturates, two monoenes, and one is a diene. These will be considered later.

Data comparing brain and spinal cord composition from similar thermal history is shown in Table 9. For cold acclimated fish, where a significant difference in composition existed, the spinal cord was overwhelmingly the tissue with the higher percentages. Of the thirteen which differed significantly, eleven were greater, in composition percentage, in the spinal cord. Following warm acclimation, only two of these eleven were found to be greater, in percent of composition, in the brain. These were the fatty acids 24:1 and 22:5. Fatty acids 20:4 and 22:6 were greater components in the brain than in the spinal cord of cold acclimated

fish. The greater compositional percentages of spinal cord material of low temperature history are in part due to the increase of so many fatty acid species. In addition, however, it represents the myelination of the spinal cord. In comparison to higher vetebrates, the fish brain is quite poor in white matter (Roots, 1968) while the spinal cord is not. As a result, differences in the fatty acid species of myelinated spinal cord and non-myelinated brain tissue should be magnified as I believe they are in the present case. Conversely, the greater percentage of 20:4 and 22:6 in brain lipids from both warm and cold acclimation groups is a reflection of the important role of these two fatty acids in the composition of the cells of the brain, i.e., the neuronal membranes themselves (Johnston and Roots, 1964) Even though several acid species of the spinal cord increase during cold acclimation, 20:4 and 22:6 remain larger components of the brain. Also attesting to their importance is the fact that although many fatty acid components of the brain decrease in low temperature, these two apparently remain unchanged.

It appears relevant to speculate on the meaning of the apparent general decrease of the several fatty acids observed in brain material from cold acclimated animals. In the following discussion I am making the assumption that an increase in amount and unsaturation of the C_{18} through C_{24} fatty acids following acclimation to low temperatures is of adaptive advantage to the organism. The ubiquitous occurrence of this phenomenon supports this assumption, as does consideration of the role of fatty acids in the structure of cell membranes.

Aequidens portalegrensis is a tropical species, originally found in central and southeastern Brazil. One would not expect, therefore, that this species would often encounter extremes of low temperature. If in its evolution, A. portalegrensis were not frequently required to tolerate lower temperatures, the species may not have developed the full range of compensatory mechanics. It is my suggestion that the results reported in this investigation may reflect this. As was stated in the INTRODUCTION, the site of cold blockage of the simple reflexes has been reported to lie in the spinal cord. If a fish were able to maintain a specific functional state of the spinal cord in the event of thermal stress, it would be a distinct advantage. The compositional changes reported in the fatty acids of the spinal cord are those usually attributed to cold acclimation. The changes occurring in the brain are contrary to what would be expected in acclimation. It is my tentative conclusion that these results represent an incomplete ability of A. portalegrensis to carry cold acclimation of the central nervous system to the next higher level, i.e., the brain. It is possible however, that the low temperature of 22°C suppressed the response of the brain. Perhaps after longer exposure to this temperature the more typical pattern would have developed. If, on the other hand, 22°C was entirely too cold and any chance of the brain response negated, then a slightly warmer temperature. might have induced more typical changes of the lipids.

It appears that the fish in this experiment were unable to alter the brain fatty acids 20:4 and 22:6 to compensate for low temperature These two acids are often assigned important roles as neuronal components

(Rossiter, 1962). It would be reasonable to expect them to change. The fish was unable to maintain several fatty acid components at a steady level in the brain, and these components were observed to decrease. Several of these fatty acids; 20:0, 20:1, 20:2, and 24:0 are thought to be precursors or intermediates of the important polyunsaturates (Mead, 1960).

Further evidence for this proposed incomplete acclimation response is seen in the results of the determination of the lipid content as percent of wet weight of tissue. The lipid content of the spinal cord increased with cold acclimation while the content of the brain decreased. The result appears especially real when one considers the observed decrease in tissue water with acclimation to low temperature (Hoar and Cottle, 1952).

There is a second and entirely different interpretation of the decrease in percent composition of the brain fatty acids due to cold thermal history. There were twenty-nine fatty acids identified in the samples. Of these, only eight showed a significant decrease with cold acclimation. The method of using percent of composition to represent the relative quantities of acids is severely restricted in that if one component decreases, another must increase. The total must equal one hundred percent. The apparent decrease in eight acids may, in fact, be due to an actual increase in most or all of the remaining twenty-one acids. The increases may be spread almost uniformly throughout these twenty-one acids and thus not appear as significant changes, or the increases may occur so variably as to become statistically obscured. In either case the apparent decrease in the eight brain fatty acids may be a statistical

accident and have little biological meaning. To determine the reality of these changes, quantitative gas chromatography would be necessary.

