L. THE BIOLOGICAL SIGNIFICANCE OF THE UNSAPONIFIABLE MATTER OF OILS.

IV. THE ABSORPTION OF HIGHER ALCOHOLS.

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In a previous paper [Channon, 1926], it was reported that the unsaturated hydrocarbon squalene is absorbed from the alimentary tract by the rat. Its absorption caused a large increase in the amount of the unsaponifiable fraction of the liver and its presence could be demonstrated in that fraction. It was suggested that the large increase in the amount of the liver cholesterol which occurred at the same time might be due to some chemical relationship existing between squalene and cholesterol. Alternatively, that increase might be due to the fact that, as the amount of the unsaponifiable fraction had increased on account of the absorption of squalene, the amount of cholesterol had also increased in order that the ratio of cholesterol to other unsaponifiable substances present might remain as constant as possible. The work described in this paper was carried out with a view to throwing further light on these two hypotheses.

Brief reference may be made to the literature concerning the absorption of alcohols and hydrocarbons. Munk and Rosenstein [1891] administered cetyl palmitate to a patient with a lymph fistula and found that the chyle which was subsequently collected for 14 hours contained neither cetyl palmitate nor cetyl alcohol, but tripalmitin only. Further experiments with amyl oleate yielded results which confirmed the previous finding that hydrolysis of esters precedes absorption of the fatty acids. Similar results were obtained by Frank [1898], who administered the ethyl esters of the higher fatty acids and found only the glyceryl esters in the chyle. More recently, Bloor [1912] sought to overcome the difficulty of detecting small quantities of such substances in the chyle by administering to dogs with thoracic fistulae "isomannide" esters of high optical activity. He found, however, no trace of optically active substance in the chyle. The results of these workers make it appear established that the esters of higher alcohols are first hydrolysed before the absorption of their constituent fatty acids. A number of experiments have been carried

out in which the absorption of unsaponifiable substances themselves have been studied. Connstein [1899] using the intake and excretion method found that almost all the 20 g. of lanoline which he administered to a dog was excreted in the faeces, and Henriques and Hansen [1900] carried out similar experiments with a mixture of equal parts of lard and vaseline and found that 95 % of the vaseline was excreted. Bloor [1913] administered a liquid hydrocarbon mixture and also vaseline, alone and in an emulsion in olive and coconut oils, and recovered in the faeces 85.5 to 100 % of the hydrocarbons. He also carried out experiments on animals with thoracic fistulae using similar hydrocarbon mixtures. The unsaponifiable fraction of the chyle fat was prepared and in every case it proved to be a solid of M.P. 110° (cholesterol, M.P. 148.5°). The only workers who claim that absorption of a hydrocarbon occurs are Bradley and Gasser [1911], who administered an emulsion of olive oil and petroleum to a dog and found that the thoracic lymph obtained by fistula contained both the oil and the hydrocarbon in about the same relative proportions as in the emulsion which was administered; they conclude that "this suggests a mechanical absorption of droplets of fatty acids and hydrocarbon oil mixtures."

In the present investigation use has been made of the difference method in which animals in two groups are fed on a given diet and to one group the unsaponifiable substance is given as a small daily supplement. The unsaponifiable matter is prepared from the faeces of the two groups of animals collected over the period of experiment, and the difference between these weights of unsaponifiable material taken as representing the amount of the unsaponifiable substance excreted. Not too great a reliance may be placed on such a figure, however, firstly because the collection of the faeces is difficult and it is impossible to be sure that some loss of material has not occurred, and secondly because the output of unsaponifiable substances in the faeces varies somewhat even among carefully chosen groups of animals on a standard diet. In this connection, reference may be made to the work of Sperrey [1927] who found that an animal on a fat-free diet excretes an appreciable amount of unsaponifiable substance in the faeces and that this excretion persists even when the bile is drawn off by means of a fistula. Hence, although every care may be taken to ensure that control animals of similar ages and weights are used, variations in the excretion of unsaponifiable material may occur. It is clearly desirable also to commence and terminate the experiment by the inclusion of charcoal or bone ash to ensure that the collection of the faeces be accurately made. Within the limitation that the difference in the amount of substance administered and the amount excreted must be considerable before any conclusions as to whether absorption occurs can be drawn, this method will serve.

