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The Effect of Vitamin A Deficiency on the Cholesterol Levels of the Plasma and Liver of the Rat

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(Received 2 February 1955)

Although much research has been devoted to various aspects of the chemistry and biochemistry of vitamin A, the systemic mode of action remains an unsolved problem.

Lowe, Morton & Harrison (1953) showed that an unidentified and perhaps hitherto unknown substance accumulates in the livers of vitamin A-deficient rats. A working hypothesis was put forward to account for this, namely, that vitamin A exerts a restraining influence on the dehydrogenation of cholesterol or steroid hormones, such restraint being lifted in vitamin A deficiency, with consequent appearance of an abnormal product or products. The new substance is, however, quantitatively only a minor constituent, and it becomes important to know whether the aberrant metabolism to which it provides a clue can result in large changes in major constituents.

Cholesterol is itself probably a precursor of most steroid hormones, and fresh significance may attach to the question as to whether or not the more active sites of cholesterol metabolism suffer marked changes in vitamin A deficiency. Several groups of workers have tried to follow plasma cholesterol levels during the development of vitamin A deficiency. Ralli & Waterhouse (1933) reported a rise in the blood-cholesterol level of four dogs maintained on a vitamin A-free diet, but the level fell somewhat in two of the dogs as the

deficiency symptoms became severe. Administration of carotene or cod-liver oil to two of the vitamin A-deficient dogs produced a marked fall in the blood cholesterol to normal levels. Smith (1934) claimed that the blood cholesterol level of rats maintained on a vitamin A-free diet was higher than that of control animals, but that in the final stages of deficiency the level became lower than that of the controls. This observation was supported by the work of Collazo, Torres & Sanchez-Rodriguez (1934), who observed that blood from rats exhibiting the full picture of vitamin A deficiency had a much lower cholesterol content than blood from control animals. More recently, Mayer & Truant (1949) referred to unpublished work by Mayer indicating a 'piling-up' of cholesterol (presumably in the plasma) in vitamin A deficiency. On the other hand, Sure, Kik & Church (1933) were unable to find any changes in blood cholesterol of rats during the development of vitamin A deficiency even to the stage of severe xerophthalmia and marked loss of weight. The evidence is conflicting and there is clearly need to reinvestigate the matter.

In the present work, particular attention has been given to the experimental design so that the results could be subjected to a satisfactory statistical analysis. Concurrently, a study of the liver cholesterol content of vitamin A-deficient and control rats was undertaken.

EXPERIMENTAL

Materials

Vitamin A solution. The vitamin A administered to the control rats was in the form of a tested cod-liver oil diluted with arachis oil, so that the daily dose per rat (12 or 20 i.u.) was contained in 0.1 ml. of the solution.

Animals and diets. Newly weaned hooded rats were placed on animal diet no. 4 (British Extracting Co. Ltd., Bromborough Port, Cheshire) until about 40 days old (wt. 60–100 g.); this allowed them to store moderate amounts of vitamin A. The animals were then transferred to a vitamin A-free diet made up of casein (Genatosan low vitamin content) 18%; maize starch, 65%; arachis oil, 5%; Marmite, 8%; salt mixture (Osborne & Mendel, 1913), 4%. Vitamin E was not added (see p. 452).

Procedure. Male and female rats from six litters were divided by card selection into two groups (I and II); each group contained twenty rats, and was arranged so that for each rat in group I there was a corresponding litter mate of the same sex in group II, there being equal numbers of males and females in each group. The animals were housed separately throughout the experiment, the cages being arranged so that each subgroup of four rats in either group consisted of two males and two females. Each animal received 10 g. of diet/day for the first week and 12 g./day thereafter. Water was freely provided. The rats in group I received, in addition, 12 i.u. vitamin A/day until day 113, when the daily dose was increased to 20 i.u. until the end of the experiment. The animals in group II received 0.1 ml. of arachis oil to balance the amount given to those in group I. Both vitaminized arachis oil and arachis oil were administered orally from a pipette. The animals grew well, and on the 58th day of the experiment fourteen rats receiving 12 i.u./day of vitamin A had increased in weight by 1448 g. (average 103.4), whereas sixteen rats not receiving vitamin A had increased in weight by 1595 g. (average 99.7 g.).