An assumption was made earlier, that a selective advantage may accrue to those organisims which are capable of appropriately adjusting membrane chemistry thus enabling them to preserve a specific functional state despite a changing of environmental temperature. Luzzati and Husson (1962) showed that temperature dictates the physical state of a biological membrane. They stated that human brain phospholipids may undergo a transition from a coagel to a liquid-crystalline state at a temperature that is close to the body temperature. The lipid component of a membrane is indispensible to membrane function. Acetone extraction of mitochondria removes the phospholipids and with them goes the electron transport activity (Fleischer et al., 1962). The phospholipid integrity is essential for maintenance of neuronal membrane resistance, capacity, excitability, and potentials (Rojas and Tobias, 1965). The membrane integrity, i.e., the kinetics of binding of phospholipids to protein is affected by the fatty acid composition of the phospholipids (De Pury and Collins, 1966a).

To understand how the fatty acid composition can affect membrane integrity, it is necessary to consider the current model of the biological membrane. At the present time, membranes are thought to be composed of a two dimensional mosaic of lipoprotein subunits. Each subunit consists of a protein molecule combined with its phospholipid complement. The lipids are believed to form two layers with the fatty acid moiety interdigitated with the tertiary structure of the protein (Benson, 1966,

1968; Korn, 1966). The lipoprotein complex is held together by hydrophobic bonds formed between the phospholipid and protein fractions. The bond strength is dependent on the composition of the fatty acids (De Pury and Collins, 1966b). In summary, a change in the degree of unsaturation of the fatty acid species tends to counteract the unfavorable affect temperature has on the interactions of membrane components. An increase in unsaturation following a decrease in environmental temperature would perform this function.

The result of the thin layer chromatography was useful only as it indicated the phospholipid composition of the central nervous system of A. portalegrensis. As has been stated in the RESULTS, the major components appear to be phosphatidyl serine and phosphatidyl choline, followed by phosphatidyl ethanolamine and phosphatidyl inositol. Cardiolipin was present in lesser amounts and only traces of sphingomyelin were found. It is not possible to draw any conclusions about the participation of phospholipids in thermal acclimation. Recent studies of Roots (1968), however, indicate that environmental temperature does not influence the percent of composition of the brain phospholipids in fish; only the fatty acid composition of the phospholipids.

A fatty acid analysis of whole earthworms allowed a comparison of the dietary fatty acid composition with that of the fish tissues studied. The objective was to learn something of the fatty acid metabolism of A. portalegrensis and the influence of diet on the composition of the fish tissues.

It is shown in the legend of Figure 4 that both tissues considered

brain and spinal cord, contained 16:3 and the polyunsaturates 22:5, 22:6, 24:5, 24:6. The earthworm samples contained none of these acids and it is assumed that the fish is able to synthesize these components. De novo synthesis of these acids has never been demonstrated in fish (Mead, 1960) and so it must be assumed that A. portalegrensis is synthesizing these acids by the extension of chain length and an increase in unsaturation of pre-existing acids derived from the diet. It has been demonstrated that fish are capable of producing the C_{20} polyunsaturates from linoleic (18:2) and the C_{22} and C_{24} polyunsaturates from linolenic (18:3) (Kelly et al., 1958; Reiser et al., 1963). In both studies it was found that such synthesis would occur only if the two precursors were present in the diet in amounts less than 5% of the total. Both fatty acids 18:2 and 18:3 comprise less than 3% of the earthworm lipids and the proposed sysnthesis is plausible.

One of the initial steps in any gas chromatographic study of fatty acids is the identification of the observed peaks. This phase of the present investigation was complicated by the large number of components observed in the C_{12} to C_{18} portion of the chromatograms. After the more conventional methods failed to identify several of these peaks, the technique of urea adduction was employed to determine whether the unknown components were branched acids. It was surprising to find branched acids present as major components of the samples. Phytanic acid, for example, represented as much as 15% of the samples. Although not unknown in biological samples, these branched acids normally appear in trace amounts (Ackman et al., 1967). The results of the separation of total

extractable lipids into fractions prior to fatty acid analysis revealed that most of the branched acid components occur in the phospholipids (Table 10 and Figure 10). Comparison of these results with the profile of earthworm fatty acids shows that the diet was a probable source of the branched acids. Why some of these acids, multibranch 16:0, Iso 16:0, and phytanic, were incorporated mainly in the phospholipids is unknown.

