LXXVII. THE UNSAPONIFIABLE FRACTION OF LIVER OILS.

V. THE ABSORPTION OF LIQUID PARAFFIN FROM THE ALIMENTARY TRACT IN THE RAT AND THE PIG.

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THE researches of a number of workers, which have been undertaken in recent years in an attempt to elucidate the question as to the chemical nature of the unsaponifiable fractions of liver oils, have revealed the fact that such fractions may contain a number of hydrocarbons and alcohols of varying chemical structure. Of the alcohols, mention may be made of the occurrence in certain fish-liver oils of batyl, selachyl and chimyl alcohols [Tsuji-moto and Toyama, 1922; Toyama, 1923, 1; 1924, 1, 2; Drummond and Baker, 1929]. The chemical nature of these dihydric alcohols was studied by Tsuji-moto and Toyama [1922] and by Toyama [1924, 1] and later by Heilbron and Owens [1928] and Drummond and Baker [1929], who showed that they were the monoglyceryl ethers of octadecyl, oleyl and cetyl alcohols respectively. The presence in fish-liver oils of cetyl and oleyl alcohols has also been reported [Toyama, 1922; 1923, 1], while the latter alcohol has been found also in arctic sperm oil [Tsuji-moto, 1925]. In addition to these alcohols, the presence of three hydrocarbons in various liver oils has been demonstrated. The unsaturated hydrocarbon, squalene, occurs in a large number of shark-liver oils [Tsuji-moto, 1906; 1917; 1920, 1, 2] and, in traces, in cod-liver oil [Drummond, Channon and Coward, 1925; Drummond and Baker, 1929], and has been shown by Heilbron and his colleagues [Heilbron, Kamm and Owens, 1926] to be a dihydrotriterpene. In mammalian liver, a hydrocarbon of similar chemical nature to squalene occurs [Channon and Marrian, 1926]. A third hydrocarbon is *isooctadecane* which has been obtained from fish-liver oils by Tsuji-moto [1917] and Toyama [1923, 2].

The question thus arises as to the source and possible function of these alcohols and hydrocarbons, and in particular whether they are present in these unsaponifiable fractions as a result of their occurrence in the food

ingested, or whether they are synthesised by the organism and play some part in the metabolism of fats or substances of allied nature. With these possibilities in mind, we have been investigating the ability of the mammal to absorb these unsaponifiable substances from the alimentary tract. The study of the absorption of substances other than fats but resembling fats in physical properties began with the researches of Munk, who found that when he administered cetyl palmitate to a patient with a lymph fistula he was unable to demonstrate the presence either of the ester or of the alcohol in the chyle [Munk and Rosenstein, 1891]. Later Connstein [1899] reported that almost all of the 20 g. of lanolin which he administered to a dog was excreted in the faeces, while Henriques and Hansen [1900] on administering a mixture of vaselin and lard to rats found that 95 % of the vaselin was excreted. Again, Bloor [1913] administered a liquid hydrocarbon mixture and also vaselin alone and in emulsions in olive and cocoanut oils, and recovered in the faeces 85–100 % of the hydrocarbons administered. He also gave similar mixtures to animals with thoracic fistulae and prepared the unsaponifiable fraction from the chyle. This was found in every case to be a solid melting about 110° (cholesterol has m.p. 149·5°). These results, therefore, show that although these unsaponifiable substances can be readily emulsified in the small intestine, they remain unabsorbed; and, since fats themselves are readily absorbed, such results provide weighty evidence against the occurrence of particulate absorption. Some of the more recent work, however, seems to suggest that unsaponifiable substances may be absorbed to some extent, at least. Thus, Thomas and Flaschenträger [1923] reported that cetyl alcohol was absorbed to some extent by the dog, and that the rat can also absorb this substance has been shown by Carter and Malcolm [1927] and Channon and Collinson [1928]. In addition, the latter authors carried out experiments with oleyl alcohol and phytol, and absorption of both these alcohols was found to occur in rats. The results of the experiments with phytol, cetyl and oleyl alcohols suggested that their absorption had caused an increase in the amount of unsaponifiable fraction of the liver, although the difficulty of identifying such substances in the small amount of material available prevented their actual isolation. The fact that they are absorbed, however, would possibly explain their appearance in the unsaponifiable fractions of certain liver oils.