Analyses of blood and tissues

Frequency of plasma-cholesterol determinations. Determinations were performed on eight rats each day (one set of four from each group) so that the forty animals were examined in 5 consecutive days. Analyses were carried out on the same eight rats at intervals of 14 or 21 days throughout the experiment. When a rat in group II was xerophthalmic and losing weight it was killed, together with the corresponding rat from group I. The rats were anaesthetized with CHCl_3 and as much blood as possible was withdrawn by cardiac puncture (Glover, Goodwin & Morton, 1947). Final duplicate cholesterol determinations were made on each plasma.

Routine collection of blood samples. Blood (0.5 ml.) was withdrawn by cardiac puncture from each rat. With this technique there is always the risk that the animal may die from shock or haemorrhage. In our experiments 212 punctures were performed and there were five deaths from haemorrhage and six from shock. Nevertheless, this procedure for obtaining samples of blood at intervals from the same rat was found to be preferable on all counts to other methods such as severing the tip of the tail. The heparinized blood was transferred to an ignition tube fitted with a rubber collar. This was then placed inside a

15 ml. centrifuge tube and the assembly centrifuged. The plasma was carefully removed by means of a capillary pipette and transferred to a watch-glass.

Estimation of cholesterol. The method employed was a modification of that described by Kenny (1952). Plasma (0.2 ml.) was pipetted into 5 ml. of ethanol-ether mixture (3 vol. of 95% ethanol: 1 vol. of ether) contained in a 10 ml. glass-stoppered test tube; the tube was then stoppered and shaken to mix the sample thoroughly. After loosening the stopper, the tube was heated in a boiling-water bath until the mixture just boiled. The stopper was replaced firmly and the tube and contents were shaken vigorously for about 4 min. The mixture was centrifuged (1800 g for 10 min.), after which the supernatant ethanol-ether mixture was decanted into a boiling tube. The precipitate was resuspended in 5 ml. of ethanol-ether mixture, heated just to boiling, shaken for 4 min. and recentrifuged. The supernatant was again decanted off completely and added to the first 5 ml. of extract. A portion (8 ml.) of this ethanol-ether extract was transferred to a boiling tube, a piece of capillary tubing sealed at the upper end was added to prevent bumping and the contents were evaporated to dryness in a boiling-water bath. The tube was then placed in an electric oven at 100° for 10 min. to remove the last traces of ethanol and water; after cooling, the residue was extracted with 7 ml. of CHCl_3 , the walls of the tube being scraped with a glass rod to remove any adhering particles. The solution was warmed on a water bath and quickly filtered through a Whatman no. 1 filter paper, the top of the funnel being covered with a watch glass to minimize evaporation. A portion (5 ml.) of the filtrate was transferred to a clean, dry glass-stoppered tube and 1 ml. of acetic anhydride was added. The tube was then placed in a water bath at 37° for 3 min. to allow temperature equilibration to occur, after which 0.1 ml. of H_2SO_4 (A.R.) was added from a micro-burette, the stopper was replaced and the contents of the tube were mixed by shaking. The tube was then incubated in the water bath at 37° for 30 min., light being rigorously excluded during this period. The extinction of the solution at 410 m μ . was then measured in a Unicam S.P. 600 spectrophotometer, using a blank prepared as described below. The amount of cholesterol present in the solution was calculated from a standard curve prepared from solutions containing known amounts of cholesterol.

Blank and standard. Cholesterol solution (5 ml.) [0.005% (w/v) in CHCl_3 , freshly prepared from a stock solution of 0.02% (w/v) cholesterol (in CHCl_3)] was evaporated to dryness in a glass-stoppered tube; 10 ml. of ethanol-ether mixture were added, heated just to boiling, shaken and allowed to cool. Part of this solution (8 ml.) was transferred to a boiling tube and evaporated to dryness as for the test. Both were then treated exactly as described for the plasma extract, except that with the blank the H_2SO_4 was omitted. As volumes of plasma in excess of 0.2 ml. could not be obtained with safety, it was necessary to use 'reagent blanks' in place of true 'plasma blanks'. Comparison of these two types of blank revealed that the extinction values were almost identical. No allowance has been made for the higher extinction given by cholesterol esters in this method (see Kenny, 1952).

