II. CONDITIONS INFLUENCING THE STORAGE OF VITAMIN A IN THE LIVER

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It is generally recognized that in most animals the liver is the chief depot of vitamin A reserves. It has long been known that these reserves are utilized when the diet of the animal contains insufficient vitamin, but an important fact was revealed when Davies & Moore [1935] showed that the considerable accumulation of vitamin which occurs in the liver when the diet contains a large excess is rapidly dispersed when an A-deficient diet is given. The rate of disappearance of this reserve was far greater than that which might reasonably represent the daily requirements of the animal.

The fate of this metabolized vitamin is unknown, although preliminary studies carried out in this laboratory [Santos Ruis, 1935] suggested that a part is excreted in the urine as an unsaturated breakdown product. Clearly the observations of Davies & Moore have an important bearing not only on the question of the cause of the variations in the vitamin A content of liver oils used for therapeutic purposes but also on the fundamental problem of the function of the reserves of vitamin A in the liver.

A large amount of work on the variations in the vitamin A potency of fish liver oil has shown that influx of fat into the liver or its subsequent mobilization will change the concentrations of vitamin, but it is not known whether this is merely a dilution effect or whether the vitamin may be directly concerned, more particularly when fat leaves the liver. The investigations of Drummond [1919], confirmed by Emerique [1932], indicated that vitamin A is not obviously concerned with the mechanisms for absorption and utilization of fat in the organism, whilst recently Green [1934] has recorded that a diet very rich in fat does not accelerate the utilization of the vitamin and that the more obvious aspects of fat metabolism appear to be unrelated directly to vitamin A.

A new experimental method of great value in investigating the function of the liver as a storage organ for fat was provided when it was discovered that choline would prevent the accumulation of fat in the liver of rats living on unbalanced diets rich in fat or cause the excess to disperse if the livers had already become fatty [Best, Huntsman & Solandt, 1932; Best & Ridout, 1933]. The action of choline is primarily on the glyceride fraction, the increased deposition of cholesterol which occurs in animals fed on fat-rich diets, or more strikingly, after administration of unusually large amounts of the sterol itself, being less affected [Best, Channon & Ridout, 1934; Channon & Wilkinson, 1935; Best & Ridout, 1935].

It was thought that a series of experiments in which fatty livers were produced in animals on high or low intakes of vitamin A and in which the fat so deposited could afterwards be dispersed by the "lipotropic" action of choline might throw some light on the influence which these changes had on the amount of vitamin A stored or utilized.

EXPERIMENTAL

Litters of rats, seven in each, from 3 weeks to a month old were used. They were fed on a balanced stock diet until they weighed from 100 to 120 g. They were then distributed, on a litter basis, between seven groups and given one of the following diets. The protein content of the diets was kept low in order to diminish the influence of the "lipotropic" effect which proteins or associated substances exert [Beeston, Channon & Wilkinson, 1935]. The casein was extracted with alcohol and ether.

Diet*	Casein	Starch	Glucose	Lard	Cholesterol	Choline hydrochloride
Control normal (N.)	10	18	50	12	—	—
High fat (H.F.)	10	10	30	40	—	—
High fat with choline (H.F.C.)	10	10	30	40	—	2
High cholesterol (H.C.)	10	10	40	28	2	—
High cholesterol with choline (H.C.C.)	10	10	40	28	2	2
Low fat (L.F.)	10	25	55	—	—	—
Low fat with choline (L.F.C.)	10	25	55	—	—	2

* We wish to acknowledge gratefully much helpful advice given by Prof. H. J. Channon with regard to the selection of appropriate diets and on other matters.

Diet for all groups contained:

Yeast extract	...	5
Salt mixture	...	5

In addition each animal received .25 mg. of a standardized cod-liver oil every week. It contained 1800 I.U. vitamin A per g. The cholesterol was dissolved in the lard used for inclusion in diets H.C. and H.C.C.

The animals were fed on these diets for 4 weeks. Growth and food consumption were recorded.