The presence of hydrocarbons in the unsaponifiable fractions of liver oils suggested that a study of the question whether hydrocarbons could be absorbed from the small intestine should be undertaken afresh, and it was shown in a previous paper [Channon, 1926] that the unsaturated hydrocarbon, squalene, was absorbed by the rat and appeared in the liver. This finding, coupled with the occurrence of *isooctadecane* in fish-liver oils, made it appear worth while to investigate the question as to whether liquid paraffin was absorbed from the alimentary tract. Our reasons for re-opening this question in the light of much contrary evidence have been that considerable difficulties arise in determining whether absorption of unsaponifiable substances

in small amounts occurs. Hitherto, evidence has been sought in one of two ways. Substances have been administered by mouth and the chyle has been collected and examined for the particular substance, or, alternatively, after administering a known amount of the substance an attempt has been made to determine the amount excreted in the faeces during the experimental period. Apart from the known experimental difficulties of the first method, there is the greater one that the identification of unsaponifiable substances generally is one necessitating a large amount of material and, although the unsaponifiable fraction of the chyle fat may be prepared, the separation of unsaponifiable substances of similar chemical and physical properties is impossible, unless an individual substance constitutes a considerable proportion of the material available. The intake and excretion method suffers from the same difficulty, and in most cases the difference between the weights of the unsaponifiable fraction of the faeces from the control group of animals and that from those receiving the substance has to be taken as the amount of the substance excreted; an uncertain proceeding when it is remembered that faeces normally contain a high and variable content of unsaponifiable substances.

Since the results with squalene showed that this hydrocarbon could be isolated from the animal's liver after absorption, and those with cetyl and oleyl alcohols and phytol also suggested that some part at least of any unsaponifiable material absorbed by an animal would be found in that organ, we decided that a study of the unsaponifiable fractions of the livers of animals which had received liquid paraffin in their diet might provide a more delicate test as to whether the latter were absorbed. As liquid paraffin is a mixture of saturated hydrocarbons, the iodine value of the unsaponifiable fraction of the livers of animals receiving it in their diet should show a fall compared with that of the livers of the animals receiving the control diet only. The unsaponifiable fraction of mammalian livers consists largely of cholesterol, which may constitute up to 85 % of the fraction. Hence it will be necessary to remove the cholesterol quantitatively by means of digitonin, and determine the iodine value of the sterol-free material so obtained, or alternatively to determine the iodine value of the unsaponifiable fraction and to estimate quantitatively the amount of sterol present in it. Calculation of the iodine value of the sterol-free material may then be made, by making allowance for the amount which the cholesterol present contributes to the iodine value. A difficulty with the latter method has hitherto been that cholesterol behaves abnormally with iodine solutions [Smedley-MacLean and Thomas, 1920]. This difficulty has now been overcome by the introduction of the pyridine sulphate dibromide method of Rosenmund and Kuhnhehn [1923] as described by Dam [1925]. This method has been shown by Dam and later by Copping [1928] to give theoretical values with cholesterol, and it possesses the further advantage that it can be applied to very small quantities.

EXPERIMENTAL.

Exps. 1 and 2. Administration of liquid paraffin to rats.

A control group of rats was fed on the synthetic diet described by Drummond and Coward [1920], while 5 % of liquid paraffin, without previous emulsification, was mixed with the diet of the experimental group. The animals were maintained on these diets for 5 weeks, after which they were kept without food for 24 hours and killed. Their livers were removed and weighed, and the unsaponifiable fractions prepared from them by the method previously described [Channon, 1925]. Aliquot portions of the unsaponifiable fractions were then used for determination of the sterol content by the digitonin method [Windaus, 1910] and of the iodine value [Dam, 1925]. In a second experiment the procedure was exactly repeated. The results so obtained are recorded in Table I.

Table I.