In our experience, when estimating small amounts of cholesterol (of the order of 0.15–0.2 mg.), it is essential to adhere strictly to this experimental procedure. This method gives results reproducible to within 5%.

Livers and kidneys

Extraction of lipids. At the end of the experiment the livers and kidneys were removed, washed, dried on filter paper and weighed. The kidney lipid was extracted and the vitamin A content determined by the method of Glover *et al.* (1947).

For two rats (I_1 and II_1) the liver lipid was extracted by the method of Glover *et al.* (1947). Parts of the livers from rats (I_3 and II_3) were examined in the Pathology Department; the lipid was extracted from the remainder by the method of Glover *et al.* (1947), except that 95% ethanol was used as the solvent instead of ether.

Saponification of livers. Most of the livers were directly saponified by the following method: to the moist tissue 60% (w/v) KOH (0.5 ml./g.) was added and digestion continued on a water bath until the mixture was homogeneous. Ethanol (2 vol.) was then added and the mixture boiled for a further 30–40 min. under reflux. The mixture was extracted six times with ether (freshly distilled over reduced iron); the extracts were bulked and washed with water until free of alkali, dried over anhydrous Na_2SO_4 , filtered through a sintered-glass funnel (G3), evaporated to dryness under N_2 and weighed. The lipids from the livers of rats I_1 , II_1 , I_3 and II_3 were saponified by this method before cholesterol determinations.

Determination of liver cholesterol. The unsaponifiable matter was dissolved in a suitable volume of $CHCl_3$ and 5 ml. portions were transferred to each of three glass-stoppered tubes (one blank, two tests). The cholesterol content was then determined by adding 1 ml. of acetic anhydride and proceeding as already described under 'Estimation of cholesterol'.

RESULTS

When the rats were killed at the close of the experiment, gross abnormalities of the liver, urinary bladder and testes were seen in several vitamin A-deficient animals. The livers of II_1 , II_3 and II_5 presented signs of fatty infiltration, since they were distinctly yellow and friable. Portions of livers I_3 and II_3 were examined histologically, and it was found that the liver of rat II_3 was indeed infiltrated with fat, whereas that of I_3 (control) was normal. The remaining portions of these livers, and the other fatty livers, were examined as usual and the results are included in the analyses. Grossly distended urinary bladders were observed in rats II_6 , II_8 and II_{15} , and the testes of rats II_{16} and II_{18} were abnormally small and discoloured. All the organs of the control rats appeared normal.

The complete series of plasma cholesterol values as determined at intervals throughout the experiment, together with the terminal plasma vitamin A levels, are presented in Table 1. The mean and standard deviation for each set of values are recorded at the foot of the respective columns.

The liver cholesterol contents for both groups of rats, together with the kidney vitamin A levels, are recorded in Table 2. Results of statistical analyses of sex differences in the liver cholesterol contents of the two groups are given in Table 3.

DISCUSSION

In order to ensure a long depletion period, the rats were maintained on the stock colony diet until they were about 40 days old; they were then placed on the vitamin A-free diet. For some rats the depletion lasted for 19 weeks, and in all animals it was long enough to allow six determinations of plasma cholesterol to be made in each survivor, together with a final duplicate determination when signs of vitamin A deficiency (severe loss of weight and xerophthalmia) were exhibited.

The kidneys from each rat were examined for vitamin A (Table 2) because at low levels of vitamin A intake the kidney exceeds the liver in its capacity to store the vitamin (Johnson & Baumann, 1947).

