TABLE III
Composition of Sesame and Safflower Oils

Reference	Safflower oil (a)	Safflower oil (b)	Safflower oil (c)	Sesame oil (a)	Sesame oil (b)	Sesame oil (c)
Arachidic	0.02	....	....	....	0.02	....
Stearic	6.06	6.36	....	2.01	2.19	....
Palmitic	6.54	5.45	....	4.42	3.83	....
Myristic	....	....	....	....	....	....
Total sat'd	12.62	11.81	15.04	6.43	9.95	6.71
Oleic	52.68	54.96	45.26	31.45	28.36	22.48
Linoleic	31.41	28.39	37.55	58.38	61.07	67.21
Linolenic	....	....	2.13	....	....	3.62

(a) Composition by urea adduct elution of methyl esters.
(b) Composition by urea adduct elution of mixed fatty acids.
(c) Composition from cumulative fractionation data.

tures while the urea method can be used at room temperatures.

Chromatographic and countercurrent distribution methods yield fairly good separations but require unwieldy equipment for large-scale separations.

Summary

The fatty acid compositions of sesame and safflower oils have been determined by two urea fractionation procedures. A simple cumulative urea fractionation procedure has been found to give results similar to those obtained by an urea adduct elution method.

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The Effect of Diet on the Fatty Acid Composition of Several Species of Fresh Water Fish[1]

PETER B. KELLY,[2] RAYMOND REISER, and DONALD W. HOOD, Agricultural and Mechanical College of Texas, College Station, Texas

In a recent report from this laboratory (6) it was shown that the nature of the body fatty acids of the common mullet, a marine teleost fish, was markedly influenced by the dietary fatty acids. It was noted that the polyunsaturated acids which remained in these animals after 104 days on a fat-free diet could have been residual pre-experimental dietary acids. That they could also have been the result of synthesis by the fish was recognized as a possibility. Certainly however the fish on the fat-free diet did not synthesize polyunsaturated fatty acids to the degree as found in their tissues immediately after removal from their natural habitat.

In a continuation of the study of the origin of the aquatic type of fats a number of fresh-water fish were fed low-fat, 10% cottonseed oil and 10% menhaden oil diets. After various periods on these rations the relative amounts of the polyunsaturated fatty acids of the whole fish were determined. These were then compared with a group analyzed immediately after capture and with the marine mullet of the earlier study (6).

Experimental

Four species of fresh water fish were maintained on a series of synthetic diets. These diets were: a low fat diet; a simulated terrestrial fat type of diet, incorporating a commercially refined and winterized cottonseed oil; and a simulated marine fat diet, incorporating menhaden oil, a typical marine oil. The diets, the same as used in the previous study (6), are based on egg albumen and starch plus generous amounts of minerals and water and fat-soluble vitamins. The starch utilized in formulating the diets contained a maximum of 0.32% fat. It was considered that this fatty material was not enough to influence the study and would prevent possible fatty acid deficiency on the low fat diet. The fats were incorporated at 10% of the dry weight of the diet.

The fish employed in these experiments were the yellow bullhead (*Ameiurus natilis natalis* LeSueur), the common bluegill (*Lepomis macrochirus macrochirus* Rufinesque), the rockbass (*Ambloplites rupestris ariommus*), the small-mouth buffalo (*Ictiobus bubalus* Rufinesque), and the rainbow trout (*Salmo gairdnerii irideus* Gibbons). The fish were kept in aged, aerated, tap water in a 45-gal. aquaria. The water was constantly filtered through glass wool and charcoal. They were fed once daily; the amount varied with the size of the fish but approximated about a twenty-fifth of the mass of the fish. Uneaten food was removed. The bluegills and rockbass were between 3 and 6 in. in length, and no growth was observed in the course of the experiment. The buffalo grew 1 in. to a final length of 3 in., and bullheads doubled their length to 6 in. from their original 3 in. The trout developed from the eyed egg stage to fishes 1 to 1¼ in. in length. The rockbass ingested less food than all others except the trout.

In the experiment with the bullheads the fish were maintained on the fat-free diet for 51 days. They were then separated into three groups. One group was continued on the fat-free diet, and the others were placed on the marine and terrestrial fat diets

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[2] Present address: Shell Chemical Corporation, Houston, Tex.

