ther hand, the eyes may be so bad that one has to deal vith them first and do a thyroidectomy afterwards.

X-ray Irradiation

Irradiation is, I believe, a valuable palliative measure, ınd irradiation both of the orbits and of the pituitary ıave led to an improvement more rapid than I should ıave expected in the ordinary course of the disorder. My ɔatient aged 80 who was also thyrotoxic was irradiated, ınd, though his eyes gave cause for much anxiety, he mproved, and three years later had been stabilised with ittle diplopia.

Orbital Decompression

My surgical colleagues have used Naffziger's trans-rontal approach for this operation, so I have no experi-ence of any other. A curious and unexplained feature of t, to which attention has been drawn before, is that sometimes, when the worse eye is operated on first, the ɔther one improves so much that it does not need opera-tion. The main indications for orbital decompression are danger to the cornea from raised orbital tension, and danger to vision from papilloedema or (less often) primary optic atrophy.

Conclusions

The treatment of endocrine exophthalmos thus presents many difficulties. Nevertheless, on the whole, time is on the patient's side, and I believe that by taking every aspect of the case into consideration it is almost always possible to obtain a reasonably satisfactory result. Complete disappearance of physical signs is rare, but the visual acuity of both eyes should be normal and double vision should not be an inconvenience. (In suitable cases, when the endocrine changes have subsided, this can be much improved by operations on the ocular muscles.)

There will usually be some residual exophthalmos; but, with disappearance of swelling of the eyelids and conjunctivæ, the patient's appearance should be greatly improved and his general health should be restored to normal.

REFERENCES

Adams, D. D., Purves, H. D. (1957) *Metabolism*, **6**, 26.
Aterman, K. (1954) *Acta endocr., Copenhagen* suppl. 20.
Barraquer, J., Canadell, J. M. (1955) *Rev. esp. Oto-neuro-oftal.* **14**, 274.
Brain, W. R. (1936) *Lancet*, i, 182.
— (1938) *Quart. J. Med.* **7**, 293.
— (1952) *Proc. R. Soc. Med.* **45**, 237.
Bristowe, J. S. (1886) *Brain*, **8**, 313.
Bruun, E. (1945) *Acta med. scand.* **122**, 13.
Burrows, H. (1949) Biological Action of the Sex Hormones. Cambridge.
Dobyns, B. M. (1945) *Surg. Gynec. Obstet.* **80**, 526.
— (1946) *ibid.* **82**, 609.
— Brown, M., Steelman, S. L. (1953) *Endocrinology*, **52**, 705.
— Wilson, L. A. (1954) *J. clin. Endocrin.* **14**, 1393.
Friedgood, H. B. (1934) *Bull. Johns Hopk. Hosp.* **54**, 48.
Gilliland, I. C. (1955) *Proc. R. Soc. Med.* **49**, 212.
— Strudwick, J. I. (1953) *Clin. Sci.* **12**, 265.
— — (1956) *Brit. med. J.* i, 378.
Greene, R., Farran, H. E. A. (1958) *ibid.* ii, 1057.
Jackson, W. P. U. (1956) *J. clin. Endocrin. Metab.* **16**, 1245.
Langford, H. G. (1957) *Endocrinology*, **60**, 390.
Marine, D., Rosen, S. H. (1934) *Amer. J. med. Sci.* **188**, 565.
— — Cipra, A. (1932–33) *Proc. Soc. exp. Biol.* **30**, 649.
— Spence, A. W., Cipra, A. (1931–32) *ibid.* **29**, 822.
Medical Research Council Committee (1955) Cortisone in exophthalmos. *Lancet*, i, 6.
Naumann, M. (1853) *Dtsch. Klin.* **5**, 269.
Prunty, F. T. G. (1958–59) *Proc. R. Soc. Med.* (in the press).
Querido, A., Lameyer, L. D. F. (1955) *ibid.* **49**, 209.
Rundle, F. F., Finlay-Jones, L. R., Noad, K. B. (1953) *Aust. Ann. Med.* **2**, 128.
— Pochin, E. E. (1944) *Clin. Sci.* **5**, 51.
Smelser, G. K. (1937) *Amer. J. Ophthal.* **20**, 1189.
— (1939) *Amer. J. Path.* **15**, 341.
— (1943) *Amer. J. Physiol.* **140**, 308.
— Ozanics, M. S. (1949) *Amer. J. Ophthal.* **32**, 1557.
— — (1954) *ibid.* **38**, 107.
— — (1955) *ibid.* **39**, 146.
Warner, F. (1883) *Med. Clin. Trans.* **66**, 107.