The ultimate source of multibranch 14:0, multibranch 16:0, and phytanic acids is probably the phytol group of chlorophyll (Patton and Benson, 1966; Ackman and Hooper, 1968). Whether the conversion of phytol to these fatty acid derivatives occurs before or after ingestion by the earthworms is unknown. In any case, it is probably due to bacterial metabolism (Benson, personal communication; Ackman and Hansen, 1967). The relatively high content of phytanic acid in the phospholipids of fish tissues studied here is similar to a pathological condition in humans called Refsum's syndrome. This disease results from a reduced ability to catabolize phytanic. It would seem that A. portalegrensis may offer a good system in which to study metabolic relationships of phytanic acid.

BIBLIOGRAPHY

Ackman R. G. and Hansen R. P. (1967) The occurrence of diastereomers of phytanic and pristanic acids and their determination by gas-liquid chromatography. Lipids 2, 357-362.

Ackman R. G., Sipos J. C. and Tocher C. S. (1967) Some linear, iso-, anteiso- and multi-branched fatty acids of marine origin: Analyses on packed and open-tubular gas-liquid chromatography columns with particular reference to pristanic and phytanic acids. J. Fish. Res. Bd. Canada 24, 635-650.

Ackman R. G. and Hooper S. N. (1968) Examination of isoprenoid fatty acids as distinguishing characteristics of specific marine oils with particular reference to whale oils. Comp. Biochem. Physiol. 24, 549-565.

Allee W. C., Greenberg B., Rosenthal G. M., and Frank P. (1958) Some effects of social organization on growth in the green sunfish, Lepomis cyanellus. J. Exptl. Zool. 108, 1-19.

Battle H. I. (1926) Effects of extreme temperature on muscle and nerve tissue of marine fish. Trans. Roy. Soc. Canada. Ser. III. 20, 127-143.

Benson A. A. (1966) On the orientation of lipids in chloroplast and cell membranes. J. Am. Oil Chem. Soc. 43, 265-270.

Benson A. A. (1968) The cell membrane: A lipoprotein mono layer. In: Membrane models and the formation of biological membranes. Bolis L. and Pethica B. A. (ed.) pp. 190-202.

Black E. C., Fry F. E. J., and Scott W. J. (1939) Maximum rates of oxygen transport for certain freshwater fish (abstract). Anat. Rec. 75 Supp. 80.

Böttcher C. J. F., Woodford F. P., Boelsma-Van Houte E. and Van Gent C. M. (1959) Methods for the analysis of lipids extracted from human arteries and other tissues. Rec. Trav. Chim. 78, 794-814.

Brett J. R. (1956) Some principles in the thermal requirements of fishes. Quart. Rev. of Biol. 31, 75-87.

Brown M. E. (1946) The growth of brown trout (Salmo trutta Linn.). I. Factors influencing the growth of trout fry. J. Exptl. Biol. 22, 118-129.

Brown M. E. (1951) The growth of brown trout (Salmo trutta Linn.). IV. The effect of food and temperature on the survival and growth of fry. J. Exptl. Biol. 28, 473-491.

Brown M. E. (1957) Experimental studies on growth. In: The physiology of fishes. Vol. I. Metabolism. Brown M. E. (ed.) pp. 361-400. Academic Press Inc., New York.

Caldwell R. S. (1967) The influence of acclimation temperature on the lipid composition of fish gill mitochondria. Thesis, Duke University.

Das A. B. and Prosser C. L. (1967) Biochemical changes in tissues of goldfish acclimated to high and low temperatures. I. Protein synthesis. Comp. Biochem. Physiol. 21, 449-467.

Das A. B. (1967) Biochemical changes in tissues of goldfish acclimated to high and low temperatures. II. Synthesis of protein and RNA of subcellular fractions and tissue composition. Comp. Biochem. Physiol. 21, 469-485.

Dawson W. R. and Bartholomew G. A. (1956) Relation of oxygen consumption to body weight, temperature, and temperature acclimation in lizards Uta stansburiana and Sceloporus occidentalis. Physiol. Zool. 29, 40-51.

De Pury G. G. and Collins F. D. (1966a) The influence of fatty acid composition on the rate of binding of lecithin by extracted mitochondria. Chem. Phys. Lipids 1, 1-19.

De Pury G. G. and Collins F. D. (1966b) The influence of fatty acid composition on the restoration of succinate-cytochrome C reductase activity by phospholipids in extracted mitochondria. Chem. Phys. Lipids 1, 20-32.

