LXVI. THE ABSORPTION OF *n*-HEXADECANE FROM THE ALIMENTARY TRACT OF THE CAT.

BY HAROLD JOHN CHANNON AND JOHN DEVINE.

From the Department of Biochemistry, The University of Liverpool.

(Received February 28th, 1934.)

IN continuance of previous studies of factors affecting the nature and amount of the unsaponifiable fractions of liver, it was shown that *n*-hexadecane is absorbed from the alimentary tract of the rat to the extent of 50 to 108 mg. daily, depending on the quantity of hydrocarbon included in the diet [Mahdi and Channon, 1933]. This result is of interest in relation to fat absorption, because it is opposed to current ideas that absorption of paraffinoid substances does not occur. A point of further importance arose in the course of this work. In one experiment, of 149 g. of hexadecane administered, only 116 g. were excreted, and of the 33 g. of hexadecane unaccounted for no more than an insignificant trace, if any, was present in the liver, whilst the unsaponifiable fraction of the carcass contained at most 1.85 g. of the hydrocarbon. The suggestion contained in this result of the ability of the rat to metabolise a normal hydrocarbon together with the surprising fact that the hydrocarbon failed to appear in the liver but did apparently find its way to the tissues, led us to investigate the matter further. Our first object was to confirm and extend these results by use of an animal which would provide more material for investigation, and also to study the distribution of the hydrocarbon in the tissues by its isolation in chemically pure form.

EXPERIMENTAL.

Hexadecane emulsified in a cream made by means of a machine for preparing a "synthetic" cream from butter and milk was administered to a cat. The amounts of hydrocarbon included, negligibly small at the start of the experiment, were gradually increased as the animal became accustomed to consuming the cream. The usual daily food provided—meat and fish—was withheld until the bulk of the cream had been consumed. At the end of each day, the vessel containing the cream residues was removed from the cage, and the contents were quantitatively transferred to a flask by means of ether. The combined residues were stored until the end of the experiment. By this means, 43.70 g. of hexadecane were presented to the cat during 29 days. A control animal received the same quantity of cream without added hexadecane.

Investigation of the tissues. At the end of the experiment and 24 hours after the last meal, the animals were killed by chloroform and skinned. The kidneys, heart, spleen, brain, liver, mesentery, omentum and perirenal fat were then removed. The muscle was then dissected from the skeleton. The tissues were heated with 5 % aqueous sodium hydroxide until dissolution occurred. The solutions were extracted quantitatively with ether. The fat obtained by

evaporation of the ethereal extract was saponified with alcoholic potassium hydroxide, and the alcoholic soap solution was poured into water and again extracted with ether. The crude unsaponifiable fractions were again saponified with sodium ethoxide to free them from possible contamination with fat, in view of the large amounts of some of the tissues used as starting material. The yields of unsaponifiable matter, together with its cholesterol content and iodine value, and the calculated iodine value of the non-sterol fraction of the unsaponifiable matter from the animal which received hexadecane are recorded in Table I.

Table I.

	Wt. of tissue g.	Unsap. matter g.	Unsap. matter %	Iodine value	Cholesterol as % of unsap. matter	Iodine value of non- sterol material (calculated)
Kidneys	56	0.2882	0.515	91.5	59.7	129.6
Heart	15	0.0827	0.551	42.4	20.0	36.5
Spleen	14	0.0919	0.656	57.1	58.9	44.6
Brain	26	0.6306	2.425	64.2	86.7	53.9
Liver	155	1.7943	1.158	66.5	58.9	67.4
Mesentery	56	0.4001	0.715	19.3	13.3	12.1
Omentum	176	1.0881	0.618	26.3	8.9	27.6
Perirenal fat	107	1.1313	1.057	28.0	13.3	22.2
Muscle	1531	3.2235	0.215	32.2	27.3	19.6
Skin	934	4.7691	0.511	23.5	26.1	8.6