	Animals receiving control diet		Animals receiving control diet plus liquid paraffin	
	Exp. 1	Exp. 2	Exp. 1	Exp. 2
No. of animals	5	6	6	6
Wt. of livers, g.	32.0	39.7	41.0	43.5
(a) Wt. of unsap. fraction, g.	0.0965	0.1447	0.1860	0.2122
(b) Wt. of cholesterol in (a), g.	0.0692	0.1200	0.0810	0.0936
(c) Wt. of material other than sterol in (a) (by difference), g.	0.0273	0.0247	0.1050	0.1186
Iodine value of (a) (Dam)	78.6	77.7	47.2	45.1

The results are rendered more lucid by the figures in Table II, in which are recorded the yields of unsaponifiable material per 100 g. of liver and the percentage of cholesterol in the unsaponifiable fraction. With the knowledge of the cholesterol percentage and that cholesterol has an iodine value of 65.8, it is possible to calculate, from these figures and the iodine value of the unsaponifiable matter, the iodine value of that part of the latter which is not sterol. Thus in the control group of Exp. 1, the unsaponifiable matter, which has an iodine value of 78.56, contains 71.69 % of sterol. The iodine value (x) of the non-sterol fraction may thus be calculated as follows:

$$\left(\frac{71.69}{100} \times 65.8\right) + \frac{28.31}{100} x = 78.56.$$

The results so obtained are also recorded in Table II, together with the mean values of the figures from the two experiments.

From the mean values it is seen that the yield of unsaponifiable matter from the livers of the animals which received liquid paraffin has increased by 40 % over that of the control groups, which suggests that some of the liquid paraffin has been absorbed and has appeared in the liver. This result seems to receive support from the degree of unsaturation of the non-sterol portion of the unsaponifiable fractions, for, whereas the iodine value of the material other than sterol is 118.8 in the control group, it is only 30.8 in the

case of the animals to which liquid paraffin had been administered. Moreover, the percentage of non-sterol material in the unsaponifiable fraction of the livers of the control group is only 22.68, as against 56.17 in the case of the animals which received paraffin.

Table II.

Exp. No.	Animals receiving	(a) Unsap. matter per 100 g. liver g.	Cholesterol in (a) %	Material other than cholesterol in (a) %	Iodine value of (a)	Iodine value of the non-sterol fraction of (a) (calc.)
1	Control diet	0.3016	71.69	28.31	78.6	110.9
2	"	0.3645	82.94	17.06	77.7	135.8
1	Control diet plus liquid paraffin	0.4536	43.56	56.44	47.2	32.7
2	"	0.4877	44.10	55.90	45.1	28.8
Mean values	Control diet	0.3330	77.82	22.68	78.1	118.8
	Control diet plus liquid paraffin	0.4707	43.83	56.17	46.1	30.8

With the other results already reported [Channon, 1926; Channon and Collinson, 1928] figures for the amount of unsaponifiable matter per 100 g. liver and its percentage content of cholesterol have been obtained from determinations on upwards of 200 rats. For this large number of determinations the mean value of the unsaponifiable fraction is of the order of 0.42 g. per 100 g. liver, of which 73 % is sterol precipitable by digitonin, while 27 % is composed of material other than sterol. These figures are mentioned here because, although the percentage of sterol in the unsaponifiable fraction of the control groups in the experiment under discussion is of the same order, 77.82, the yield of unsaponifiable matter per 100 g. of liver, 0.3330 g., is considerably lower than the average value worked out on the large numbers of livers to which reference has just been made. As to the reasons why the yields of unsaponifiable matter per 100 g. of liver in the control groups of these two experiments are considerably below the mean value found for the large number of animals, we have no information, for the diet in this and previous experiments has been the same, although the ages of the rats have varied. Hence no reliance can be placed on conclusions drawn from the figures obtained in these experiments for the yield of unsaponifiable matter per 100 g. of liver only. On the other hand, the significant result of these experiments is that, in the control animals, the material other than sterol in the unsaponifiable fraction constitutes but 22.68 % of the whole and has an iodine value of 118.8 as against 56.17 % and 30.8 for the corresponding figures in the animals which received paraffin. These results seem fairly convincing proof that some liquid paraffin has been absorbed and has found its way into the liver, to cause an increase in the amount of the unsaponifiable fraction and a consequent reduction in the percentage of cholesterol present and the iodine value of the material other than cholesterol. On the other hand, as has been pointed out already, the iodine values of the material

other than sterol have been calculated from the iodine value of the whole unsaponifiable matter by making allowance for the amount of cholesterol present. Since cholesterol gives theoretical results by the iodine method employed [Copping, 1928], this calculation seems to be sound. On the other hand, sterols tend to behave abnormally towards iodine solutions and, since these results seem directly opposed to those of the workers discussed in the opening paragraph of this paper, we have carried out a further experiment on larger animals (pigs) and attempted to isolate the hydrocarbon fraction in some state of purity, to avoid the doubts which calculated results inevitably raise.

