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The fatty acids of coho salmon were identified and then a study was conducted to determine the effects of exercise on the fatty acids of salmon forced to swim against water velocities of 52, 54, 56, and 59 cm/sec. The shorter and lighter salmon were less competent at a given velocity than were the longer and heavier salmon. The metabolism of exercise, the unexpected selection by the exercise procedure of long and short fish, and time of holding in the laboratory were involved in the differences noted between control and exercised salmon.

Seven saturated and twenty unsaturated fatty acids were found in the lipids of the coho salmon. At all four velocities between 13 and 18 fatty acids were present in significantly smaller quantities after swimming than in control salmon. At 52 and 54 cm/sec the greater changes were in acids 16:0, 16:1, and 18:2; at 56 cm/sec in 22:6, 20:4, and 18:2; and at 59 cm/sec in 14:0, 18:2, and 18:4.

Salmon swimming for shorter distances at high velocities preferentially metabolize a higher percentage of unsaturated fatty acids.

The average swimming times for salmon swimming at velocities of 52, 54, 56, and 59 cm/sec were 1141, 645, 469, and 398 minutes respectively. The average distances traveled for salmon swimming at 52, 54, 56, and 59 cm/sec were 26.0, 16.9, 13.7, and 12.7 miles respectively. The average weight of control salmon was 5.22 grams and the average weights of swimming salmon were 4.39, 4.28, 4.81, and 4.74 grams, respectively, for water velocities of 52, 54, 56, and 59 cm/sec. The average amount of lipid for control salmon was 233 mg and the average amounts of lipids for swimming salmon were 179, 206, 245, and 223 mg respectively. The average amount of fatty acid methyl esters for control salmon was 189 mg and the average amounts of fatty acid methyl esters for swimming salmon were 149, 169, 192, and 178 respectively.

Brett (21) has provided information relating swimming velocity and oxygen consumption for sockeye salmon. The estimated average oxygen consumption for salmon swimming at 52 cm/sec was 98.4 mg and at 59 cm/sec was 53.7 mg. Since 1 mg of oxygen will burn 0.38 mg of lipid, the lipid equivalent of the total estimated oxidative costs for exercise are 37.0 mg at 52 cm/sec and 20 mg at 59 cm/sec. The observed lipid losses noted were 54 mg at 52 cm/sec and 10 mg

at 59 cm/sec and are considerably higher at 52 cm/sec than calculated values even when all oxidative losses are attributed to lipids.

At 52 cm/sec the weight loss for swimming salmon was 830 mg and at 59 cm/sec was 480 mg. Of the total loss, lipids represent 54 and 10 mg respectively. Approximately 80% or 664 and 384 mg may have been water. The protein plus carbohydrate losses were approximately 112 mg and 86 mg respectively. The lipid losses represent 502 and 93 calories, the protein plus carbohydrate losses approximately 616 and 473 calories, and the total caloric losses were 1118 and 566 calories at 52 and 59 cm/sec respectively. Similar losses calculated from the oxygen consumption data of Brett (21) on Oncorhynchus nerka give 344 and 188 calories respectively.

The caloric cost as estimated from material losses are three times as great as the losses calculated from the oxygen consumption data of Brett. However, the oxygen consumption data of Brett were collected during one, and at the most two hours at high velocity. It seems reasonable that when a maximum effort is involved, that each succeeding mile and each succeeding hour is more difficult and more costly to the salmon.

Brett (21) found a ratio for maximum active metabolism to standard metabolism of 8:1 for a 5 gram fish. If the data in this

study on material balance are supported by further bioenergetic data, for prolonged exercise the ratio of active to standard metabolism may even reach a value of 24:1 on the basis of calories lost.

Fatty Acids of Exercised and Non-Exercised Salmon

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HISTORICAL INTRODUCTION

For centuries, fish oils have been of interest and importance to man, and considerable information is available concerning fish lipids. Lipids constitute a miscellaneous group of biological compounds having in common the property of solubility in fat solvents. Fish lipids may include triglycerides, phospholipids, sterols, sterol-esters, fatty acids and carotenoids. The majority of the lipids found in fish may be related to one or more of the fatty acids. For this study, the individual fatty acids of coho salmon (Oncorhynchus kisutch Walbaum) were identified as far as possible, and the quantitative alterations in fatty acid composition of exercised fish and non-exercised fish were investigated.

Our present views concerning the occurrence and structure of fats and fatty acids were formulated during the first quarter of the nineteenth century (65). Since that time, considerable information has been obtained with respect to the identification of lipid substances. In this study, mainly those papers having a direct bearing on the identification of fatty acids in fish, on the lipid composition of fish and related organisms, and on exercise of fish will be reviewed. Background information will be presented here and additional

references will be introduced in the outline of techniques, in the presentation of the information developed, and in the discussion.

Lipids

Lipids constitute important components of the individual cell. Phospholipids function as structural materials in the cell membrane and in the membranes of organelles. Fish lipids may also be important sources of vitamins A and D. Lipids serve to lower specific gravity and thus to buoy floating animals due to their lower specific gravity. Lipids sometimes accumulate and function as important energy storage materials. Fish accumulate significant lipid reserves prior to breeding and migration. Fat is an ideal storage material because on combustion it liberates considerably more energy per unit of weight than either carbohydrate or protein. There is a decrease in lipid content during starvation.

Properties of Fatty Acids

The acids occurring in natural fats and oils are aliphatic carboxylic acids, and are called fatty acids. Naturally occurring saturated acids are invariably associated with unsaturated acids and often with hydroxy acids. The fatty acids range from volatile liquids to waxy solids. The intermediate members show all gradations

between these extremes. These acids which contain from 6 to 9 carbon atoms are oily liquids, while those with 10 or more carbon atoms are solids with wax-like properties increasing as the molecular weight increases. When one member of the series of saturated acids is present in relatively high concentrations, the next lower and the next higher homologs will also be found. This is one of the reasons why the separation of the higher acids has been so difficult and the final determination of their structures so long delayed.

Structure of Fatty Acids

All saturated straight chain acids from 3 to 30 carbons have been prepared synthetically (Ralston, 65, p. 14-18). In the nitrile method a fatty acid is reduced to the alcohol, converted into an alkyl iodide and then to the cyanide which on hydrolysis gives a fatty acid with one more carbon in the chain. Synthesis involving malonic-acid or a Grignard reagent may also be used to increase the chain length by one carbon. Since the saturated fatty acids have been synthetically prepared in a pure form their physical properties can be established and used for subsequent identification. Excellent summaries of the physical properties of the fatty acids are given by Ralston (65, p. 18-61). The purity of separation for a fatty acid is provided in the determination of its elemental composition. The

elemental composition will usually allow derivation of the number of carbons.

Pure unsaturated fatty acids can be converted to the corresponding saturated acids. The molecular weights can be determined by saponification number. Saponification number and elementary composition will usually provide the number of carbons, hydrogens, and oxygens in the molecule and the relations between numbers of carbons and numbers of hydrogens will provide the numbers of double bonds. When an ambiguity exists it can be clarified by determining the iodine number. Thus with relatively simple information it is possible to ascribe the degree of purity, the number of carbons, hydrogens, oxygens, molecular weight and the number of double bonds in fatty acids obtainable in a chemically pure form. The position of the double bonds, however, is more difficult to evaluate.

The information available to 1946 on the structure of fatty acids is summarized in the excellent text of Ralston (65) on fatty acids and their derivatives. In this study will be included only information germane to the identification of the fatty acids in salmon.

The action of strong oxidizing agents on the saturated acids has not been extensively investigated. The major oxidative reaction of the saturated acids is undoubtedly an oxidative degradation, the oxidation taking place initially at the alpha carbon atom, and yielding

large amounts of shorter-chain acids. Biological oxidative degradations apparently involve the β-carbon atom.

The unsaturated acids are reactive toward a large number of oxidizing agents, action taking place at the unsaturated linkages. Identification of the oxidative products is a recognized method for establishing the position of the double bonds. However, the reliability is open to question, because the products are dependent on the oxidizing agent used. Secondary oxidative reactions are frequently due to a shift in position of the double bond prior or during actual cleavage (65).

The varied effects of oxidation have led to many difficulties in the assignment of structures to many of the unsaturated fatty acids. Oleic acid is one of the simpler unsaturated fatty acids. Undoubtedly the evidence supports the view that natural oleic acid is composed of only one stereoisomeride. Oxidation under certain conditions is accompanied by molecular rearrangment; however, isomerization does not necessarily accompany bromination and debromination (65).

According to Ralston (65) the first rigid proof of the position of the double bond in oleic acid was developed by Baruch in 1894, who brominated oleic acid to give dibromostearic acid, and this was treated with alcoholic potassium hydroxide to give the acetylenic acid, stearolic acid. When stearolic acid was treated with

concentrated sulfuric acid it yielded a ketostearic acid which formed a ketoxime capable of existing as two sterioisomers. On hydrolysis the isomeric ketoximes yielded nonanoic acid, 9-amino nonanoic acid, octylamine and sebacic acid respectively. Thus, the acetylenic bond in stearolic acid was between the 9th and 10th carbon atoms, and consequently oleic was 9-octadecenoic acid.

In acids which contain two or more double bonds the position of those bonds is often a determinative factor for physical and chemical properties. If the ethylenic linkages form a conjugated system, compounds display chemical properties quite distinct from those displayed by unconjugated systems.

Linoleic acid is probably the most important acid with two double bonds. Animals require small amounts of linoleic acid for maintenance of proper growth. The animal body may be incapable of synthesizing linoleic acid or cannot synthesize it in the amounts demanded during growth. Linoleic acid is found in large amounts in vegetable oils and has been identified as 9, 12-octadecadienoic acid. But the great difficulty in obtaining linoleic acid free from other unsaturated fatty acids led to uncertainties concerning its structure (65).

The relationship which exists between the linoleic acid in vegetable fats and that in the marine and animal oils has not been definitely established. Although small amounts of ordinary linoleic

appear in animal fats, the presence of other octadecadienoic acids seemed probable to Ralston (65).

The oxidation of linoleic acid yields tetrahydroxystearic acid which can then undergo further oxidation with the formation of cleavage products. The cleavage products show that linoleic acid is 9, 12-octadecadienoic acid (Ralston, 65). Klenk and Bongard obtained propionic acid, hexanoic, nonanoic acid, and nonanedioic acid on oxidative ozonolysis of linoleic acid (42). Presumably the linoleic acid used by Klenk and Bongard was contaminated with linolenic and oleic acid.

Ralston indicated that acids C_{20} and C_{22} dominate the polyethenoic acids and show little resemblance to the unsaturated fatty acids of the vegetable kingdom. Divinyl-methylene and divinyl-ethylene groups characterize the polyethenoic acids of marine oils. Conjugation is apparently absent in spite of their high degree of unsaturation (65).

Fatty acid 20:4 has frequently been reported in fish and whale oils. Ozonolysis of the amyl ester indicated the acid to be 4, 8, 12, 16-eicosotetraenoic acid (65).

Identification of Fatty Acids

In recent years, the development of techniques of gas-liquid

chromatography, thin-layer chromatography, and column chromatography has permitted the separation and subsequent identification of complex mixtures of fatty acids. Roubal (68) in 1963 extracted the lipids from the musculature of the bluefin tuna, Thunnus thynnus, and analyzed the fatty acid methyl esters by gas-liquid chromatography. Roubal found 19 fatty acids ranging in chain length from 14 to 22 carbons, and the unsaturated fatty acids contained up to and including 6 double bonds. Identification of the fatty acids was accomplished by plotting the log of the retention times against the chain length or degree of unsaturation and comparing these plots with plots prepared from known standards. Further identification was obtained by comparing the chromatographic record from the fish fatty acids with chromatograms obtained from isolated reference fatty acids. Roubal did not assign carbon number positions for the double bonds in the unsaturated fatty acids.

In 1964, Ackman and Sipos (9) analyzed the fatty acids in the oil of the Pacific sardine (Sardinops sagax) by gas-liquid chromatography. They found 34 fatty acids ranging in chain lengths from 12 to 24 carbons, and the unsaturated acids contained from 1 to 6 double bonds. Identification was obtained by plotting the logarithms of retention times against the number of carbons in the chains. Additional identification was obtained by separation factors, defined

by Ackman (2) in 1963 as the quotient on dividing the retention time of one fatty acid methyl ester by the lesser retention time or relative retention time of another fatty acid of the same chain length. Ackman and Sipos assigned carbon number positions to double bonds of the unsaturated fatty acids; however, question marks indicated the uncertainty of the identification of several of the fatty acids.

Gruger, Nelson, and Stansby (34) in 1964 compared the fatty acid composition of the oils from 14 species of saltwater fish, three species of fresh water fish, and four species of shellfish. The fatty acid methyl esters were analyzed by gas-liquid chromatography. A total of 24 fatty acids were found with chain lengths from 14 to 24 carbons and double bonds from 1 to 6. Identification was made by comparing the sample chromatograms with chromatograms of methyl esters of pure fatty acids, and by plotting the logarithms of the retention times of the fatty acid methyl esters against the number of carbon atoms. These semilog plots resulted in nearly linear relations for homologous series of fatty acids. Gruger, Nelson, and Stansby did not attempt to isolate individual fatty acids or to determine the positions of double bonds.

The occurrence in natural fats of fatty acids containing an odd number of carbon atoms has been questioned by some investigators. Ralston (65) in 1948 commented that it was rather universally accepted that the fatty acids in natural fats contained an even number

of carbon atoms. In 1957, Love (49) reiterated that the fatty acids present in fish lipids were straight chained and contained an even number of carbon atoms.

Morice and Shorland (59) in 1956 isolated fatty acids containing 15 and 17 carbon atoms from the liver lipids of the New Zealand school shark (Galeorhinus australis). Isolation of the fatty acids containing 15 and 17 carbons in their chains was accomplished by high-vacuum fractional distillation and subsequent crystallization. Further identification was obtained by melting point determinations and combustion analysis.

Sen and Schlenk (70) in 1964 found that in the common mullet (Mugil cephalus) more than 25% of the total fatty acids were of the odd numbered type. Both saturated and unsaturated fatty acids containing 15, 17, and 19 carbon atoms in their chains were identified in mullet oil. Identification was accomplished by distillation, liquid-liquid chromatography, and gas-liquid chromatography. Ozonization and hydrogenation procedures were employed for identification of the unsaturated fatty acids.

To study the lipids of some marine organisms, Ackman and Sipos (10) in 1965 isolated the saturated fatty acids by silver nitrate silicic acid column chromatography. Especial attention was focused on the odd-numbered carbon fatty acids present in diatoms, squid,

cod, herring, seal, and finback whale. Fatty acids containing 13, 15, 17, and 19 carbon atoms were found. Identification of the fatty acids was by gas-liquid chromatography, semilog plots, and separation factors (2).

Procedures utilizing gas-liquid chromatography have permitted the separation of fatty acids from complex mixtures; however, establishment of the number of double bonds and the localization of position of the double bonds in the molecules of the unsaturated fatty acids have been difficult. Ozonolysis of the unsaturated fatty acids has proven to be a useful method for the determination of the positions of double bonds. Oxidative and reductive methods have been used to cleave ozonides of unsaturated fatty acids.

Klenk and Bongard (43) in 1953 used oxidative ozonolysis to determine the structure of unsaturated fatty acids of blue skate and cod liver oil. If the double bonds were interrupted by methylene groups, malonic acid would be the product formed from oxidative ozonolysis; and if the double bonds were ethylene interrupted, succinic acid would be formed.

Klenk and Bongard (43) emphasize that no succinic acid was recovered on oxidative ozonolysis of methyl esters of cod liver oil and only very small amounts on the oxidative ozonolysis of skate liver oil. The ratio of malonic to succinic acid recovered was given as

160:1. They felt that the small amounts of succinic acid formed were due to hydrolysis of succinic acid methyl ester, and interpreted their data as indicating that the fatty acids of both cod and skate liver oils were methylene interrupted. In the protocol for skate liver oil, however, the recoveries listed were 3.99 grams of malonic acid and 0.5 grams of succinic acid or a ration of 8:1 instead of 160:1.

In 1957, Klenk and Eberhagen (45) isolated eicosapentaeneoic acid from cod liver oil by vacuum distillation and counter-current distribution. They reported that ozonolysis of the isolated fatty acid and subsequent identification of the fragments indicated that the double bonds were methylene interrupted.

Their tables indicated that malonic and succinic acids were recovered in a molar ration of 55:1. No malonic acid was recovered on ozonolysis of cod liver oil (43), but succinic acid was obtained to the extent of two per cent on ozonolysis of the eicosapentaenoic acid obtained from cod liver oil. Thus, their methods of separation led to the formation of ethylene interrupted double bonds.

Klenk and Brockerhoff (44) in 1957 isolated an octadecatetraenoic acid from herring oil and on the basis of malonic acid recovered, found the double bonds to be methylene interrupted. Recovery of malonic acid ranged from 50-80% of theory.

Stein and Nicolaides (75) in 1962 introduced reductive ozonolysis as a simpler method because the aldehyde products do not require subsequent modification before gas chromatographic analysis. They further stated that procedures of reductive ozonolysis using triphenylphosphine to reduce the ozonide gave no indication that by-products were formed by alternative oxidative pathways and that impurities detectable by gas chromatography were less than one percent. Stein and Nicolaides analyzed both naturally occurring unsaturated fatty acids and synthetically prepared octadecenoic acids. Malondialdehyde was the product formed by reductive ozonolysis of polyunsaturated fatty acids containing double-bonded carbons separated by a methylene group.

Privett and Nickell (64) in 1962 emphasized the potential of reductive ozonolysis by recovery of fragments obtained from Hormel standards. Oleic acid was $\omega 9\ \Delta 9$, linoleic acid $\omega 6\ \Delta 9$, linolenic $\omega 3\ \Delta 9$ and arachidonic $\omega 6\ \Delta 5$. Malonaldehyde was identified by gas-liquid chromatography of the products from the reductive ozonolysis of methyl linoleate. A significant feature of reductive ozonolysis was the complete analysis of all fragments obtained because both the ozonization and reduction were uncomplicated by side reactions. Gas-liquid chromatograms of the fragments obtained from linoleate, linolenate, and arachidonate all contained malonaldehyde, indicating

that the double bonds were methylene interrupted (see discussion).

Complex lipid mixtures may contain unsaturated fatty acids of varied chain lengths with different geometries and degrees of unsaturation. Privett et al. (63) in 1965 described the use of silver nitrate impregnated silicic acid thin-layer plates for the fractionation of fatty acid methyl esters on the basis of geometry and degree of unsaturation. Ozonolysis and gas chromatographic analysis of the fractions from the thin-layer plates provide additional information concerning the number and positions of the double bonds.

Physiological Alterations of Fatty Acids

Of importance to this study on fatty acids are papers concerning the influence that diet, temperature, and other environmental conditions may possibly have on the fatty acid composition of fish.

Diet

Reiser et al. (57) in 1963 studied the influence of diet on both marine and fresh water fish. The fish were depleted of tissue fatty acids to various degrees and subsequently presented with linoleic and linolenic acid at different dietary levels. The ingestion of linoleic and linolenic acids either in the form of isolated acids or as cottonseed or linseed oil did not induce the return of the more

highly polyunsaturated acids. It was especially significant that the most typical marine polyunsaturated acid, 22:6, did not return. Reiser and his co-workers concluded that neither linoleic nor linolenic acid was the precursor of 22:6 in marine fish. At low levels of linoleic and linolenic acids in the tissues, there was a significant, but slight conversion to the longer chain acids at low environmental temperatures. Examination of the tissue fatty acids suggested that marine and fresh water fish did not differ between themselves or from other classes of animals in basic mechanisms of deposition and interconversions of dietary fatty acids.

In 1963, Brenner, Vazza, and DeTomás (17) fed a fresh water fish (Pimelodus maculatus) on a semisynthetic fat-deficient diet and another fresh water fish (Parapimelodus valenciennesi) first a fat-deficient diet and then a diet containing methyl palmitate, methyl oleate, and methyl linoleate. Percentages of linoleic acid and arachidonic acid decreased and palmitoleic acid, oleic acid, and eicosatrienoic acid increased in Pimelodus maculatus when fed a fat-deficient diet. The duration of the experiment was 15 months with sampling at 7 months and 15 months.

In Parapimelodus valenciennesi, the fat-deficient diet was offered for 55 days and then the fish were fed a diet supplemented with methyl palmitate, methyl oleate, and methyl linoleate for 15 days.

Palmitic and oleic acids were stored primarily in the glyceride fraction while linoleic acid was stored in both the glycerides and phospholipids. Some linoleic acid appeared to have been transformed into arachidonic acid. Under the conditions of their experiment, the concentration of palmitic acid seemed to be very constant in the fish. Brenner, Vazza, and DeTomás suggested that the metabolic paths of palmitic, oleic, and linoleic acids probably do not differ substantially between fish and mammals, but that the degree and rates of anabolism and catabolism may be different.

Lasker and Theilacker (48) in 1962 compared the fatty acids of Pacific sardines (Sardinops caerulea) fed a commercial trout diet rich in oleic and linoleic acids with those of sardines fed a natural crustacean diet. Comparing the fatty acids present in the mesenteric fat of sardines, Lasker and Theilacker found that the percentage of 18:1 was 27%, 22:6 was 1% and 18:2 was 9% in fish fed the trout diet, while the percentage of 18:1 was 25%, 22:6 was 0.8%, and 18:2 was 26% in the trout food. Fish fed the crustacean diet had 30% of 18:1, 7% of 22:6, and 1.3% of 18:2, while the diet contained 7% of 18:1, 15% of 22:6, and 1% of 18:2. The individual percentages of 18:1, 18:2, and 22:6 present in the fish fed the two diets were substantially different. The percentage of individual fatty acids present in the mesenteric fat of the Pacific sardines were influenced

by the commercial trout diet and the natural crustacean diet.

Temperature

Reiser et al. (57) depleted marine and fresh water fish of tissue fatty acids and subsequently presented the fish with linoleic and linolenic acid at different dietary levels. At low levels of linoleic and linolenic acids in the tissues, there was a significant, but slight conversion to the longer chain acids at low environmental temperatures.