These figures show that in the majority of these fractions the percentage of cholesterol in the unsaponifiable material is low, and this suggests the presence of excess of some unusual non-sterol constituent. That this is a saturated compound appears from the very low iodine values of the non-sterol fractions, particularly of the unsaponifiable materials of the mesentery, omentum, perirenal fat, muscle and skin which are 12.1, 27.6, 22.2, 19.6 and 8.6 respectively. The unsaponifiable fraction of the omentum was a deep yellow oil, those of the muscle and perirenal fat were almost liquid at room temperature, while the materials from the mesentery and skin were low-melting solids. In view of the small amounts of materials available, isolation of any hexadecane present could only be attempted in the case of the omentum, muscle, perirenal fat, skin and liver. These fractions were submitted to distillation *in vacuo* in Claisen flasks of suitable size. At the end of the distillations, the flasks were cooled and the residues quantitatively transferred by ether to small flasks, dried at 100° *in vacuo* and weighed. Because of its high cholesterol content (58.9 %), the liver material was dissolved in light petroleum and much of the cholesterol removed by crystallisation at 0° before distillation. The remainder of the cholesterol was quantitatively removed by means of digitonin. In Table II, the results of the

Table II. *Distillation of the unsaponifiable fractions.*

	Unsap. matter g.	Distillate g.	Yield as % of unsap. matter	Residue g.	Wt. of distillate referred to the whole of each unsap. fraction g.
Omentum	0.541	0.259	47.9	0.284	0.521
Muscle	2.49	1.54	61.8	0.900	1.99
Perirenal fat	0.681	0.329	48.3	0.360	0.547
Skin	4.21	1.27	30.2	2.91	1.44
Liver*	0.619	Trace	—	0.609	—
					4.5 g

* Sterol-free.

distillations are recorded, and the yields of distillate in the whole of each unsaponifiable fraction are shown in the last column.

The distillates were all obtained over a range of 152–166° at 18–21 mm. The hexadecane administered had B.P. 152°/12 mm., and bearing in mind the difficulties of distilling small amounts of materials such as these, the distillates could be considered as reasonably homogeneous. All the corresponding unsaponifiable fractions from the control cat were submitted to an identical distillation procedure, but in no case was any distillate obtained. Analysis of these fractions gave the following figures: Skin: C, 85.6; H, 14.47 %. Omentum: C, 84.23; H, 15.06 %. Muscle: C, 85.35; H, 14.52 %. Perirenal fat: C, 84.90; H, 14.98 %. $C_{16}H_{34}$ requires: C, 84.85; H, 15.15 %. These analytical figures indicated that all the fractions were fairly pure hexadecane. The fractions were therefore mixed, transferred quantitatively to a test-tube and treated twice with an equal bulk of concentrated sulphuric acid at 130° for 15 minutes with constant shaking to remove any contaminating oxygen compounds. The oil was then frozen out by cooling to 0°, transferred to a funnel with ether and washed, first with dilute alkali to remove sulphuric acid and sulphonic acids, and finally with water until the washings were neutral. After removal of the solvent the residue was dried at 100° *in vacuo*; 2.70 g. colourless oil were obtained having the following constants:

	M.P.	B.P.	Mol wt. (Rast)	C	H	Δ_4^{24}	Δ_4^{18}
Found	18	288	230, 234	84.73	14.97	0.772	—
Required for $C_{16}H_{34}$	18	288	226	84.85	15.15	—	0.775

These data show that the 2.70 g. of purified material was hexadecane. Unavoidable losses occurred in the treatment with sulphuric acid, but the isolation of the greater part of the material in chemically pure form and the carbon and hydrogen figures on the crude distillates leave little doubt that the latter were almost entirely hexadecane.

The amount of hexadecane absorbed.

Preparation of the unsaponifiable fraction from the cream residues yielded 12.06 g. of a pale yellow mobile liquid. Treatment with sulphuric acid by the method already described yielded 11.04 g.

The faeces, which had been collected throughout the experiment and stored under alcohol, were worked up for their unsaponifiable matter by the method used by Mahdi and Channon [1933]. The yield was 7.88 g. of a brown liquid which had an iodine value of 12.2 and contained 26.6 % of sterol precipitable by digitonin. The residue, which weighed after analysis 7.4213 g., was subjected to three successive treatments with sulphuric acid and yielded finally 3.6 g. of hexadecane. This was equivalent to 3.81 g. from the whole of the faecal excretion. Thus of 43.70 g. of hexadecane presented to the animal, 12.06 remained in the unconsumed food and 3.81 g. were excreted. The cat thus absorbed 27.83 g. of the hydrocarbon in 29 days, or approximately 1 g. per day.

DISCUSSION.