Exp. 3. Administration of liquid paraffin to pigs.

Two pigs received a diet consisting of a mixture of barley meal 12 parts, white fish meal 1 part, and sharps 6 parts. Before the morning feed, one of the animals received in addition 100 cc. of liquid paraffin administered in the following way. To 100 cc. of gum acacia mucilage (gum 4 parts, water 6 parts) were added 100 cc. of liquid paraffin, and the mixture after very vigorous shaking was thoroughly incorporated in a small portion of the dry diet and given before the morning meal; it was readily consumed by the animal. After 54 days, the animals were killed 36 hours after their last meal. The unsaponifiable fraction of their livers was then prepared and the amount of the sterol present in an aliquot portion estimated by the digitonin method. The results obtained are recorded in Table III.

Table III.

	Animal receiving control diet	Animal receiving control diet plus liquid paraffin
Wt. of liver, g.	1537	1730
„ unsaponifiable fraction, g.	5.6231	8.2420
„ cholesterol, g.	4.231	4.267
„ material other than cholesterol, g.	1.3921	3.975

Note. As to the normal yield of unsaponifiable material from pig liver, we have little information. Two samples bought from shops at different times were worked up for their unsaponifiable content; in one case 935 g. liver yielded 3.47 g., and in the other 659 g. yielded 2.40 g., *i.e.* 3.71 and 3.64 g. per kg. respectively as against the control figure in the present experiment of 3.66 g. per kg.

With a view to the later isolation of the hydrocarbon mixture, it was decided to remove the sterol quantitatively. Accordingly the greater part was obtained by crystallisation of the fractions from light petroleum, boiling below 60°, with which liquid paraffin is miscible. Each fraction was dissolved in 110 cc. of light petroleum and cooled to 0°. The crystalline material obtained by filtration was washed with light petroleum cooled to 0° and the combined mother-liquors were concentrated to 50 cc., and the process was repeated. In this way, there were obtained 3.35 g. and 3.40 g. of sterol from the fractions from the livers of the control animal and the animal which received the liquid paraffin respectively. The remaining sterol was removed

with digitonin. To each of the residues obtained by evaporation of the light petroleum mother-liquors were added 50 cc. of 95 % alcohol, and the mixtures heated to boiling and vigorously shaken. A considerable portion of the material from the liver of the animal which received paraffin failed to dissolve. To each there was then added 3.1 g. of digitonin dissolved in 240 cc. of boiling 90 % alcohol. After gently boiling the mixtures with vigorous shaking, they were allowed to stand overnight. They were then filtered through paper, and the residues, consisting of cholesterol digitonide, excess digitonin and any substances insoluble in 90 % alcohol, were washed with the latter solvent and the washings were united with the mother-liquor, which was then evaporated to small bulk and poured into water. The mixture was then ether-extracted, and the extract washed with water, evaporated to dryness and dried *in vacuo* on the water-bath. The residues on the filters were then thoroughly washed with ether and the ether extract was filtered into a separating funnel. This ether extract was also washed with water and evaporated to dryness, and dried on the water-bath. By this process, each fraction was freed from cholesterol, and divided into two portions, one soluble in about 350 cc. of 90 % alcohol and the other insoluble in that volume of this solvent. The object of the procedure, apart from removing cholesterol, was to concentrate any liquid paraffin present in the insoluble fraction, for the liquid paraffin used in the feeding of the animals was found to have a solubility in 90 % alcohol at room temperature of only 0.18 g./100 cc. Iodine values were then determined by Hübl's method on the four fractions obtained. The weights of the four sterol-free fractions and their iodine values are recorded in Table IV.

Table IV.