Diet	Animals		Av. daily food intake g.	Av. increase g.
	♂	♀		
N.	4	6	11.8	17.0
H.F.	5	7	11.4	11.7
H.F.C.	5	6	7.7	15.7
H.C.	4	6	9.6	12.3
H.C.C.	3	8	7.8	17.7
L.F.	7	5	11.2	14.9
L.F.C.	4	7	11.0	13.4

At the end of the period the animals were starved overnight and then killed by a blow on the head. The livers were drained and weighed individually. Those belonging to rats of the same diet group were pooled, thoroughly minced and a small sample taken for moisture determination. The rest was divided into two weighed portions, each of which was ground with anhydrous sodium sulphate and the dry powder immediately extracted with chloroform in a Soxhlet apparatus.

The extract was evaporated under reduced pressure. This gave the "fat" content of the liver. The "fat" of one extract was used for estimation of total phosphorus by Tisdall's method as modified by Peters & Van Slyke [1931-2]. The "fat" from the other sample was used for estimation of the unsaponifiable fraction. One-half of the unsaponifiable matter so obtained was used for estimating cholesterol by precipitation with digitonin, whilst the other half was examined for vitamin A by means of the Adam Hilger "Vitamer A". It was originally intended to estimate the vitamin A in the whole oil as well as in the unsaponifiable matter but it was soon realized that the results thus obtained

ERRATA

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page 6 *for* 0.25 mg. of a standardized cod-liver oil
read 25 mg. of a standardized cod-liver oil

page 49, footnote *for* Buttle *read* Butler

page 58 *for* —, Hostler & Cretcher
read Butler, Hostler & Cretcher

OMISSION

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page 12, after line 4 *read*:

The authors wish to thank the Medical Research Council for a grant towards the expenses of this work, including a personal grant to one of them (A. J. P. M.).

were useless, owing to the large amount of irrelevant absorption. The "Vita-meter A" was standardized against fish liver oils previously examined spectroscopically for absorption at 3280 Å.

The results of the analyses are given below.

Composition of rat liver oils and rat livers

Diet	N.	H.F.	H.F.C.	H.C.	H.C.C.	L.F.	L.F.C.
Av. wt. of livers, g.	5.60	7.05	4.97	11.71	5.74	5.47	4.20
Fat content % of livers	8.95	26.21	5.52	41.28	18.13	5.31	4.68
Water content % of livers	67.20	54.80	69.20	44.00	60.70	69.70	69.25
Lecithin g. per 100 g. liver	3.00	2.73	3.10	1.88	2.99	2.97	2.55
Unsaponifiable % of oil	7.81	5.63	8.90	18.64	40.39	9.26	11.97
Sterol % of oil	4.71	3.30	4.78	16.9	33.3	4.82	6.00
$E_{1\text{ cm}}^{1\%}$ 328 mμ	0.18	0.11	0.39	0.048	0.14	0.34	0.40

Most of the figures are in agreement with the observations of Best & Channon and their associated workers; for example, the lecithin percentages confirm the observations of Best, Channon & Ridout [1934] that the proportion of phosphatide falls with fatty infiltration.

The vitamin A potency of the liver oils is, generally speaking, inversely proportional to the amount of fat present. These figures may, however, merely be an expression of dilution and concentration of the vitamin. They do not tell us what total amount was present. This may be estimated from the product $E_{1\text{ cm}}^{1\%} \times \% \text{ fat} \times \text{wt. of liver (E.F.L.)}$, which for the seven groups was

	N.	H.F.	H.F.C.	H.C.	H.C.C.	L.F.	L.F.C.
E.F.L.	9.64	20.4	10.7	23.3	14.5	9.85	7.87

This calculation does not take into account the size of the animals, but since they were all of the same age, had received the same quantity of vitamin A during the 4 weeks of experimental feeding and had been kept under identical conditions, this is regarded as unimportant. The figures reveal that the storage of fat or of cholesterol in the liver tends to increase the total amount of vitamin. This cannot be merely a question of better absorption when much fatty material is present in the diet, because the animals receiving no other fat than the 3.6 mg. cod liver oil daily (L.F. and L.F.C.) stored as much vitamin as did the control group (N.); moreover, there is no reason to think that the cod liver oil was not fully absorbed in all cases.