In 1966 Knipprath and Mead (47) studied the influence of temperature on the fatty acids of the mosquito fish (Gambusia affinis) fed a diet of brine shrimp, and the guppy (Lebistes reticulatus) fed a diet of commercial trout chow. The two species of fish were maintained in separate aquaria at 14-15 C and 26-27 C. At the lower temperature, there was an increase in the relative percent of longer chain polyunsaturated fatty acids in both species of fish. Individual differences existed between the two species of fish in their fatty acid distributions; however, some of the differences observed were partly due to the diets used during the experiment.

Biogenesis

Some studies have been devoted to the origin and biogenesis of fatty acids in aquatic organisms. Information obtained by using radio-tracer methods and the study of food-chain organisms in aquatic eco-systems will be discussed next.

Farkas and Herodek (31) in 1964 studied selected species of algae, cladocerans, copepods and fish with respect to their fatty acid composition. On the basis of controlled feeding experiments, Farkas and Herodek suggested that algae produce large amounts of C_{16} and C_{18} polyenes. The crustaceans, mainly the copepods, elongate these C_{16} and C_{18} polyenes to C_{20} and C_{22} fatty acids, and fish readily take up the largest part of their C_{20} and C_{22} fatty acids from the crustaceans and store them in their tissues.

Ackman et al. (7) in 1964 studied the fatty acid composition of the diatom Skeletonenma costatum. They identified 27 fatty acids that contained 14 to 22 carbons and from one to six double bonds. Results of their study after 10 days of growth indicated that 80% of the total fatty acids contained 18 carbons or less.

In 1958 Kelly, Reiser, and Hood (41) studied the effect of diet on the mullet Mugil cephalus. They fed a synthetic fat-free diet containing carbohydrate, protein and all necessary known dietary supplements, but devoid of fats and fatty acids. Tissue levels of

polyunsaturated fatty acids decreased markedly when mullet were fed the fat-free diet. Mullet apparently were not able to synthesize the large amounts of polyunsaturated fatty acids normally found in their tissues.

Mead, Kayama, and Reiser (55) in 1960 studied the biogenesis of polyunsaturated fatty acids in female Tilapia mossambica. Six mature fish were maintained on Purina Trout Chow for five days and then each was given an intraperitoneal injection of sodium acetate-1-C^{14} for a total of 0.5 microcuries. After six hours the fish were sacrificed and their lipids were extracted. On the basis that each received 0.5 microcuries, out of 110,000,000 d.p.s. administered, 2,300,000 were recovered in the fatty acids.

The d.p.s. per mg in the total fatty acids were 600. When a sample was methylated and separated on a gas chromatographic column, methyl palmitate with an activity of 261 d.p.s. per mg, methyl linoleate with an activity of 33 d.p.s. per mg, and methyl arachidonate with an activity of 60 d.p.s. per mg were obtained. Analysis of the arachidonate indicated that 71% of the activity was in the alpha carbon, 6% in the beta carbon and 23% in the remaining 18 carbon chain.

The tracer studies indicated that the fish rapidly synthesized large amounts of fatty acids, mainly saturates; however, some

unsaturated acids were also produced. Mead, Kayama, and Reiser quote a personal communication from Klenk that some tracer activity was found in the malonic acid fraction following ozonolytic degradation of the polyunsaturated acids.

In 1963 Kayama et al. (40) studied the incorporation of linolenic-1-C^{14} acid into the eicosapentaenoic and docosahexaenoic acids in the kelp bass (Paralablax clathratus). The polyunsaturated fatty acids were concentrated by low temperature crystallization and the eicosapentaenoic and docosahexaenoic acids were isolated from the concentrate by reversed-phase chromatography, and hydrogenated. The resulting arachidic and behenic acids were degraded stepwise to margaric acid, and the distribution of activity was determined. Their results indicated that linolenic acid was incorporated, at least partially undegraded into the molecules of eicosapentaenoic and docosahexaenoic acids. They further suggested that linolenic acid may undergo partial degradation and resynthesis before elongation.

Exercise

In recent years, the exercise of fish under controlled conditions has yielded correlations between the magnitude of activity and quantitative biochemical changes. In 1955 Black (12) compared the lactic acid in two-year-old kamloops trout (Salmo gairdneri

kamloops) before and after exercise. Black exercised the trout for 15 minutes by continuously chasing the fish in a standard hatchery trough. In unexercised trout the average blood levels of lactic acid were 8.0 ± 0.95 mg percent. When the trout were exercised by continuous chasing for 15 minutes the average blood levels of lactic acid had increased to 82 ± 10 mg percent. Significant increases in the blood levels of lactic acid were also found in other species of fish following exercise.

Black (13) in 1958 reviewed the information concerning hyperactivity as a lethal factor in the death of fish. Black concluded that deaths sometimes occurred in fish following severe activity. The precise cause of death could not be determined.

Katz, Pritchard, and Warren (39) in 1959 studied the ability of juvenile coho salmon to swim against known water velocities in water where the oxygen content was reduced. The young salmon were forced to swim against a current of water in an apparatus where temperature, oxygen concentration, and velocity could be controlled. Juvenile coho salmon between 95 mm and 124 mm were able to swim for two days against a water velocity of 0.8 ft/sec (24 cm/sec) with a mean dissolved oxygen concentration of 2.96 mg/l. When the oxygen concentration was between 2.03-2.72 mg/l, some of the fish were unable to swim against the velocity of 0.8 ft/sec (24 cm/sec) for two days.

Heath and Pritchard (35) in 1962 followed the changes in metabolic rate of the bluegill (Lepomis macrochirus) after severe muscular activity. The fish were forced to swim against a fast stream of flowing water. The interval of swimming activity required to fatigue individual fish ranged from 5 to 45 minutes. The maximum post-exercise metabolic rate was 166% of the pre-exercise rate and this was reached within one hour following exercise. Measurements taken after 24 hours indicated that the metabolic rate of bluegill sunfish returns to the pre-exercise level sometime between 10 and 25 hours.

To study the time over which maximum velocities could be maintained, Smith (72) determined swimming times until failure of coho salmon that were suddenly forced to swim at selected water velocities at 18 C. The mean swimming times of salmon of nearly uniform size (80 mm to 85 mm) were determined and found to increase from two minutes at 82 cm/sec to 30 minutes at 55 cm/sec as the water velocity was reduced. Fish that were able to maintain velocities of 58 cm/sec (or less) for 30 minutes usually were able to continue swimming for much longer periods of time up to 24 hours or more. Thus velocities between 52 cm/sec and 59 cm/sec covered a range where a large proportion of the fish were able to swim for 24 hours or more but some fish were not able to swim for as long

as 15 minutes. Hence, many fish would be able to swim between 15 minutes and 24 hours before failing.

When swimming velocities were increased gradually, the fish appeared to be able to maintain considerably higher velocities for longer periods than those that could be maintained after a sudden increase in velocity (72).

In 1964 Brett (20) studied the respiratory metabolism and swimming performance of young sockeye salmon (Oncorhynchus nerka). The swimming performance apparatus was designed as a recirculating water tunnel with a maximum velocity of 3.7 ft/sec (113 cm/sec). To test the swimming performance of the salmon, a system of applying successive 75-minute increments of velocity was used. This enabled salmon to maintain a final swimming velocity for some period of time. The relationship between respiratory metabolism and swimming velocity in young sockeye salmon was described by the general equation $Y = ae^{bX}$, where Y equals the rate of oxygen consumption ($mgO_2/kg/hr$) and X equals the velocity (lengths/sec), a equals the metabolism when the velocity is zero, and b equals the change in the log of the metabolism per unit increase in velocity. The relationship held for acclimation temperatures from 5 C to 24 C and indicated that when velocities were increased, oxygen consumption increased exponentially.

Brett (21) in 1965 extended his studies and investigated the relation of size to rate of oxygen consumption and sustained swimming velocity of sockeye salmon (Oncorhynchus nerka). The sockeye salmon were exercised in a mechanical apparatus where the flow of water could be controlled.

The smallest fish tested weighed 3.38 g and had standard and active metabolic rates of 230 and 920 mg O_2kg/hr whereas the largest tested weighed 1432 g and had standard and active metabolic rates of 44 and 717 mg O_2/kg/hr respectively at 15 C. The maximum sustained 60-minute swimming velocity for the smallest fish was 1.7 ft/sec (52 cm/sec) and for the largest fish was 4.7 ft/sec (143 cm/sec). When converted to lengths/sec, these velocities were 6.65 for the smallest and 2.65 for the largest.

MATERIALS AND METHODS

The experimental animals used in this study were silver salmon (Oncorhynchus kisutch Walbaum). They were taken by seine from the Yaquina River near the town of Nashville, Oregon. Approximately 100-150 fish ranging in total length from 7.5 cm to 8.5 cm were obtained each month between June 1964 and September 1964.

The captive fish were held at the Oak Creek Water Pollution Laboratory of Oregon State University and the United States Public Health Service in large aquaria supplied with oxygenated running water at 18 C. During the time the fish were retained in the laboratory, they were fed exclusively on small aquatic worms of the genus Tubifex. Fish to be used for exercise experiments were not fed during the 24 hours prior to placement in the exercising apparatus.

Food for Experimental Animals

Small aquatic worms of the genus Tubifex were collected periodically from the fish rearing ponds at the Oregon State Game Commission's Roaring River Trout Hatchery, near Scio, Oregon. The worms were held outdoors at the Oregon State University Oak Creek Laboratory in wooden troughs containing gravel and supplied with flowing water from a nearby stream. Dry fish food was

distributed in the troughs periodically to provide the tubificids with food. The tubificids could be held for months under these conditions. The tubificids, before being fed to the fish, were cleaned with running water until they were free from silt and other detritus. The partially cleaned worms were placed on a 3 mm mesh screen over a pan of water with a light source directly above the screen. The worms passed through the screen and into the water in a few minutes, leaving behind most of the detritus. The relatively clean worms could then be collected and fed to the fish.

Solvents and Absorbents

Chloroform, methanol, hexane, and dichloromethane were re-distilled from drum stock. Ether was not re-distilled. Anhydrous ether was used for thin-layer and column chromatography.

Mallinckrodt silicic acid mesh size 100-200 was used for column chromatography and Mallinckrodt silicic acid mesh size 200 and finer was used for thin-layer chromatography. The silicic acid was washed with ether to remove lipid material.

Methanol-HCl for Methylation

A half gram of Iodine, 5.0 grams of Magnesium, and 75 ml of reagent grade anhydrous methyl alcohol were refluxed in a two-liter

flask until the magnesium ceased to react. Anhydrous methyl alcohol, 900 ml, was then added and refluxing was renewed for 30 minutes. Distillation of the anhydrous methanol was accomplished using care to avoid adsorption of water.

Thirty grams of oven dried reagent grade NaCl was placed in the 500 ml bottle of a gas generator (Figure 1). Eighty ml of concentrated H_2SO_4 was added slowly through the delivery tube of the gas generator to permit a regulated evolution of HCl gas into 100 ml of prepared methanol. This process gave anhydrous methanol with an HCl content of 3.8% - 4.6%.

Fatty Acid Standards

Fatty acid methyl esters were purchased from the fatty acid project of the Hormel Institute, University of Minnesota, Austin, Minnesota. A personal letter from John D. Nadenicek of the Hormel Institute stated that the general method of preparation was to employ such physical techniques as low temperature crystallization, urea adduct formation, column chromatography and, finally, high vacuum fractionation by distillation. The final product was analyzed both by gas-liquid chromatography and thin-layer chromatography to observe any trace components present. When structure determination of unsaturated compounds was necessary, it was accomplished

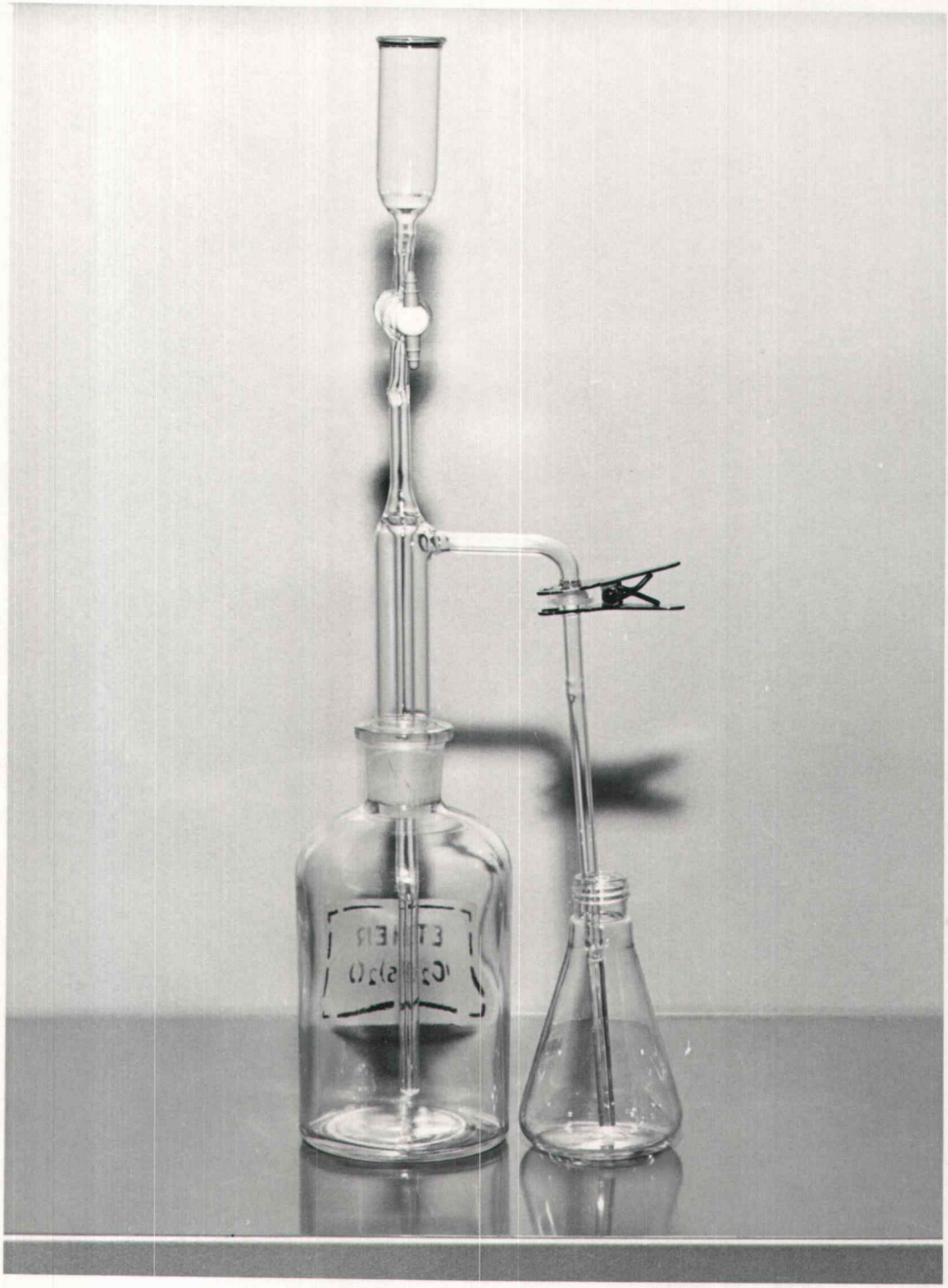

Figure 1. Apparatus for preparation of methanol-HCl with HCl gas and anhydrous methanol.

with the aid of infra-red spectrophotometry and ozonolysis.

Swimming Performance Apparatus

Fish were tested for their swimming ability in an apparatus described by Dahlberg (28). The test chamber consisted of a Pyrex glass tube, 60 inches long and 4 inches in diameter. Water was recirculated through the tube by a centrifugal pump, and the velocity was regulated by a gate valve. Water temperature was controlled by a system containing heating and refrigerating units. An exchange flow of about one liter per minute of aerated, 18 C water was maintained. The average dissolved oxygen content was 9.60 ppm with a range from 9.45 to 9.84 ppm.

Gas Chromatography

Methyl esters of individual fatty acids and of mixtures of fatty acids were analyzed with a Beckman GC-2 chromatograph equipped with a hydrogen flame detector. Hydrogen was added to the effluent from the column of the chromatograph, the mixture was burned in air, and the resulting ions were collected by a small electrode above the flame. Detection was based on the measurement of the ion current, and the ion current was a function of the number of carbon atoms. The ion current was then amplified and its

magnitude indicated on the recorder.

The aluminum column used was 1/8 inch by 6 foot, packed with 15% ethylene glycol succinate on chromosorb "P" mesh size 35-80. The operating conditions of the chromatograph were as follows: oven temperature 190 C; helium flow rate 60 ml/minute; sample size 0.2 mg/10 microliters and solid sample injection (Lowry, 51). The ten microliters were injected into a solid sample spoon with a Hamilton micro syringe, the solvent was evaporated and the residue injected was placed against the end of the column distal to the hydrogen flame. Inorganic compounds such as hydrogen, nitrogen, carbon dioxide and water were not ionized at the flame temperature and thus were not detected. Hydrogen was used as a fuel because of the low ion density of its flame.

The separation and quantification of fatty acid methyl esters was repeatedly checked with mixtures of fatty acid methyl esters of known content. Variability of chromatograms increased with the age of columns. When the separation of the mixtures of known fatty acid methyl esters failed to reproduce the expected retention times, the source of error was checked. A frequent source of error was change in the column with age. Unsatisfactory columns were replaced.

Swimming Performance

Between June 1964 and September 1964, 16 separate swimming performance tests were conducted using 10 fish per test. Two fish were used as controls and eight were introduced into the test chamber of the swimming performance apparatus. The fish were not weighed or measured prior to their introduction into the exercising apparatus. All unnecessary handling of the fish was avoided to decrease the excitement of the salmon as much as possible.

Fish were selected with lengths between 75 mm and 85 mm. The two control fish were placed in water containing ice to retard their activity; later they were measured, weighed and placed in a freezer. Fat extractions of the frozen fish were made the same day that the fish were sacrificed, and usually within 1-2 hours after freezing.

The remaining eight fish were placed in the test chamber of the swimming performance apparatus (Figure 2). The water velocity was adjusted to 8 cm/sec and the fish were allowed to remain in the test chamber for 20 hours. After this initial 20-hour period, the velocity was increased to 15 cm/sec for 1 hour, then immediately following, the velocity was increased to the desired test velocity. Test velocities used were: 52 cm/sec, 54 cm/sec, 56 cm/sec, and 59 cm/sec. Fish were allowed to swim against the test velocities

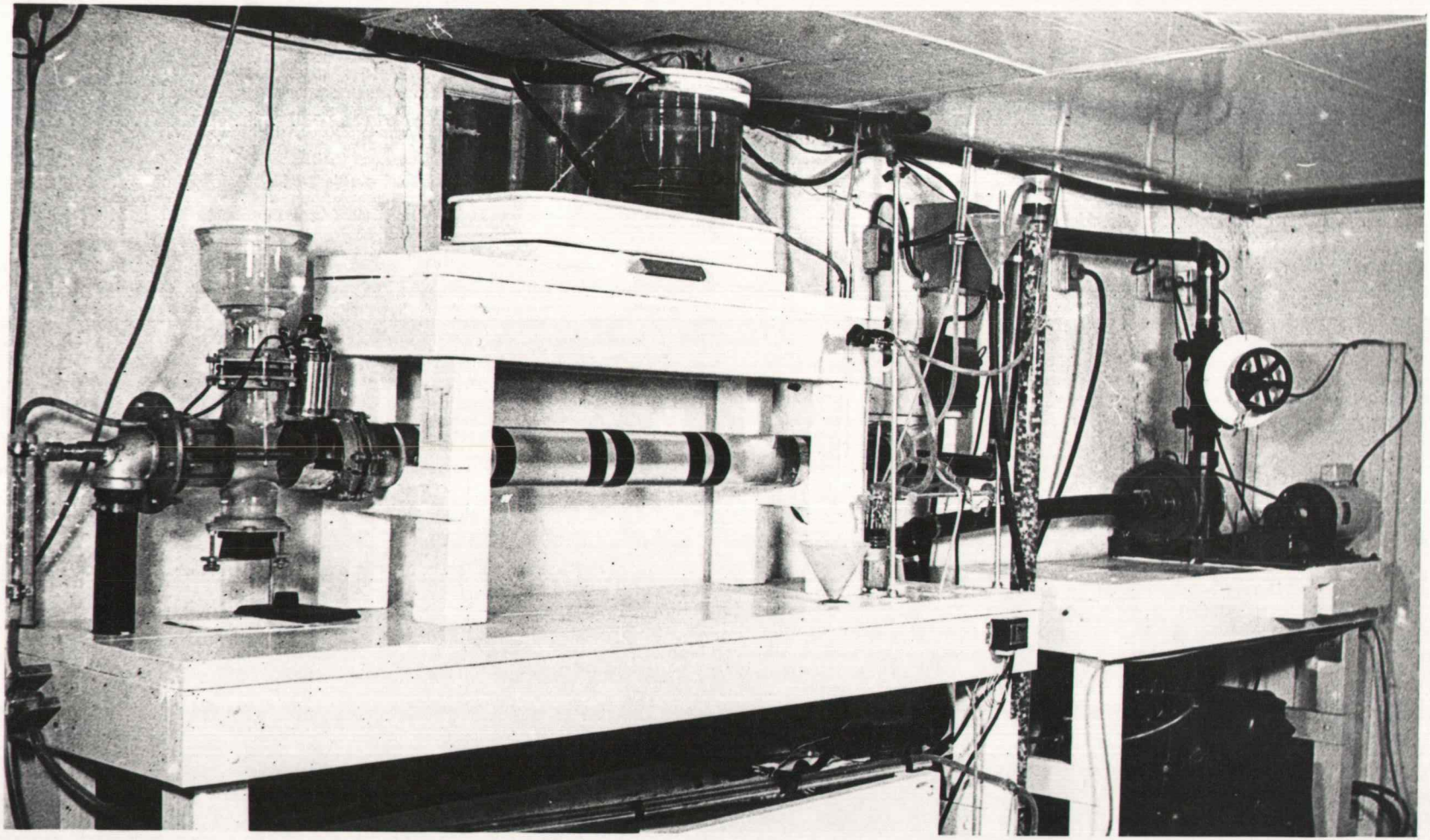

Figure 2. Swimming performance apparatus used for exercise experiments.

for a maximum of 24 hours.