TABLE I
The Extinction Coefficients ($E^{1\%}_{1cm.}$) at the Wave Lengths of Maximum Absorption of the Fat of Fish on Natural and Artificial Diets

Fish	Diet	Time (days)	Diets																			
			Natural					Low fat					10% Cottonseed oil					10% Menhaden oil				
			Double bonds					Double bonds					Double bonds					Double bonds				
			2	3	4	5	6	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
Mullet No. 1 (Marine)	Fat free Test diets	14 90	16.1	12.6	11.2	7.0	1.0	5.5	3.9	3.0	1.6	0.6	14.7	5.9	3.1	2.2	0.6	14.4	11.0	11.3	7.7	1.8
Mullet No. 2 (Marine)	Fat free Test diets	90 14	....	....	....	....	....	7.4	3.6	1.4	0.6	0.2	13.2	11.4	4.5	2.4	0.4	9.2	13.1	3.2	0.4	0.2
Buffalo (Fresh water)	.... Test diets	 143	16.4	14.7	6.5	3.0	0.8	....	....	....	....	....	14.5	5.2	1.6	0.7	0	16.2	15.1	9.2	5.0	1.7
Bullhead (Fresh water)	Fat free Test diets	51 43	9.4	7.0	2.3	0.8	0	8.7	6.1	1.9	0.8	0	9.5	5.8	1.0	0.2	0	11.2	14.5	3.3	0.8	0.3
Bluegill (Fresh water)	Fat free Test diets	64 34	10.6	8.2	1.7	0.4	0	11.3	8.7	2.6	0.8	0	12.9	9.8	2.3	0.5	0	13.7	10.8	2.6	1.3	0.3
Rockbass (Fresh water)	Fat free Test diets	64 34	16.3	11.5	3.6	1.5	0.4	14.4	11.3	4.2	1.6	0.5	19.4	12.9	6.0	2.7	0.9	15.3	12.2	5.3	2.5	0.9
Rainbow trout (Fresh water)	.... Test diet from egg to 38 days	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	13.6	17.1	8.5	4.2	1.5
Cottonseed oil			....	....	....	....	....	....	....	....	....	....	17.5	0	0	0	0	....	....	....	....	....
Menhaden oil			....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	12.8	14.3	12.2	7.1	1.5

for an additional 43 days. In the experiment with bluegills the time periods were 64 days on the fat-free diet, followed by 34 days on the terrestrial and marine fat diets. The conditions of the rockbass study duplicated exactly those of the bluegills. In fact, these two species were maintained together in the same aquaria.

The experiment with buffalo was somewhat different. These fish were divided into three groups and placed on the test diets immediately after capture. A fungus infection occurred in the tank containing the fish on the fat-free diet, killing all the fish. Since this occurred after only one month on the diet, the data from this group are not reported. Those on the fat diets were maintained for 125 days.

Rainbow trout were raised from the eyed eggs and fed only the menhaden diet for 38 days, following the mean hatching time. At the end of this time the fish had no trace of yolk sac.

A modification of the American Oil Chemists' Society spectrophotometric method (1) was used in the estimation of the relative amounts of polyunsaturated acids. Because this procedure was not designed for the quantitative determination of the various polyunsaturated acids in fish oils, only the extinction coefficients, $E^{1\%}_{1cm.}$, were recorded and no effort was made to calculate the percentage composition. The method of extraction of the total body fat was the same as in the earlier report (6).

Results and Discussion

The extinction coefficients of the oils from the various fish, at the wave lengths of maximum absorption, are summarized in Table I. For comparative purposes this table also contains the values of the earlier study of mullet. With a few possible exceptions there were no pronounced differences in the composition of any one fresh water fish on the natural, low-fat, or cottonseed rations. The failure of this low-fat ration, after more than 100 days, to cause a decrease in the polyunsaturated fatty acids, similar to that which one finds in the marine mullet (6) and land animals, is of considerable interest. A first thought that the constituent fatty acids are utilized at rates proportional to their composition cannot explain the occurrence since any residual acids must be constantly diluted with the endogenously produced acids. One is forced to conclude that the fish must synthesize fatty acids with the degrees of unsaturation indicated by the extinction coefficients of the acids extracted from the fish on this low-fat diet. If such is the case, the immediate suspicion is that the dienoic, trienoic, and tetraenoic acids may not be linoleic, linolenic, and arachidonic acids. Earlier work has indicated that the essential acids are lower in concentration than one might expect from the total polyunsaturated fatty acid content since it has been shown by Holman (5) that cod liver oil is comparatively poor in relief of essential fatty acid deficiency.

The conclusion that teleost fish probably synthesize polyunsaturated acids may also be deduced from published studies. Lovern (7) performed a series of investigations with herring and salmon during natural fasting periods. Lovern's work involved the determination of the mean degree of unsaturation of acids of various chain lengths. In the analyses herein reported, emphasis has been placed on the relative amounts of the acids of various degrees of unsaturation. The two sets of analyses cannot therefore be directly compared. Nevertheless the persistence of long-chain acids of about two double bonds in Lovern's study of starved fish and the persistence of the polyunsaturated fatty acids in the bodies of the fish on a low-fat diet force the conclusion that these acids must have been synthesized by the fish.