Doudoroff P. (1942) The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with Girella nigricans. Biol. Bull. 83, 219.

Evans C. D., Cooney P. M. and Panek E. J. (1962) Graphic aid for interpreting gas chromatograms. J. Am. Oil Chem. Soc. 39, 210-213.

Farkas T. and Herodek S. (1964) The effect of environmental temperature on the fatty acid composition of crustacean plankton. J. Lipid Res. 5, 369-373.

Fleischer S., Brierley G. P., Klouwen H., and Slautterback D. B. (1962) Studies of the electron transfer system XLVII. The role of phospholipids in electron transfer. J. Biol. Chem. 237, 3264-3272.

Folch J., Lees M. and Sloane Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.

Fraenkel G. and Hopf H. S. (1940) The physiological action of abnormally high temperatures on poikilothermic animals. I. Temperature adaptation and the degree of saturation of the phosphatides. Biochem. J. 34, 1085-1092.

Freeman J. A. (1950) Oxygen consumption, brain metabolism and respiratory movements of goldfish during temperature acclimatization, with special reference to lowered temperatures. Biol. Bull. 99, 416-424.

Fry F. E. J. and Hart J. S. (1949) Cruising speed of goldfish in relation to water temperature. J. Fish. Res. Bd. Canada 7, 169-175.

Fry F. E. J. (1957) The aquatic respiration of fish. In: The physiology of fishes. Vol. I. Metabolism. Brown M. E. (ed.) pp. 1-55. Academic Press Inc., New York.

Greenberg B. (1947) Some relations between territory, social hierarchy, and leadership in the green sunfish (Lepomis cyanellus). Physiol. Zool. 20, 267-299.

Hammerstrand K. (1966) Gas chromatographic analysis of fatty acids. Varian Aerograph (Publisher), Walnut Creek, California.

Henriques V. and Hansen C. (1901) Vergleichende untersuchungen über die chemische Zusammensetzung des thierischen Fettes. Skand. Arch Physiol. 11, 151-156.

Hoar W. S and Cottle M. K. (1952) Effects of temperature acclimatization on the chemical composition of goldfish tissues. Canad. J. Zool. 30, 49-54.

Hochachka P. W. and Hayes F. R. (1962) The effect of temperature acclimation on pathways of glucose metabolism in the trout. Canad. J. Zool. 40, 261-270.

Hubbs C. L. and Cooper G. P. (1935) Age and growth of the long-eared and green sunfishes in Michigan. Papers Mich. Acad. Sci. 20, 669-696.

Jagaard P. M., Ackman R. G. and Sipos J. C. (1967) Seasonal changes in fatty acid composition of cod liver, flesh, roe, and milt lipids. J. Fish. Res. Bd. Canada 24, 613-627.

Johnston P. V. and Roots B. I. (1964) Brain lipid fatty acids and temperature acclimation. Comp. Biochem. Physiol. 11, 303-309.

Kanungo M. S. and Prosser C. L. (1960) Physiological and biochemical adaptation of goldfish to cold and warm temperatures. II. Oxygen consumption of liver homogenate; oxygen consumption and oxidative phosphorylation of liver mitochondria. J. Cell. Comp. Physiol. 54, 265-274.

Kayama M., Tsuchiya Y. and Mead J. F. (1963) A model experiment of aquatic food chain with special significance in fatty acid composition. Bull. Japanese Soc. Sci. Fish. 29, 452-458.

Keith A. (1966) Analysis of lipids in Drosophila melanogaster. Comp. Biochem. Physiol. 17, 1127-1136.

Kelly P. B., Reiser R. and Hood D. W. (1958) The effect of diet on the fatty acid composition of several species of fresh water fish. J. Am. Oil Chem. Soc. 35, 503-505.

Knipprath W. G. and Mead J. F. (1965) Influence of temperature on the fatty acid pattern of muscle and organ lipids of the rainbow trout, Salmo gairdnerii. Fish. Indust. Res. 3, 23-27.

Knipprath W. G. and Mead J. F. (1966) Influence of temperature on the fatty acid pattern of mosquitofish (Gambusia affinis) and guppies (Lebistes reticulatus). Lipids 1, 113-117.

Knipprath W. G. and Mead J. F. (1968) The effect of the environmental temperature on the fatty acid composition and on the in vivo incorporation of 1- ^{14}C- acetate in goldfish (Carassius auratus L.). Lipids 3, 121-128.

Konishi J. and Hickman C.P. (1964) Temperature acclimation in the central nervous system of rainbow trout (Salmo gairdnerii). Comp. Biochem. Physiol. 13, 433-442.