	Animal receiving control diet	Animal receiving control diet plus liquid paraffin
Sol. in 90 % alcohol, g.	0.9589	1.3182
Insol. in 90 % alcohol, g.	0.4969	2.5785
<i>Iodine values:</i>		
Sol. in 90 % alcohol	92.8	57.7
Insol. in 90 % alcohol	151.2	25.7

From the figures recorded in this table, the iodine values of the combined sterol-free fractions for each animal may be calculated. In the case of the control animal, the material other than sterol has an iodine value of 112.8 and for the animal which received the paraffin 36.6. These figures may be compared with those obtained in the experiments on rats, the mean values of which were 118.8 and 30.8 respectively, and confirm the results obtained on those animals by a less direct method.

Before describing the attempts made to isolate the hydrocarbons, it will be well to consider the results recorded in Table IV and to discuss the nature of the substances which present-day knowledge suggests may be present. If liquid paraffin be present, it would be expected that the greater

part of the fraction containing it would remain insoluble in the volume of 90 % alcohol used for precipitation of the cholesterol by digitonin. In addition, the fact that liquid paraffin has a slight solubility in 90 % alcohol should cause the amount of the soluble fraction in the case of the animal receiving paraffin to be greater than that in the control. The figures in the table record such a result, for the amount of the insoluble material in the animal receiving paraffin is about five times as great as in the control animal, while there is a corresponding increase of about 0.36 g. in the soluble fraction. Since the paraffin has a solubility of 0.18 %, it might have been thought that this latter difference would be greater considering the volume of solvent used. The reason for it not being so is probably that the separation was carried out in the presence of the flocculent precipitate of cholesterol digitonide which carried down the paraffin with it. It was in the hope of this happening that the procedure adopted was used. A consideration of the iodine values should tell us something of the nature of the substances present. If the soluble material in the case of the animal receiving paraffin contains some hydrocarbon, it should have a proportionately low iodine value. This is so, for the control material has an iodine value of 92.8 as compared with 57.7 for that of the animal receiving paraffin. The differences shown in the insoluble fractions are still more considerable, for the control material has an iodine value of 151.2 as opposed to one of 25.7 for the animal receiving paraffin. It was decided, therefore, to attempt to isolate the saturated hydrocarbons from the fractions insoluble in 90 % alcohol.

Fractions insoluble in 90 % alcohol. It was shown in a previous paper [Channon and Marrian, 1926] that there was present in mammalian liver a highly unsaturated hydrocarbon, similar to squalene. This hydrocarbon was not obtained in pure form, but its presence was deduced from the fact that it was possible to isolate it as a bromide and hydrochloride. Since it is very insoluble in any solvent containing water, it should appear in this experiment in the fractions insoluble in 90 % alcohol, and the iodine values of those fractions will be due mainly to the presence of this unsaturated hydrocarbon. It was decided to remove it by taking advantage of the fact that the bromide to which it gives rise is insoluble in ether. Accordingly, the fractions were dissolved in anhydrous ether at 0° and excess of a solution of bromine in ether was added. After standing for a short time, the insoluble bromide from the two fractions was removed by filtration and washed with ether. Each was shown to be the bromide of the unsaturated hydrocarbon, to which reference has just been made, by its bromine content (68 %) and by the fact that it charred at 180° without melting. In this way there were isolated 0.37 g. and 0.5 g. of insoluble bromide from the material from the liver of the control animal and the animal which received paraffin respectively. Since the fraction from the liver of the latter animal had an iodine value of 25.75, 2.45 g. should absorb 0.3975 g. of bromine, while the bromine content of the insoluble bromide isolated is 0.34 g. Hence the bromine content of the

material from which the unsaturated hydrocarbon has been removed is 0.0575 g., or 2.45 % bromine, and hence, prior to bromination, the material present other than the unsaturated hydrocarbon had an iodine value of 4 only. The filtrate and washing from the insoluble bromide were then shaken with sodium thiosulphate solution in order to remove the excess of bromine; and the ether solutions, after washing with water and drying with anhydrous sodium sulphate, were evaporated to dryness. In view of the low bromine content (2.45 %) it was decided to distil the material without debromination. The 2.2 g. were transferred with ether to an 8 cc. flask but, unfortunately, about 20 % of the material was lost during the removal of the ether by evaporation. The material was distilled at 1 mm. pressure. There were obtained two fractions and a residue: fraction (1), 0.3 g. boiling up to 160°, fraction (2), 0.9 g. boiling from 160°–210° and 0.32 g. of residue. The distillates were amber-coloured oils of viscosity similar to that of liquid paraffin.