That animals synthesize polyunsaturated fatty acids has been unequivocally demonstrated. It has been shown (14) that hens on a fat-free diet deposited more dienoic acids in their eggs than could be accounted for in their tissues. Other investigations have shown that pentaenoic and hexaenoic acids, absent in land plants, occur in the adrenal (3), brain (2), testicular (5), and liver (11) tissues of herbivorous terrestrial animals.

There are two ways that long-chain highly unsaturated acids may be synthesized. One is by primary synthesis and the other by extension of chain length and an increase in the unsaturation of pre-existing acids. The first of these has never been demonstrated for animals. The mechanism of the conversion of shorter, less highly unsaturated acids to long-chain polyunsaturated acids has been adequately demonstrated by Mead and his co-workers (9, 10, 15, and 16). This observation presents the possibility that highly unsaturated acids may arise ultimately from saturated acids since in 1932 Quagliariello (12) noted the conversion of a palmetic acid to a monoethylenic acid and Shoenheimer and associates in the late

1930's (17) demonstrated the interconversion of saturated and monounsaturated acids by isotope labelling. These considerations make it conceivable that the difference between the abilities of land animals and fish to synthesize polyunsaturated acids is simply one of degree.

There still remains to be explained the differences between the level of the polyunsaturated fatty acids of fresh water and marine forms. Lovern (8) has indicated that this difference is also found in the zooplankton indigenous to those environments. The data in Table I indicate that within the limits imposed by normal variations between species and the limits of the analytical procedures no differentiation can be made between fresh water and marine forms after diets containing cottonseed and menhaden oil, that is, the differences within the two experiments with mullet and within the four species of fresh-water fish are as great as the differences between the mullet and the fresh-water fish. One can therefore conclude that, at least for the fish studied, the differences exhibited by the fresh-water and marine fish in their natural habitat are caused by differences in their natural diets. It is probable that this difference lies ultimately with the phytoplankton of those environments. A report of the work done in this laboratory on this problem is now in preparation.

Summary

Four kinds of fresh water fish were captured in the young stage, maintained on a low-fat diet for about two months, and either continued on that diet or transferred to test diets containing 10% cottonseed or menhaden oil for about five weeks. The fish were then sacrificed, and their total body fatty acids were examined for relative amounts of 2, 3, 4, 5, and 6 double bonds. It was found that no significant change from the natural diet occurred in the fatty acids on the low-fat or cottonseed oil diets while on the menhaden oil diet the fatty acid composition changed to resemble the composition of that oil.

These changes differed from those of the marine mullet in that the body fat of the latter lost much of its naturally occurring polyunsaturated acids, when placed on the low-fat regimen, and regained it on the menhaden oil diet.

Conclusion

1. Both marine and fresh-water fish appear able to synthesize polyunsaturated though not necessarily essential fatty acids from nonfatty precursors.
2. The differences between the fatty acid composition of the body fat of marine and fresh-water fish largely result from differences in their dietary fatty acids.

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Research on the Effects of Detergents (Alkylbenzenesulfonates and Phosphates) in Sewage Systems. A Progress Report [1,2]

J. D. JUSTICE, Lever Brothers Company, Research and Development Division, Edgewater, New Jersey

In 1956 about 700 million pounds of synthetic surface-active agents were produced in the United States. Combined with the appropriate quantities of polyphosphates, the commercial syndets are eventually discharged into the country's sewers and sewage-treatment plants. Sewage-treatment plant operators occasionally see foam in the treatment basins. Aided by publicity in the lay press, the notion has come into being that the detergents seriously interfere with efficient treatment of sewage. The detergent question is only one of the many problems which have arisen during the past few years to challenge the water-works and sewage-treatment people. Detergents are only one of the many chemical, organic, and bacterial substances which find their way into surface waters from municipal sewage effluents or industrial wastes, or which might result from the natural plant and animal processes which take place in surface waters.

The program established by the American Association of Soap and Glycerine Producers is aimed at learning something of the effects of formulated detergents on sewage and water treatment. Most of the formulated detergents consist of two principal components: the organic surfactant and inorganic phosphates. The most widely used organic active is sodium alkylbenzenesulfonate, ABS. The research projects have therefore been concerned with the effect of the two principal components, ABS and phosphates. In particular, the questions which these projects have sought to answer are these.

1. Do the complex phosphates find their way into natural water supplies? If so, does their presence interfere with the usual water-purification processes?
2. In addition to possible effects on drinking water, what effects, if any, do phosphates and ABS have an aquatic life?
3. What is the fate of ABS in the actual sewage-treatment process? How much is there in the effluent from the treatment plants?
4. Is the presence of ABS responsible for the frothing which is occasionally observed in treatment plants? If so, can the treatment be modified to reduce or eliminate this phenomenon?
5. Is there any truth to the belief that ABS interferes with

[1] This report covers work supported by the American Association of Soap and Glycerine Producers.
[2] Presented at the fall meeting of the American Oil Chemists' Society. September 30, 1957.