Korn E. D. (1966) Structure of biological membranes. Science 153, 1491-1498.

Krog J. (1954) The influence of seasonal environmental changes upon the metabolism, lethal temperature, and heart rate of Gammarus limnaeus (Smith) taken from an alaskan lake. Biol. Bull. 107, 397-410.

Luzzati V. and Husson F. (1962) The structure of the liquid-crystalline phases of lipid-water systems. J. Cell. Biol. 12, 207-219.

Magnuson J. J. (1962) An analysis of aggressive behavior, growth, and competition for food and space in medaka (Oryzias latipes). Canad. J. Zool. 40, 313-363.

Mather K. (1951) Statistical analysis in biology. Methuen Co. Ltd., London pp. 267.

Mead J. F. (1960) The metabolism of the polyunsaturated fatty acids Am. J. of Clin. Nutr. 8, 55-61.

Morris R. W. (1962) Body size and temperature sensitivity in the cichlid fish, Aequidens portalegrensis (Hensel). Am. Nat. 96, 35-50.

Morris R. W. (1963) A modified Barcroft respirometer for study of aquatic animals. Turtox News 41, 22-23.

Morris R. W. (1965a) Thermal acclimation in an eleotrid fish, Carassiops compresus (Krefft). Am. Nat. 99, 81-87.

Morris R. W. (1965b) Seasonal changes in metabolism of four south temperate marine fishes. Trans. Roy. Soc. New Zealand 6, 141-152.

Morris R. W. (1965c) Thermal acclimation of metabolism of the yellow bullhead, Ictalurus natalis (Le Sueur). Physiol. Zool. 38, 219-227.

Nicolaides N. and Ray T. (1965) Skin lipids. III. Fatty chains in skin lipids. The use of Vernix caseosa to differentiate between endogenous and exogenous components in human skin surface lipid. J. Am. Oil Chem. Soc. 42, 702-707.

Orr P. R. (1955) Heat death. I. Time-temperature relationships in marine animals. Physiol. Zool. 28, 290-294.

Patton S. and Benson A. A. (1966) Phytol metabolism in the bovine. Biochem. Biophys. Acta 125, 22-32.

Pitt T. K., Garside E. T. and Hepburn R. L. (1956) Temperature selection of the carp (Cyprinus carpio Linn.). Canad. J. Zool. 34, 555-557.

Precht H., Christophersen J. and Hensel H. (1955) Temperature und leben Berlin, Springer-verlag, pp. 514.

Prosser C. L. and Brown F. A. (1962) Comparative animal physiology, 2nd Ed., Chapter 9, pp. 238-284. Saunders, Philadelphia.

Prosser C.L., Precht H. and Jankowsky H. D. (1965) Nervous control of metabolism during temperature acclimation of fish. Naturwissenschaften 52, 168-169.

Reiser R., Stevenson B., Kayama M., Choudbury R. and Hood D. (1963) The influence of dietary fatty acids and environmental temperature on the fatty acid composition of teleost fish. J. Am. Oil Chem. Soc. 40, 507-513.

Rojas E. and Tobias J. M. (1965) Membrane model: Association of inorganic cations with phospholipid momolayers. Biochem. et Biophys. Acta 94, 394-404.

Roots B. I. and Prosser C. L. (1962) Temperature acclimation and the nervous system in fish. J. Exp. Biol. 39, 617-629.

Roots B. I. (1968) Phospholipids of goldfish (Carassius auratus L.) brain: The influence of environmental temperature. Comp. Biochem. Physiol. 25, 457-466.

Rose S. M. (1959) Failure of survival of slowly growing members of a population. Science 129, 1026.

Rossiter R. J. (1962) Chemical constituents of brain and nerve. In: Neurochemistry: the chemistry of brain and nerve. Elliot K. A. C., Page I. H. and Quastel J. H. (ed.). pp. 10-54. Charles C. Thomas, Springfield.

Schlenk H. (1954) Urea inclusion compounds of fatty acids. In: Progress in the chemistry of fats and other lipids. Vol. 2, pp. 243-267.

Suhrman R. (1955) Weitere versuche über die temperaturadaptation der Karauschen, Carassius vulgaris. Biol. Zentr. 74, 432-447.

Tsukuda H. (1961) Temperature acclimatization on different organization levels of fishes. J. Biol. Osaka City Univ. 12, 15-45.

West E. S. and Todd W. E. (1964) Textbook of biochemistry, 3rd Ed., Chap. 6, pp 126-176., Macmillan Co., New York.