Influence of fat mobilization

A second series of experiments was then carried out to ascertain what happens to vitamin A already present in the liver when associated fat is caused to leave under the "lipotropic" influence of choline.

The main comparison was between basal diets containing much (H.F.) and little (L.F.) fat. Instead of giving cod liver oil as a source of vitamin A, a concentrate was employed, so as to enable a larger dose to be given. The amount given to the rats of the appropriate groups was 2 mg. daily. Spectroscopic measurements showed that this corresponded to an intake of approximately 3500 I.U. a day. It was dissolved in 0.025 g. of coconut oil and administered directly to the animals.

The basal "high-fat" diet (H.F.) was of the same composition as that given on p. 6; the composition of the "low-fat" diet was, however, modified so that it included 30 % of casein and 50 % of starch. It did not contain glucose.

Seven groups of animals were studied. They were treated as follows:

Group 1 was fed for 4 weeks on the "low-fat" (L.F.) diet. This was regarded as a control group.

Group 2 received the L.F. diet supplemented with vitamin A for 4 weeks after which it was divided into three subgroups.

One, *Group 3*, was transferred to the basal L.F. diet for 9 days.

Another, *Group 4*, was given the low-fat-choline (L.F.C.) diet for the same period, whilst the third was killed.

Group 5 received the "high-fat" (H.F.) diet supplemented with vitamin A for 4 weeks and was then similarly divided into three subgroups.

One, *Group 6*, was given the basal H.F. diet.

The second, *Group 7*, was transferred to the "low-fat" diet supplemented with choline (L.F.C.), whilst the third was killed. In all cases the period was 9 days.

Group	Original diet 4 weeks	Subsequent diet for 9 days	No. of animals	Av. wt. of rats g.	% fat in livers	% Unsap. in liver fat	$E_{1\text{ cm.}}^{1\%}$ 328 m μ in liver fat	E.F.L.
1	L.F.	—	5	143	4.98	9.00	0.49	13
2	L.F. + Vit. A	—	5	145	5.13	9.55	12.96	400
3	L.F. + Vit. A	L.F.	6	139	5.17	8.20	1.03	26
4	L.F. + Vit. A	L.F. + choline	4	185	4.76	9.09	1.42	48
5	H.F. + Vit. A	—	6	139	11.12	9.57	14.66	900
6	H.F. + Vit. A	H.F.	6	125	8.71	7.96	7.83	300
7	H.F. + Vit. A	L.F. + choline	6	150	6.28	11.26	2.79	120

E.F.L. represents, as before, the product of the $E_{1\text{ cm.}}^{1\%}$ (328 m μ) $\times$ % fat $\times$ wt. of liver, i.e. it is an expression of the total amount of vitamin stored. The first point to observe is that the percentage of fat in the liver was not so high in the animals fed on the H.F. diet as it had been in the previous experiment. Such variations are not uncommon.

The results again show that storage of vitamin A in the liver is facilitated by the presence of fat in the diet and its subsequent deposition in the liver. Considerable storage of the vitamin occurs as a consequence of giving a large excess in the diet, but such reserves are rapidly depleted, as Davies & Moore demonstrated, when the vitamin is no longer administered. In as short a period as 9 days the reserves accumulated on a diet rich in vitamin A but poor in fat are almost completely dispersed. This loss occurs without there being any material change in the fat content of the liver. A similar and striking reduction in the amount of vitamin A stored in the liver occurs in animals fed on a diet rich both in the vitamin and fat when the vitamin is withheld. The depletion appears to be accelerated, however, by causing a partial dispersal of fat in the liver by the administration of choline. This indicates that fat leaving the liver may take with it vitamin A. The matter requires further study but it is interesting to note that Dann [1934] has recorded that female rats fed on a fat-rich diet (20%) appear to pass on more vitamin A to the foetuses during gestation than those fed on a diet containing only 1%.

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