THE EFFECT ON HUMAN SERUM-LIPIDS OF A DIETARY FAT, HIGHLY UNSATURATED, BUT POOR IN ESSENTIAL FATTY ACIDS

EDWARD H. AHRENS, JR. M.D.
WILLIAM INSULL, JR. M.D.
JULES HIRSCH M.D.
WILHELM STOFFEL M.D.
MALCOLM L. PETERSON M.D.
JOHN W. FARQUHAR M.D.
OF THE ROCKEFELLER INSTITUTE, NEW YORK

THEODORE MILLER
OF MARINE CHEMURGICS, INC., MOREHEAD CITY, NORTH CAROLINA

H. J. THOMASSON
MED. DRS.
OF THE UNILEVER RESEARCH LABORATORIES, VLAARDINGEN, HOLLAND

NUMEROUS studies (Kinsell et al. 1952, Bronte-Stewart et al. 1956, Ahrens et al. 1957, Malmros and Wigand 1957, Keys et al. 1957) have shown that exchanges of dietary fats have striking effects upon the concentration of lipids in the serum. Thus, unsaturated fats, when substituted isocalorically for fats of more saturated types, appear to produce definite decreases in the serum level of cholesterol and phospholipids. On the basis of long-term clinical feeding experiments in this laboratory (Ahrens et al. 1957) we have suggested that the effects observed are consistently related to the net unsaturation of the dietary fat. But it has been postulated by others (Kinsell and Sinclair 1957) that the serum-lipid effects are due to the content of essential fatty acids (E.F.A.) in the dietary fat. No specific fatty-acid requirement has been demonstrated for adult human beings, but it is conceivable that hypercholesterolæmia in man might represent a biochemical expression of E.F.A. deficiency. Although it is important to distinguish clearly whether the serum-lipid effects described above are due to the E.F.A.-content of dietary fats, or simply to net unsaturation, this question cannot be answered by feeding experiments with natural vegetable and animal fats (Ahrens et al. 1958). However, a partial answer might be obtained with marine oils, since these fats are rich in unsaturation but reputedly poor in E.F.A.

In the present study, a practical procedure was developed for preparing a highly unsaturated marine oil (menhaden oil) which patients found acceptable over many weeks. Bioassay showed that this oil had an exceedingly low content of E.F.A., and chemically the contents of linoleic and arachidonic acid were correspondingly low. The oil was given as the sole dietary fat to two patients for 8-week periods, and the serum-lipid responses were compared with those during periods in which the dietary fat was corn (maize) oil, which is rich in E.F.A. but lower in net unsaturation.

Menhaden Body Oil *

Rendering and Storage

Fresh body oil was prepared from 500 lb. of body portions of menhaden (*Brevoortia tyrannus*) caught in December, 1956, off the North Carolina coast of the United States.

These gill feeders are thought to feed heavily on plankton in more northerly waters before beginning their rapid southward migration. The fish were 12–17 in. long and were in spawning condition. Immediately after being brought aboard

* The generous cooperation of Mr. Charles Butler, of the United States Fish and Wildlife Service, Washington, D.C., is gratefully acknowledged.

the fishing vessel, they were decapitated, eviscerated, and washed with sea water. To decrease bacterial and auto-oxidative changes, the bodies were held in crushed ice mixed with a solution containing 1% sodium bisulphite and 1% phosphoric acid. The temperature was kept at 4°C by repeated additions of ice. The processing of the fish was started as soon as the vessel arrived at the wharf, and it was completed within 15 hours of the time the fish were netted. The bodies were placed in trays in a barrel cooker, steamed for 5 minutes, and immediately transferred to a press. The press liquors were centrifuged to concentrate the crude oil, which was then thoroughly agitated with water at 82°C in an atmosphere of carbon dioxide, before recentrifuging. The washed oil was subjected to a vacuum at 60°C, transferred to pint bottles, flushed with carbon dioxide, and immediately capped and frozen. The containers were stored at −15°C until the oil was required on the metabolic ward.

The final product was pale yellow and semisolid at room temperature. Although unmistakably "fishy" in odour and flavour, it was more bland than any other fish oil tested to date. In separate tests this flavour was not noticeably reduced by steam deodorisation or molecular distillation.

Chemical Data

The iodine value of this oil was 179, peroxide value less than 1, and Gardner colour 10. The oil was subjected to chromatography on silicic acid columns (Hirsch and Ahrens 1958). The total non-triglyceride material was less than 0·2%. Free fatty acids (as oleic) formed 0·1%, lipid phosphorus was present only in trace amounts, and the tocopherol content was 3·2 mg. per 100 g.