From Table 1 it can be seen that there was no significant difference (1% level) between the mean plasma cholesterol levels of the deficient and control animals at any time during the experiment. Moreover, statistical analysis (Table 2) of the liver cholesterol (whether expressed as total cholesterol/liver or as mg. cholesterol/g. moist liver) reveals that there was again no significant difference between the two groups ($P=0.05$).

The results of Lowe *et al.* (1953) suggested the possibility that cholesterol metabolism was drastically deranged in vitamin A deficiency. Such a derangement could be pictured as a sequence of abnormal metabolites, only one of which could be detected by ultraviolet spectrophotometry; in the aggregate a substantial proportion of the steroid content might be affected. Alternatively the deranged metabolism might be quantitatively of little significance in the sense that the proportion of steroid molecules affected would be quite small, although the new materials might be qualitatively sharply different, e.g. in their physiological effects. The conflicting reports on the behaviour of plasma cholesterol levels in vitamin A deficiency do not permit a decision to be made between these alternatives.

Plasma cholesterol levels are profoundly affected by alterations in the rate of conversion and destruction of cholesterol (Byers, Friedman & Rosenman, 1952). In our experiments the synthetic diet was virtually sterol-free. Hence, the fact that there is no significant difference in the plasma and liver cholesterol levels between the two groups indicates that no quantitatively major alteration in endogenous cholesterol metabolism occurs during vitamin A depletion. If therefore the abnormal metabolite which appears in the livers of vitamin A-deficient rats is indeed a dehydrogenation product of cholesterol or of steroid hormones, the amounts of this compound are seen to be too small to make a demonstrable contribution to the

Table 1. Serial plasma cholesterol and terminal plasma vitamin A levels in vitamin A-deprived (G II) and control (G I) rats

Rat Sex	Plasma cholesterol (mg./100 ml.)														Terminal vitamin A (i.u./100 ml.)	
	Week 1		Week 3		Week 5		Week 7		Week 10		Week 13		Terminal		G I	G II
	G I	G II	G I	G II	G I	G II	G I	G II	G I	G II	G I	G II	G I	G II		
1 M.	106	103	103	116	102	103	103	103	89	111	98	112	80	116	73.4	Nil
2 M.	90	107	103	102	90	—	102	—	102	—	97	—	88	—	53.7	—
3 F.	—	128	114	113	120	109	120	102	114	108	103	74	125	157	109	Nil
4 F.	146	92	137	97	119	120	138	112	—	—	—	—	—	—	—	—
5 M.	107	136	107	125	114	115	100	118	109	102	104	114	89	137	—	Nil
6 F.	141	113	136	127	121	130	131	108	—	112	—	103	—	77	—	Nil
7 M.	103	131	107	133	102	113	97	114	86	114	103	124	101	112	—	Nil
8 F.	129	149	114	130	103	141	119	138	137	144	98	126	100	132	45.8	Nil
9 M.	131	125	114	114	123	103	108	113	—	84	—	90	—	—	—	—
10 F.	115	121	129	125	123	102	129	83	109	—	111	—	103	—	59.8	—
11 M.	143	120	155	102	—	102	—	102	—	87	—	121	—	—	—	—
12 F.	143	127	154	—	132	—	141	—	107	—	115	—	111	—	85	—
13 F.	111	121	114	102	107	114	129	103	113	141	125	95	96	58	48.5	Nil
14 M.	119	114	119	114	121	120	118	108	114	131	—	115	—	92	—	Nil
15 F.	—	108	109	125	117	103	114	117	107	90	95	82	94	37	120	Nil
16 M.	88	107	—	131	109	100	88	98	91	97	81	71	76	69	52.7	Nil
17 F.	109	100	111	131	102	147	120	131	129	124	121	80	115	52	80	Nil
18 M.	105	114	98	143	127	129	103	125	131	137	102	89	114	85	101	Nil
19 M.	124	114	109	108	119	113	—	109	—	103	—	126	—	—	—	—
20 F.	—	113	—	132	—	124	—	120	—	121	—	82	—	103	—	Nil
Mean	118.2	117.2	118.5	119.5	113.9	116.0	115.3	111.3	109.8	112.9	104.1	100.3	99.4	94.4	—	—
s.d. ±	18.32	13.29	16.9	13.1	11.0	13.9	15.2	12.7	15.28	18.94	11.68	19.58	14.33	35.88	—	—
F	0.039		0.698		0.243		0.223		0.384		7.68		7.64		7.47	
Required for significance (1%)	7.42		7.42		7.44		7.47		7.64		7.68		7.64		7.47	

