CCI. THE ABSORPTION OF *n*-HEXADECANE FROM THE ALIMENTARY TRACT OF THE RAT.

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THE degree to which unsaponifiable substances are absorbed from the alimentary tract has assumed considerable importance in the last few years on account of the biological significance of the fat-soluble vitamins and carotene. In previous papers the absorption of squalene, phytol, oleyl and cetyl alcohols, cholesterol and a medicinal liquid paraffin has been investigated [Channon, 1926; Channon and Collinson, 1928; 1929]. In the last case, it was found that the liquid paraffin was absorbed to some extent by the rat. Further, from consideration of the cholesterol content of the liver unsaponifiable fractions and of the iodine values of the non-sterol portion of those fractions, it was deduced that the liquid paraffin was present in the liver. By further experiments on pigs, it was found possible chemically to isolate the paraffin from the liver of that animal. These results were of interest because firstly they demonstrated the absorption of hydrocarbons from the alimentary tract to a limited extent at least, even though previous investigations had led to the current view that such hydrocarbons were not absorbed, and secondly they focused attention on the apparent rôle of the liver in storing unsaponifiable substances generally. Demonstration of the former fact raised the question as to the mechanism whereby such inert substances as hydrocarbons are absorbed. Since chemical investigation of the liquid paraffin used showed that it consisted of a mixture of polycyclic hydrocarbons, it was felt that the positive results obtained for its absorption, which seemed at variance with previous work, might be attributable to its particular chemical nature. For this reason, it was deemed necessary to carry out further work using a synthetic normal hydrocarbon.

EXPERIMENTAL.

Preparation of hexadecane.

n-Hexadecane was chosen for this work because it can be prepared readily from palmitic acid and secondly, the fact that it is liquid at room temperature (M.P. 18°) should facilitate its absorption. Pure ethyl palmitate was prepared either from palm oil or commercial palmitic acid. In the main preparation 348 g. of ester dissolved in the minimum volume of warm alcohol (which had been previously distilled after dissolution in it of 2 % of sodium and then boiled for a day with metallic calcium and again distilled) were slowly run on to 209 g. of sodium at 130°. The cetyl alcohol isolated weighed 280 g. (94 %

of theory). The alcohol was converted into the iodide by means of red phosphorus and iodine and the iodide converted into *n*-hexadecane by treatment with zinc dust in glacial acetic acid into which hydrochloric acid gas was occasionally bubbled for a few minutes. The hexadecane was then distilled. The first preparation (B.P. 287°, M.P. 18°) gave C 84.48, H 14.68 % (calc. C 84.85, H 15.15 %) and this was used for exp. I. For exp. II, part of this material was again distilled and the product used gave C 84.60, H 15.1 %. For exp. III, the large preparation after purification with concentrated sulphuric acid at 130° gave C 84.81, H 15.15 %.

Animal experiments.

In each of exps. I and II, two groups of rats, containing the same number of animals of each sex, were fed on a synthetic diet (caseinogen 20, butter 10, starch 60, marmite 5, salt mixture 5) for 21 and 20 days respectively. The experimental groups received in addition, immediately before feeding, doses of hydrocarbon delivered into the mouth from a micro-burette, the dose being gradually increased from 0.035 to 0.12 cc. daily. The animals were then killed by chloroform after being without food for 24 hours. Their livers were removed and the unsaponifiable fractions prepared as described by Channon [1926].

The faeces had been collected twice weekly and stored under absolute alcohol and at the close of the experiments the cages and trays were rinsed with ether, which was added to the main extracts. The faeces from each batch of rats were then boiled separately with a litre of absolute alcohol. This alcohol extraction was repeated three times and followed by three extractions with ether under reflux. The finely powdered residues were then further treated with ether for 12 hours in a Soxhlet extractor. After evaporation to dryness of the combined alcohol-ether extracts, the material was saponified with strong alcoholic potassium hydroxide and the unsaponified material extracted by ether. After a second saponification of this material, the faecal unsaponifiable fraction was obtained. Cholesterol was determined by the digitonin method and the iodine value by the Rosenmund and Kuhnhehn [1923] methods on the unsaponifiable fractions from both livers and faeces.