The fatty-acid composition was defined by gas/liquid chromatography according to James and Martin (1956), utilising retention-volume data described previously (Stoffel et al. 1958b). Because of the extreme complexity of the fatty-acid pattern, with numerous unsaturated components in each chain-length group, it was necessary to chromatograph the mixed methyl esters on two different stationary phases—Apiezon M (James and Martin 1956) and Reoplex 400 (Orr and Callen 1958). The complementary use of Apiezon M and Reoplex 400 for preparative and analytical chromatography

TABLE I—FATTY-ACID COMPOSITION OF DIETARY FATS AND SERUM-ESTER GROUPS DEFINED BY GAS/LIQUID CHROMATOGRAPHY

(Retention-volume data, calculated in relation to that of methyl stearate, are shown for esters resolved on one or both of two stationary phases. Double-bond content of each acid is shown under "shorthand" designation, i.e., 18 : 2 = a C_{18}-acid with two double bonds. Definitive structures of all even numbered acids have been established [Stoffel and Ahrens 1958, and unpublished].)

Fatty acids					Dietary fats		Patient I						Patient II					
							Menhaden oil (week 14)			Corn oil (week 21)			Menhaden oil (week 16)			Corn oil (week 21)		
No.	Familiar name	Shorthand designation	Retention volumes relative to methyl stearate: Apiezon M	Retention volumes relative to methyl stearate: Reoplex 400	Menhaden oil	Corn oil	CE	TG	PL	CE	TG	PL	CE	TG	PL	CE	TG	PL
1	Lauric	12 : 0	0·075	0·18	0·1	0·1		0·1										
2		13 : 0	0·112	0·22	0·1			0·3			0·1							
3		13 : br	0·098		tr													
4		13 : br	0·085		tr													
5	Myristic	14 : 0	0·174	0·32	7·2	0·3	2·1	2·9	1·2	0·3	0·8	0·3	1·1	1·3	0·4	0·2	1·0	0·3
6		14 : br	0·155		0·1													
7		14 : 1	0·159	0·36	0·1			0·2										
8		15 : 0	0·270	0·43	0·5		0·8	0·4	0·5	0·2			0·1	0·5	0·2	0·2		
9		15 : br	0·230		0·2			0·2					tr			0·2		
10		15 : br	0·218		0·2													
11	Palmitic	16 : 0	0·419	0·57	17·0	12·7	30·4	22·4	42·1	9·8	16·9	31·4	23·3	23·7	35·0	12·6	19·9	30·7
12	Palmitoleic	16 : 1	0·366	0·64	9·8		8·4	5·5	2·0	1·0	1·6	0·2	5·6	5·2	1·0	1·6	3·2	0·6
13		16 : 2	0·36	0·77	2·0		0·4	1·8	1·3	0·1	0·2	0·1	tr	0·4	0·2		tr	0·1
14		16 : 2	0·33	0·77														
15		16 : 3	0·324	0·88	1·3													
16		16 : 4	0·314		2·0													
17		17 : 0	0·649	0·74	0·4		0·4	0·3	0·8			0·3			1·3			0·5
18		17 : br	0·500		0·3			0·6						0·5	1·6			0·6
19		17 : br	0·554		0·4													
20	Stearic	18 : 0	1·00	1·00	3·1	2·7	4·2	4·5	23·9	1·0	2·1	14·7	1·0	7·3	15·6	1·0	3·1	14·3
21	Oleic	18 : 1	0·860	1·11	14·5	30·7	21·9	22·2	11·9	17·8	33·4	12·5	21·9	27·7	11·0	18·9	38·1	11·9
22	Linoleic	18 : 2	0·790	1·32	2·0	53·5	9·9	8·4	3·0	62·1	37·4	31·3	16·8	13·4	6·3	59·1	29·3	26·1
23		18 : 2	0·760	1·40	0·7						3·8							
24	Linolenic	18 : 3	0·801	1·66	1·3													
25		18 : 4	0·720	1·91	3·2			1·1		0·4	0·4	0·7				0·3		1·2
26		19 : 0	1·54	1·32	tr													
27		19 : br		1·50	0·4													
28		19 : un		1·59	0·4													
29		20 : 0	2·36	1·78	0·1													
30		20 : 1	1·99	1·96	2·1													
31		20 : 2	1·76	2·24	0·6													
32		20 : 2	1·85	2·24	0·6													
33		20 : 3	1·64	2·68	2·0					0·7		1·7				0·1		1·3
34	Arachidonic	20 : 4	1·46	2·90	0·6		3·1	2·6	1·8	3·9	0·8	3·5	4·2	0·6	4·5	2·6		3·7
35		20 : 5	1·46	3·67	12·5		18·9	19·4	7·2	1·7	0·4	0·8	24·3	11·3	12·9	1·9	1·9	2·0
36		20 : un		4·00				2·8									2·1	
37		20 : un		5·01	0·7													
38		20 : un		5·80	0·5													
39		21 : 0	3·65	2·36	tr													
40		20 : un		3·33	1·7													
41		22 : un		6·30	tr													
42		22 : 5	3·30	6·67	2·0				2·5	1·0		0·7	0·2	2·0	1·8			1·8
43		22 : 6	3·02	7·40	8·9			5·1	1·8		1·0	1·8	1·5	6·0	8·1	0·6	0·9	4·7
44		24 : un			tr													
Saturated acids (% of total)					30·6	15·8	37·9	31·0	68·6	11·3	19·7	46·7	25·5	33·3	52·5	14·6	24·4	46·5
Unsaturated acids (% of total)					69·4	84·2	62·5	69·6	31·4	88·8	80·0	53·3	74·5	66·7	47·5	85·4	75·6	53·5
Mean molecular weight of fatty acids					278·5	277·8	276·1	281·3	273·9	279·9	276·6	276·1	280·3	279·7	280·7	277·2	275·3	278·2
Average no. of double bonds per fatty acid molecule					2·14	1·38	1·58	2·08	0·89	1·76	1·29	1·17	2·09	1·66	1·67	1·64	1·26	1·37
Iodine values: Calculated					189	122	139	144	78	157	113	103	186	148	149	145	110	121
Iodine values: Determined					179	126												
Serum-lipid levels (mg. per 100 ml. serum)							176	180	151	228	530	229	370	155	226	391	175	274