Table 2. *Liver cholesterol and kidney vitamin A levels in vitamin A-deficient (group II) and control (group I) rats*

Rat	Sex	Total liver cholesterol (mg.)		Liver cholesterol (mg./g. tissue)		Kidney vitamin A (i.u./g.)	
		Group I	Group II	Group I	Group II	Group I	Group II
1	M.	32.72	32.08	3.976	3.565	9.8	Nil
2	M.	18.88	—	2.289	—	7.0	—
3	F.	10.08	35.33	2.045	4.087	4.9	Nil
5	M.	16.35	33.36	2.296	3.848	—	—
6	F.	—	20.00	—	3.378	—	—
7	M.	29.10	13.92	4.163	2.611	12.6	Nil
8	F.	16.16	17.20	2.887	3.532	3.5	—
10	F.	13.84	—	2.057	—	5.0	—
12	F.	13.04	—	1.836	—	7.8	—
13	F.	16.64	11.40	2.586	1.786	9.5	Nil
14	M.	—	24.40	—	4.014	—	Nil
15	F.	16.30	14.64	2.358	1.920	8.8	Nil
16	M.	22.96	11.80	2.375	1.825	8.2	Nil
17	F.	16.01	7.79	2.185	1.306	4.5	Nil
18	M.	19.28	14.80	2.180	2.210	8.5	Nil
20	F.	—	17.50	—	3.301	—	Nil
Mean		18.57	19.56	2.556	2.876	—	—
S.D.		±6.34	±9.01	±0.660	±0.967	—	—
<i>t</i> (Found) =		0.3251		0.9552			
<i>t</i> (Tables) =		2.064		2.064			

Level of probability = 0.05.

For significance *t* (found) must exceed *t* (tables) at the desired level of probability (Snedecor, 1946).Table 3. *Comparison of liver cholesterol in vitamin A-deficient (group II) and control (group I) rats*

		Total cholesterol (mg.)	<i>t</i> (found)	<i>t</i> (tables)	Concentration (mg./g. tissue)	<i>t</i> (found)	<i>t</i> (tables)
Males	Group I	23.215 ± 6.426*	0.317	2.228	2.880 ± 0.926*	0.494	2.228
	Group II	21.727 ± 9.562			3.012 ± 0.919		
Females	Group I	14.58 ± 2.412	0.9025	2.179	2.279 ± 0.360	1.129	2.179
	Group II	17.69 ± 8.791			2.759 ± 1.064		
Group I	Males	23.215 ± 6.426	3.534	2.201	2.880 ± 0.926	1.675	2.201
	Females	14.58 ± 2.412			2.279 ± 0.361		
Group II	Males	21.727 ± 9.562	0.794	2.201	3.012 ± 0.919	0.4542	2.201
	Females	17.69 ± 8.791			2.759 ± 1.064		

* S.E.M.

For significance *t* (found) must exceed *t* (tables) at the desired level of probability (taken as 0.05) (Snedecor, 1946).

total liver and plasma cholesterol levels. If this compound is itself toxic or is the precursor of other toxic substances which, carried to other parts of the body, induce the derangement of normal epithelial metabolism so characteristic of the vitamin A-deficiency state, then the amount of this compound formed need only be relatively small. For these reasons, the results reported here are not necessarily incompatible with the working hypothesis, previously advanced, about the systemic mode of action of vitamin A.