Fraction (2) analysed as follows:

0.1021 g. gave 0.3202 g. CO_2 and 0.1262 g. H_2O : C, 85.53; H, 13.73.

0.1482 g. absorbed 0.31 cc. of 0.1036 *N* iodine: Iodine value 2.7.

These figures show that fraction (2) approximates sufficiently closely to a saturated hydrocarbon as to render it unnecessary to attempt to purify the remainder further, and the object of this experiment, namely the isolation of the paraffin from the liver, was thus achieved.

Fractions soluble in 90 % alcohol. It was considered desirable to attempt to demonstrate whether the increase in the amount of the fraction soluble in 90 % alcohol derived from the liver of the animal which received the liquid paraffin was caused by the presence of that substance. Accordingly, these fractions, 0.864 g. and 1.186 g. from the control and the animal receiving paraffin respectively, were converted into acetyl derivatives by boiling with acetic anhydride. The acetyl derivatives were then saponified by boiling with excess of 0.2 *N* alcoholic potassium hydroxide for 4 hours and the acetyl values so determined. After saponification, the fractions were recovered by ether extraction and brominated in cold dry ether as described in the previous section. After removal of the insoluble bromide of the small amount of the unsaturated hydrocarbon appearing in these fractions, the excess bromine of the mother-liquor was removed by washing with sodium thiosulphate solution. The ether solutions were then dried with anhydrous sodium sulphate and evaporated to dryness, and the bromine content of the materials so obtained determined. The results obtained are given in Table V.

Table V.

	Animal receiving control diet	Animal receiving control diet plus liquid paraffin
Acetyl value	116.6	91.3
Wt. of insol. bromide (g.)	0.14	0.05
Acetyl value corrected for the unsat. hydrocarbon	123.5	92.5
Bromine content after removing the insol. bromide, %	25.68	22.14

It will be seen from the above table that both the acetyl values and the bromine contents of the fractions after bromination are considerably lower in the case of the fraction from the animal which received paraffin, and it seems clear, therefore, that the increased amount of the latter over that of the control animal is due to liquid paraffin appearing in it, which would lower its acetyl value and the percentage of bromine in the brominated product.

DISCUSSION.

The results obtained from the experiment on the pigs in which hydrocarbon has been isolated in nearly pure form leaves no doubt that some part of the paraffin administered has been absorbed and has appeared in the liver. Further, the figures obtained showing the effect of the administration of liquid paraffin on the yields of the unsaponifiable fractions and their iodine values are similar in both the rat experiments and those with the pigs, and if due allowance be made for the biological nature of the experiment, these figures may be used to gain some insight into the question as to how much paraffin has accumulated in the liver of the animals. These figures are set out in Table VI, where the mean values of the two rat experiments are given.

Table VI.

Animals	Diet	Sterol g./100 g.	Non-sterol g./100 g.	Iodine value of non-sterol
Rats (mean values)	Control diet	0.2591	0.0755	118.8
	Control diet plus liquid paraffin	0.2063	0.2643	30.8
Pigs	Control diet	0.2752	0.0905	112.8
	Control diet plus liquid paraffin	0.2467	0.2298	36.6

In the rat experiments, the non-sterol fraction of the livers of the animals which received paraffin is roughly 3.5 times greater than that of those of the control animals, and the iodine value has shown a fall corresponding closely with this increase. In the pig experiment, the increase in the non-sterol fraction is about 2.5 times that of the control, while the iodine value is about one-third as great as the control iodine value, a result in not quite such good accordance as the rat experiments provide.

As to the mechanism by which the absorption of the paraffin has occurred, we have little information. In a previous paper [Channon and Collinson, 1928], the absorption of cholesterol, phytol, cetyl and oleyl alcohols was discussed. The results of Moore and Parker [1901], which extended the earlier observations that bile salt solutions would dissolve fatty acids, by demonstrating that such acids were very much more soluble in bile salt solutions in which lecithin was present, made it worth while to determine the solubilities of these alcohols together with that of squalene and liquid paraffin in bile salt solutions with and without lecithin. Not too great a reliance can be placed on these results on account of the difficulties of such determination and the very low solubilities obtained, but it was found that the alcohols were soluble to an extent in rough proportion to the degree to which they