The results are recorded in Table I.

Table I.

Exps. I and II.

	I		II	
Duration of experiment (days)	20		21	
Amount of hexadecane administered (g.)	12		14	
	Animals receiving the control diet		Animals receiving control diet <i>plus</i> hexadecane	
	I	II	I	II
The unsaponifiable matter of the livers				
No. of animals	9	10	9	10
Wt. of livers (g.)	69.4	77.2	79.9	82.3
(a) Wt. of unsap. fraction (g.)	0.226	0.320	0.238	0.3835
Wt. of sterol in (a) (g.)	0.162	0.150	0.190	0.1788
(b) Wt. of non-sterol fraction of (a) (g.)	0.064	0.170	0.048	0.2047
Iodine value of (a)	94.7	87.3	89.1	90.7
The unsaponifiable matter from the faeces				
(a) Wt. of unsap. fraction (g.)	2.60	2.26	5.80	6.40
Iodine value of fraction	69.0	62.6	32.8	30.1
Wt. of sterol in unsap. fraction (g.)	0.78	0.72	1.00	1.02
Wt. of non-sterol fraction of (a) (g.)	1.82	1.54	4.80	5.62

The results are rendered more lucid by the figures in Table II in which are recorded the yields of unsaponifiable material and sterol per 100 g. of liver, together with the iodine values of the non-sterol fraction calculated from the cholesterol content.

Table II. *Yields of unsaponifiable matter and cholesterol mg./100 g. of liver.*

<i>Exp. I</i>	Unsap. matter	Sterol	Non-sterol	Iodine value of non-sterol fraction
Control	325	233	92	169
Control + hexadecane	298	238	60	181
<i>Exp. II</i>				
Control	415	194	221	106
Control + hexadecane	466	217	249	111

The mean values calculated from 11 control groups of animals in results reported by Channon [1926] and Channon and Collinson [1928] in similar experiments are: unsap. matter 414, cholesterol 296, non-sterol 118 mg. per 100 g. liver, and the figures recorded in Table II all lie within the range of normal variation in those 11 experiments.

Thus the administration of the hydrocarbon has had no significant effect on the yield of the unsaponifiable material of the livers, or on its sterol content. Further, the iodine values of the non-sterol part of the unsaponifiable fractions show no significant difference between the control and experimental animals. With these figures may be contrasted the results of the two experiments of Channon and Collinson [1929] with the polycyclic hydrocarbon mixture. As a mean value for two experiments, these authors found the significant result that in the control animals the material other than sterol in the unsaponifiable fraction constituted but 22.7 % of the whole and had an iodine value of 118.8, as against 56.2 % and 30.8 for the corresponding figures for the animals which received the polycyclic paraffin. It is to be noted however that they too observed no increase in the actual amount of the unsaponifiable matter in the livers.

The unsaponifiable matter of the faeces.

From the figures recorded in Table I on the unsaponifiable fraction prepared from the faeces, certain deductions may be made. In exp. I the weight of the non-sterol fraction in the control group is 1.82 g., and in the faeces of the animals which received the hydrocarbon, 4.80 g. The difference between these two values, namely 2.98 g., gives an approximate value for the amount of the hydrocarbon excreted. Further, an approximate check on this value may be obtained from calculations made from the iodine values of the unsaponifiable fractions and their sterol content, provided it be remembered that the use of the iodine value of cholesterol, 65.8, for the mixture of sterols present in the faeces is arbitrary. Calculation on this basis shows in the control group of exp. I that the fraction of the unsaponifiable matter which is not sterol, 1.82 g., has an iodine value of 70.4, whilst the corresponding fraction for the animals fed with hexadecane, 4.80 g., has an iodine value of 25.9. If the increased amount of the faecal unsaponifiable matter in the latter group is due to the presence of hexadecane, of iodine value zero, and if that part of the unsaponifiable matter which is neither sterol nor hydrocarbon has the same iodine value as the corresponding fraction from the control group, namely 70.4, the amount of the latter may be calculated. The figure so obtained for the amount of hexadecane in the

faecal material is $4.80 - 1.75 = 3.05$ g. which agrees well with the figure obtained directly by difference between the weights of the non-sterol fractions, *i.e.* 2.98 g. Therefore in exp. I, of 12 g. of hexadecane administered, 3 g. only have been excreted. Similar calculations may be applied to exp. II, and the combined results are set out in Table III.