Closer study of Table 1 reveals that the mean value for each set of plasma cholesterol determinations in both groups I and II undergoes a gradual decline during the experimental period. This observation is not unexpected, since the animals

were on a diet virtually free of sterols. In general, the plasma cholesterol levels of the vitamin A-deprived rats showed only minor fluctuations before the very late stages of the deficiency. When the rats in group II were avitaminotic they could be subdivided into three groups according as the plasma cholesterol tended to rise, fall or remain constant. In rats 3 and 5 there was a sharp rise, in 13, 14, 15, 17 and 18 a severe fall (in 6, 9 and 16 the decline was more gradual), and in 1, 7, 8 and 20 there was little change in plasma cholesterol values.

In view of these variations, it might be possible to reach erroneous conclusions if a small number of rats were used in the experimental design. It is therefore interesting to note that our findings on

plasma cholesterol levels confirm those of Sure *et al.* (1933), who used thirty-four test and twenty-seven control rats, but they are not in agreement with those of Collazo *et al.* (1934), Ralli & Waterhouse (1933) and Smith (1934), none of whom used more than six test animals.

Our results on liver cholesterol content are in full agreement with those of Gillum & Okey (1936*a*) but not with those of Javillier, Rousseau & Emerique (1929) and Collazo *et al.* (1934). These last two groups of workers claimed that the livers of vitamin A-deficient animals had a lower cholesterol content than those of controls. However, since liver cholesterol levels are affected by dietary sterols, it should be noted that although Gillum & Okey (1936*a*) employed a sterol-free diet, Collazo *et al.* (1934) used such a diet for the deficient animals but not for the controls, whereas the diet employed by Javillier *et al.* (1929) was not fully reported.

Rats II₁, II₂ and II₃, which all had fatty livers, merit special mention. In II₂ and II₃, the plasma cholesterol levels rose sharply, but in II₁ no marked change was observed, although all three livers had a high cholesterol content (Table 2). In this Laboratory, fatty livers have been observed previously in several of our vitamin A-deficient rats, and it would appear that in the vitamin A-deficient state the liver is rendered more susceptible to fatty infiltration. When tocopherol was added to the vitamin A-deficient diet the abnormalities recorded in the present paper were observed (unpublished work). It is interesting to note that the plasma cholesterol levels of the control rats lie within a fairly restricted range, whereas the terminal liver levels exhibit marked variation. The deficient rat plasma cholesterol levels remain fairly constant until the terminal stages are reached, when they become very unstable. The terminal liver levels of these rats, however, vary even more widely than the terminal plasma levels (see S.D. values, Tables 1 and 2).

Since sex hormones are known to influence cholesterol metabolism (see below) and distribution of vitamin A (Moore, Sharman & Ward, 1951), it seemed of interest to compare the liver cholesterol contents of group I males with those of the males in group II, a similar comparison being made of the females of the two groups. However, in neither case did statistical analysis reveal any significant difference, whether the cholesterol was expressed as mg./g. of moist liver or as total/liver (Table 3).

Our results show that the livers of the control male rats contained a higher absolute amount of cholesterol than the livers of the control females, this difference being statistically significant (Table 3) ($P=0.05$). However, there was no significant difference when a similar comparison

was made on the vitamin A-deficient animals. These findings confirm those of Okey, Gillum & Yokela (1934) and Gillum & Okey (1936*b*); moreover, like these two groups of workers and like Chamberlain (1929), we found that when the liver cholesterol was expressed as mg./g. moist liver the mean figure for the control male rats was higher than the mean for the control females, but this difference was not statistically significant (Table 3).

Although the livers of the control females were on average lighter than those of the males (6.435 g. female and 8.18 g. male) and the male livers tended to decline in weight during vitamin A deficiency, whereas those of the females did not (6.387 g. female, 7.041 g. male), it is unlikely that the differences in liver cholesterol can be due entirely to the weight factor. It seems probable that a full explanation must include an influence exerted by sex hormones. Since androgens have little effect on plasma cholesterol levels (Entenman, Lorenz & Chaikoff, 1940; Rosenman, Friedman & Byers, 1952) or on the rate of hepatic cholesterol synthesis (Rosenman *et al.* 1952), the sex differences found in the liver cholesterol content of the normal rats cannot be explained as an effect of the male sex hormones. On the other hand, although oestrogens do not readily affect plasma cholesterol levels in the rat (Horlick & Havel, 1948; Rosenman *et al.* 1952), they do decrease the hepatic synthesis of cholesterol (Rosenman *et al.* 1952). Since our diet had a negligible sterol content (a factor favouring endogenous sterol production), the effect of oestrogens on hepatic cholesterol synthesis might be exaggerated, and in consequence the difference in the liver cholesterol content of male and female control animals would be at least partially accounted for.