In all water flows used, some fish were capable of swimming for the 24-hour period; however, in each of the water flows used, some fish were fatigued in less than one hour.

When the fish failed to swim against the flow of water or at the end of 24 hours, they were removed from the swimming chamber, placed in an ice water bath, measured, weighed, and placed immediately in a freezer. The elapsed time between removal from the swimming apparatus and freezing was normally two minutes or less.

Extraction of Lipids

The lipids were extracted utilizing the procedure of Bligh and Dyer (15). A frozen fish was placed in a stainless steel omni- mixer cup with 52 ml of chloroform and 26 ml of methanol, and homogenized for two minutes in a water bath at room temperature. The homogenate was passed through sharkskin filter paper into a 250 ml separatory funnel fitted with a teflon stopcock. To the filtrate was added 16 ml of redistilled water. The contents of the separatory funnel were shaken briskly, but not enough to form an emulsion. The contents of the separatory funnel were then allowed to separate for four hours at room temperature. During this time an upper layer, containing water-methanol, and a lower layer, containing the

lipids in chloroform, separated.

The lower chloroform layer was drained into a coarse porosity sintered glass funnel containing chloroform-washed anhydrous sodium sulfate. The chloroform-lipid mixture was placed in a screw-cap bottle with a polyethylene seal. The volume of the chloroform-lipid mixture was recorded to the nearest tenth of a milliliter. The bottles containing the chloroform-lipid mixture were held at -15 C until nitrogen evaporation of the chloroform could be initiated.

Lipid Determination

A five milliliter aliquot of the chloroform-lipid mixture was pipetted into a small aluminum pan that had been dried in a desicator over phosphoric acid anhydride, weighed and re-dried. The aliquot in the aluminum pans was evaporated at 50 C, the pans were dried again in the desicator and re-weighed. The lipid was calculated by:

$$\text{lipid content} = \frac{\text{chloroform volume}}{5 \text{ ml}} \times \text{weight of pan residue}$$

Methylation of the Fatty Acids

The remaining chloroform-lipid mixture was evaporated in a water bath at 50 C using a constant nitrogen gas flow through tapered glass tubing. The lipid residue in the bottle was treated with 4 ml of hexane and the bottle was rotated to insure dissolving of the lipid

in hexane. To the hexane-lipid mixture was added 4 ml of methanol-HCl (3.8 - 4.6% HCl). The closed tubes were then placed in a water bath at 80 C for three hours for methylation of the fatty acids (76).

The tubes were taken from the water bath, cooled, and the hexane solution removed with a pipette. The contents of the tubes were extracted three times using 2 ml portions of hexane. The hexane solutions of the fatty acid methyl esters were then stored at 0 C. The residue of the methylation process was discarded.

Following gas-liquid chromatographic analysis of the fatty acid methyl esters, the remaining hexane solutions of the fatty acid methyl esters were weighed to determine the total amount of esters present.

Silicic Acid Columns for Removal of Non-Ester Material

The fatty acid methyl esters were further purified using silicic acid columns. An apparatus was designed to accommodate 6 columns with valves to adjust air pressure individually. Mallinckrodt silicic acid mesh size 100-200 was placed in the columns (Figure 3) to a depth of approximately 5 cm. The silicic acid was washed once with 20 ml of 3% ethyl ether in hexane. Then, 4 ml of the fatty acid methyl ester mixture was placed on the column and eluted with two 20 ml portions of 3% ethyl ether in hexane with an air pressure of 3 pounds per square inch. The ethyl ether-hexane liquid eluate was

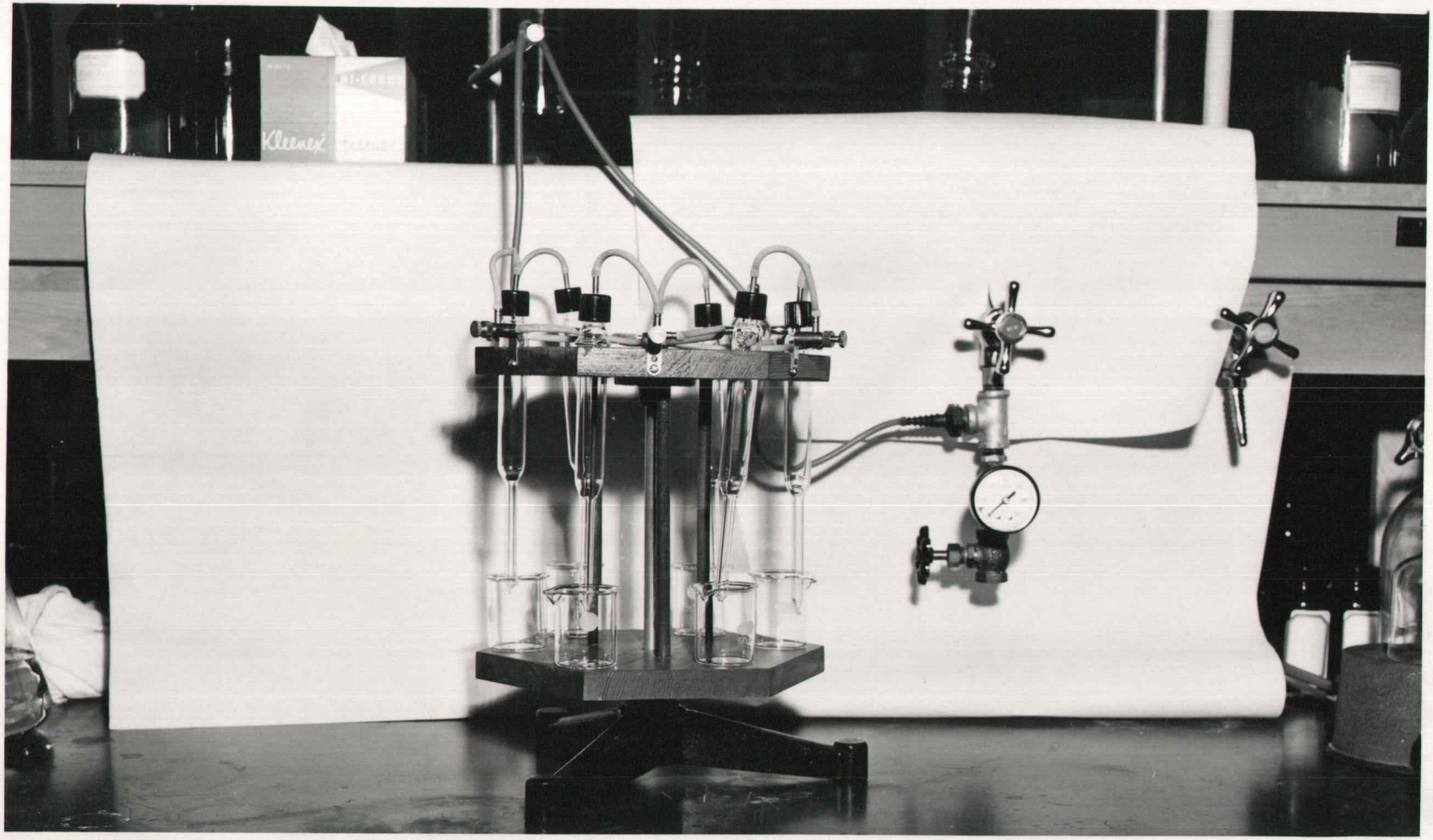

Figure 3. Column chromatography utilizing individual flow control.

evaporated in a water bath at 50 C using nitrogen gas. The residue of purified fatty acid methyl esters was redissolved in 4 ml of hexane (73).

Separation of the Fatty Acid Methyl Esters by Thin-Layer Chromatography

Plates for separation of the fatty acid methyl esters were made using 6.25 grams of silver nitrate, 25 grams of Mallinckrodt silicic acid mesh size 200 and finer, and 50 ml of water. These ingredients were sufficient for the preparation of 5 eight-inch square plates. The plates were activated in an oven at 100 C for one hour and used the same day (63).

The samples of fatty acid methyl esters were applied to the plates with a spotter developed by Robert Lowry of the Agricultural Chemistry Department of Oregon State University. The plates were covered with a plexiglass lid which permitted the maintenance of an atmosphere of nitrogen gas (Figure 4). The hexane solution of the mixture of fatty acid methyl esters was held in a micropipette and delivery was controlled by a thumb screw. The pipette was held perpendicular to the plane of the plate, and the plate was propelled slowly by a motor driven screw aligned parallel to the spotting edge.

This device allowed an even spotting of the sample while at the same time utilizing nitrogen gas to prevent oxidation of the

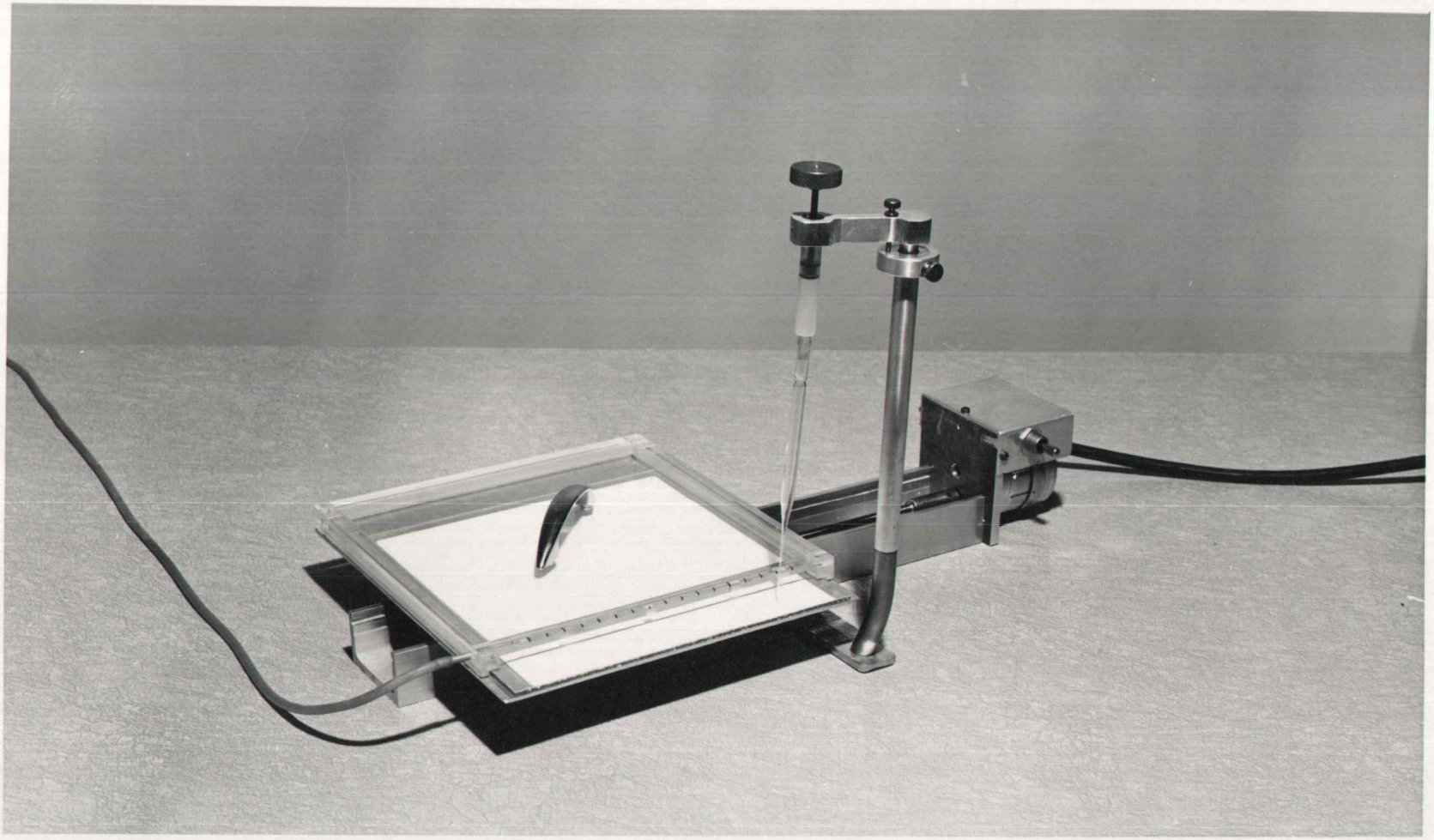

Figure 4. Motor driven micro-spotter for thin-layer chromatographic plates maintained under an atmosphere of nitrogen gas.

unsaturated fatty acids. The plates were developed with 20% ethyl ether in hexane for 25 minutes. Detection of the separated masses was accomplished by lightly spraying with 2,7 dichlorofluorescein. The sprayed plates were viewed under ultraviolet light. The fatty acid methyl esters formed bands according to their degree of unsaturation, saturated fatty acid methyl esters being the greatest distance from the spotting edge and fatty acids with 6 double bonds nearest the spotting edge (63).

The bands from the thin-layer plates were removed by outlining the border and carefully scraping off the band into a small funnel and re-extracting the mixture with chloroform. A higher degree of separation was obtained by re-spotting the mixture removed from the bands of a thin-layer plate onto another thin-layer plate. Gas chromatographic analysis gave evidence concerning the degree of separation achieved by the thin-layer plates and the composition of each band.

Hydrogenation of the Fatty Acid Methyl Esters

The unsaturated fractions removed from the thin-layer plates were subsequently hydrogenated by the method of Farquhar (32). The hydrogenation apparatus consisted of a pyrex flask, a hydrogen source and a water reservoir (Figure 5). A sample of 15 mg of a

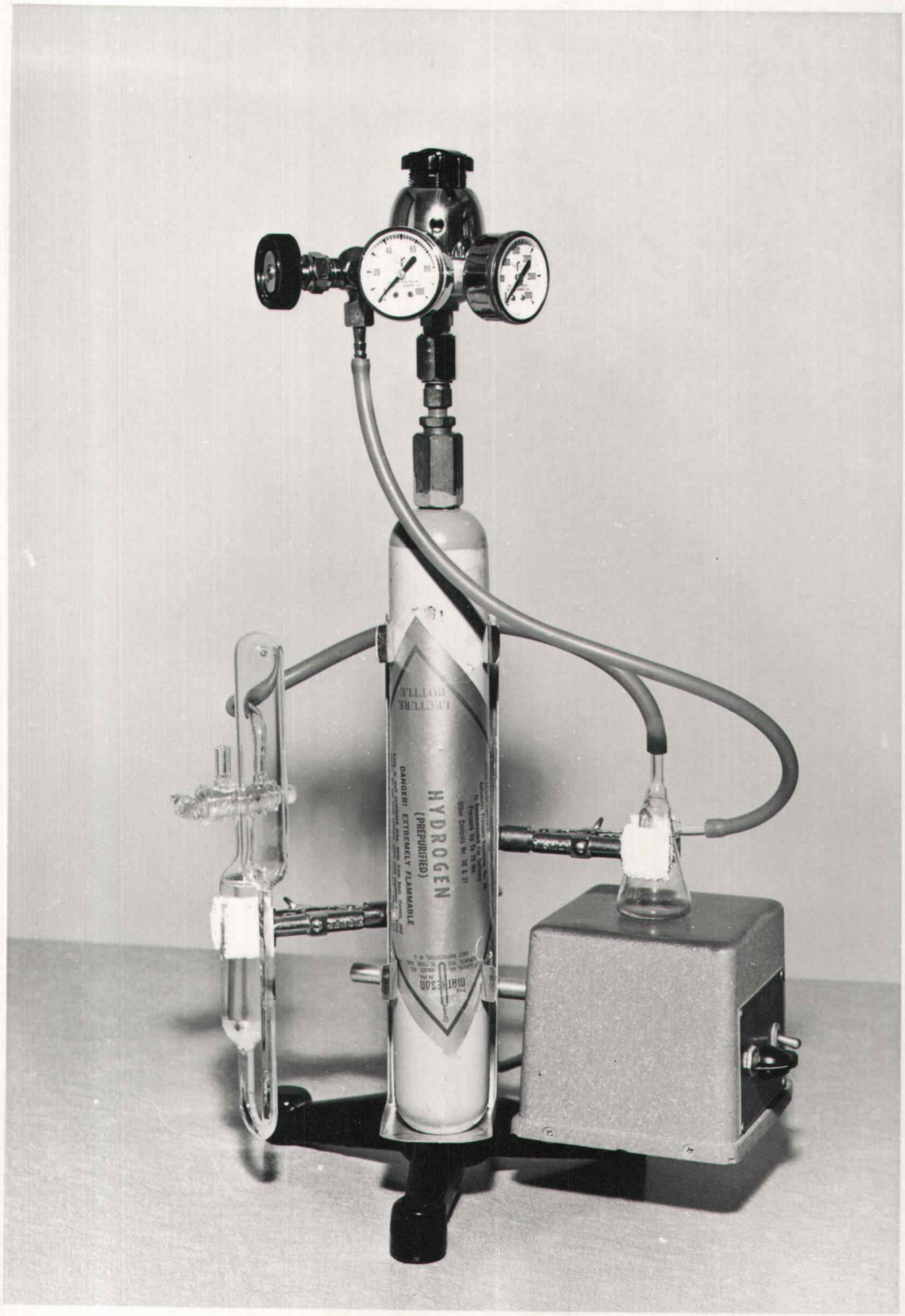

Figure 5. Micro-hydrogenator for the hydrogenation of unsaturated fatty acid methyl esters.

fatty acid methyl ester mixture in hexane was placed in the pyrex flask containing an outlet for the attachment to the hydrogen source and a second outlet attached to the water reservoir. The hydrogenation was accelerated with 20 mg of Adams platinum oxide catalyst. The contents of the flask were flushed five times with hydrogen gas, after which the sample was maintained in a hydrogen environment for 30 minutes.

After hydrogenation, the sample was filtered to remove the catalyst. The hydrogenated samples were injected into the gas chromatograph for identification of the fatty acids present after hydrogenation. Hydrogenation converted the unsaturated acids to saturated acids and thus the chain length of the unsaturated acids associated with given peaks on the chromatograms could be confirmed. Hydrogenation of the salmon fatty acid methyl ester mixture reduced the number of peaks and the peaks remaining were those of the straight chain series of fatty acids.

Ozonolysis of the Unsaturated Fatty Acid Methyl Esters

Location of the ethylenic groups of the unsaturated fatty acid methyl esters were investigated by reductive ozonolysis. An apparatus modified after Bonner (16) was used for the generation of ozone and for ozonolysis. Ten mg of a fatty acid methyl ester

mixture were placed in 2 ml of dichloromethane in a test tube. A disposable pipette was added and the tube was placed in a dry ice-acetone bath at -65 C. Oxygen was bubbled through the solution to expel air before ozone generation. Ozonolysis required approximately 3 minutes and then the odor of ozone could be detected at the mouth of the test tube (Figure 6). After ozonolysis was complete, nitrogen was bubbled through the solution to remove the excess ozone. Excess ozone may be detected by blue coloration in the solution or its presence can be made more vivid by use of Sudan III.

The ozonide formed from each unsaturated fatty acid methyl ester on triphenylphosphine reduction yielded an aldehyde and an aldehyde-ester (Figure 7). The mixture resulting from reductive ozonolysis was placed on silicic acid columns, the aldehydes were eluted using dichloromethane, and the aldehyde-esters were eluted using ethyl formate. The aldehyde and aldehyde-ester portions were subjected to gas chromatographic analysis at oven temperatures of 100 C and 160 C respectively.

Identification of the Fatty Acids

Comparison of the retention times of the fatty acid methyl esters obtained from the fish lipids with the retention times of fatty acid methyl esters of known purity from the Hormel Institute aided

Figure 6. Apparatus for the generation of ozone and the ozonolysis of unsaturated fatty acid methyl esters. Shown are a rheostat, transformer, Bertholet tube, oxygen tank, and a low temperature bath.

Ozonolysis of Methyl Linoleate

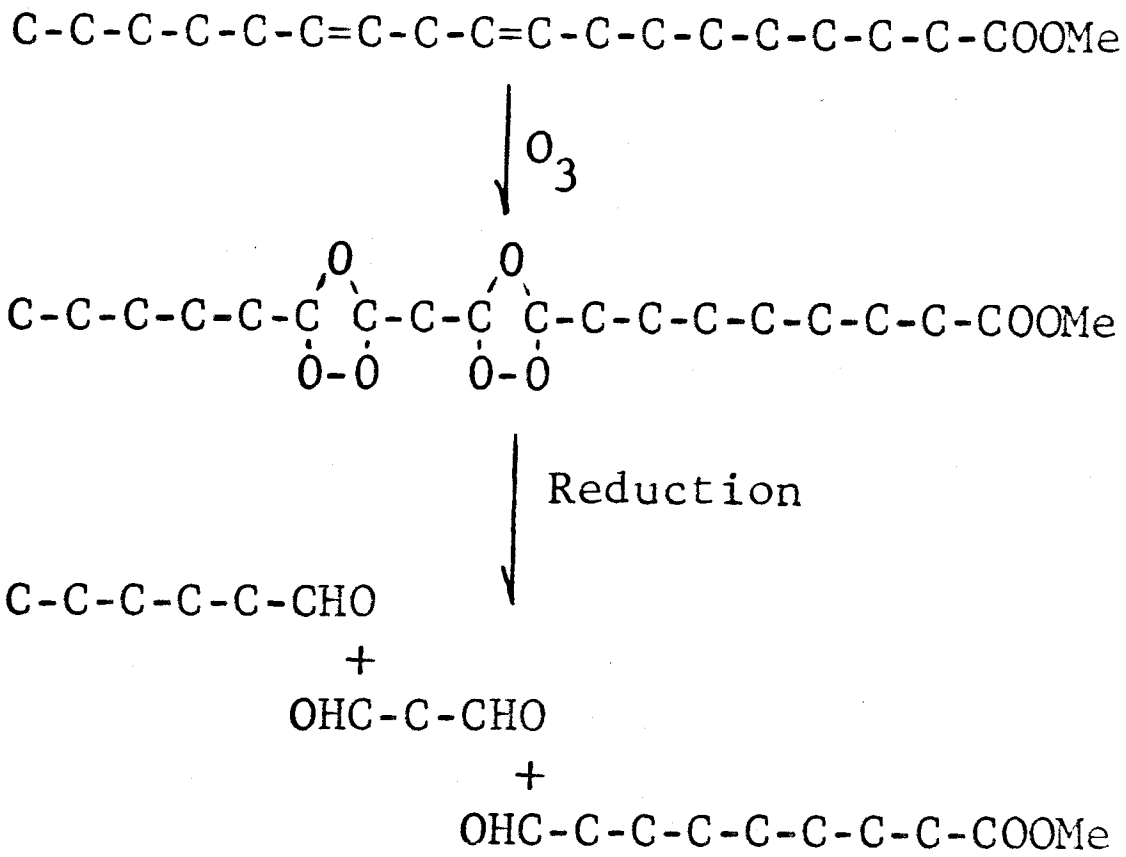

Figure 7. The ozonolysis of methyl linoleate to produce a six carbon aldehyde and a nine carbon aldehyde-ester.

in the direct identification of peaks on the chromatographic record. There may have been peaks for trace amounts (less than 0.01% of the total fatty acids) of hydroxy and branched chain fatty acids or of fatty acids with chain lengths greater than 22 carbon atoms. These were neglected because of the difficulty of separation from residual noise in the apparatus. The fatty acids from the Hormel Institute were 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 16:1, 18:1, 18:2, 20:4, 20:5, and 22:6.