Table III. *Excretion of hexadecane in the faeces.*

	Animals receiving control diet		Animals receiving control diet <i>plus</i> hexadecane	
	Exp. I	Exp. II	Exp. I	Exp. II
(a) Wt. of non-sterol fraction (g.)	1.82	1.54	4.80	5.62
(b) Iodine value of non-sterol fraction (calc.)	70.4	61.2	26.3	22.3
(c) Wt. of material other than hydrocarbon in (a) (g.) (calc. from (b))	—	—	1.75	2.05
(d) Wt. of hydrocarbon (g.):				
(1) By difference from (a)	$4.80 - 1.82 = 2.98$		$5.62 - 1.54 = 4.08$	
(2) By calc. from (b)	$4.80 - 1.75 = 3.05$		$5.62 - 2.05 = 3.57$	
(e) Wt. of hexadecane (g.) (Mean values of (d) 1 and (d) 2)	Exp. I		Exp. II	
Hexadecane administered (g.)	3.00		3.83	
Hexadecane not recovered (g.)	12.00		14.00	
	9.00		10.17	

In the two experiments therefore 9.0 and 10.17 g. of the hexadecane administered were not recovered in the faeces. This corresponds with an absorption of 50 and 49 mg. per rat per day respectively. Hence, although there has been no alteration in the amount or character of the unsaponifiable matter of the livers, a definite amount of the hydrocarbon has been absorbed. In view of the fact that Channon [1926] isolated squalene from the livers of rats which had received squalene in their diet, and that Channon and Collinson isolated the polycyclic hydrocarbon from the liver of a pig which had received liquid paraffin, the failure of the hydrocarbon to appear in the liver is unexpected. Since the liver seems to fulfil the rôle of storing unsaponifiable substances, the absence of hexadecane suggested that the 19 g. absorbed by 19 rats in 21 days may have been completely oxidised or less probably stored in part elsewhere. It was accordingly decided to carry out a further experiment more closely akin to the conditions of that of Channon and Collinson [1929] and at the end to attempt to determine whether the hydrocarbon were present not only in the livers but also in the carcasses. In this experiment, the hydrocarbon was incorporated in a similar diet to the extent of 4 % for 15 days which was increased to 6 % for a further 9 days, the percentage of starch being correspondingly reduced. Every care was taken to keep the experiment as quantitative as possible and the following method was adopted for incorporating the hexadecane in the diet: one-half the butter required for a given quantity of diet was melted and run in to a weighed amount of hydrocarbon in another vessel. After mixing, the butter-hydrocarbon mixture was incorporated in the dry diet, the remainder of the butter being used successively to rinse out the vessel in which the hexadecane and butter had been mixed.

The unsaponifiable fractions of the livers and faeces were prepared as in the previous experiments, and after removal of the entire alimentary tract the carcasses were also worked up. They were warmed on a water-bath with 5 % aqueous sodium hydroxide until digestion was complete, when the fat was exhaustively extracted by ether. The fat was then resaponified with alcoholic

potash and the unsaponified material obtained. In view of the large amount of fat present prior to the second saponification, the resultant product was again submitted to saponification with alcoholic sodium ethoxide. The results of exp. III are set out in Table IV.

Table IV.

Exp. III.

Duration of experiment 25 days. Hexadecane administered 149 g.

	Animals receiving the control diet	Animals receiving the control diet <i>plus</i> hexadecane
The unsaponifiable matter of the livers		
No. of animals	13	13
Wt. of livers (g.)	107.2	111.0
Wt. of unsap. fraction (g.)	0.3403	0.3276
Wt. of sterol in unsap. fraction (g.)	0.2327	0.2402
Iodine value of unsap. fraction	83.3	65.8
Wt. of material other than sterol (g.)	0.1076	0.0874
The unsaponifiable matter of the carcasses after removal of the alimentary tract and liver		
Wt. of unsap. fraction (g.)	5.01	8.04
Wt. of sterol in unsap. fraction (g.)	2.68 (53.5 %)	3.63 (45.1 %)
Iodine value of unsap. fraction	73.8	58.2
Wt. of material other than sterol (g.)	2.33	4.41
The unsaponifiable matter of the faeces		
Wt. of unsap. fraction (g.)	3.90	118.3
Iodine value of unsap. fraction	53.7	9.4

The results calculated on a percentage of the weight of liver are recorded in Table V.