It will be remembered that the unsaturated fatty acids are more readily absorbed than the saturated, probably because they are more readily emulsified but possibly by virtue of their chemical properties. The alcohols used in these experiments—cholesterol, phytol, cetyl and oleyl alcohols—were chosen

with a view to seeing whether chemical structure as opposed to physical properties were a factor involved in the absorption. Although it is known that cholesterol (M.P. 148.5°) is absorbed to some extent, that alcohol was used because the general distribution of sterols in tissues necessitates the daily ingestion of a definite quantity of those substances and, secondly, cholesterol can be accurately estimated by the digitonin method. Phytol, $C_{26}H_{53}OH$ (B.P. 202° , 10 mm.), which is present in all plant tissues containing chlorophyll must likewise be ingested in small amounts. Cetyl alcohol, $C_{16}H_{33}OH$ (M.P. 51°), previously used as the palmitate by Munk and Rosenstein [1891] and the unsaturated oleyl alcohol, $C_{18}H_{33}OH$ (B.P. 207° 13 mm.), are natural products. The results obtained with squalene [Channon, 1926] suggested that it was desirable to determine firstly, whether these alcohols were absorbed and if the absorption could be related to their chemical or physical properties, and secondly, whether their absorption caused an increase in the amount of the unsaponifiable fraction of the liver, and if so, to determine whether there was an increase at the same time in the amount of the liver cholesterol.

EXPERIMENTAL.

Two groups of rats were fed on a complete artificial diet, one of the groups receiving, in addition, a small quantity of the particular alcohol daily. The diet used was that described by Drummond and Coward [1920] with the replacement of the vegetable oil by butter, and the groups of animals were chosen as alike as possible in age, weight and sex. The daily supplements of alcohol were given prior to the food. Oleyl alcohol and phytol were given as drops direct into the animal's mouth. Cetyl alcohol and cholesterol were dispersed in olive oil and given in this way, the control animals in these experiments receiving similar amounts of olive oil daily. In order to facilitate absorption, it was considered desirable to give small doses for some time rather than to administer larger doses over shorter periods.

Other experiments were carried out with cetyl alcohol and cholesterol in which the animals received a diet containing 1 % of these substances. Throughout the experiments the faeces were collected daily and stored in alcohol until the end of each experiment.

Treatment of livers. At the end of the experiment the animals were killed by chloroform and the combined livers of each group were weighed, heated with 5 % potassium hydroxide until solution was effected, and the hydrolysed product was extracted six or seven times with ether in a separating funnel after adding sufficient alcohol to ensure rapid separation of the two layers. The combined ether extracts of each batch of livers were washed with water to remove soap and alcohol, evaporated to dryness and finally dried *in vacuo* at 100° . The material so obtained was then resaponified with sodium ethoxide dissolved in alcohol, and the solution poured into water and extracted with ether as before. The combined ether extracts were washed with water, great care being taken at this stage to ensure by many washings that no soap was

left behind in the ether extract. The ether extract was dried *in vacuo* at 100° and the weight of the unsaponifiable matter obtained. Cholesterol was estimated in an aliquot part by the digitonin method.

Treatment of faeces. The faeces, which had been stored in alcohol, were ground up in the alcohol and filtered; two more grindings with absolute alcohol were made and the residue sucked as dry as possible. The residue was then extracted with ether in a Soxhlet apparatus. All the alcohol washings and ether extracts were combined, and the solvents removed. Two saponifications followed by ether extractions were carried out, as already described for the livers.

RESULTS.

Oleyl alcohol. The oleyl alcohol used in these experiments was prepared by the reduction of ethyl oleate by the method of Bouveault and Blanc [1904]. In both experiments it was administered before the daily food in drops.

	No. of rats	Time days	Weight of livers g.	Weight of unsap. matter g.	Weight of cholesterol g.	Unsap. matter of faeces g.	Oleyl alcohol administered cc.
<i>Exp. 1</i>							
Control diet	6	30	56	0.2174	0.1880	1.8044	—
Control diet + oleyl alcohol	6	30	60	0.3052	0.2200	3.4416	11.23
<i>Exp. 2</i>							
Control diet	6	15	41.8	0.2195	0.1161	1.2960	—
Control diet + oleyl alcohol	6	15	45.8	0.2636	0.1711	1.5918	5.26

Thus, in Exp. 1, of 9.7 g. of oleyl alcohol administered, there has been excreted about 1.6 g., and in Exp. 2, of 4.53 g., about 0.3 g. It may be calculated from these figures that the amount of oleyl alcohol absorbed by the rat per day is 0.045 g. in Exp. 1, and 0.047 g. in Exp. 2.

Phytol. The phytol used was in part prepared from nettle leaves by the method of Willstätter and Stoll [1913]. The remainder was kindly supplied by Prof. I. M. Heilbron. The experiments were carried out as described for oleyl alcohol.

	No. of rats	Time days	Weight of livers g.	Weight of unsap. matter g.	Weight of cholesterol g.	Unsap. matter of faeces g.	Phytol administered cc.
<i>Exp. 3</i>							
Control diet	6	15	45.4	0.2308	0.1322	1.5586	—
Control diet + phytol	6	15	43.2	0.2400	0.1577	1.6338	4.80
<i>Exp. 4</i>							
Control diet	4	28	35.4	0.1316	0.1004	1.762	—
Control diet + phytol	7	28	61.0	0.3972	0.2762	1.828	7.47

From these results it appears that the phytol administered has been completely absorbed, and taking the figure of the first experiment, it is seen that the rat can absorb 0.046 g. of phytol per day.