Abbreviations: tr = trace (<0·05%); br = branched acid; un = unsaturated acid; CE = cholesterol esters; TG = triglycerides; PL = phospholipids. Weight concentrations of cholesterol esters calculated from ratios of free/total cholesterol, and from mean molecular weights of cholesterol-ester fatty acids. Iodine values calculated from mean molecular weights of fatty acids and from mean number of double bonds per fatty-acid molecule.

has been discussed elsewhere (Stoffel et al. 1958b). In addition, chromatograms were obtained on the mixed esters after complete hydrogenation (to obtain an accurate chain-length analysis), and after complete bromination (to assure precise analysis of the saturated components). The data on saturated and unsaturated acids in the even-numbered series (left side of table I) are presented with considerable assurance; the odd-numbered acids have not yet been positively identified, but are named according to theoretical considerations (James and Martin 1956). The retention-volume data listed in table I may assist other workers in identifying the numerous components present in other biological mixtures.

Table I shows that menhaden oil is composed of at least 44 different acids. In view of the technical difficulties encountered in defining the quantitative relationships of this complex mixture, it is gratifying to note the close agreement between determined and calculated iodine values for menhaden oil shown at the bottom of the table. This close correspondence validates both the identification of the acids and their quantitation.

C_{18}-dienes constituted 2·0% of the total acids. By degradation studies (Stoffel and Ahrens, unpublished data) this material was shown to be linoleic acid (octadeca-9,12-dienoic acid). C_{20}-tetraene was 0·6% of the total acids; characterisation of the degradation products proved that it was arachidonic acid (eicoso-5,8,11,14-tetraenoic acid). The C_{16}-acids comprised the largest single chain-length group (32% of total acids by weight); the palmitic-acid content of 17% is similar to that reported in other marine oils (Lovern 1950) and in other samples of menhaden oil (Smith and Brown 1945). The C_{16}, C_{18}, and C_{20} unsaturated acids have been fully characterised (Stoffel and Ahrens 1958, Stoffel and Ahrens, unpublished data). Although saturated acids constituted 30% of the total acids by weight, acids with 4–6 double bonds totalled 32%. Thus, the mean number of double bonds per molecule of fatty acid was 2·14, and the mean molecular weight 279 (cf. linoleic acid with 2·0 double bonds, and a molecular weight of 280).

Bioassay

The E.F.A.-content of this sample of menhaden oil was assayed in 60 E.F.A.-deficient rats by the method of Thomasson (1953), and was 4 units per g. of oil (pure linoleic acid is assigned a potency of 100 units per g.). In this 4-week assay of growth, two groups of rats were fed the menhaden oil at dosage levels of 2 and 10 ml. (total per rat) during the first 2 weeks of the test; none of the rats showed signs of toxicity.

Metabolic Studies

Methods

The selection of patients, their management on the metabolic ward, the procedures of feeding, and the clinical laboratory methods have been described fully elsewhere (Ahrens et al. 1957). Two patients were studied for 21 and 22 weeks continuously on the metabolic ward. During this time oral feeding of a liquid mixture (Ahrens et al. 1954) was the sole source of calories, and this was given 5 times per day in amounts such that the body-weight remained constant. Mineral and vitamin supplements were given daily (Ahrens et al. 1957). The protein/fat/carbohydrate composition of the liquid mixture was 15/40/45% of the total calories at all times, and in the two experiments corn oil and menhaden oil were the sole dietary fats in three successive feeding periods. The patients became accustomed in 1–2 days to the corn-oil mixture, and in 3–5 days to the menhaden-oil mixture. While both patients found the menhaden oil unpleasant in taste, at no time was there nausea, vomiting, or diarrhœa.