Table V. *Yields of unsaponifiable matter and cholesterol mg./100 g. of liver.*

	Unsap. matter	Sterol	Non-sterol	Iodine value of non-sterol
Control diet	317	217	100	122
Control diet + hydrocarbon	295	216	79	66

These figures also show no increase in the amount of the unsaponifiable matter in the livers of the experimental group although the iodine value of the non-sterol fraction is 66 compared with 122 for that of the control animals, a result similar to that of Channon and Collinson, previously mentioned. The amount of hydrocarbon in the faeces, about 114 g., leaves 35 g. unaccounted for. This corresponds to a daily absorption per rat of 108 mg. an amount rather more than twice as great as was obtained in exps. I and II, a result to be anticipated because in those experiments the animals received the hexadecane in drops before being fed in the morning, while in exp. III the considerably greater amount of hydrocarbon administered was dissolved in the butter before admixture with the rest of the diet.

On the other hand, although increase in the amount of unsaponifiable matter of the livers has occurred, study of the figures recorded in Table IV reveals the fact that the amount of unsaponifiable matter yielded by the carcasses of the experimental animals, 8.04 g., is some 60 % greater than that of those of the control group, 5.01 g.

Further the non-sterol fraction present is 4.41 g. compared with 2.33 g. of the control group. Assuming that the increase is due to the presence of hexadecane only, simple subtraction suggests the presence of 2.08 g. of that substance.

Further, if the iodine value of the material which is not sterol or hydrocarbon in the unsaponifiable fraction from the animals which received hexadecane is the same as that of the non-sterol material from the control group, calculation shows that the former must contain 1.65 g. of a saturated substance. This figure is in fair agreement with that obtained by simple subtraction, 2.08 g. The presumptive evidence of the presence of the hydrocarbon in the carcasses was strengthened by the semi-liquid appearance of the unsaponifiable fraction from the experimental group and its low acetyl value, but it was not finally confirmed by chemical isolation of the hexadecane. We believe, however, that the evidence is sufficiently convincing to enable us to say that from 1.5 to 2 g. of hexadecane or some other saturated substance derived from it has been stored in the tissues other than the liver as a result of feeding. Thus of 149 g. of hexadecane administered, about 114 g. has been excreted in the faeces, and of the remaining 35 g., about 1.85 g. has appeared in the tissues, leaving about 33 g. unaccounted for.

DISCUSSION.

The degree to which the hexadecane has been absorbed needs to be assessed with caution from these results. In exps. I and II the amount of hydrocarbon not recovered in the faeces corresponds to 49 and 50 mg. per rat per day respectively and in exp. III to 108 mg. per day. These figures are open to two possible sources of error: firstly that the accuracy of measurement as determined by the intake and excretion method is limited for the reasons discussed in a previous paper [Channon and Collinson, 1928] and secondly it is not impossible that part of the hexadecane may have been attacked by bacteria in the large intestine, since, as is mentioned later, certain moulds at least are able to utilise higher *n*-hydrocarbons as a sole source of carbon. We may perhaps dismiss the latter possibility as one unlikely seriously to impair the results on account of the slowness with which such an action is likely to take place, while the former should not in any case render the results markedly inaccurate. If therefore the results are accepted at their face value, a number of deductions may be made.

(1) That *n*-hexadecane is absorbed from the alimentary tract of the rat to the extent of between 50 and 100 mg. per day.

This result is essentially similar to those previously obtained with squalene [Channon, 1926] and with a liquid paraffin which was proved to be a mixture of polycyclic hydrocarbons [Channon and Collinson, 1929] in that absorption is definite although the degree of absorption is very limited. It is to be noted that previous experiments suggest that squalene is absorbed even to a less extent than is hexadecane. So far as appears at present therefore the chemical nature of the hydrocarbon has little effect on its degree of absorption which is possibly conditioned by its solubility in bile salt solutions of fatty acids.