APPENDIX I

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CCCCCCCCCCCCCCCCCCCCNOVEMBER26,1968CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
C                                FATTY ACID ANALYSIS                                C
C REGRESSION COEFFICIENT CALCULATIONS WITH T TESTS - PROGRAMMER D.M.ALLEN            C
C WITH M.J.SCHNEIDER AND R.W.MORRIS                                                 C
C CALCULATES FOR UP TO 50 PEAKS ON GAS CHROMATOGRAPHY FATTY ACID ANALYSES           C
C UP TO 100 OBSERVATIONS/GROUP, AND UP TO 5 EXPERIMENTAL GROUPS.                    C
C CONTROL CARDS PRECEDE EACH SET OF OBSERVATIONS AND FORMAT STATEMENT 100            C
C MUST BE CHANGED TO READ THE DESIRED NO. AND PRECISION OF DATA (PEAKS)             C
C X(=SIZE) FOLLOWED BY UP TO 50 Y VARIABLES(=PEAKS). THE CONTROL CARD MUST          C
C HAVE THE FOLLOWING DATA:                                                          C
C COL 1-4 NO. OF OBSERVATIONS IN GROUP                                              C
C COL 6-7 NO. OF VARIABLES INCLUDING DEPENDENT (SIZE)                               C
C COL 10-70 TITLE OR OTHER IMFORMATION WISHED AS PRINTOUT HEADING                   C
C                                                                                   C
C A CONTROL CARD MUST PRECEDE EACH GROUP OF OBSERVATIONS                            C
C                                                                                   C
C   THE FOLLOWING IS CALCULATED FOR EACH GROUP:                                     C
C    SAMPLE SIZE...REGRSSION OF EACH Y ON X...RANGE AND MEAN OF EACH VARIABLE       C
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C     VARIANCE...VARIANCE RATIO(SNEDECOR'S F)...STANDARD ERROR OF B
C          ALSO CALCULATED FOR COMPARING GROUPS:                              C
C          VARIANCE OF EACH MEAN...STANDARD DEVIATIONS...T-VALUES FOR MEAN OF X  C
C          T-VALUES FOR EACH REGRESSION OF Y ON X...T-VALUES FOR MEANS OF Y    C
C          VARIABLES...NOTE...THESE LAST CALCULATIONS MAY BE BYPASSED IF DESIRED C
C                                                                             C
CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
      DIMENSION YSUM(50),YSQ(50),XYSUM(50),X(100),Y(100,50),          FATT0010
     1 YMEAN(50,10),SYSQ(50),SXY(50),REGCOF(50,10),A(50),F(50),RPRIME(50FATT0020
     2 ),SEB(50,10),TITLE(35),YTEST(50),NFRDM(5),VX(10),VY(50,10),    FATT0030
     3 XMEAN(10),RTEST(50)                                            FATT0040
      TTEST(T1,T2,T3,T4)=ABS((T1-T2)/(SQRT(T3**2+T4**2)))             FATT0050
      KT=0                                                            FATT0060
    5 KT=KT+1                                                         FATT0070
C
C READ A CONTROL CARD FOR A GROUP
C
      READ(1,100,END=99)NOBS,NOVAR,TITLE                              FATT0080
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  100 FORMAT(I4,1X,I2,2X,35A2)                                          FATT0090
      WRITE(3,107) TITLE                                                FATT0100
  107 FORMAT('1',//'0',35A2)                                            FATT0110
C
C INITIALIZE FOR THIS PASS
C
      XSUM=0.                                                           FATT0120
      XSQ=0.                                                            FATT0130
      DO 22 K=1,NOVAR                                                   FATT0140
      YSUM(K)=0.                                                        FATT0150
      XYSUM(K)=0.                                                       FATT0160
      YSQ(K)=0.                                                         FATT0170
   22 CONTINUE                                                          FATT0180
      DO 33  J=1,NOBS                                                   FATT0190
      READ(1,101)X(J),(Y(J,K),K=1,NOVAR)                                FATT0200
  101 FORMAT(F5.2,12F6.2/F5.2,12F6.2/F5.2,3F6.2)                        FATT0210
      XSUM=XSUM+X(J)                                                    FATT0220
      XSQ=XSQ+X(J)**2                                                   FATT0230
```