Supplementary vitamin E was not given, in view of the difficulty in producing E-deficiency in human adults (Horwitt et al. 1956). However, since E-deficiency in animals has been precipitated by feeding highly unsaturated oils (Mason 1954), we were constantly alert to the possibility of the development of creatinuria, myopathy, or encephalopathy. No such symptoms of toxicity nor changes in the sense of well-being were observed. Creatinuria did not occur. Both patients showed lower levels of serum-tocopherol † when taking menhaden oil than when taking corn oil, probably because of decreased intake during the fish-oil period.

Changes in Serum-lipid Levels

Patient I, a 30-year-old white male, showed hyperlipæmia and xanthomatosis of tendons and skin, but no manifestations of cardiovascular disease. He required 2800 calories per day (35 calories per kg.) to maintain constant body-weight. The course of the experiment is shown in fig. 1 and table II. As expected from previous experience (Ahrens et al. 1957), substitution of the corn-oil mixture for an ad-libitum diet produced a striking decrease in all serum-lipid levels; a new steady state was reached in about 3 weeks. When menhaden oil replaced corn oil, there was further depression of cholesterol, phospholipid, and triglyceride values. On reinstitution of corn oil these levels rose, but not to their previous height.

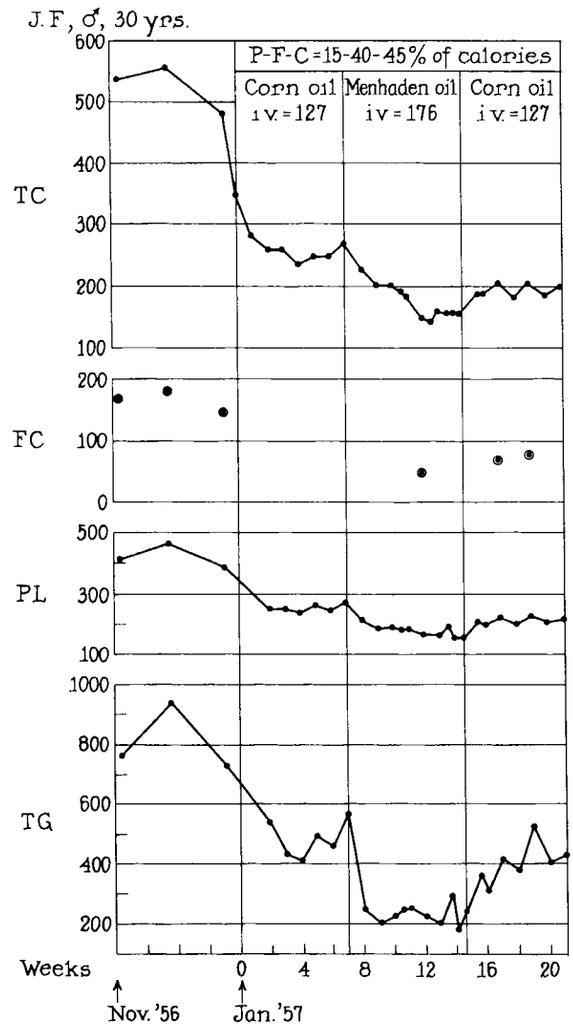

Fig. 1—Serum-lipid changes in patient I when on an ad-libitum diet as an outpatient and when on three dietary regimens in a metabolic ward.

The entire dietary fat intake was composed of corn oil or menhaden oil in successive periods as shown. TC=total cholesterol, FC=free cholesterol, PL=phospholipids, TG=triglycerides, all expressed as mg. per 100 ml. serum; i.v.=iodine value. Each new steady state was preceded by a 2–4 week transition period.

† For these determinations we are deeply indebted to Dr. M. K. Horwitt, of the Elgin State Hospital, Elgin, Illinois.

TABLE II—SERUM-LIPID DATA DURING SUCCESSIVE STUDY PERIODS
(Transitional data were omitted in calculating mean ± standard deviation. During each dietary period calculations were based only on steady-state data obtained in *n* successive determinations.)

Patient	Dietary fat	*n*	Steady-state data (mg. per 100 ml. serum)			
			Total cholesterol	Free cholesterol	Total phospholipids	Triglycerides
I 30 yr. M	Ad-libitum diet	3	504±44	166±17	423±37	810±114
	Corn oil	6	253±12	..	257±12	486±62
	Menhaden oil	6	158± 5	48	169±15	230±45
	Corn oil	5	197±11	67, 74	214±13	435±60
II 38 yr. F	Ad-libitum diet	3	499±21	138± 2	388±23	299± 8
	Corn oil	6	306± 7	82, 76	270±13	194±35
	Menhaden oil	5	305±28	82, 84	234±12	161±17
	Corn oil	4	325± 5	89, 87	268± 8	182± 9

Skin xanthomata began to disappear within a month of starting oil feeding, and they continued to shrink throughout all experimental periods.