(2) In exp. III, in which the unsaponifiable matter from the entire bodies of the animals was prepared, of 35 g. of hexadecane not recovered in the faeces, only about 1.5 g. were present in the tissues, and hence 13 rats have apparently metabolised 33.5 g. in 25 days. Such a finding that the animal appears to possess the power to metabolise a normal aliphatic hydrocarbon is an unexpected one. On the other hand the work of Tausson [1925] and Hopkins and Chibnall [1932] has shown that certain moulds can utilise higher paraffins as a sole source of carbon, while Tausz and Peter [1929] showed that certain soil bacteria possess similar powers. The utilisation of higher normal hydrocarbons by moulds shows that these substances, even though possessing no polar group, are not immune from attack at least by this form of living matter, and hence the disappearance

of some 33 g. of hexadecane in our experiments on the rat may be less surprising than appears at first sight.

(3) The third point worthy of note is that the absorption of hydrocarbon has not resulted in an increase in the percentage of the unsaponifiable matter in the liver. This result is the same as was obtained by Channon and Collinson [1929] when they administered a liquid paraffin to rats, where the percentage of unsaponifiable matter did not rise although the iodine value of the non-sterol fraction was modified in a similar way to that in exp. III of this paper. It was this finding which led these workers to continue the work on pigs from the liver of which the hydrocarbon was isolated. On the other hand, when squalene was administered to rats Channon [1926], the liver unsaponifiable matter rose to about 1 %. It is difficult to suggest why the effects of the administration of squalene which is apparently less well absorbed should cause such a marked rise in the liver unsaponifiable matter, when that of hexadecane causes no rise whatever. Similarly, of the alcohols previously investigated in this experiment phytol, oleyl and cetyl alcohols and cholesterol only the last caused a significant rise in the liver unsaponifiable matter.

(4) The failure of the hydrocarbon to raise the liver unsaponifiable matter significantly is the more surprising in view of the presence of the hydrocarbon in other tissues of the animals, presumably the fat of adipose tissues. During recent years work on the fat-soluble vitamins and the investigations which have been carried out on other substances of peculiar biochemical interest which occur in various fish-liver oils (batyl, selachyl, chimyl and other alcohols, squalene and other hydrocarbons) has tended to focus attention on the liver as the tissue where unsaponifiable substances are generally to be found. This is in part because other tissues, so far as the slight extent to which they have been investigated shows, contain unsaponifiable substances in relatively insignificant amount only. Further, cholesterol is the main constituent of this fraction. Hence it may appear surprising that the hydrocarbon should not be obviously present in the liver. On the other hand, presumably the capacity of the liver for unsaponifiable substances generally must be limited by their nature and therefore it would perhaps be that such a saturated substance as a hydrocarbon would find its way into the fat depots, if it were not readily oxidised.

SUMMARY.

1. Synthetic *n*-hexadecane has been administered to two groups of 9 and 10 rats for periods of 21 days respectively and the degree of absorption measured by the preparation and analysis of the unsaponifiable fractions of the faeces of these and the control groups of animals.
2. The results indicate an absorption by the rat of 50 mg. of hexadecane daily. This absorption had no effect on the amount of the unsaponifiable matter of the livers, nor on their cholesterol content, nor on the iodine value of the non-sterol fraction.
3. In a third experiment 149 g. of synthetic *n*-hexadecane were administered to 13 rats for 25 days, being incorporated in the diet to the extent of 5 %.
4. The degree of absorption measured by the intake and excretion method corresponded to each rat having absorbed 108 mg. per day and resulted in the iodine value of the non-sterol fraction of the unsaponifiable matter of the livers falling from the control value of 122 to 66.
5. After removal of the livers and alimentary tracts, the carcasses of the animals were examined for the hydrocarbon, and of the 35 g. of the latter not

recovered from the faeces, evidence was obtained of the presence of 1.85 g. in the tissues.

6. The disappearance of the remaining 33.15 g. suggests that the animal organism can metabolise normal aliphatic hydrocarbons.

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