The amounts of individual fatty acids present in a mixture are proportional to the product of peak height by the retention time of the fatty acids (Carroll, 23, 24; Bartlett and Smith, 11). The peak height by retention time products were totaled and the fractional contribution of each fatty acid to the total was computed. Thus was obtained the percentage contribution of each fatty acid to the total fatty acids in the sample.

Tentative identification of both the saturated and unsaturated fatty acids was accomplished by plotting the log of the retention times against the number of carbons in the chain (Evans, 30; Ackman, 1), and by comparison with Hormel Institute standards. For fatty acids with the same number of double bonds, the graph of log retention time against the number of carbon atoms gives a straight line and allows the tentative establishment of the number of carbons and the number of double bonds for fatty acids contributing

to the chromatographic record. However, errors can be made by relying simply upon retention time as a means of fatty acid identification since retention time varies not only with the number of double bonds, but also with the location of the double bonds in the molecule and with the presence or absence of substituent groups or side chains in the molecule.

Further identification of the saturated fatty acids was obtained by comparison with the saturated fatty acids from the Hormel Institute, by their continued presence on chromatograms of hydrogenated products of the original samples, and by linear relationship between the number of carbons and the log of their retention times for both the original and hydrogenated samples.

A comparison of chromatograms before and after hydrogenation allows identification of the peaks associated with fatty acids containing double bonds. Further identification of the unsaturated fatty acids was obtained by the hydrogenation and ozonolysis of the unsaturated fractions obtained from the thin-layer plates. By hydrogenation of the fatty acids, the number of carbons in the chains were established. Ozonolysis aided in the determination of the position of the double bonds in the molecule.

Statistical Analysis

Numerical data obtained in this study were processed by the Computer Center, Department of Statistics, Oregon State University. Means, standard deviations, and assorted regression lines were computed.

RESULTS

The information developed in this study required identification of the fatty acids found in salmon, classification of the fatty acids, quantitative estimation of the amounts of specific fatty acids, and studies of fatty acid changes relative to swimming performance of salmon. Statistical studies were made of some of the interrelationships between total fatty acid methyl esters, total lipid content, specific amounts of individual fatty acids, body weight, body length, swimming velocity and swimming time of salmon.

Identification of Fatty Acids in Salmon Lipids

To identify the individual fatty acids present in salmon lipids, methyl esters were prepared and the fatty acid methyl esters were detected by gas-liquid chromatography. Comparison of chromatographic records of individual fatty acid methyl esters of known purity obtained from the Hormel Institute with the chromatograms of salmon fatty acid methyl esters identified some of the salmon fatty acids. Chromatograms of the individual Hormel acids had only one peak and indicated a very high degree of purity of these standards.

In the procedure yielding the chromatograms, fatty acids with N carbon atoms came off the column before acids with N+1 carbons; acids of the same number of carbons with N double bonds came off

before acids with N+1 double bonds. Thus, if the positions of some of the fatty acids were known by comparison with the Hormel standards, a reasonably accurate identification of all fatty acid peaks could be made by simple inspection. A straight line was obtained by plotting the logs of the retention times of individual Hormel saturated fatty acid methyl esters against the number of carbons in their respective chains. Another straight line was drawn through the log retention time:carbon number points of 16:1 and 18:1. When fatty acid ester retention times were marked on these lines a verification or negation of the carbon numbers assigned by inspection could readily be obtained.

Separation of the salmon fatty acid methyl ester mixture by silver nitrate thin-layer plates according to the number of double bonds allowed the production of chromatograms with peaks for only saturated fatty acids or for unsaturated fatty acids with the same number of double bonds. Hydrogenation of the unsaturated fractions separated by the thin-layer plates allowed precise determination of the number of carbons for the unsaturated fatty acids. Ozonolysis of the unsaturated fractions separated by the thin-layer plates and subsequent identification of the ozonolytic products aided in the determination of the position of some of the double bonds in the unsaturated fatty acids.

A sample chromatogram showing the detector response to the fatty acid methyl ester mixture obtained from a non-exercised salmon is given in Figure 8. Inspection shows 27 peaks and suggests that 27 fatty acids are present in salmon lipids. Subsequent analysis indicated that some of the 27 fatty acids were saturated, some unsaturated, and that there were between 8 and 22 carbons in the chains.

Saturated Fatty Acids

Seven saturated fatty acids were identified in the lipids of coho salmon. They were 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, and 18:0. Comparison of the chromatographic response from Hormel standards 8:0, 10:0, 12:0, 14:0, 16:0, and 18:0 with the chromatogram from the salmon fatty acid methyl esters gave direct identification of these saturated fatty acids. The straight line relating the log retention time to the number of carbons allowed the identification of fatty acid 15:0 (Figure 9).

Further evidence confirming the identity of the saturated fatty acids was obtained by separating the fatty acid methyl esters on silver nitrate thin-layer plates. The fatty acid methyl esters separated into distinct bands according to the number of double bonds in the molecule, with the saturated acids displaced the most from distribution point and the unsaturated acids displaced less as

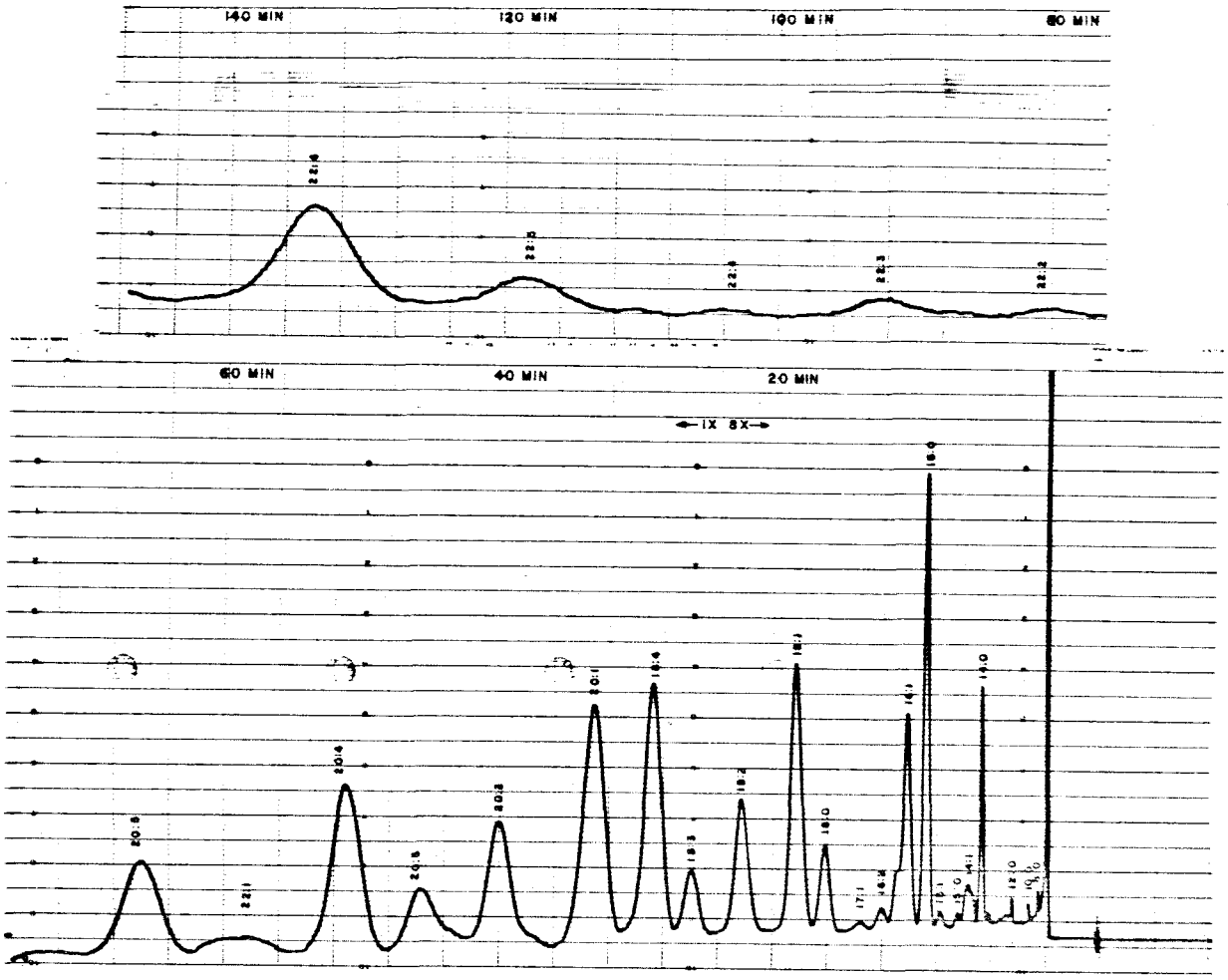

Figure 8. Gas chromatogram of the fatty acid methyl esters of a coho salmon.

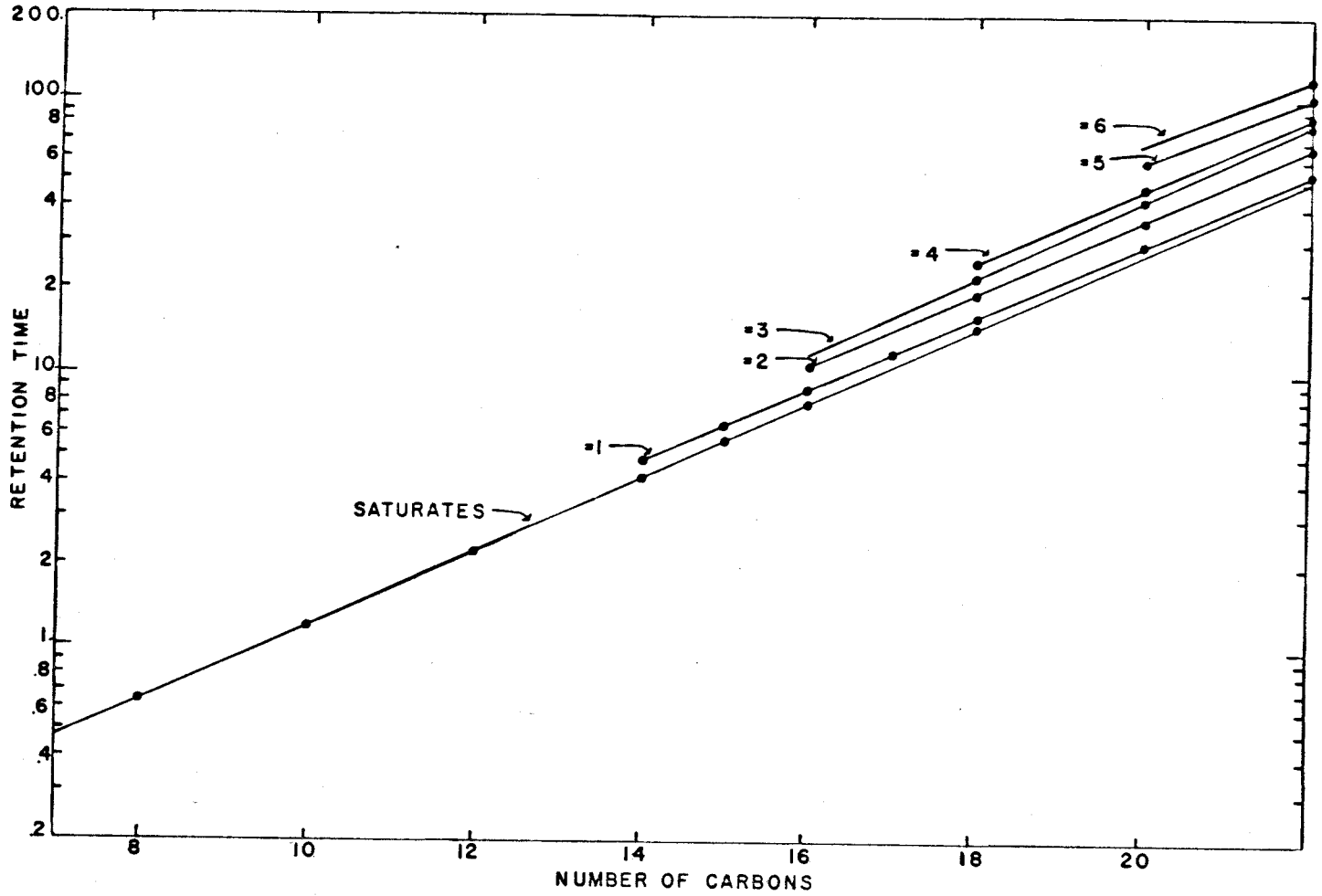

Figure 9. Semilog plot of the retention times of the fatty acid methyl esters of a coho salmon against the number of carbons in the fatty acid chain.

the number of double bonds increased (Privett et al., 63).

Seven peaks were found in the chromatogram obtained from the saturated fatty acid fraction. Comparison of the original chromatogram, the chromatograms of the Hormel standards, and the chromatogram of the saturated fatty acids verified the efficacy of the thin-layer separation and added additional identification for the peaks associated with the saturated fatty acids. Hydrogenation of the thin-layer fraction containing the saturated fatty acids did not alter their retention times.

Unsaturated Fatty Acids

Twenty unsaturated fatty acids were identified in the lipids of coho salmon. Comparison of the chromatographic peaks obtained from Hormel standards 16:1, 18:1, 18:2, 18:3, 20:4, 20:5, and 22:6 with the chromatographic peaks of salmon fatty acid methyl esters gave direct preliminary identification of seven of the unsaturated fatty acids.

Tentative assignation of chain length and number of double bonds for the remaining unsaturated fatty acids were derived from the position of the peaks with reference to identified peaks. The semilog plot of the log retention time against assigned numbers of carbons gave a series of parallel straight lines for acids with no,

one, two, three, four and five double bonds. The line for N+1 double bonds lay above the line for N double bonds (Figure 9).

Hydrogenation of the fractions from the thin-layer plates changed the retention times of unsaturated fatty acid methyl esters to the retention times of corresponding saturated methyl esters. Thus, a comparison between the original chromatogram, the chromatogram of the thin-layer fractions, and the chromatogram after hydrogenation of the fractions provided evidence for the number of carbons in the chains of the unsaturated fatty acids.

Ozonolysis of the unsaturated fatty acids aids in the determination of the position of the double bonds. Previous work by Stein and Nicolaides (75) had indicated that treatment of unsaturated fatty acid methyl esters with ozone under restricted conditions leads to the formation of an ozonide, which, upon triphenylphosphine reduction, produces an aldehyde and an aldehyde-ester. Stein and Nicolaides also identified malondialdehyde, a product expected from the ozonolysis of unsaturated fatty acids when double bonded carbons are separated by a methylene group.

To clarify the position of the double bonds in the unsaturated salmon fatty acids, Hormel fatty acid methyl esters 16:1, 18:1, 18:2, 18:3, 20:4, 20:5, and 22:6 were ozonized separately and the products were analyzed by gas-liquid chromatography. Each Hormel fatty acid ester gave only one aldehyde-ester peak and only one

aldehyde peak. The aldehyde-esters of 16:1, 18:1, 18:2, and 18:3 were chromatographically identical and indicated identity of the structure of the aldehyde-ester portion of the four fatty acids. The aldehyde-esters of 20:4 and 20:5 gave identical chromatographic responses while the aldehyde-ester of 22:6 gave a different response. Fatty acid 20:4 yielded an aldehyde chromatographically identical to 18:2 while fatty acids 20:5 and 22:6 yielded an aldehyde identical to 18:3.

The reductive ozonolysis of a fatty acid methyl ester with one double bond produces an aldehyde and an aldehyde-ester, and the nature of the reaction requires that the sum of the chain lengths of the aldehyde and aldehyde-ester equals the chain length of the fatty acid methyl ester. Naturally occurring oleic acid, 18:1, has the double bond between carbon 9 and carbon 10 (Ralston, 65, p. 105). If the aldehyde-esters for fatty acids 16:1, 18:1, 18:2, and 18:3 contain 9 carbons, then the aldehyde for 16:1 would contain 7 carbons and the aldehyde for 18:1 would contain 9 carbons.

When Hormel standard methyl esters 16:1, 18:1, 18:2, 18:3, 20:4 and 20:5 were ozonized the peaks on the chromatograms of the aldehydes had four different retention times and those of the aldehyde-esters had two different retention times. If a line is drawn through the points of log retention time:number of carbons for

Table 1. Computations of Constants for Straight Line Relating Log Retention Time and Number of Carbons for Aldehyde-esters.

Fatty Acid	Aldehyde-ester *Retention Time (cm)	Log Retention Time	Interval Difference $B(n_j - n_i)$	Ratio to Lower Interval $(n_j - n_i)$	Aldehyde-ester length
18:1	10.50	1.021			9 C
			0.632	4	
20:4, 20:5	2.45	0.389			9-4=5C
			0.159	1	
22:6	1.70	0.230			5-1=4C

*Retention Time is Measured in Distance on the Chromatogram

the 9 carbon nonaldehyde from 18:1 and the 7 carbon heptaldehyde from 16:1, and if the retention times for the other two aldehyde peaks are located on this line, the positions correspond to 3 and 6 carbon aldehydes. Hormel fatty acid methyl esters 18:2 and 20:4 yielded aldehydes corresponding to the 6 carbon aldehyde; and 18:3, 20:5 and 22:6 yielded an aldehyde corresponding to the 3 carbon aldehyde.

Aldehyde-esters follow the general rule that

$$\text{Log } t = A + nB \qquad (1)$$

where t is the retention time, n is the number of carbons, B is the increment in log t with each unit increase in the number of carbons, and A +2B is the retention time for the two carbon aldehyde-ester (HCOCOOR). Hence

$$\text{Log } t_j - \text{Log } t_i = (n_j - n_i)B \qquad (2)$$

Equation (2) can be used to establish A and B of equation (1). This is done in Table 1. Columns 2 and 3 of Table 1 give the retention times and the log retention times of the acids in column 1. The fourth column of figures in Table 1 gives the interval between the log retention times of aldehyde-esters 18:1 and 20:4, and between 20:4 and 22:6; and the fifth column gives the ratio of the longer to the shorter interval. The gap in number of carbons between the aldehyde-esters from 18:1 and 20:4 is 4 times as long as the gap between the aldehyde-esters from 20:4 and 22:6. As the aldehyde-

ester from 18:1 has 9 carbons and only integer numbers of carbons are allowed, the aldehyde-ester from 20:4 and 20:5 must have four less than nine, or five carbons, and that from 22:6 must have one less than five, or four carbons, and the formula for the aldehyde-ester line of equation (1) becomes

$$\log t = -0.402 + 0.158B \qquad (3)$$

As any carbon compound would have to have at least one carbon, A has no clear physical meaning. A + 2B, however, gives the log of retention time of the two carbon aldehyde-ester as -0.086 and the retention time as 0.82 cm. The value of B indicates that the retention time is to be multiplied by 1.44 for each carbon added to the aldehyde-ester chain.

Salmon Fatty Acids Containing One Double Bond

Seven fatty acids were identified in the lipids of coho salmon as containing one double bond. They were 14:1, 15:1, 16:1, 17:1, 18:1, 20:1, and 22:1. Comparison with retention times of Hormel standards gave direct identification of fatty acids 16:1 and 18:1. The other fatty acids containing one double bond were tentatively identified on the basis of their chromatograph positions relative to known fatty acids. After plotting the log of the retention times of 16:1 and 18:1 against the number of carbons in their molecules and

drawing a straight line through the two points, fatty acids 14:1, 15:1, 17:1, 20:1, and 22:1 could again be identified by reading the number of carbons corresponding to the position of their log retention times on the straight line. Finally, the semilog plot of the retention time against assigned carbon number for fatty acids containing one double bond gave a straight line parallel to and above the straight line derived for the saturated fatty acids.

Further identification of the fatty acids containing one double bond was obtained by separation of the fatty acid methyl esters on silver nitrate thin-layer plates. Gas-liquid chromatographic analysis of the fraction containing the monene fatty acids revealed seven peaks with positions that verified the carbon numbers originally assigned and established the efficacy of the separation (Table 2).

Hydrogenation did not alter the number of peaks for the monene fatty acids, but their individual retention times were changed to the retention times of the corresponding saturated acids. Thus a comparison between the original chromatogram, the chromatogram containing the fatty acid methyl esters with one double bond, and the chromatogram after saturation, provided convincing evidence for the number of carbons in the chains of the monene fatty acids.