Patient II, a 38-year-old white female with hypercholesterolæmia and tendon xanthomata, also was free of symptoms or signs of atherosclerosis. She required 2250 calories per day (37 calories per kg.) to maintain her body-weight. " Steady-state " data from three dietary periods are listed in table II. The levels of total cholesterol, phospholipids, and triglycerides were not significantly different during the corn-oil and menhaden-oil periods. However, in all three test periods the levels of cholesterol and phospholipids were significantly lower than when taking an ad-libitum diet. There was no change in tendon xanthomata during the experimental study.

Changes in Serum-fatty Acids

The composition of the fatty-acid mixture of each lipid group in the serum of both patients was determined after equilibrium had been established in the menhaden-oil and in the second corn-oil dietary periods. The three serum-lipid ester groups (cholesterol esters, triglycerides, and phospholipids) were isolated by preparative chromatography on silicic-acid columns (Hirsch and Ahrens 1958) from chloroform/methanol extracts of 8–16 ml. of serum (Folch et al. 1951). The methyl esters of the fatty acids were formed and isolated (Stoffel et al. 1958b) and were analysed by gas/liquid chromatography on Apiezon M and Reoplex 400 columns.

The fatty-acid compositions in the right side of table I are calculated as percentages of the total mixture in each ester group. When corn oil replaced menhaden oil as the sole dietary fat, the fatty acids of all serum-lipid groups were strikingly affected. In both patients the saturated acids and C_{20-22}-polyenes, which were characteristic of menhaden oil, were correspondingly higher in all lipid groups during the menhaden-oil diet. On the other hand, in both patients during corn-oil diets the C_{18}-dienes increased considerably in all groups.

At the bottom of table I are shown the levels of the three serum-lipid groups in the serum at the designated times. From these data the content of individual acids in each group was calculated (e.g., mg. of 16 : 0 per 100 ml. serum in the cholesterol-ester fraction). Five acids comprised more than 80% of the total in each group; changes in these acids are shown graphically in fig. 2. The ratios of the five major acids to each other in the cholesterol esters were very similar in the two patients in comparable feeding periods (i.e., patterns of cholesterol-ester fatty acids during corn-oil diets in patient I versus patient II). Likewise, the patterns of acids in the phospholipids also were similar in the two patients in respective feeding periods, but they differed considerably from the cholesterol-ester patterns. There were striking dissimilarities in the distribution of major acids in the triglyceride fractions of the two patients on the two diets.

In both patients the fatty-acid mixtures of the three ester groups showed distinctive inter-group differences during the two dietary regimens.

Phospholipids.—About 50% of the acids were saturated in three of four dietary periods, and when patient I was on menhaden oil nearly 70% of the acids were saturated. The phospholipids appear to have the most unchanging fatty-acid pattern of any serum-lipid group. The triglycerides and cholesterol esters, however, more strongly mirror the changes in the dietary fats.

Cholesterol esters.—Of the three ester groups, the cholesterol-ester fatty acids showed the smallest proportion of saturated acids in three of four periods; hence the highest calculated