SUMMARY

1. A modified method for the estimation of cholesterol in small volumes of plasma is described.
2. By the use of this method, plasma cholesterol levels were determined at intervals on rats undergoing depletion of vitamin A and on their litter-mate controls.
3. At the end of the depletion period, when the deficient animals exhibited severe xerophthalmia and loss of weight, total liver cholesterol contents were determined on both groups of rats. There was no significant difference between the plasma cholesterol levels of the deficient and control rats at any stage during the depletion, neither was there any difference between the terminal liver cholesterol contents of the two groups.
4. The results are discussed in relation to earlier work and it is concluded that in a major and quantitative sense cholesterol metabolism is not responsive to vitamin A depletion.

We wish to thank Dr J. C. Davis, Pathology Department, University of Liverpool, for histological examination of livers, and Dr Jaspersen for valuable help.

This work was carried out during the tenure of a Medical Research Council Scholarship (B.G.) and an I.C.I. Fellowship (J.S.L.).

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The Isolation of *n*-Pentadecanoic and *n*-Heptadecanoic Acids from Shark (*Galeorhinus australis* Macleay) Liver Oil

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(Received 17 March 1955)

Although the occurrence in natural fats of normal odd-numbered fatty acids, including *n*-pentadecanoic and *n*-heptadecanoic acids, has been reported by various investigators, subsequent work has invariably shown that the acids described were in fact mixtures of adjacent members of even-numbered fatty acids. The generally accepted view until recently is indicated by Hilditch (1947) as follows: 'with the solitary exception of *iso*-valeric acid (found only in the depot fats of the dolphin and porpoise) the molecules of all natural straight-chain fatty acids, saturated or unsaturated, contain an even number of carbon atoms.' This view, however, now requires modification as, after the isolation of pure *n*-heptadecanoic acid from hydrogenated mutton fat (Hansen, Shorland & Cooke, 1954*a, b*), *n*-pentadecanoic acid has been found in hydrogenated mutton fat (Hansen, Shorland & Cooke, 1954*c*), in hydrogenated shark-liver oil (Morice & Shorland, 1954) and in butterfat (Shorland, Gerson & Hansen, 1955). In addition, *n*-undecanoic acid was shown to be present in hydrogenated butterfat (Hansen, Shorland & Cooke, 1955); also, by means of liquid-gas partition chromatography (James & Martin, 1952),

Hansen & McInnes (1954) demonstrated the occurrence in hydrogenated ox tallow of all the odd-numbered carbon acids from C₃ to C₉ inclusive.

In this paper is described the isolation of *n*-pentadecanoic and *n*-heptadecanoic acids from shark-liver oil by the combined processes of fractional distillation and crystallization without recourse to hydrogenation.

EXPERIMENTAL

The X-ray spacings were determined with a Philips Geiger X-ray spectrometer, Fe-filtered K α radiation being used. The samples were melted on a glass slide and quickly cooled. Melting points are uncorrected. The specific rotations were determined on pure fractions, except where these melted above 24°. Such fractions were diluted with CHCl₃. C and H analyses were by Drs G. Weiler and F. B. Strauss, Oxford.

The oil (saponification equiv. 320.0, iodine value 161.9, unsaponifiable matter 8.4%, acid value 5.2) was prepared by solvent extraction (cf. Shorland, Bruce & Jessop, 1952) of the fresh livers of thirty-one male sharks caught in Cook Strait on 21 June 1944. The crude methyl esters prepared from the oil were distilled under high vacuum (at approx. 0.001 mm.) in a falling-film still at 140–160°, the residue being recycled twice to separate the volatile methyl esters