The chromatogram of the aldehyde-esters from ozonolysis of the monene acid thin-layer fraction had four peaks and the

Table 2. Theoretical Percentage Contributions of Salmon Monene Fatty Acids to Aldehydes and Aldehyde-esters Formed by Ozonolysis. (Theory from Chromatograms of Monenes; Experimental from Chromatograms of Aldehydes and Aldehyde-esters).

	Fatty Acids			Aldehydes			Aldehyde-esters			
Fatty Acid	% in Total Fatty Acids	% in Unseparated Monene Acids	% from Monene Chromatogram							
$14{:}1\ \omega_6^{6\,8}\Delta_9$	0.86	2.58	2.70	2.45			2.97			
$15{:}1\ \omega_6^{7}\Delta_9$	0.26	0.78	0.91	0.79				1.03		
$16{:}1\ \omega_9^{7}\Delta_8^{9}$	9.94	29.87	27.40		25.49			29.10		
$17{:}1\ \omega_9^{9}\Delta_9$	0.56	1.68	1.28			1.42	1.19			
$18{:}1\ \omega_9^{9}\Delta_{11}$	18.32	55.05	55.49			58.20		53.34		
$20{:}1\ \omega_9^{9}\Delta_{13}$	3.13	9.41	11.46			10.98			11.56	
$22{:}1\ \omega^{9}\Delta^{13}$	0.20	0.60	0.77			0.68				0.81
*Theory Total		100	100	3.24	25.49	71.28	4.16	83.47	11.56	0.81
**Experimental Total				3.2	25.4	71.2	3.9	88.0	8.6	0.5

* = Theory from Formulation and Percentage Contribution to Monene Acids

** = Experimental from Aldehyde and Aldehyde-ester Chromatograms

chromatogram of the aldehydes had three peaks. The Hormel standards allowed definite identification of the 6, 7, and 9 carbon aldehydes. The Hormel standards also allowed identification of the 9 carbon aldehyde-ester, and the retention times of the other three peaks suggested 8, 11, and 13 carbon aldehyde-esters when placed on the straight line through the 4, 5, and 9 carbon points provided by the aldehyde-esters from the Hormel standards. Figure 10 depicts the semilog plot of the retention times of the aldehydes and aldehyde-esters as a function of chain length. The values plotted were derived from Hormel standards and from the salmon monene fatty acids.

The only possible combination of 6, 7, and 9 carbon aldehydes with 8, 9, 11, and 13 carbon aldehyde-esters from the monene fatty acids that would meet the chain length requirement for 14:1 was a 6 carbon aldehyde and an 8 carbon aldehyde-ester. Using standard nomenclature, the terminal chain containing the methyl group would be ω6 while the chain containing the carboxyl group would be Δ8, and the acid would be identified as 14:1 ω6, Δ8 (25, 54).

Similarly, 16:1 was uniquely determined from the possibilities available to have yielded a 7 carbon aldehyde and a 9 carbon aldehyde-ester while 22:1 yielded a 9 carbon aldehyde and a 13 carbon aldehyde-ester.

Fatty acid 18:1 probably yielded a 9 carbon aldehyde and a

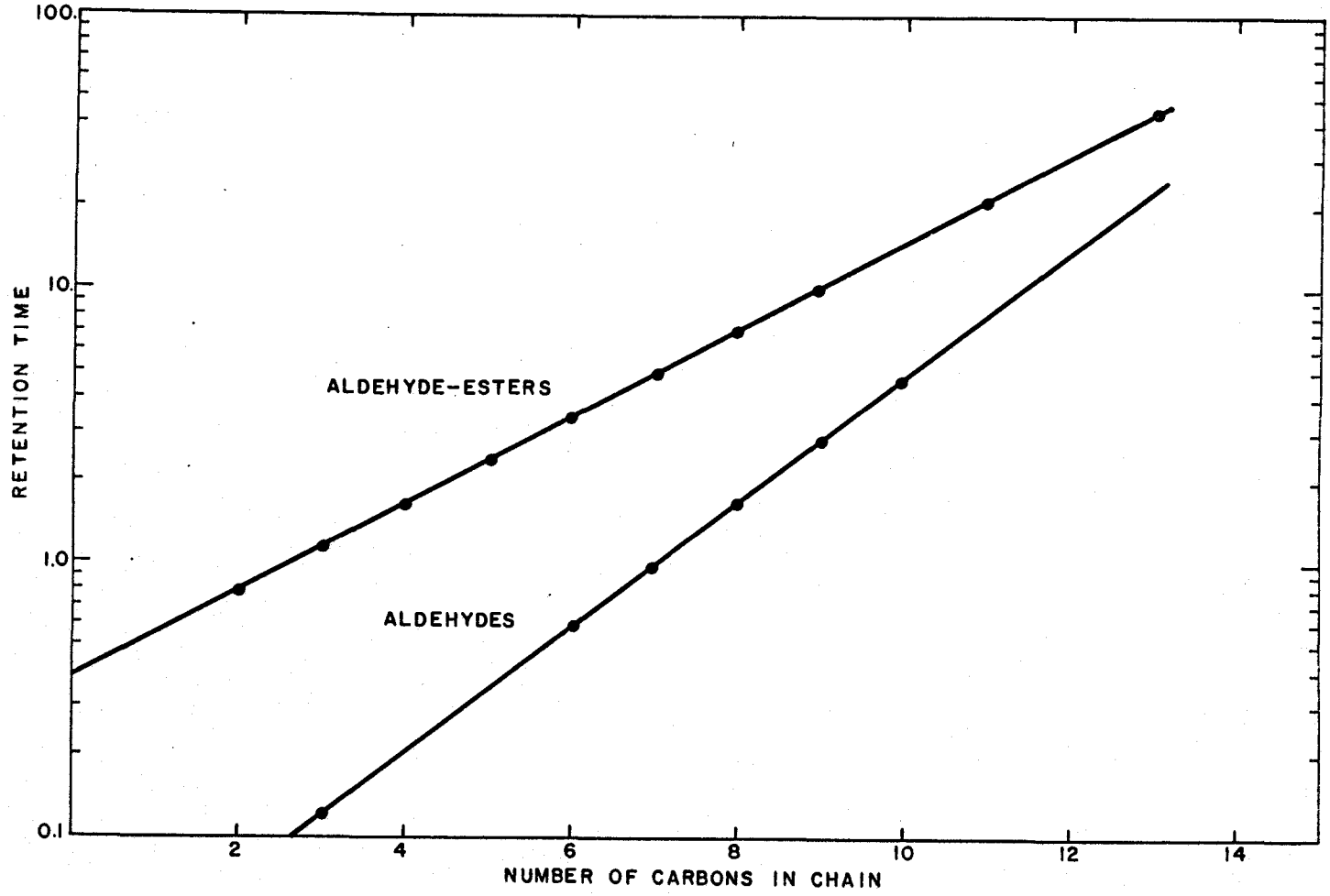

Figure 10. Semi-log plot of the retention times of the aldehydes and aldehyde-esters produced by the ozonolysis of the unsaturated fatty acid methyl esters of a coho salmon and Hormel esters against the number of carbons in the chains.

9 carbon aldehyde-ester on the basis of the ozonolysis products from the Hormel standard 18:1. This was verified by the amounts of aldehyde and aldehyde-ester produced from the ozonolysis of the salmon 18:1, since 18:1 constituted 55% of the fatty acids with one double bond. Therefore, the aldehyde and aldehyde-ester peak areas to which 18:1 contributed would have to be the largest peak area on the chromatogram of the aldehydes and of the aldehyde-esters. These were the peak areas for the 9 carbon aldehyde and the 9 carbon aldehyde-ester.

The only possible combinations of aldehyde and of aldehyde-ester fragments available that could yield 20:1 are a 7 carbon aldehyde with a 13 carbon ester, or a 9 carbon aldehyde with an 11 carbon ester. Fatty acid 20:1 would have to be $\omega 7$, $\Delta 13$ or $\omega 9$, $\Delta 11$. Since 20:1 constituted 9.4% of the fatty acids with one double bond, the amount of the 13 carbon aldehyde-ester was insufficient (0.5% of the total : Table 2) to account for 20:1; also, the 13 carbon aldehyde was required for 22:1. Therefore, the aldehyde-ester from 20:1 had 11 carbons and 20:1 is $\omega 9$ $\Delta 11$.

Fatty acid 15:1 could be $\omega 6$, $\Delta 9$ or $\omega 7$, $\Delta 8$ and fatty acid 17:1 could be $\omega 6$, $\Delta 11$ or $\omega 9$, $\Delta 8$. However, 20:1 is $\omega 9$, $\Delta 11$, and requires all of the availble 11 carbon aldehyde-ester. Thus fatty acid 17:1 would have to be $\omega 9$, $\Delta 8$. Fatty acid 16:1 must be $\omega 7$ $\Delta 9$; hence

all of the ω7, but not all of Δ9 is required for 16:1. Therefore, 15:1 must be ω6, Δ9.

In summary, the fatty acids found in salmon lipids having one double bond were 14:1 ω6, Δ8; 15:1 ω6, Δ9; 16:1 ω7, Δ9: 17:1 ω9, Δ8; 18:1 ω9, Δ9; 20:1 ω9, Δ11; and 22:1 ω9, Δ13.

Column two of Table 2 gives the percent of each monene acid that contributed to the total salmon monene acids. Column three is calculated from the chromatogram of the salmon fatty acid methyl esters and gives expected percentage contribution of each monene acid to the total monene acids. The fourth column gives the percentage contribution of each monene acid to the total monene acids as calculated from the chromatogram of the monene acids.

If one takes a sample of the original mixture of the methyl esters of the salmon fatty acids sufficient to contain 100 grams of the monene acids, the masses of the aldehydes and aldehyde-esters expected to be formed can be calculated by

$$\frac{\text{Molecular weight of aldehyde} \times \text{Percent of monene fatty acid}}{\text{Molecular weight of fatty acid}}$$

or

$$\frac{\text{Molecular weight of aldehyde-ester} \times \text{Percent of monene fatty acid}}{\text{Molecular weight of fatty acid}}$$

The masses of the aldehydes can be summed and the percentage contribution of each aldehyde to the sum can be calculated;

the aldehyde-esters can be treated similarly. The expected percent contribution of each acid to the total percentages of specific aldehydes and aldehyde-esters is tabulated on the right side of Table 2. There is close agreement between specific fatty acid percentages as calculated from salmon fatty acids and from monene chromatograms. There is also close agreement between calculated and experimental percentages for both aldehydes and aldehyde-esters. The agreement lends considerable confirmation to the correctness of the assignment of the position for the double bonds in the monenes from salmon fatty acids and to the assignment of carbon numbers for the aldehydes and aldehyde-esters involved.

Salmon Fatty Acids Containing Two Double Bonds

Four fatty acids were identified in the lipids of coho salmon as containing two double bonds. They were 16:2, 18:2, 20:2, and 22:2. Comparison with the Hormel standard 18:2 provided direct identification of this acid. Fatty acids 16:2, 20:2, and 22:2 were identified by the position of their peaks on the chromatographic record with respect to the positions of the fatty acids with one double bond and the saturated fatty acids. Further verification was obtained by plotting the log of their retention times against the number of carbons assigned to their molecules as determined by their positions on the

chromatographic record. The semilog plot of the retention times of the fatty acids containing two double bonds against the number of carbons assigned gave a straight line which was parallel to and above the straight lines produced from the saturated fatty acids and the fatty acids containing one double bond (Figure 9).

A gas chromatogram of the diene acids, separated by thin-layer chromatography, revealed three peaks with retention times corresponding with the retention times of 16:2, 18:2, and 20:2 as identified on the chromatogram of the total salmon fatty acids. Fatty acid methyl ester 22:2 was less than 0.01% of the total salmon acids and was not recovered from the thin-layer plate. The chromatogram after saturation of the diene acid esters had three peaks but with the shorter retention times required for fatty acids 16:0, 18:0 and 20:0.

Ozonolysis of the diene fatty acid methyl esters produced 6 and 7 carbon aldehydes and 6, 9 and 11 carbon aldehyde-esters. The nature of the reaction requires that the sum of the chain lengths of the aldehyde and aldehyde-esters be less than the number of carbons in the original fatty acid.

Among the combinations between aldehydes of 7 or 9 carbons and aldehyde-esters of 6, 9, and 11 carbons that might be possible for 16:2 are $\omega 7$, $\Delta 6$ and $\omega 9$, $\Delta 6$. Fatty acid 16:2 $\omega 9$, $\Delta 6$ would require a twin double bond or allene structure and this would spontaneously rearrange to give a conjugated double bond structure (5, 7

diene) or to a methylene interrupted double bond (5, 8 diene). As no 5 carbon aldehyde-ester or 8 carbon aldehyde was indicated on the chromatograms, it is extremely unlikely that 16:2 ω9, Δ6 was present in the original sample or in the double bonded fraction from the thin-layer plate. Hence, 16:2 was ω7, Δ6.

Fatty acid 18:2 yielded a 6 carbon aldehyde and a 9 carbon aldehyde-ester on the basis of the products of Hormel standard 18:2. Fatty acid 18:2 constituted 75% of the salmon diene fatty acids. Thus, the aldehyde and aldehyde-ester peak areas to which 18:2 contributed would have to be the largest peak areas on the chromatogram of the aldehydes or of the aldehyde-esters. These were the 6 carbon aldehyde and the 9 carbon aldehyde-ester.

The seven carbon aldehyde and the six carbon aldehyde-ester are required for 16:2 ω7, Δ6. The nine carbon aldehyde-ester and some of the six carbon aldehyde are required for 18:2 ω6, Δ9. Hence, fatty acid 20:2 was probably ω6, Δ11 (Table 3).

In the first column of Table 3 are tabulated the diene salmon fatty acids and the length of the initial and terminal fragments thus far assigned. In the second column is given the percentage contributions of these acids to the total fatty acids, and in column three is given the percentage contribution to the diene acids as calculated from the total fatty acid chromatogram. In the fourth column are the percentage contributions of each diene acid to the total diene

Table 3. Theoretical Percentage Contributions of Salmon Diene Fatty Acids to Aldehydes and Aldehyde-esters Formed by Ozonolysis. (Theory from Chromatograms of Dienes; Experimental from Chromatograms of Aldehydes and Aldehyde-esters).

	Fatty Acids			Aldehydes		Aldehyde-esters		
Fatty Acid	% in Total Fatty Acids	% in Unseparated Diene Acids	% from Diene Chromatogram					
$16{:}2\omega^{7}{}_{\Delta}{}^{6}$	1.48	9.02	9.7		5.63	8.36		
$18{:}2\omega^{6}{}_{\Delta}{}^{9}$	12.43	75.79	76.2	35.10			76.3	
$20{:}2\omega^{6}{}_{\Delta}{}^{11}$	2.49	15.18	14.0	59.29				14.92
22:2	0.005	0.03	0.00					
Total	16.40	100	100					
Theory from Diene Chromatogram				94.39	5.63	8.36	76.3	14.92
Theory from Fatty Acid Chromatogram				94.9	5.03	7.92	77.7	14.36
Experimental from Aldehyde and Aldehyde-ester Chromatogram				93.8	6.1	9.9	74.6	15.4

fatty acids as calculated from the chromatogram of the diene acids. Columns three and four give the diene contributions as evaluated from two widely (from the technical point of view) separated chromatograms.

The next two columns give the calculated contributions of each diene fatty acid to the percentages of the aldehydes on the basis of the assigned positions for the double bonds and of the relative mass of each diene acid as reflected in the diene chromatogram. Columns 6, 7, and 8 give the contributions of each diene acid to the percentages of the aldehyde-esters. Similar calculations were made on the basis of the diene acid contribution to the total salmon fatty acids. The calculations are summarized on the lower three lines. There is close agreement between fatty acid percentages as calculated from the total fatty acid ester chromatogram and from the chromatogram of the diene acid esters. There is also close agreement between calculated and experimental percentages of aldehydes and aldehyde-esters. The data are highly consistent with the assigned positions of the double bonds in the diene fatty acids. It will be noted that the assigned positions in 16:2, 18:2, and 20:2 indicate the double bonds to be methylene interrupted.

Fatty Acids Containing Three and Four Double Bonds

Three fatty acids were identified in the lipids of the coho salmon as containing three double bonds and three fatty acids were identified as containing four double bonds. They were 18:3, 18:4, 20:3, 20:4, 22:3, and 22:4. Comparison with the Hormel acids provided direct identification of 18:3 and 20:4. Fatty acids 18:4, 20:3, 22:3, and 22:4 were identified by the position of their peaks on the chromatographic record with respect to previously identified fatty acids. Further identification was obtained by plotting the log of their individual retention times against the number of carbons assigned to their molecules as determined by their positions on the chromatographic record. The semi-log plot of the retention times of the fatty acids containing three and four double bonds against the number of carbons assigned gave separate straight lines which were parallel to and above the straight lines developed from saturated fatty acids, fatty acids with one double bond, and fatty acids with two double bonds (Figure 9).

Generally, the silver nitrate plates provided separation into groups of acids with the same number of double bonds. The separation of compounds with three and four double bonds was not clear and the bands of the trienes and tetraenes were combined. Gas chromatographic analysis of the fraction containing three and four

double bonds revealed four peaks with retention times corresponding to the retention times of 18:3, 18:4, 20:3, and 20:4 as identified on the chromatogram of the total salmon fatty acids. The chromatogram after saturation had two peaks with retention times corresponding to saturated fatty acids 18:0 and 20:0. Fatty acid methyl esters 22:3 and 22:4 were less than 0.01% of the total salmon fatty acids and were not recovered from the thin-layer plate.

Ozonolysis of the triene and tetraene fatty acid methyl esters produced 3 and 6 carbon aldehydes and 5, 6, 8, and 9 carbon aldehyde-esters. Fatty acid 18:3 was probably $\omega 3$, $\Delta 9$ and 20:4 was probably $\omega 6$, $\Delta 5$ based upon the analysis of the Hormel standards. Fatty acid 18:4 was 45% of the fatty acids containing three and four double bonds and 20:3 was 10%. Ozonolytic analysis revealed 45% of the aldehyde-esters present contained 6 carbons and 10% contained 8 carbons. Therefore, 18:4 was probably $\Delta 6$ and 20:3 was probably $\Delta 8$ (Table 4).

The three carbon aldehyde is volatile and was not completely recovered following ozonolysis. Thus the relative amounts of the 3 carbon aldehyde and the 6 carbon aldehyde produced were not certain.

Although the amount of $\omega 3$ recovered was low, much more was recovered than was required for 18:3 $\omega 3$, $\Delta 9$. Hence 18:4 or 20:3 had to be $\omega 3$. If the amount of $\omega 3$ was more than 27.4% of the

Table 4. Theoretical Percentage Contributions of Salmon Polyene Fatty Acids to Aldehydes and Aldehyde-esters Formed by Ozonolysis. (Theory from Chromatograms of Polyenes; Experimental from Chromatograms of Aldehydes and Aldehyde-esters).

Fatty Acids				Aldehydes		Aldehyde-esters			
Fatty Acid	% in Total Fatty Acids	% in Unseparated Polyenes	% from Polyene Chromatogram						
18:3 $\omega^3\Delta^9$	1.05	10.48	10.13	8.64					13.35
18:4 $\omega^3\Delta^6$	4.39	43.81	45.25	36.25			46.49		
20:3 $\omega^6\Delta^8$	0.84	8.38	9.79		12.25			10.88	
20:4 $\omega^6\Delta^5$	3.74	37.33	34.82		42.86	29.28			
22:3	Trace	Trace	None						
22:4	Trace	Trace	None						
Total	10.02	100	100						
Theory from Polyene Chromatogram				43.01	56.98	29.28	46.49	10.88	13.35
Theory from Fatty Acid Chromatogram				44.89	55.11	31.67	45.13	9.34	13.86
Experimental from Aldehyde and Aldehyde-ester Chromatogram				27.4	72.5	32.84	45.62	10.47	11.06

aldehydes produced from the triene and tetraene acids, and the combination of 18:3 and 20:3 would only give 21% ω3, the remainder of ω3 would have to be produced from fatty acid 18:4. Therefore, fatty acid 18:4 was ω3, Δ6 and 20:3 was ω6, Δ8.

In the first column of Table 4 are tabulated the salmon fatty acids containing three and four double bonds. In the second column is given the percentage contributions of these acids to the total fatty acids. In column three the percentage contribution to the triene and tetraene fatty acids was calculated from the total fatty acid methyl ester chromatogram. In the fourth column are the percentage contributions as calculated from the chromatogram of trienes and tetraenes as separated on silver nitrate plates. Columns three and four give the triene and tetraene contributions as evaluated from two widely (from the technical point of view) separated chromatograms. The agreement is satisfactory.

The next columns give the calculated contributions of each acid to the percentages of the aldehydes and aldehyde-esters derived from the triene and tetraene acids on the basis of the assigned position of double bonds and on the relative mass of each acid as reflected in the polyene chromatogram. Similar calculations were made on the basis of triene and tetraene contribution to the total salmon fatty acids. The calculations are summarized on the lower three lines.

Except for the aldehydes, the data are highly consistent with the assigned positions of the double bonds in the triene and tetraene fatty acids.

Fatty Acids Containing Five and Six Double Bonds

Two fatty acids were identified in the lipids of the coho salmon as containing five double bonds and one fatty acid was identified as containing six double bonds. They were 20:5, 22:5, and 22:6. Comparison with Hormel acids provided direct identification of 20:5 and 22:6. Fatty acid 22:5 was identified by its position on the chromatographic record with respect to 22:6. When the log of the retention times of 20:5 and 22:5 were plotted against the number of carbons assigned, a straight line was produced which was parallel to and above the straight lines previously developed. The log of the retention time of 22:6 against the number of carbons gave a point above the straight lines developed previously (Figure 9).

The gas chromatographic analysis of the silver nitrate thin-layer fraction containing the pentene acids had two peaks and the fraction containing acids with six double bonds had only one peak. The retention times of the peaks on the two chromatograms corresponded to fatty acids 20:5, 22:5, and 22:6. After hydrogenation, the chromatogram of the pentenes had peaks corresponding to

saturated acids 20:0 and 22:0; and the chromatogram of the hexenes had one peak, corresponding to 22:0.