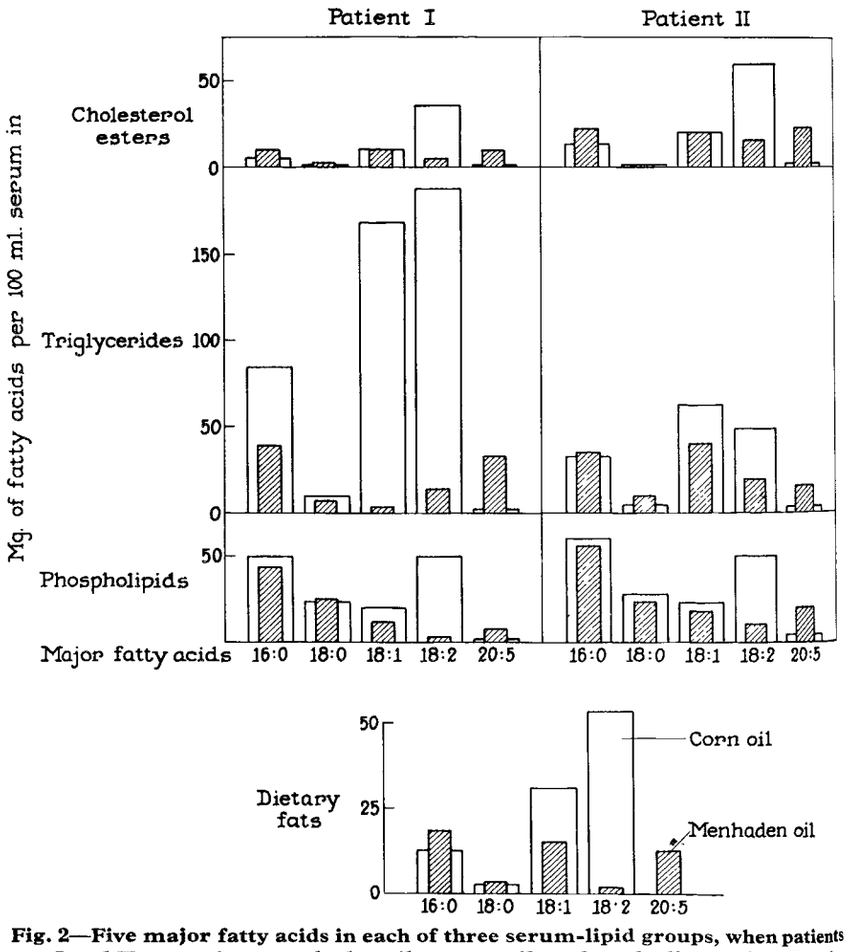

Fig. 2—Five major fatty acids in each of three serum-lipid groups, when patients I and II were given menhaden oil or corn oil as the sole dietary fat. Results are expressed as mg. of each acid in each group, per 100 ml. serum.

This quantitative representation differs from that in table I, where all fatty acids are expressed as % of total acids of each serum-lipid group. At the bottom, the compositions of the two dietary fats are compared. The 5 major acids made up 99% of the acids in corn oil. But in menhaden oil these same acids comprised only 50% of the total acids; four other major components, not shown (14 : 0, 16 : 1, 18 : 4, and 22 : 6), totalled 29%, while at least 35 minor acids made up the remaining 21%.

iodine value. Moreover, during respective dietary periods this fraction contained a higher proportion of linoleic acid than was present in the corn oil (table I). There was also a higher proportion of C_{20}-pentaenes and C_{20}-tetraenes than was present in menhaden oil, but in contrast C_{22}-pentaenes and C_{22}-hexaenes of menhaden oil did not appear in the cholesterol-ester fatty acids.

Triglycerides.—The acid mixtures of the triglycerides were altered by the two dietary fats, particularly in patient I. There was considerable resemblance between the triglyceride fatty acids in patient I to corn oil, and in patient II to menhaden oil during those dietary periods. The C_{22}-polyenes appeared more strikingly in the triglycerides than in the cholesterol esters.

TABLE III—SUMMARY OF DIETARY EXPERIMENT

	Characteristics of dietary fats			Effect on serum-lipids	
	E.F.A.	Sterols	Iodine value	Patient I	Patient II
Corn oil	54%	1·5%	126	↓↓	↓
Menhaden-body oil	4%	<0·2%	179	↓↓↓	↓

In the four dietary periods, acids shorter in chain-length than C_{14} formed an insignificant proportion of all ester groups. Although myristic acid (14 : 0) constituted 7% of the menhaden-oil fatty acids, it made up only a small part of the serum-fatty acids. The predominance of palmitic acid over stearic acid was striking in the cholesterol esters and triglycerides; however, in the phospholipids, stearic acid assumed much greater importance in all dietary periods. The odd-numbered acids in menhaden oil did not appear in the serum in significant concentrations.

From table I it is apparent that all serum-ester groups are influenced by the pattern of acids in the dietary fat. However, we lack precise knowledge of the turnover of dietary fatty acids in man, and it would be misleading at this time to assume that alterations in fatty-acid patterns in the serum indicate significant changes in utilisation of the individual acids. Moreover, the differences in triglyceride fatty acids of the two patients may reflect a fundamental metabolic difference between patients with hyperlipæmia and those with hypercholesterolæmia.

Discussion

Despite efforts to produce a completely bland oil, the menhaden oil was somewhat distasteful. Nevertheless, amounts of oil equal to 40% of the total calories (100 to 125 g. per day) incorporated in the liquid mixture of protein, carbohydrate, and water caused no gastrointestinal complaint. The isocaloric substitution of menhaden oil for the bland and relatively tasteless corn oil produced no change in total body-weight; presumably both oils were equally well absorbed. Physical activity and sense of well-being also were unchanged.

The content of E.F.A. in the menhaden oil used in these experiments was found to be exceedingly low by two independent methods. The chromatographic analyses defined the upper limits of the levels of linoleic and arachidonic acids. However, since menhaden oil might contain essential acids other than these two (Thomasson 1956), it was necessary also to determine the E.F.A. activity of this oil biologically. The bioassay data are compatible with the chemical data, showing a very low titre of E.F.A., in agreement with values found previously (Thomasson 1953) for samples of menhaden and other marine oils.