```
C
C CALCULATE THE SUMS
C
      DO 33  K=1,NOVAR                                              FATT0240
      XYSUM(K)=XYSUM(K)+X(J)*Y(J,K)                                  FATT0250
      YSQ(K)=YSQ(K)+Y(J,K)**2                                        FATT0260
       YSUM(K)=YSUM(K)+Y(J,K)                                        FATT0270
   33 CONTINUE                                                       FATT0280
C
C FIND THE RANGE OF EACH VARIABLE, AND THE MEANS
C
      CALL MAXMIN(X,Y,NOBS,NOVAR)                                    FATT0290
      DIV=NOBS                                                       FATT0300
      XMEAN(KT)=XSUM/DIV                                             FATT0310
      WRITE(3,102)XMEAN(KT)                                          FATT0320
      DO 44 K=1,NOVAR                                                FATT0330
      L=K                                                            FATT0340
      YMEAN(K,KT)=YSUM(K)/DIV                                        FATT0350
```

```
      WRITE(3,103)L,YMEAN(K,KT)                                        FATT0360
   44 CONTINUE                                                         FATT0370
  102 FORMAT('0','MEAN SIZE=',F6.2)                                    FATT0380
  103 FORMAT(' ','MEAN OF PEAK #',I2,' = ',F6.2)                       FATT0390
C
C CALCULATE THE SUM OF THE SQUARES OF THE DEVIATIONS
C
      SXSQ=XSQ-XSUM**2/DIV                                             FATT0400
      DO 55 K=1,NOVAR                                                  FATT0410
      SYSQ(K)=YSQ(K)-YSUM(K)/DIV                                       FATT0420
      SXY(K)=XYSUM(K)-(XSUM*YSUM(K))/DIV                               FATT0430
   55 CONTINUE                                                         FATT0440
C
C FIND THE REGRESSION COFFFICIENT OF EACH Y ON X
C
      DO 66 K=1,NOVAR                                                  FATT0450
      REGCOF(K,KT)=SXY(K)/SXSQ                                         FATT0460
      A(K)=YMEAN(K,KT)-REGCOF(K,KT)*XMEAN(KT)                          FATT0470
```

```
      L=K                                                                FATT0480
      WRITE(3,104)L,REGCOF(K,KT),A(K)                                    FATT0490
   66 CONTINUE                                                           FATT0500
  104 FORMAT('0'///' ','REGRESSION OF Y',I2,' ON X=',E15.8,4X,           FATT0510
     C 'Y INTERCEPT=',E15.8)                                             FATT0520
      DIV=NOBS-2                                                         FATT0530
      WRITE(3,110)                                                       FATT0540
C
C FIND THE VARIANCE RATIOS (F)
C FIND STANDARD ERROR OF B
C
      DO 77 K=1,NOVAR                                                    FATT0550
      L=K                                                                FATT0560
      F(K)=REGCOF(K,KT)*SXY(K)/((SYSQ(K)-REGCOF(K,KT)*SXY(K))/DIV)       FATT0570
      RPRIME(K)=(SYSQ(K)-REGCOF(K,KT)*SXY(K))/DIV                        FATT0580
   77 SEB(K,KT)=SQRT(RPRIME(K))*SQRT(1./SXSQ)                            FATT0590
      DO 88 K=1,NOVAR                                                    FATT0600
      L=K                                                                FATT0610
```

```
      WRITE(3,105)L, F(K),RPRIME(K)                                    FATT0620
  105 FORMAT('0','VARIANCE RATIO OF Y',I2,'=',E15.8,'   R=',E15.8)     FATT0630
      WRITE(3,106)SEB(K,KT)                                            FATT0640
  106 FORMAT(' ','THE STANDARD ERROR OF B= ', E15.8)                   FATT0650
   88 CONTINUE                                                         FATT0660
      DIV=(NOBS-1)*NOBS                                                FATT0670
      NFRDM(KT)=NOBS                                                   FATT0680
      VX(KT)=SXSQ/DIV                                                  FATT0690
      STDEV=SQRT(VX(KT))                                               FATT0700
      WRITE(3,299)VX(KT),STDEV                                         FATT0710
  299 FORMAT('0',///,'0','VARIANCE OF X VARIABLE=',E15.8,'    STANDARD  FATT0720
     1DEVIATION=',E15.8)                                               FATT0730
      DO 122 I=1,NOVAR                                                 FATT0740
      VY(I,KT)=SYSQ(I)/DIV                                             FATT0750
      STDEV=SQRT(VY(I,KT))                                             FATT0760
      L=I                                                              FATT0770
      WRITE(3,300)L,VY(I,KT),STDEV                                     FATT0780
  300 FORMAT('0','VARIABLE ',I2,':   VARIANCE=',E15.8,                 FATT0790
```