Reductive ozonolysis of the pentenoic acid fraction produced a 3 carbon aldehyde and 5 and 7 carbon aldehyde-esters. Relative percentages of the fatty acid methyl esters (Table 5) and of the derived aldehyde-esters indicated that 20:5 was ω3, Δ5 and 22:5 was ω3, Δ7.

Reductive ozonolysis of the fatty acid methyl ester with six double bonds produced a 3 carbon aldehyde and a 4 carbon aldehyde-ester. Therefore, 22:6 was ω3, Δ4.

Table 5 shows good agreement of the relative percentages of Δ5 and Δ7 fragments as calculated from the aldehyde-ester chromatogram and the percentages as calculated from the chromatogram of the total salmon fatty acids, and from the fraction containing only 20:5 and 22:5.

If two concentrations of pure preparation of a fatty acid are separately converted into an aldehyde and an aldehyde-ester by ozonolysis, the ratio of the concentrations of the original acid, of the aldehyde, and of the aldehyde-ester obtained should be identical within the limits of error involved in the procedures. Relative retention times for 22:6, the 3 carbon aldehyde, and the 4 carbon aldehyde-ester from the Hormel acid and from the salmon acid respectively were the same; therefore, the masses of aldehydes and aldehyde-

Table 5. Theoretical Percentage Contributions of Salmon Pentene Fatty Acids to Aldehydes and Aldehyde-esters Formed by Ozonolysis. (Theory from Chromatograms of Pentenes; Experimental from Chromatograms of Aldehydes and Aldehyde-esters).

Fatty Acids				Aldehydes	Aldehyde-esters	
Fatty Acid	% in Total Fatty Acids	% in Unseparated Pentenes	% from Pentene Chromatogram			
20:5 $\omega^3\Delta^5$	4.88	72.08	74.6	76.2	72.5	
22:5 $\omega^3\Delta^7$	1.89	27.92	25.3	23.8		27.5
Total	6.77	100	100			
Theory from Pentene Chromatogram				100	72.5	27.5
Theory from Fatty Acid Chromatogram				100	69.8	30.2
Experimental from Aldehyde and Aldehyde-ester Chromatogram				100	73.6	26.3

esters would be in direct proportion to the peak heights. These peak heights are given in Table 6. The aldehydes and aldehyde-ester ratios agree within 11%. This agreement gives additional weight to the conclusion that 22:6 from the Hormel Institute and from salmon oil were identical.

Summary

Thus, the procedures used identify the fatty acids of the coho salmon as:

8:0, 10:0, 12:0, 14:0, 15:0, 16:0 and 18:0

$14{:}1\omega^{6}\Delta^{8}$ $15{:}1\omega^{6}\Delta^{9}$ $16{:}1\omega^{7}\Delta^{9}$ $17{:}1\omega^{9}\Delta^{8}$

$18{:}1\omega^{9}\Delta^{9}$ $20{:}1\omega^{9}\Delta^{11}$ $22{:}1\omega^{9}\Delta^{13}$

$16{:}2\omega^{7}\Delta^{6}$ $18{:}2\omega^{6}\Delta^{9}$ $20{:}2\omega^{6}\Delta^{11}$ 22:2

$18{:}3\omega^{3}\Delta^{9}$ $20{:}3\omega^{6}\Delta^{8}$ 22:3

$18{:}4\omega^{3}\Delta^{6}$ $20{:}4\omega^{6}\Delta^{5}$ 22:4

$20{:}5\omega^{3}\Delta^{5}$ $22{:}5\omega^{3}\Delta^{7}$

$22{:}6\omega^{3}\Delta^{4}$

Table 6. Peak Heights of Aldehydes and Aldehyde-esters from Hormel 22:6 and from Salmon 22:6.

	Hormel	Salmon	Ratio
Three Carbon Aldehyde	58mm	35mm	1.66
Four Carbon Aldehyde-ester	116mm	77mm	1.50
Ratio	0.50	0.45	1.11

Swimming Performance of Coho Salmon

The first portion of the data developed in this manuscript was concerned with identification of fatty acids found in the coho salmon. This portion is concerned with quantitative alterations of fatty acids during exercise. The procedures will be briefly reviewed, then data on fatty acid percentages and amounts in controls will be presented. Next, data will be provided for salmon exercised at each of the selected water velocities. Finally, the net alterations in specific fatty acids with exercise will be discussed. The salmon exercised at each velocity will be divided into a group that was unable to swim for 24 hours (these salmon will be designated as failing salmon) and a group that was still swimming at 24 hours. Tables 7 and 8 give data on control salmon, Tables 9 through 37 give data from exercised salmon. Some of the tables give data on the differences between control and exercised salmon.

To investigate swimming performance, coho salmon were forced to swim against sustained water velocities. Ten salmon were randomly selected for each of 16 swimming performance experiments; two salmon were used as controls and eight salmon were introduced into the test chamber. On the basis of the work by Smith (72) cited earlier, the salmon were forced to swim against water velocities of 52 cm/sec, 54 cm/sec, 56 cm/sec, and 59 cm/sec for a maximum of

Table 7. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Control Coho Salmon

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.05						
10	0.11						
12	0.50						
14	4.62	1.09					
15	0.45	0.44					
16	14.70	9.03	1.35				
17		0.55					
18	6.10	19.26	11.66	0.98	4.26		
20		3.91	2.40	1.10	4.02	3.82	
22		0.50	TR	TR	TR	1.78	7.39

TR = Trace
Number of Salmon = 32

Table 8. Mean Amounts of Individual Fatty Acid Methyl Esters Derive d from Control Coho Salmon.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.08						
10	0.18						
12	0.83						
14	8.38	2.03					
15	0.87	0.81					
16	27.85	17.55	2.64				
17		1.02					
18	11.15	36.73	22.05	1.81	7.93		
20		7.69	4.73	2.11	7.65	7.20	
22		0.92	TR	TR	TR	3.31	13.19

TR = Trace
Number of Salmon = 32

Table 9. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming less than 24 Hours at 52 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.04						
10	0.10						
12	0.38						
14	4.80	1.15					
15	0.39	0.34					
16	14.38	8.48	1.24				
17		0.54					
18	6.42	18.99	11.71	0.89	3.91		
20		3.52	2.42	1.18	3.72	3.45	
22		0.58	TR	TR	TR	2.04	8.97

TR = Trace
Number of Salmon = 8

Table 10. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming less than 24 Hours at 52 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.04						
10	0.11						
12	0.54						
14	7.35	1.71					
15	0.59	0.51					
16	21.47	12.83	1.88				
17		0.83					
18	9.10	28.82	17.30	1.42	5.87		
20		5.08	3.52	1.75	5.23	4.75	
22		0.75	TR	TR	TR	2.44	11.01

TR = Trace
Number of Salmon = 8

Table 11. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming less than 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 52 cm/sec. Table 10 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	1.03	—					
15	—	—					
16	6.38	4.72	0.76				
17		—					
18	2.05	7.91	4.70	—	2.06		
20		2.61	1.21	—	2.42	3.45	
22		—	—	—	—	0.87	2.18

Table 12. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming for 24 Hours at 52 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.05						
10	0.12						
12	0.33						
14	4.82	1.20					
15	0.44	0.45					
16	13.88	8.24	1.29				
17		0.54					
18	6.42	18.83	12.26	0.97	3.27		
20		3.74	3.07	1.40	4.23	3.48	
22		0.45	TR	TR	TR	1.78	8.82

TR = Trace
Number of Salmon = 20

Table 13. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming for 24 Hours at 52 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.06						
10	0.15						
12	0.48						
14	7.29	1.80					
15	0.67	0.68					
16	19.85	12.49	1.98				
17		0.81					
18	9.57	28.17	18.48	1.42	4.84		
20		5.77	4.74	2.13	6.41	5.24	
22		0.66	TR	TR	TR	2.62	12.89

TR = Trace
Number of Salmon = 20

Table 14. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming for 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 52 cm/sec. Table 13 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	1.09	—					
15	—	—					
16	8.00	5.06	—				
17		—					
18	1.58	8.56	3.57	—	2.09		
20		1.92	—	—	1.24	1.96	
22		—	—	—	—	0.68	—

Table 15. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of All Salmon Swimming at Velocity of 52 cm/sec from Comparable Amounts from Control Salmon

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	1.07	—					
15	—	—					
16	7.53	4.96	0.22				
17		—					
18	1.76	8.37	3.89	—	2.08		
20		2.12	0.34	—	1.58	2.38	
22		—	—	—	—	0.73	0.62

Table 16. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming less than 24 Hours at 54 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.04						
10	0.11						
12	0.30						
14	4.86	1.25					
15	0.49	0.51					
16	14.27	8.91	1.35				
17		0.50					
18	6.08	18.87	12.43	0.89	3.10		
20		4.01	3.08	1.32	4.33	3.71	
22		0.36	TR	TR	TR	1.73	7.56

TR = Trace
Number of Salmon = 10

Table 17. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming less than 24 Hours at 54 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.05						
10	0.14						
12	0.45						
14	7.31	1.91					
15	0.74	0.79					
16	21.78	13.66	2.07				
17		0.82					
18	9.20	28.62	18.97	1.34	4.78		
20		6.17	4.67	1.96	6.61	5.70	
22		0.56	TR	TR	TR	2.63	11.36

TR = Trace
Number of Salmon = 10

Table 18. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming less than 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 54 cm/sec Table 17 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	1.07	—					
15	—	—					
16	6.07	3.89	—				
17		—					
18	1.95	8.11	3.08	—	3.15		
20		1.52	—	—	1.04	1.50	
22		—	—	—	—	0.68	1.83

Table 19. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming for 24 Hours at 54 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.04						
10	0.10						
12	0.41						
14	4.37	1.22					
15	0.50	0.51					
16	14.45	8.87	1.38				
17		0.49					
18	5.98	19.57	12.37	0.77	3.23		
20		4.50	3.00	1.20	4.22	3.75	
22		0.46	TR	TR	TR	1.93	7.10

TR = Trace
Number of Salmon = 6

Table 20. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming for 24 Hours at 54 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.06						
10	0.19						
12	0.82						
14	8.47	2.38					
15	0.97	0.96					
16	28.23	17.31	2.70				
17		0.98					
18	11.64	38.39	24.14	1.51	6.38		
20		8.91	5.82	2.28	8.20	7.37	
22		0.93	TR	TR	TR	3.76	13.78

TR = Trace
Number of Salmon = 6

Table 21. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming for 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 54 cm/sec. Table 20 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	—	—					
15	—	—					
16	—	—	—				
17		—					
18	-0.49	-1.66	-2.09	—	1.55		
20		-1.22	-1.09	—	0.55	—	
22		—	—	—	—	—	0.59

Table 22. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of All Salmon Swimming at Velocity of 54 cm/sec from Comparable Amounts from Control Salmon.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	0.63	—					
15	—	—					
16	3.75	2.43	—				
17		—					
18	1.03	4.45	1.14	—	2.55		
20		0.49	0.41	—	0.86	0.94	
22		—	—	—	—	0.42	1.36

Table 23. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming less than 24 Hours at 56 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.10						
10	0.14						
12	0.47						
14	4.84	1.03					
15	0.45	0.39					
16	14.98	9.18	1.28				
17		0.53					
18	5.99	19.17	11.85	1.02	4.24		
20		3.88	2.27	1.07	3.91	3.80	
22		0.50	TR	TR	TR	1.85	7.23

TR = Trace
Number of Salmon = 20

Table 24. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming less than 24 Hours at 56 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.17						
10	0.24						
12	0.82						
14	8.46	1.80					
15	0.79	0.68					
16	26.17	16.04	2.24				
17		0.93					
18	10.46	33.49	20.70	1.78	7.41		
20		6.78	3.96	1.87	6.83	6.64	
22		0.87	TR	TR	TR	3.23	12.63

TR = Trace
Number of Salmon = 20

Table 25. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming less than 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 56 cm/sec. Table 24 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	—	—					
15	—	—					
16	1.68	1.51	0.40				
17		—					
18	0.69	3.24	1.35	—	0.52		
20		0.91	0.77	—	0.82	0.56	
22		—	—	—	—	—	0.56

Table 26. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming for 24 Hours at 56 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.05						
10	0.08						
12	0.30						
14	4.77	1.30					
15	0.48	0.60					
16	13.80	9.00	1.20				
17		0.60					
18	5.93	18.57	12.65	1.07	3.02		
20		4.35	3.27	1.45	4.64	3.77	
22		0.50	TR	TR	TR	1.90	6.75

TR = Trace
Number of Salmon = 6

Table 27. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming for 24 Hours at 56 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.11						
10	0.18						
12	0.75						
14	11.51	3.31					
15	1.18	1.55					
16	34.09	22.33	3.00				
17		1.49					
18	14.72	46.03	31.37	2.54	7.49		
20		10.75	8.14	3.67	11.49	9.29	
22		1.22	TR	TR	TR	4.78	16.51

TR = Trace
Number of Salmon = 6

Table 28. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming for 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 56 cm/sec. Table 27 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	-3.13	-1.28					
15	—	-0.74					
16	-6.24	-4.78	—				
17		—					
18	-3.57	-9.30	-9.32	-0.73	—		
20		-3.06	-3.41	-1.56	-3.84	-2.09	
22		—	—	—	—	-1.47	-3.32

Table 29. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of All Salmon Swimming at Velocity of 56 cm/sec from Comparable Amounts from Control Salmon.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	-0.72	-0.30					
15	—	-0.17					
16	-0.15	0.06	0.31				
17		—					
18	-0.29	0.35	-1.11	-0.17	0.40		
20		-0.01	-0.19	-0.36	-0.26	-0.05	
22		—	—	—	—	-0.34	-0.34

Table 30. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming less than 24 Hours at 59 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.05						
10	0.12						
12	0.57						
14	4.87	1.00					
15	0.43	0.37					
16	14.47	9.47	1.50				
17		0.50					
18	6.23	20.63	11.50	1.10	4.27		
20		4.00	2.03	0.97	3.70	3.60	
22		0.60	TR	TR	TR	1.87	6.57

TR = Trace
Number of Salmon = 30

Table 31. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming less than 24 Hours at 59 cm/sec. (Unable to Swim for 24 Hours).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.06						
10	0.17						
12	0.86						
14	7.46	1.59					
15	0.70	0.59					
16	24.34	15.76	2.48				
17		0.87					
18	9.98	33.80	18.42	1.66	7.08		
20		6.61	3.39	1.53	5.85	5.80	
22		0.89	TR	TR	TR	2.91	10.15

TR = Trace
Number of Salmon = 30

Table 32. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming less than 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 59 cm/sec. Table 31 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	1.08	—					
15	—	—					
16	3.51	1.79	—				
17		—					
18	1.17	2.93	3.63	—	0.85		
20		1.08	1.34	—	1.80	1.40	
22		—	—	—	—	—	3.04

Table 33. Mean Percentage Contribution of Individual Fatty Acids to Total Fatty Acids of Coho Salmon Swimming for 24 Hours at 59 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.05						
10	0.10						
12	0.40						
14	4.10	1.00					
15	0.50	0.40					
16	15.50	9.90	1.70				
17		0.50					
18	5.80	20.20	11.30	1.10	3.40		
20		4.30	2.50	1.10	3.80	3.80	
22		0.40	TR	TR	TR	1.80	5.90

TR = Trace
Number of Salmon = 8

Table 34. Mean Amounts of Individual Fatty Acid Methyl Esters Derived from Coho Salmon Swimming for 24 Hours at 59 cm/sec.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	0.11						
10	0.17						
12	0.88						
14	9.71	2.33					
15	1.17	0.86					
16	36.53	23.49	6.38				
17		1.13					
18	13.79	48.14	26.90	2.55	7.93		
20		10.17	6.24	2.55	8.95	8.96	
22		0.75	TR	TR	TR	4.04	13.54

TR = Trace
Number of Salmon = 8

Table 35. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of Salmon Swimming for 24 Hours from Comparable Amounts Derived from Control Salmon (Velocity: 59 cm/sec. Table 34 minus Table 8).

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	-1.33	—					
15	—	—					
16	-8.68	-5.94	-3.74				
17		—					
18	-2.64	-11.41	-4.85	-0.74	—		
20		-2.48	-1.51	—	-1.30	-1.76	
22		—	—	—	—	-0.73	—

Table 36. Significant Differences Obtained by Subtracting Amounts of Fatty Acid Methyl Esters of All Salmon Swimming at Velocity of 59 cm/sec from Comparable Amounts from Control Salmon.

Number of Carbons	Double Bonds						
	0	1	2	3	4	5	6
8	—						
10	—						
12	—						
14	0.57	—					
15	—	—					
16	0.94	0.16	-0.79				
17		—					
18	0.37	-0.09	1.84	-0.16	0.67		
20		0.59	0.74	—	1.15	0.74	
22		—	—	—	—	-0.15	2.40

Table 37. Average Values for Length, Weight, Weight of Lipid, Percent of Lipid, and Weight of Fatty Acid Methyl Esters of Coho Salmon Used for Swimming Performance Experiments

Swimming Velocity cm/sec	Length cm	Weight grams	Weight of Lipid mg	Weight of Lipid %	Weight of Ester mg
		Control Salmon			
0	8.06	5.22	233	4.38	189.2
		Salmon Swimming Less than 24 Hours			
52	7.79	4.16	177	4.20	145.0
54	7.60	4.40	187	4.58	152.4
56	7.94	4.58	224	4.83	174.7
59	7.82	4.48	203	4.45	161.8
		Salmon Swimming for 24 Hours			
52	7.98	4.44	180	4.07	150.3
54	7.98	4.69	238	5.06	195.8
56	8.30	5.51	313	5.75	247.6
59	8.25	5.14	299	5.82	237.1
		Total Salmon Swimming			
52	7.91	4.39	179	4.10	148.8
54	7.74	4.28	206	4.76	168.7
56	8.03	4.81	245	5.04	191.5
59	7.90	4.74	223	4.74	177.7

24 hours or until they ceased to swim.

When a salmon ceased to swim, (or after 24 hours), it was removed from the swimming chamber and its swimming time, length, and wet weight recorded and the fatty acid composition determined. Intercurrent technical and analytical problems interfered with collection of complete data on each of the salmon originally selected thereby preventing an even distribution of the performance tests among the velocities. Only those salmon on whom complete data were obtained were used for this study.

Control Salmon

Two salmon were taken at random from each of 16 swimming performance experiments for a total of 32 controls. The average weight of the 32 control salmon was 5.22 grams and the average length was 8.06 cm. The average total lipid was 233 mg and the lipids averaged 4.38% of the wet weight of the salmon. The average amount of total derived fatty acid methyl esters was 189 mg per salmon.

Twenty-seven fatty acids were identified in the lipids of all salmon whether control or exercised and, in the controls, ranged in quantity from trace amounts (22:4) to an average of 19.3% (18:1) of the total fatty acids. Table 7 gives the mean percentage contribution

of the individual fatty acids to the total fatty acids of the control salmon. Table 8 gives the mean amounts of individual fatty acid methyl esters derived from control salmon. Saturated fatty acids with 8, 10, 12, and 15 carbons occurred in small amounts and together only constituted approximately 1% of the total acids. Fatty acids with 14, 16, and 18 carbons constituted the major portion of the saturated acids with the 16 carbon acid occurring in the greatest amount.

Monene acids ranged from less than 1% for 15 and 22 carbon acids to a maximum of 19% for the 18 carbon acid. In the diene acids, the 18 carbon acid was again the most abundant. Of three triene acids, the most abundant had 20 carbons. Among the tetraenes, acids with 18 and 20 carbons occurred in nearly equal percentages. Of pentenes with 20 and 22 carbon acids, the most abundant was the 20 carbon acid. One hexene contained 22 carbons and constituted 7% of the total fatty acids. There was a general tendency for the unsaturated compounds to have relatively higher percentages of longer chains as the number of double bonds increased (Table 8).

Salmon Swimming at 52, 54, 56, and 59 cm/sec.

Groups of 28, 16, 26, and 38 salmon were forced to swim against water velocities of 52, 54, 56 and 59 cm/sec respectively. In each group, some salmon were unable to swim for 24 hours and

some salmon were still swimming at 24 hours. The percentage of failing salmon for water velocities of 52, 54, 56, and 59 cm/sec were 29, 62, 77 and 79% respectively. Conversely, 71, 38, 23, and 21% respectively were still swimming at 24 hours.

Salmon swimming less than 24 hours. The 8.06 cm average length of control salmon was greater than the average length of failing salmon at all velocities. The average lengths were 7.79 cm, 7.60 cm, 7.94 cm, and 7.82 cm respectively for water velocities of 52, 54 56, and 59 cm/sec. The average length of failing salmon at 54 cm/sec differed the most from the average length of control salmon. This maximum difference was 5% of the control value. The differences between swimming and control salmon were statistically significant at the 1% level for velocities of 52, 54, and 59 cm/sec; however, at 56 cm/sec the difference was not statistically significant.

The 5.22 gram average weight of control salmon was greater than the average weight of failing salmon at all velocities. The average weights of the salmon failing to swim for 24 hours were 4.16, 4.40, 4.58, and 4.48 grams respectively for water velocities of 52, 54, 56, and 59 cm/sec. The average weight of failing salmon at 52 cm/sec differed the most (20%) from control salmon while failing salmon at 56 cm/sec differed the least, but still 12% from control salmon. The differences between swimming and control salmon were statistically significant at the 1% level for all velocities.

The 233 mg average amount of lipid for control salmon was greater than the average amount for failing salmon at all velocities. The average amount of lipids were 177, 187, 224, and 203 mg, respectively, for water velocities of 52, 54, 56, and 59 cm/sec. The average amount of lipid for failing salmon at 52 cm/sec differed the most from control salmon while the amount of lipid for failing salmon at 56 cm/sec differed the least from control salmon. The differences between swimming and control salmon were statistically significant at the 1% level for water velocities of 52, 54, and 59 cm/sec; however, the difference was not statistically significant at velocity of 56 cm/sec.