The results of our dietary tests in two patients are summarised in table III. Since it is seen that isocaloric substitution of E.F.A.-poor menhaden oil for E.F.A.-rich corn oil produced as low or lower levels of serum-lipids, it may be concluded that the presence of E.F.A. is not required to produce these serum-lipid alterations. Findings reported by other laboratories are consistent with the present results: pilchard oil fed by Bronte-Stewart et al. (1956), sardine oil used by Keys et al. (1957), and whale oil tested by Malmros and Wigand (1957) caused significant depressions in levels of serum-cholesterol. Although the highly unsaturated fats used in those tests were not completely characterised, chemically or biologically, it is probable (Thomasson 1956) that they had a low E.F.A.-content. Our results fail to confirm Beveridge's postulate (Beveridge et al. 1958) that the effectiveness of corn oil in lowering serum-cholesterol levels is mainly due to its sitosterol content (about 1·5%). The menhaden-body oil used in this study contained only negligible amounts of sterols, yet the serum-lipid effects were comparable, whether menhaden oil or corn oil was given.

Conclusions

A menhaden-body oil, acceptable for human consumption in large quantities over a long period of time, was prepared for use in dietary experiments on a metabolic ward.

The content of essential fatty acids in the oil, determined chemically and by bioassay, was found to be less than 4% of the total fatty acids. The content of total non-saponifiable material was less than 0·2%.

When menhaden oil was substituted isocalorically for corn oil in the diet of two patients (one with hyperlipæmia, the other with hypercholesterolæmia) serum levels of cholesterol, phospholipids, and triglycerides remained depressed. The fatty-acid patterns of all three ester groups of the serum-lipids were considerably affected by the strikingly different composition of the two dietary fats.

The effects on serum-lipid levels were unrelated to the essential-fatty-acid and sterol contents of the dietary fats under consideration.

This study was supported in part by grants from the United States Public Health Service (H-2539) and the Nutrition Foundation.

REFERENCES

Ahrens, E. H., Jr., Dole, V. P., Blankenhorn, D. H. (1954) *Amer. J. clin. Nutr.* **2**, 336.
— Hirsch, J., Insull, W., Jr., Tsaltas, T. T., Blomstrand, R., Peterson, M. L. (1957) *Lancet*, i, 943.
— — — Peterson, M. L. (1958) *in* Chemistry of Lipids as Related to Atherosclerosis (edited by I. H. Page); p. 222.
Beveridge, J. M. R., Connell, W. F., Mayer, G. A., Haust, H. L. (1958) *Canad. J. Biochem. Physiol.* **36**, 895.
Bronte-Stewart, B., Antonis, A., Eales, L., Brock, J. F. (1956) *Lancet*, i, 521.
Folch, J., Ascoli, I., Lees, M., Meath, J. A., Le Baron, F. N. (1951) *J. biol. Chem.* **191**, 833.
Hirsch, J., Ahrens, E. H., Jr. (1958) *ibid.* **233**, 311.
Horwitt, M. K., Harvey, C. C., Duncan, G. D., Wilson, W. C. (1956) *Amer. J. clin. Nutr.* **4**, 408.
James, A. T., Martin, A. J. P. (1956) *Biochem. J.* **63**, 144.
Keys, A., Anderson, J. T., Grande, F. (1957) *Lancet*, i, 66.
Kinsell, L. W., Partridge, J., Boling, L., Margen, S., Michaels, G. D. (1952) *J. clin. Endocrin.* **12**, 909.
— Sinclair, H. M. (1957) *Lancet*, i, 883.
Lovern, J. A. (1950) *J. Soc. Leath. Tr. Chem.* **34**, 7.
Malmros, H., Wigand, G. (1957) *Lancet*, ii, 1.
Mason, K. E. (1954) *in* The Vitamins (edited by W. H. Sebrell, Jr., and R. S. Harris); vol. III, p. 514.
Orr, C. H., Callen, J. E. (1958) *J. Amer. chem. Soc.* **80**, 249.
Smith, F. A., Brown, J. B. (1945) *Oil & Soap*, **22**, 321.
Stoffel, W., Ahrens, E. H., Jr. (1958) *J. Amer. chem. Soc.* (in the press).
— Chu, F., Ahren, E. H., Jr. (1958a) *Analyt. Chem.* (in the press).
— Insull, W., Jr., Ahrens, E. H., Jr. (1958b) *Proc. Soc. exp. Biol.* **99**, 238.
Thomasson, H. J. (1953) *Int. Rev. Vitam. Res.* **35**, 62.
— (1956) *in* Third International Conference on the Biochemical Problems of Lipids (edited by G. Popjak and E. Le Breton); p. 452.