These results confirm and extend those previously obtained on rats. The failure to isolate the hydrocarbon from the liver is of interest, and there seems little doubt that it was present, if at all, in traces only. The quantitative removal of the sterol before distillation of the liver-unsaponifiable fraction was carried out in order to facilitate the isolation of any hydrocarbon present, and yet the

0.619 g. of sterol-free material yielded on distillation only a trace of an oil, too small an amount for investigation. Further, 0.541 g. of the material from the omentum, which still contained its sterol, readily yielded 0.259 g. of hexadecane on distillation. This seems therefore proof of the absence of the hydrocarbon from the liver. With this result may be contrasted those previously obtained with squalene [Channon, 1926], and with a medicinal liquid paraffin [Channon and Collinson, 1929]. In the former experiments, squalene was isolated from the livers of rats, and in the latter, definite though indirect evidence of the presence of liquid paraffin in the livers of rats was obtained, and this was confirmed by the isolation of about 1 g. of the paraffin from the liver of a pig which had received the paraffin for a period of 54 days.

Hence the unsaturated hydrocarbon squalene, and a liquid paraffin which was shown to be a mixture of polycyclic hydrocarbons, both appear in the liver when administered in the diet, whereas the normal aliphatic hydrocarbon hexadecane does not. It is difficult to interpret the significance of this finding, especially as work on the fat-soluble vitamins and allied substances of recent years has shown that the liver appears to be the tissue in which these substances are predominantly stored. Equally difficult of interpretation is the presence of the hexadecane in the other tissues. Although sufficient material was not available for its isolation from all the tissues, inspection of Table I makes it appear not impossible that it may have been present in all of them. Be that as it may, hexadecane constituted from 30.2 to 61.8 % of the unsaponifiable fraction of the omentum, muscle, perirenal fat and skin, with an average of 47 %. Hence hexadecane does not appear in the liver, but does appear in the tissues. One point worthy of notice is that if the results are referred to yields of hexadecane per kg. of fresh tissue, they become: perirenal fat 3.08 g., omentum 1.47 g., skin 1.36 g. and muscle 1.00 g. per kg. The result with muscle is rendered uncertain, however, because the pad of fat ventral to the xiphisternum was included with the muscle tissue. Thus, so far as these results can be interpreted at present, they suggest provisionally that the hydrocarbon is distributed in the tissues in proportion to the amount of fat which the tissue contains.

We have no positive evidence as to whether squalene and liquid paraffin, which do appear in the liver on administration, also find their way into the fat depots, and we are giving attention to this problem. If the suggestion that the concentration of hexadecane in the tissues depends on their fat content is correct, it would be in keeping with its absence from the liver, which contains an amount of neutral fat of the order of 1 % only. The unsaponifiable fractions of depot fat are so small in amount that they have been little investigated, and these results suggest that they would repay further study.

Of the 28.14 g. of hexadecane absorbed, 4.50 g. only were isolated from the tissues, and hence 24.74 g. were metabolised in 29 days. As to the intermediate steps involved in this process we have no information at present, and we are investigating this problem further by another line of approach. No positive information is as yet available as to how the body finally metabolises any of the unsaponifiable substances. The general tendency for such substances to appear in the liver may suggest that that tissue plays a predominant part in their metabolism, as it does in the case of the carotene-vitamin A conversion. On the other hand, the latter may be a process peculiar to the liver which may act merely as a temporary place of storage for unsaponifiable substances generally. If the liver were concerned in the breakdown of these substances, it is conceivable that its ability to deal with a chemically inert material such as an aliphatic hydrocarbon is limited. The accumulation in the liver of the amounts

of hexadecane found in the tissues of the cat used in this experiment could obviously not be allowed to occur, if for no reason other than that of the solvent action of such quantities of the hydrocarbon on the lipoid constituents of the liver cells.

SUMMARY.

1. A cat absorbed 28.14 g. of *n*-hexadecane when 33.64 g. were administered to it in cream over a period of 29 days.

2. Consideration of the iodine values and cholesterol contents of the unsaponifiable fractions prepared from the different tissues suggested that hexadecane was generally present in them.

3. The hydrocarbon was isolated in chemically pure form from the omentum, perirenal fat, muscle and skin; it could not be isolated from the liver. Its distribution in the tissues appears to be related to their fat content.

4. The relationship of these findings to those previously obtained in experiments with squalene and with a liquid paraffin which was a mixture of polycyclic hydrocarbons is discussed.

5. The animal can metabolise *n*-hexadecane.

REFERENCES.

- Channon (1926). *Biochem. J.* **20**, 400.
— and Collinson (1929). *Biochem. J.* **23**, 676.
Mahdi and Channon (1933). *Biochem. J.* **27**, 1487.