The 4.38% average lipid content of control salmon was greater than the 4.20% of failing salmon at 52 cm/sec; however, the average lipid contents of failing salmon at 54, 56, and 59 cm/sec, respectively, were 4.58%, 4.83%, and 4.45%. The differences between swimming and control salmon were not statistically significant at the 1% level for water velocities of 52, 54, and 59 cm/sec; however, at 56 cm/sec the difference was statistically significant.

The 189 mg average amount of fatty acid methyl esters for control salmon was greater than the average amounts for failing salmon at all velocities. The average amounts for failing salmon

were 145, 152, 175, and 162 mg, respectively for water velocities of 52, 54, 56, and 59 cm/sec (Table 37). The differences between swimming and control salmon were statistically significant at the 1% level for water velocities of 52, 54, and 59 cm/sec; however, at 56 cm/sec the difference was not significant.

Failing salmon at 52, 54, and 59 cm/sec differed significantly from control salmon in that they had shorter lengths, smaller weights, and smaller amounts of lipids and of fatty acids. The salmon failing at 56 cm/sec were similar to controls with respect to length, amount of lipids, and amount of fatty acids; these salmon differed significantly from controls in body weight and lipid percent.

Salmon swimming for 24 hours. The 8.06 cm average length of control salmon was greater than the average lengths of salmon swimming for 24 hours at 52 and 54 cm/sec, but less than the average lengths of salmon swimming for 24 hours at 56 and 59 cm/sec. The average lengths for salmon swimming for 24 hours were 7.98 cm, 7.98 cm, 8.30 cm, and 8.25 cm, respectively for water velocities of 52, 54, 56, and 59 cm/sec. The differences between swimming and control salmon were statistically significant at the 1% level for velocities of 56 and 59 cm/sec, but the differences were not significant for velocities of 52 and 54 cm/sec.

The 5.22 gram average weight of control salmon was greater than the average weight of salmon swimming for 24 hours at 52, 54, and 59 cm/sec, but less than the average weight of salmon swimming for 24 hours at 56 cm/sec. The average weights of salmon swimming for 24 hours were 4.44, 4.69, 5.51, and 5.14 grams, respectively, for water velocities of 52, 54, 56, and 59 cm/sec. The average weight of swimming salmon at 52 cm/sec differed the most (15%) from control salmon, while salmon swimming at 59 cm/sec differed the least (2%) from control salmon. The average weight of salmon swimming at 56 cm/sec was 6% greater than the average weight of control salmon. The differences between control and swimming salmon was statistically significant at the 1% level for the water velocity of 52 cm/sec; however, the differences were not statistically significant at velocities of 54, 56, and 59 cm/sec.

The 233 mg average amount of lipid for control salmon was greater than the average amount for salmon swimming for 24 hours at 52 cm/sec, but less than the average amounts for salmon swimming for 24 hours at 54, 56, and 59 cm/sec. The average amounts of lipids were 180 mg, 238 mg, 313 mg, and 299 mg for salmon swimming for 24 hours, respectively, at 52, 54, 56, and 59 cm/sec. The salmon swimming at 52 cm/sec when compared with controls showed a 23% less lipid while salmon swimming at velocities 54, 56,

and 59 cm/sec had increased amounts of lipids. The difference between control and swimming salmon was not statistically significant at the 1% level for water velocity of 54 cm/sec; however, the differences were statistically significant at velocities of 52, 56, and 59 cm/sec.

The 4.38% average lipid content for control salmon was greater than the 4.07 of the salmon swimming for 24 hours at 52 cm/sec; however, the average lipid contents of salmon swimming for 24 hours at 54, 56, and 59 cm/sec, respectively, were 5.06%, 5.75%, and 5.82%. The differences between control and swimming salmon were statistically significant at the 1% level for water velocities of 56 and 59 cm/sec, but were not statistically significant for velocities of 52 and 54 cm/sec.

The 189 mg average amount of fatty acid methyl esters for control salmon was greater than the average amounts for salmon swimming for 24 hours at 52 cm/sec, but less than the amounts for salmon swimming for 24 hours at 54, 56, and 59 cm/sec. The average amounts of fatty acid methyl esters for salmon swimming 24 hours were 150 mg, 196 mg, 248 mg, and 237 mg, respectively, at 52, 54, 56, and 59 cm/sec (Table 37). The difference between control and swimming salmon was not statistically significant for water velocity 54 cm/sec; however, the differences were statistically significant for velocities of 52, 56, and 59 cm/sec.

Total Salmon Swimming

Within the limits of cognizance, the salmon were randomly distributed between controls and salmon swimming at the four selected velocities. An examination of the data on length may allow an evaluation of randomness with respect to the eight categories of swimming salmon. It seems unlikely that body length would be changed by one day of exercise as length is primarily related to bone and cartilage structure. The mean lengths obtained in five out of eight of the swimming categories differed significantly from the control lengths at the 1% level. Thus, on the basis of length, at least five of the eight swimming populations were not random selections from the same population as the controls (Table 37).

If the salmon used were considered to have been divided into five subpopulations (control and four swimming velocities), the fish chosen to swim at 52, 56, and 59 cm/sec can be considered as random variations of the control population, but the group chosen for 54 cm/sec was significantly different from controls even at the 1% level. However, data were not obtained on all salmon chosen for 54 cm/sec, therefore, consideration of this group can be deferred.

Since, on the basis of length, the control salmon and the salmon placed to swim at 52, 56, and 59 cm/sec can be considered as random samples of the same population, but the mean lengths of

the salmon swimming 24 hours and those unable to swim differed significantly from each other, one or more factors in addition to the metabolism of exercise were involved. The length and time data indicate that at each velocity, the exercise problem divided the salmon into a group that was able to swim at a given velocity for short periods of time and a group that was able to swim for longer periods of time. The shorter and lighter salmon were less competent at a given velocity than were the longer and heavier salmon.

Since selection for size, the metabolism during exercise, and perhaps even other factors contribute to the differences (implicit in Table 9-35) between controls and swimmers, and to the differences among the swimming groups, an evaluation of the effects of exercise is difficult to obtain. Tables 9-35, however, have been included because they are valid descriptions of the fatty acid status of the different sample populations of salmon, and valid descriptions of the differences between these populations even if the factors inducing the differences are complex.

In order to evaluate the changes during exercise, the failing salmon and salmon swimming for 24 hours have been combined to form a single population and information has been presented for total salmon swimming at each of the selected velocities. When failing salmon and salmon that were able to swim for 24 hours were

combined, differences in lengths between controls and swimming salmon at the water velocities of 52, 56 and 59 cm/sec were not significant at the 1% level. Therefore, combination of the data eliminated some of the selection problem induced by swimming and allowed the salmon to be compared on the basis of a sample population of controls and sample populations that were forced to swim but with no consideration of the swimming ability of individuals within a sample (Table 37).

Comparison of Control and Swimming Salmon

The 5.22 gram average weight of control salmon was greater than the average weight of salmon swimming at all velocities. The average weights of swimming salmon were 4.39, 4.28, 4.81, and 4.74 grams, respectively, for water velocities of 52, 54, 56, and 59 cm/sec. The differences between control and swimming salmon were statistically significant at the 1% level for water velocities of 52 and 54 cm/sec, but the differences were not statistically significant for velocities of 56 and 59 cm/sec.

The 233 mg average amount of lipid for control salmon was greater than for salmon swimming at water velocities of 52, 54, and 59 cm/sec, but less than the amount of salmon swimming at 56 cm/sec. The average amounts of lipids were 179, 206, 245, and

223 mg, respectively, for velocities of 52, 54, 56, and 59 cm/sec. The differences between control and swimming salmon were statistically significant at the 1% level for water velocities of 52 and 54 cm/sec, but the differences were not statistically significant for velocities of 56 and 59 cm/sec.

The 4.38% average lipid content of control salmon was greater than the average 4.10% of salmon swimming at 52 cm/sec, but less than the 4.76%, 5.04%, and 4.74%, respectively, for salmon swimming at 54, 56, and 59 cm/sec. The differences between control and swimming salmon were not statistically significant at the 1% level for water velocities of 52, 54, and 59 cm/sec; however, the difference was statistically significant for the velocity of 56 cm/sec.

The 189 mg average amount of fatty acid methyl esters for control salmon was greater than the average amounts for salmon swimming at velocities of 52, 54, and 59 cm/sec, but less than the amounts for salmon swimming at 56 cm/sec. The average amounts of fatty acid methyl esters were 149, 169, 192, and 178 mg, respectively, for salmon swimming at 52, 54, 56, and 59 cm/sec. The difference between control and swimming salmon was statistically significant at the 1% level for water velocity 52 cm/sec; however, the differences were not statistically significant for velocities of 54, 56, and 59 cm/sec.

Differences in Fatty Acids Induced by Swimming at 52 cm/sec.

Table 15 gives the significant differences obtained on subtracting amounts of fatty acid methyl esters of all salmon swimming at velocity of 52 cm/sec from comparable amounts for control salmon. The total loss of fatty acid methyl esters for salmon swimming at 52 cm/sec was 40.4 mg. The amounts of saturated fatty acids with 14, 16, and 18 carbons were significantly different from comparable amounts in controls. The total loss in saturated fatty acids was 10.4 mg or 27.5% of the total loss in fatty acids for salmon swimming at 52 cm/sec.

Monene fatty acids accounted for 15.5 mg or 41% of the total loss. Fatty acids with 16, 18, and 20 carbons were significantly different from controls and the decrease for the 18 carbon acid was 8.4 mg. In the diene acids, the 18 carbon acid again showed the largest decrease from controls. For the diene acids, the total loss was 4.5 mg or 11.8% of the total. The triene acids were not significantly different from controls.

Among the tetraenes, the decrease was 3.4 mg or 8.9% of the total. The 18 carbon acid decreased the most. Of pentenes with 20 and 22 carbons, the 20 carbon acid decreased the most. The total decrease for the pentenes was 3.1 mg or 8.3% of the total. One hexene with 22 carbons decreased 0.6 mg or only 1.6% of the total.

There was a general tendency for the greatest decrease to occur in the acids with 16 and 18 carbons. Saturated fatty acids and monene fatty acids accounted for 68.5% of the total decrease. Fatty acids with 22 carbons decreased the least amount.

Differences in Fatty Acids Induced by Swimming at 54 cm/sec

Table 22 gives the significant differences obtained by subtracting amounts of fatty acid methyl esters of all salmon swimming at velocity of 54 cm/sec from comparable amounts for control salmon. The total loss of fatty acid methyl esters for salmon swimming at 54 cm/sec was 20.5 mg. The amounts of saturated fatty acids with 14, 16, and 18 carbons were significantly different from comparable amounts in controls. The total loss in saturated fatty acids was 5.4 mg or 27% of the total loss in fatty acids for salmon swimming at 54 cm/sec.

Monene fatty acids accounted for 7.4 mg or 36.8% of the total loss. Fatty acids with 16, 18, and 20 carbons contributed to the decrease with the 18 carbon acid decreasing the most. For the diene acids, the 18 carbon acid decreased 1.2 mg while the 20 carbon acid increased 0.4 mg. The triene acids were not significantly different from controls.

Among the tetraenes, the decrease was 3.4 mg or 17.0% of the

total. The 18 carbon acid again was decreased the most. Of pentenes with 20 and 22 carbons, the 20 carbon acid decreased the most. The total decrease for the pentenes was 1.3 mg or 6.8% of the total. One hexene with 22 carbons decreased 1.3 mg or 6.8% of the total. For salmon swimming at 54 cm/sec, the general tendency again was for the greatest decrease to occur in the acids with 16 and 18 carbons. Saturated fatty acids and monene fatty acids together accounted for 63.8% of the total acids lost.

The average swimming time for salmon at 52 cm/sec was 1141 minutes and the average total fatty acids lost during swimming was 37.7 mg when compared with controls. The average swimming time for salmon at 54 cm/sec was 645 minutes and the average total fatty acids lost during swimming was 20.1 mg when compared with controls. The ratio of swimming time for salmon swimming at 52 and 54 cm/sec was 1.8:1 and the ratio of fatty acids lost by swimming was 1.9:1. (It should be noted that all fish had been forced to swim for 1200 minutes at 8 cm/sec before swimming at the experimental velocities).

Differences in Fatty Acids Induced by Swimming at 56 cm/sec

Table 29 gives the significant differences obtained by subtracting amounts of fatty acid methyl esters of all salmon swimming at 56 cm/sec from comparable amounts for control salmon. The

total difference in fatty acid methyl esters for salmon swimming at 56 cm/sec was 2.3 mg with the value for swimmers higher than for controls. Individual fatty acids, in most instances, were present in higher amounts in swimmers than in controls. Differences between swimming and control salmon were less than 1 mg for all fatty acids except 18:2 whether the difference was positive or negative. Acid 18:2 was 1.1 mg higher in swimmers than in controls.

The total excess of fatty acids was 4.5 mg for those present in greater amounts in swimmers; and the total excess of fatty acids was 1.1 mg for those in greater amounts in controls. Thus, a net excess of 3.4 mg over controls was found in salmon swimming at 56 cm/sec. An excess cannot be explained on the basis of lipid losses in metabolism during exercise. The significantly higher total lipid contents for swimming salmon also needs further explanation. Failing salmon at 56 cm/sec were longer, heavier, and contained a higher lipid percentage than the other categories of failing salmon. Salmon swimming for 24 hours at 56 cm/sec were again longer, heavier, and contained a higher lipid percentage than the other categories of salmon swimming for 24 hours. Also, the average for all salmon swimming at 56 cm/sec was higher than the lipid percentage of controls.

Differences in Fatty Acids Induced by Swimming at 59 cm/sec

Table 36 gives the significant differences obtained by subtracting amounts of fatty acid methyl esters of all salmon swimming at 59 cm/sec from comparable amounts from control salmon. The total loss of fatty acid methyl esters for salmon swimming at 59 cm/sec was 11.5 mg. The amounts of saturated fatty acids with 14, 16, and 18 carbons were significantly different from comparable amounts in controls. The total loss in saturated fatty acids was 1.9 mg or 18.5% of the total loss in fatty acids for salmon swimming at 59 cm/sec.

Monene acids accounted for 7.4% of the loss. Fatty acids with 16 and 20 carbons decreased while the fatty acid with 18 carbons increased slightly. In the diene acids, the 18 carbon acid decreased 1.8 mg and the total loss for the diene acids was 25.4% of the total. One triene acid with 18 carbons increased by 0.2 mg.

The 18 and 20 carbon tetraene acids accounted for 18% of the total decrease in fatty acids while the 20 and 22 carbon pentene acids accounted for 7.3% of the total fatty acid loss. One hexene acid with 22 carbons decreased 2.4 mg or 23.6% of the total loss. (Elimination of differences not statistically or technically significant contribute to rounding-out errors so that the sum of the fatty acid difference was only 9.13 mg while the total difference on direct subtraction was 11.5 mg).

DISCUSSION

A major portion of this study was devoted to the identification of the fatty acids in salmon lipids. Subsequent to the identification of the salmon fatty acids, alterations in fatty acids produced by exercise were studied. Many questions were raised by the data presented and only a few are discussed here.

Purity of Standard Fatty Acids

Identification of saturated salmon fatty acids proceeded without complications since all straight chain saturated fatty acids containing from 3 to 30 carbons had been synthetically prepared (65). Thus reliable data on physical properties for specific saturated fatty acids are available in the literature (65). Hence the degree of purity of fatty acid standards can readily be established. Currently the requirement for a high degree of purity of a fatty acid is the presence of only one peak when a chromatogram of the fatty acid is obtained by gas chromatography. Seven unsaturated fatty acid standards were available from the Hormel Institute. The stated purities of the Hormel standards ranged between 90% and 99% and the position of the double bonds was not given. Chromatographic analysis revealed that each unsaturated fatty acid standard produced only one peak, indicating a high percentage of purity for each compound.

Methylene Interruption of Double Bonds

Unsaturated salmon fatty acids required extensive chemical and chromatographic analysis in order to determine the positions of the double bonds. Reductive ozonolysis (75) of available standards and salmon unsaturated acids permitted the identification of the terminal aldehydes and aldehyde-esters formed and thus provided information concerning the position of the first and last double bond in the unsaturated acids. The formulations adopted for the available Hormel unsaturated standards and salmon unsaturated fatty acids were consistent with a methylene interruption between double bonds when two or more were present in a fatty acid. However, proof of methylene interruption has not been obtained and will probably await the development of methods allowing good recovery of formaldehyde, glyoxal, malondialdehyde, and succinaldehyde on reductive ozonolysis and of carbon dioxide, oxalic acid, malonic acid, and succinic acid on oxidative ozonolysis. Statements will have to be made that the methods used could identify these substances if present and that they were looked for and only malondialdehyde or malonic acid were found before it can be proven that only methylene interruption occurs in salmon fatty acids with multiple double bonds.

Comparative Distribution of Fatty Acids in Fish

Roubal (68) found 19 fatty acids in the tuna, 8 less than the number here identified for coho salmon. The fatty acids identified in the salmon that were not found in the tuna by Roubal were 8:0, 10:0, 12:0, 14:1, 15:1, 17:1, 20:2, and 20:3. Ackman and Sipos (9) found 34 fatty acids in pilchard oil or 7 more than the number identified for coho salmon. Fatty acids reported for pilchard oil that were different from the lipids of coho salmon were 16:3, 16:4, 17:0, 19:0, 19:1, 20:0, 21:5, 22:0, 24:1, 24:5, 24:6. Ackman and Sipos indicated that 3 of the fatty acids occurred in much less than 0.1% and that they were possibly present. Eight fatty acids were listed as being present but occurring in trace amounts or less than 0.1%. Seven unsaturated fatty acids had question marks placed next to them, apparently indicating uncertainty in their identification. Fatty acids identified in coho salmon that were not found in pilchard oil by Ackman and Sipos were 8:0, 10:0, 22:2, and 22:3.

Gruger, Nelson, and Stansby (34) found 24 fatty acids in coho salmon as compared to 27 fatty acids identified in coho salmon in this study. Gruger, Nelson, and Stansby did not report any 8:0, 10:0, 12:0, or 14:1, and question marks were placed by fatty acids 15:1, 20:2, 20:3, 22:1, 22:4, and 24:1.

The uncertainty in the identification of some of the fatty acids

reported by Ackman and Sipos, and Gruger, Nelson, and Stansby might be explained by the use of semilog plots as the primary method of identification. Ackman and Sipos list more than one value for 11 fatty acids, apparently signifying the occurrence of more than one peak; they further suggest that an additional 5 fatty acids may have more than one isomer present. It is probable that isomerization may have occurred during preparation of the pilchard oil.

Fatty Acids with Odd-Numbers of Carbons

Fatty acids containing 15 and 17 carbons were first reported by Morice and Shorland (59) in the liver lipids of shark. Ackman and Sipos (10) found fatty acids containing 13, 15, 17 and 19 carbons in the lipids of certain marine organisms. In the mullet, Sen and Schlenk (70) reported more than 25% of the fatty acids contained 15, 17, and 19 carbons in their chains. In this study, fatty acids containing 15 and 17 carbons were identified. Differences in the odd numbered fatty acids present in fish may possibly be due to species, diet, temperature, habitat, and other undetermined factors.

Octadecadienoic Acids

Ralston (65) stated that the relationship which exists between linoleic acid in vegetable fats and that in marine and animal fats had not been definitely established and that the occurrence of

octadecadienoic acids other than 9, 12-octadecadienoic seemed probable. Rouba (68) only listed one value for 18:2 in tuna fatty acids; Gruger, Nelson, and Stansby (34) only listed one value for 18:2 in the oils of the 21 species of fish and shellfish they examined. Thus only one isomer is suggested in each of these papers. Neither Roubal nor Gruger, Nelson and Stansby assigned positions for the double bonds in 18:2.

Ackman and Sipos (9) in their study of pilchard oil gave two values for 18:2. Ackman and Sipos suggested that 18:2 ω6, Δ9 represented 1.3% of the fatty acids in pilchard oil and that 18:2 ω9, Δ6 was possibly present to an extent less than 0.1%. Ackman and Burgher (4) suggest that 18:2 ω9, Δ6 existed in turtle oil, but gave no value for the amount of 18:2 ω9, Δ6 in their table of turtle oil fatty acids. Adjacent to the tabular listing of 18:2 ω9, Δ6, was the statement that no evidence existed for this material. Hence, the significant of 18:2 ω9, Δ6 in turtle oil was not clear.

In this study of the fatty acids of coho salmon, only one peak was observed for 18:2. Ozonolytic procedures identified the 18:2 in salmon lipids as 18:2 ω6, Δ9.

Total Lipid Alterations of Salmon in Laboratory

Salmon used for exercise experiments were retained in the

laboratory and fed tubificid worms. The minimum length of time was 8 days and the maximum length of time was 33 days. During the time the salmon were retained in the laboratory, their total lipids increased. The average time in the laboratory for control salmon was 18 days, and their average lipid content was 4.38%. Figure 11 gives the relationship between total lipid content for control salmon and the length of time they were retained in the laboratory. The average length of time that swimming salmon were retained in the laboratory for velocities of 52, 54, 56, and 59 cm/sec were 14, 15, 25, and 17 days respectively. The average time in the laboratory for salmon swimming at 56 cm/sec was 25 days or 7 days longer than the average of 18 days for controls. Figure 11 gives the regression line developed for the total lipid percentage of control salmon as influenced by the number of days retained in the laboratory. Inspection of Figure 11 suggests that control salmon retained in the laboratory for 25 days would have a total lipid content of 5.30%. Thus, one factor inducing a high lipid content for salmon swimming at 56 cm/sec was the long time they were retained in the laboratory. Thus the metabolism of exercise, selection for long and short fish, and time of holding in the laboratory were involved in the differences between control and salmon exercised at 56 cm/sec water velocity.