were absorbed. The solubility of squalene in 5 % bile salt solution at 37° was found to be as low as 0.024 %, while liquid paraffin was found to possess no detectable solubility in this medium. In this connection, the results of Mellanby [1927, 1] are of interest. Mellanby introduced various fat emulsions into the duodenum of a cat and observed fat absorption by inspection of the lacteals in the mesentery, cannulae having been previously inserted into the gall bladder, common bile and pancreatic ducts. He found that emulsified fat to which bile had been added was rapidly absorbed, and absorption caused a secretion of pancreatic juice and an increased flow of bile. On the other hand, neither emulsified fat nor fat previously digested by lipase was absorbed in the absence of bile, and the injections of such mixtures caused no secretion of pancreatic juice and no increase in bile secretion. If there be no available source of lipase other than pancreatic juice, therefore, emulsified fat according to these results may be absorbed without previous hydrolysis. Continuing these experiments, Mellanby [1927, 2] found that in a similar experiment in which an emulsion of paraffin and bile was injected, there was neither absorption of the hydrocarbon nor secretion of pancreatic juice nor an increase in the bile secretion. An experiment which the same author kindly carried out with squalene showed that in contrast to liquid paraffin squalene was absorbed to a small extent, and that the injection caused a large secretion of pancreatic juice and bile. Mellanby's results, both with emulsified fat and squalene, suggest that particulate absorption may occur in spite of much contrary evidence. The results with liquid paraffin recorded in this paper are opposed to those just cited, and in this connection the work of Clark and Clark [1917] may be mentioned. These authors studied the results of the injection of fine emulsions of fat, such as olive oil, oleic acid, egg-yolk and cream, into the tissue of tadpoles' tails. They found that the fat injected was soon surrounded by leucocytes, and lymph vessels grew out to the oil droplet in a time varying from a few hours to two or three days. No reaction, however, was obtained with substances such as mineral oil.

A word may be said here as to the chemical nature of the liquid paraffin used in the experiments. The main fraction boiled at 210° at 1 mm., and since no solid products could be obtained by distillation, and by attempts at crystallisation of the undistilled residues, we were led to the view that the hydrocarbons present in the mixture could not be normal aliphatic substances. This view was confirmed by the figures obtained on combustion, for the mean values showed that the liquid paraffin contained C, 86.6 %; H, 13.3 %. On the other hand, $C_{10}H_{22}$ requires C, 84.50; H, 15.50 and $C_{20}H_{42}$ requires C, 85.1; H, 14.9, and hence a mixture of aliphatic hydrocarbons C_nH_{2n+2} of which liquid paraffin might be composed would have figures intermediate between these such as C, 84.8; H, 15.2, a carbon content definitely lower than that actually found. We consulted Dr W. R. Ormandy on this point and he informed us that, although the popular idea is that medicinal paraffin is composed entirely of aliphatic compounds, strong grounds exist for believing that a large

proportion of it is naphthenes and that some authorities go so far as to consider that the whole of ordinary liquid paraffin is composed of ring compounds. A naphthene $(\text{CH}_2)_n$ requires C, 85.7; H, 14.3, while our liquid paraffin corresponds to $(\text{CH}_{1.84})_n$. From the combustion figures, therefore, the liquid paraffin appears to consist of a mixture of polycyclic paraffins. This point is important because the nature of the hydrocarbons present in the paraffin may be a factor involved in the absorption¹.

We have no information as to how much liquid paraffin was absorbed by the animals in the experiments described in this paper, but the results of previous workers suggest that it can have been absorbed in small amount only. The fact that some absorption occurs, however, suggests that a study should be made as to whether liquid paraffin administered to a lactating animal can be detected in the milk. This is a question of some practical importance to which we are now giving attention.

SUMMARY.

1. The iodine value of the non-sterol fraction of the unsaponifiable matter of the livers of rats which receive liquid paraffin in their diet was found to be 31 as against 119 for that from the control animals. This result suggests that liquid paraffin is absorbed to some extent.
2. This was confirmed by an experiment on pigs, in which the hydrocarbon was isolated from the liver.
3. The factors concerned in the absorption are discussed.

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¹ The question as to whether unsaturated material was present in the paraffin was investigated by determination of the iodine value (Wijs). The average value found was 0.3.

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