```
     1'    ST. DEV. = ', E15.8)                                          FATT0800
  122 CONTINUE                                                          FATT0810
      GO TO 5                                                           FATT0820
C
C CONDUCT T-TESTS FOR THE MEANS OF Y'S AND X BETWEEN EACH GROUP
C CONDUCT T-TEST ON THE REGRESSION COEFFICIENT (REGCOF) FOR SAME
C TO BYPASS, MOVE STATEMENT LABEL 99 TO STOP
C
   99 KT=KT-1                                                           FATT0830
      DO 133 I=1,KT                                                     FATT0840
      DO 133 J=1,KT                                                     FATT0850
      IF(J.LE.I) GO TO 133                                              FATT0860
      XTEST=TTEST(XMEAN(I),XMEAN(J),VX(I),VX(J))                        FATT0870
      DO 144 K=1,NOVAR                                                  FATT0880
      YTEST(K)=TTEST(YMEAN(K,I),YMEAN(K,J),VY(K,I),VY(K,J))             FATT0890
  144 RTEST(K)=TTEST(REGCOF(K,I),REGCOF(K,J),SEB(K,I),SEB(K,J))         FATT0900
      NF=NFRDM(I)+NFRDM(J)-2                                            FATT0910
      NFR=NF-2                                                          FATT0920
```

```
      WRITE(3,401)XTEST                                                 FATT0930
      WRITE(3,404)NF                                                    FATT0940
      WRITE(3,110)                                                      FATT0950
      WRITE(3,400)I,J                                                   FATT0960
      WRITE(3,405)NF,NFR                                                FATT0970
      DO 155 K=1,NOVAR                                                  FATT0980
      L=K                                                               FATT0990
  155 WRITE(3,403)L,YTEST(K),RTEST(K)                                   FATT1000
  133 CONTINUE                                                          FATT1010
  400 FORMAT('0','      T-VALUES FOR GROUP',I2,'     VS.      GROUP',I2)FATT1020
  401 FORMAT('1','ON MEANS OF X VARIABLE',F15.8)                        FATT1030
  403 FORMAT('0','T-VALUES FOR Y',I2,' ON MEANS =',F15.8,'    ON REG. COFATT1040
     1F. =',F15.8)                                                      FATT1050
  404 FORMAT('0','NO. OF DEGREES OF FREEDOM=',I3)                       FATT1060
  405 FORMAT('0','                ',I3,' DEGREES F.',6X,I3,' DEGREES   FATT1070
     1F.')                                                              FATT1080
      STOP                                                              FATT1090
  110 FORMAT('0',///)                                                   FATT1100
```

```
      END                                                              FATT1110
      SUBROUTINE MAXMIN( X,Y ,NOBS,NOVAR)                              FATT1120
C
C THIS SUBROUTINE CALCULATES THE RANGE OF X AND EACH
C Y (ARRAY SIZE = NOVAR) FOR NO. OF OBSERVATIONS (NOBS).
C
      DIMENSION X(100),Y(100,50),BY(50),SY(50)                         FATT1130
      SX=X(1)                                                          FATT1140
      BX=X(1)                                                          FATT1150
      DO 2 K=1,NOVAR                                                   FATT1160
      BY(K)=Y(1,K)                                                     FATT1170
    2 SY(K)=Y(1,K)                                                     FATT1180
      DO 4 J=2,NOBS                                                    FATT1190
      IF (X(J).GT.BX) BX=X(J)                                          FATT1200
      IF(X(J).LT.SX) SX=X(J)                                           FATT1210
      DO 3 K=1,NOVAR                                                   FATT1220
      IF(Y(J,K).GT.BY(K))BY(K)=Y(J,K)                                  FATT1230
      IF(Y(J,K).LT.SY(K))SY(K)=Y(J,K)                                  FATT1240
```

```
  3 CONTINUE                                                              FATT1250
  4 CONTINUE                                                              FATT1260
    WRITE(3,10) BX,SX                                                     FATT1270
    DO 6 K=1,NOVAR                                                        FATT1280
    L=K                                                                   FATT1290
  6 WRITE (3,12) L,BY(K),SY(K)                                            FATT1300
 10 FORMAT('0','RANGE OF X =', F6.2,' TO ',F6.2)                          FATT1310
 12 FORMAT(' ','RANGE OF Y',I2,' = ',F6.2,' TO ',F6.2)                    FATT1320
    RETURN                                                                FATT1330
    END                                                                   FATT1340
```

Typed by Myrtle May
Multilithed by
Margaret Pluid