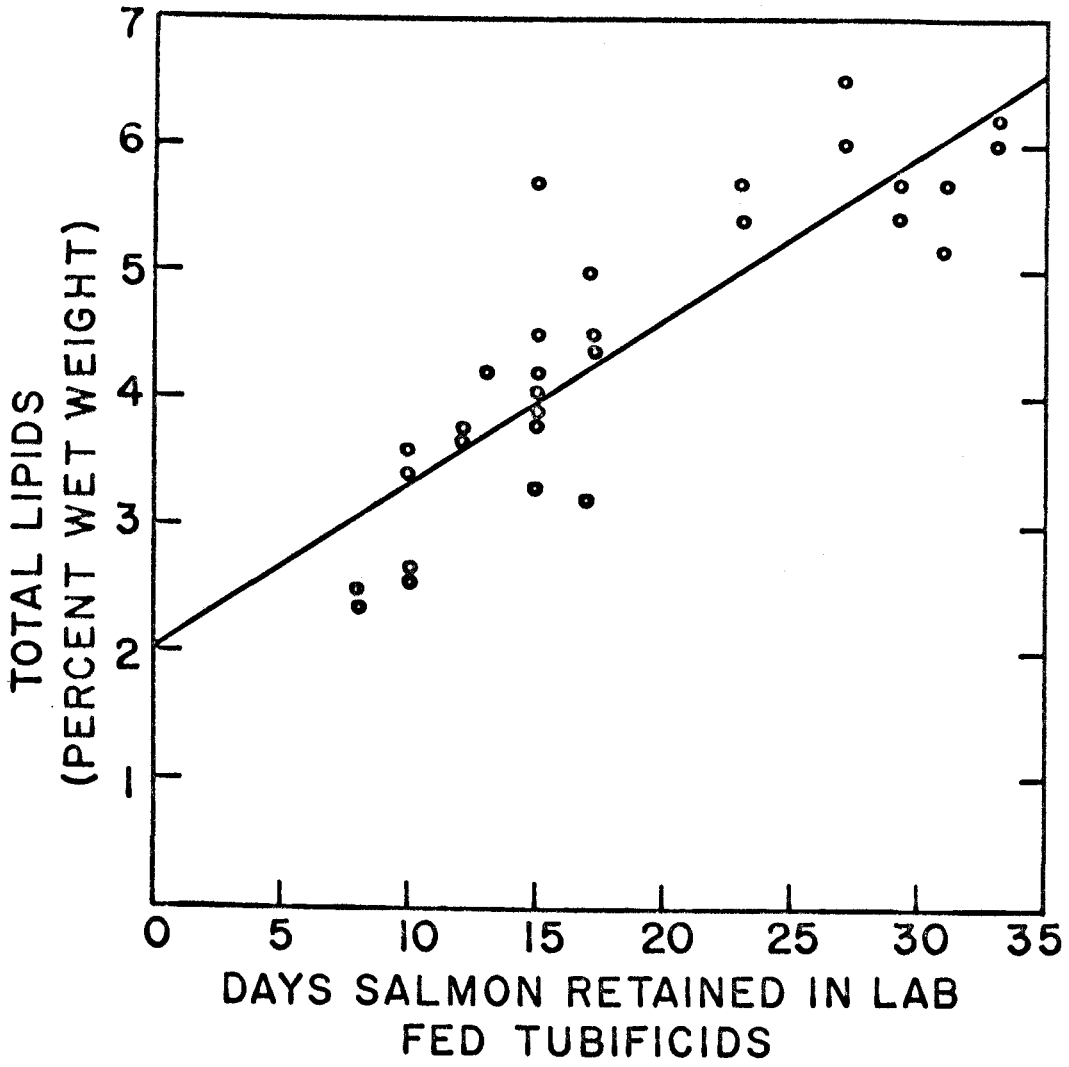

Figure 11. The relation between percent of total lipids and the number of days that control salmon were retained in the laboratory.

Average Distance Traveled by Swimming Salmon

All salmon used in this study were forced to swim for 1200 minutes at 8 cm/sec and 60 minutes at 15 cm/sec; then, the salmon were forced to swim against test velocities of 52, 54, 56 and 59 cm/sec for a maximum of 24 hours. At each test velocity, some salmon failed to swim for the maximum 24 hours and some salmon were able to swim for the maximum 24 hours. The average swimming times in minutes for salmon swimming at velocities of 52, 54, 56, and 59 cm/sec were 1141, 645, 469, and 398 respectively.

The average distances traveled for salmon swimming at 52, 54, 56, and 59 cm/sec were 26.0, 16.9, 13.7, and 12.7 miles respectively. (These distances include approximately 3.6 miles at 8 cm/sec and 0.3 miles at 15 cm/sec). Thus, the total metabolic cost for salmon swimming at 52 cm/sec could be expected to exceed the total metabolic cost of salmon swimming at 59 cm/sec because the average distance traveled by salmon swimming at 52 cm/sec was more than twice the average distance traveled by salmon swimming at 59 cm/sec. An increase in velocity from 52 to 59 cm/sec reduced the average distance traveled by 52%, because average swimming time was reduced from 1141 to 398 minutes.

Average Weight Loss by Swimming Salmon

The average weights of swimming salmon were less than the average weight of control salmon for water velocities of 52, 54, 56, and 59 cm/sec (Table 37).

The average weight loss for all salmon swimming at 52 cm/sec was 830 mg and the average weight loss for all salmon swimming at 59 cm/sec was 480 mg (weight loss obtained by subtracting the weight of swimming salmon from the weight of control salmon). Hence, the weight loss for salmon swimming at 52 cm/sec was approximately two times the weight loss for salmon swimming at 59 cm/sec. Salmon swimming at 52 cm/sec traveled approximately 26.0 miles and the salmon swimming at 59 cm/sec traveled only 12.7 miles.

The interpretation of the weight loss for salmon swimming at 54 and 56 cm/sec is complicated. Salmon swimming at 54 cm/sec were significantly shorter than control salmon. Therefore, the initial weight of salmon selected to swim at 54 cm/sec could be expected to be less than the weight of control salmon. On the basis of length, salmon swimming at 56 cm/sec can be considered as from the same population as controls; however, salmon selected for swimming at 56 cm/sec had been retained in the laboratory for a longer period of time during which the lipid percentage increased

(Figure 11). Hence, the weight of salmon before swimming at 54 cm/sec was probably less than controls and the initial weight of salmon before swimming at 56 cm/sec was more than controls.

Fatty Acid Loss by Swimming Salmon

The average amounts of total fatty acids for swimming salmon at 52 54, and 59 cm/sec were less than the amount of total fatty acids for control salmon. The amount of total fatty acids for salmon swimming at 56 cm/sec exceeded the amount of total fatty acids of control salmon.

The total loss of fatty acids for salmon swimming at 52 and 59 cm/sec (total amount of fatty acids for swimming salmon subtracted from total amount of fatty acids for control salmon) was 40 mg and 11 mg respectively. The weight loss and average distance traveled for salmon swimming at 52 cm/sec was approximately two times the weight loss and average distance traveled for salmon swimming at 59 cm/sec but the fatty acid loss for salmon swimming at 52 cm/sec was nearly four times the fatty acid loss for salmon swimming at 59 cm/sec.

Saturated fatty acids accounted for 27.5% of the total fatty acid loss for salmon swimming at 52 cm/sec while the saturated fatty acid loss for salmon swimming at 59 cm/sec accounted for

only 18% of the total loss (saturated fatty acids in control salmon were 26.6% of the total). The relationship suggests that salmon swimming for shorter distances at high velocities preferentially metabolize a higher percentage of unsaturated fatty acids.

Figure 12 gives the relationship between fatty acid content and the number of days control salmon were retained in the laboratory. Salmon swimming at 56 cm/sec were retained in the laboratory for an average of 25 days. Inspection of Figure 12 suggests that the average fatty acid content for salmon selected to swim at 56 cm/sec would have been approximately 250 mg before swimming and thus somewhat greater than the 189 mg average for control salmon. Thus, the higher level of fatty acids noted for salmon swimming at 56 cm/sec as compared with controls does not represent an exercise effect and the analysis of the fatty acid alterations for salmon swimming at 56 cm/sec cannot easily be developed.

The average 7.7 cm length for salmon swimming at 54 cm/sec is significantly different from the control salmon and other categories of swimming salmon. Hence, it can be assumed that the initial weight (before swimming) and the initial amount before swimming of fatty acids for salmon selected to swim at 54 cm/sec would have been less than comparable values in controls. Thus, evaluation of the effects of exercise for salmon swimming at 54 cm/sec is

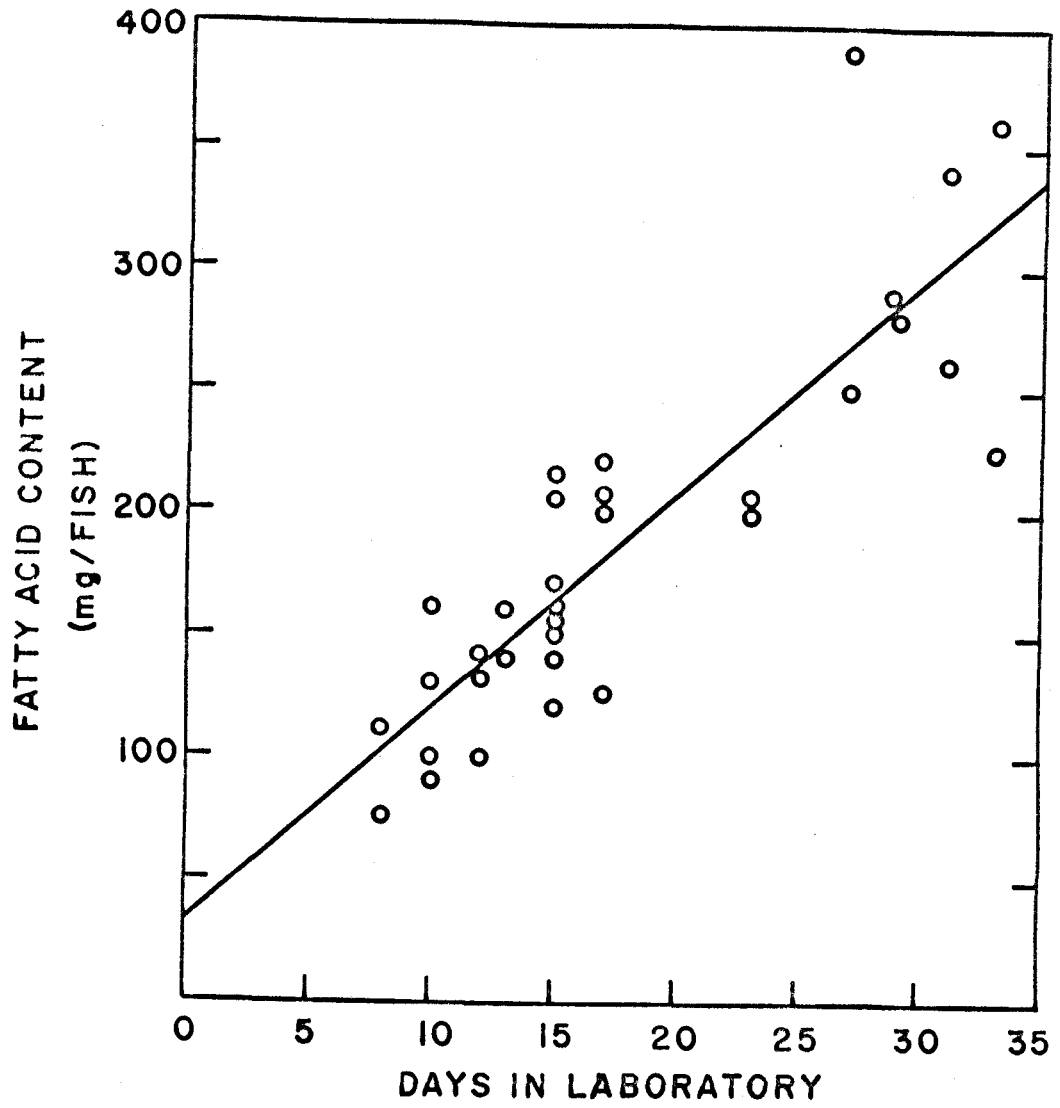

Figure 12. The relation between the total fatty acid content and the number of days that control salmon were retained in the laboratory.

difficult and will not be further attempted in this thesis.

Analysis of Metabolic Costs

Brett (21) has provided information relating swimming velocity and oxygen consumption for sockeye salmon. From this information, it is possible to calculate the expected heat production and obtain estimates of material losses with reasonable reliability for coho salmon forced to swim at high velocities.

From Figure 2 of the paper by Brett (21) the estimated oxygen consumption for a 5.2 gram salmon swimming against a water velocity of 8 cm/sec for 20 hours would be 18.2 mg and for one hour at 15 cm/sec would be 1.0 mg. For 1141 minutes at 52 cm/sec the oxygen requirement would be 79.1 mg and for 398 minutes at 59 cm/sec, 34.5 mg. Thus, the average oxygen consumptions for the fish swimming at 52 cm/sec should equal 98.4 mg (18.2 + 1.0 + 79.1) and at 59 cm/sec, 53.7 mg (18.2 + 1.0 + 34.5). Since 1 mg of oxygen will burn 0.376 mg (3.5/9.3) of lipid, the lipid equivalents of the total estimated oxidative costs for exercise are 37.0 mg at 52 cm/sec and 20 mg at 59 cm/sec. The observed lipid losses noted were 54 mg at 52 cm/sec and 10 mg at 59 cm/sec and are considerably higher at 52 cm/sec than calculated values even when all oxidative losses are attributed to lipids.

Analysis of Material Losses During Exercise

At 52 cm/sec the weight loss for swimming salmon was 830 mg and at 59 cm/sec was 480 mg. Of the total loss, lipids represent 54 and 10 mg respectively. Losses other than lipids were 776 mg and 470 mg and possibly involved water, protein, carbohydrate, and salt modifications. In general, the water content of juvenile salmonid type fish is approximately 80% (62). Thus, of the 830 mg and 480 mg total losses, approximately 80% or 664 and 384 mg would have been water. On subtracting both water and lipid losses, the protein plus carbohydrate would be approximately 112 mg and 86 mg respectively. The lipid losses represent 502 and 93 calories, the protein plus carbohydrate losses approximately 616 and 473 calories, and the total caloric losses were 1,118 and 566 calories at 52 and 59 cm/sec respectively. Similar losses calculated from the oxygen consumption data of Brett (21) on Oncorhynchus nerka give 344 and 188 calories respectively.

The caloric costs as estimated from material losses are three times as great as the losses calculated from the oxygen consumption data of Brett. The species differences and possible errors in materials since losses are not measured within animals but between controls and exercised salmon, account for some of the differences. The possibility of greater water losses and some salt

loss also exists. Some additional material losses may be attributed to excretion of noncombusted metabolites via the gills and kidneys. However, even gross errors cannot account for the entire differences.

In the procedure of Brett (21) the salmon swam a maximum distance of 5.2 miles with approximately one-half of the distance at velocities below 45 cm/sec and approximately one-quarter each at velocities of 52 and 60 cm/sec. In this study, the average swimming distance for salmon selected to swim at 52 cm/sec was 26.0 miles and at 59 cm/sec was 12.7 miles with three miles of the distance for both at low velocities. The caloric data calculated from the oxygen consumption graph of Brett (21) and from material losses have taken time, distance, and velocity factors into consideration. However, the oxygen consumption data of Brett were collected during one, and at the most, two hours at high velocity. It is still a moot question as to whether oxygen consumption during a third, a fifth, or tenth, or even 24th hour of swimming at high velocities would be the same, or more, or less, than the oxygen consumption during the first hour. However, it seems reasonable that when a maximum effort is involved, that each succeeding mile and each succeeding hour is more difficult and more costly to the salmon.

In general, Brett (21) found a ratio for maximum active metabolism to standard metabolism of 8:1 for a 5 gram fish. If

the data in this study on material balance are supported by bio-energetic data, one presumes that for prolonged exercise the ratio of active to standard metabolism may even reach a value of 24:1 if computed on the basis of calories lost.

SUMMARY

The fatty acids of coho salmon were identified and then a study was conducted to determine the effects of exercise on the fatty acids of salmon between 75 mm and 85 mm in length, and forced to swim against water velocities of 52, 54, 56, and 59 cm/sec.

Twenty-seven fatty acids were found in the lipids of the coho salmon. Seven were the saturated acids 8:0, 10:0, 12:0, 14:0, 15:0, 16:0, and 18:0. Twenty were unsaturated; they were 14:1 ω6, Δ8; 15:1 ω6, Δ9; 16:1 ω7, Δ9; 17:1 ω9, Δ8; 18:1 ω9, Δ9; 20:1 ω9, Δ11; 22:1 ω9, Δ13; 16:2 ω7, Δ6; 18:2 ω6, Δ9; 20:2 ω6, Δ11; 18:3 ω3, Δ9; 20:3 ω6, Δ8; 18:4 ω3, Δ6; 20:4 ω6, Δ5; 20:5 ω3, Δ5; 22:5 ω3, Δ7; and 22:6 ω3, Δ4. Fatty acids 22:2, 22:3, and 22:4 occurred in less than 0.01% and the positions could not be assigned for the double bonds.

Formulations adopted for the available Hormel unsaturated standards and salmon unsaturated fatty acids were consistent with a methylene interruption between double bonds when two or more were present in a fatty acid.

Salmon placed to swim against water velocities of 52, 54, 56, and 59 cm/sec can be divided into a group that was unable to swim for 24 hours (failing salmon) and a group that was still swimming at 24 hours. Failing salmon at each velocity had shorter lengths and weighed less than control salmon or salmon still swimming after

24 hours at the same velocity. Salmon swimming for 24 hours at 56 and 59 cm/sec had greater lengths and weights than control salmon. Hence, the shorter and lighter salmon were less competent at a given velocity than were the longer and heavier salmon. The metabolism of exercise, the selection by the exercise procedure of long and short fish, and time of holding in the laboratory were involved in the differences between control and exercised salmon.

Groups of 28, 16, 26, and 38 salmon were forced to swim against water velocities of 52, 54, 56, and 59 cm/sec respectively. The percentage of failing salmon were 29, 62, 77, and 79% respectively. Conversely, 71, 38, 23, and 21% respectively were still swimming at 24 hours.

The average weight of control salmon was 5.22 grams and the average weights of swimming salmon (failing + 24 hour swimmers) were 4.39, 4.28, 4.81, and 4.74 grams, respectively, for water velocities of 52, 54, 56, and 59 cm/sec. The average amount of lipid for control salmon was 233 mg and the average amounts of lipids for swimming salmon were 179, 206, 245, and 223 mg respectively. The average amount of fatty acid methyl esters for control salmon was 189 mg and the average amounts of fatty acid methyl esters for swimming salmon were 149, 169, 192, and 178 respectively.

At all four velocities, between 13 and 18 fatty acids were present in significantly smaller quantities after swimming than in control salmon. At 52 and 54 cm/sec the greater changes were in 18:2, 16:1, and 16:0; at 56 cm/sec in 22:6, 18:2, and 20:4; and at 59 cm/sec in 14:0, 18:2, and 18:4. Saturated fatty acids accounted for 27.5% of the total fatty acid loss for salmon swimming at 52 cm/sec while the saturated fatty acid loss for salmon swimming at 59 cm/sec accounted for only 18.5% of the total loss (saturated fatty acids in control salmon were 26.6% of the total). The relationship suggests that salmon swimming for shorter distances at high velocities preferentially metabolize a higher percentage of unsaturated fatty acids.

All salmon were forced to swim for 1200 minutes at 8 cm/sec and 60 minutes at 15 cm/sec; then, the salmon were forced to swim against test velocities of 52, 54, 56 and 59 cm/sec for a maximum of 24 hours. The average swimming times for salmon swimming at velocities of 52, 54, 56, and 59 cm/sec were 1141, 645, 469, and 398 minutes respectively.

The average distances traveled for salmon swimming at 52, 54, 56, and 59 cm/sec were 26.0, 16.9, 13.7, and 12.7 miles respectively. (These distances include approximately 3.6 miles at 8 cm/sec and 0.3 miles at 15 cm/sec). An increase in velocity

from 52 to 59 cm/sec reduced the average distance traveled by 52% because average swimming time was reduced from 1141 to 398 minutes.

Brett (21) has provided information relating swimming velocity and oxygen consumption for sockeye salmon. The estimated average oxygen consumption for salmon swimming at 52 cm/sec was 98.4 mg and at 59 cm/sec was 53.7 mg. Since 1 mg of oxygen will burn 0.38 mg of lipid, the lipid equivalents of the total estimated oxidative costs for exercise are 37.0 mg at 52 cm/sec and 20 mg at 59 cm/sec. The observed lipid losses noted were 54 mg at 52 cm/sec and 10 mg at 59 cm/sec and are considerably higher at 52 cm/sec than calculated values even when all oxidative losses are attributed to lipids.

At 52 cm/sec the weight loss for swimming salmon was 830 mg and at 59 cm/sec was 480 mg. Of the total loss, lipids represent 54 and 10 mg respectively. Losses other than lipids were 776 mg and 470 mg. Of the 830 mg and 480 mg total losses, approximately 80% or 664 and 384 mg may have been water. The protein plus carbohydrate losses were approximately 112 mg and 86 mg respectively. The lipid losses represent 502 and 93 calories, the protein plus carbohydrate losses approximately 616 and 472 calories, and the total caloric losses were 1118 and 566 calories at 52 and 59

cm/sec respectively. Similar losses calculated from the oxygen consumption data of Brett (21) on Oncorhynchus nerka give 344 and 188 calories respectively.

The caloric cost as estimated from material losses are three times as great as the losses calculated from the oxygen consumption data of Brett. However, the oxygen consumption data of Brett were collected during one, and at the most two hours at high velocity. It seems reasonable that when a maximum effort is involved, that each succeeding mile and each succeeding hour is more difficult and more costly to the salmon.

Brett (21) found a ratio for maximum active metabolism to standard metabolism of 8:1 for a 5 gram fish. If the data in this study on material balance are supported by bioenergetic data, one presumes that for prolonged exercise the ratio of active to standard metabolism may even reach a value of 24:1 if computed on the basis of calories lost.

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