Cetyl alcohol.

	No. of rats	Time days	Weight of livers g.	Weight of unsap. matter g.	Weight of cholesterol g.	Unsap. matter of faeces g.	Cetyl alcohol administered g.
<i>Exp. 5</i>							
Control diet	8	21	50.9	0.2206	0.1697	0.5703	—
Control diet + cetyl alcohol	8	21	46.1	0.1890	0.1290	1.1455	5.60

In Exps. 6 and 7, the animals received the basal diet plus 1 % of cetyl alcohol in an attempt to increase the amount of the unsaponifiable fraction of the liver.

	No. of rats	Time days	Weight of livers g.	Weight of unsap. matter g.	Weight of cholesterol g.	Unsap. matter of faeces g.
<i>Exps. 6 and 7</i>						
Control diet	6	16	45.4	0.1868	—	0.9546
	6	16	45.3	0.1561	0.1036	0.8419
Control diet + cetyl alcohol	6	16	42.7	0.2104	—	2.5166
	6	16	41.1	0.1928	0.1044	2.1488

The results of Exp. 5 show that 8 rats have absorbed about 5 g. of cetyl alcohol in 21 days, or an absorption of 0.030 g. per day. If we take 10 g. per day as the figure for the food intake of each rat, the total amount of cetyl alcohol ingested in Exps. 6 and 7 by 12 rats in 16 days is 19.2 g., whereas about 1.5 g. has been excreted. This would give an average absorption of about 0.09 g. per rat per day as against 0.03 g. in Exp. 5.

Cholesterol. A known weight of cholesterol was suspended in olive oil at 37° and three or four drops of the suspension were given to each animal daily. At the end of the experiment, the unused cholesterol was determined by the digitonin method, and the weight of cholesterol so determined subtracted from the weight taken.

	No. of rats	Time days	Weight of livers g.	Weight of unsap. matter g.	Weight of cholesterol g.	Unsap. matter of faeces g.	Weight of cholesterol given g.
<i>Exp. 8</i>							
Control diet	12	21	76.3	0.3308	0.2545	0.8556	—
Control diet + cholesterol	12	21	77.2	0.9657	0.9122	4.9013	8.30

These results show that about 4 g. or 50 % of the cholesterol administered has been absorbed, which corresponds to about 0.016 g. per rat per day, and that it has caused an increase in the amount of liver cholesterol to about three and a half times the control value.

In a repetition of Exp. 8, animals were given the basal diet with the addition of 1 % of cholesterol for a period of 16 days instead of administration of a daily supplement.

	No. of rats	Weight of livers g.	Weight of unsap. matter g.	Weight of cholesterol g.	Unsap. matter of faeces g.
<i>Exp. 9</i>					
Control diet	6	50.6	0.2341	0.1779	0.7494
Control diet + 1 % cholesterol	6	51.1	0.6755	0.5934	8.7934

It is seen that the liver cholesterol has again risen to about three times the control value. No records were kept of food intake, but allowing that each

animal consumed 10 g. of the diet per day, the amount of cholesterol ingested by the experimental group is about 9.6 g. with an excretion of 8 g., or an absorption of 1.6 g. by 6 animals in 16 days.

From these two experiments, it is clear that cholesterol is absorbed by the rat to the extent of about 0.017 g. per day, and that much of this cholesterol finds its way into the liver.

The results of these experiments show that all the alcohols administered have been absorbed but that the degree to which they are absorbed varies. It is naturally not easy to state with any accuracy from the results of such experiments as these the actual amount of any one substance which the rat can absorb, because a number of factors, such as whether the substance was given alone, as a suspension in olive oil, or mixed in the diet, and the ages and weights of the rats, need to be taken into consideration. There seems little to choose between the amounts of oleyl alcohol and phytol which have been absorbed and the results obtained in the experiments with these substances may be well compared, for each was given in drops. Apparently none of the phytol administered in either experiment has been excreted, so that the figure obtained for the absorption, namely 0.046 g. per day, must necessarily be a minimum. On the other hand, some of the oleyl alcohol has been excreted in both experiments with that substance, and hence the figure obtained for the absorption, namely 0.046 g. per rat per day, the mean of Exps. 1 and 2, is a maximum. This seems to show definitely that phytol is more readily absorbed than oleyl alcohol.

The results of the experiments with cetyl alcohol differ from each other. Where the alcohol was given as a suspension in olive oil, the rat absorbed 0.03 g. per day: on the other hand, in Exps. 6 and 7 of this series, in which the alcohol constituted 1 % of the diet, an average figure of 0.09 g. per day is obtained. There seems little doubt that the degree to which such substances as these are absorbed depends to a considerable extent on the method of administration and on what other fatty substances are present in the diet. Thus in their paper on Mutton Bird oil, which contains 38 % of unsaponifiable matter consisting chiefly of cetyl alcohol together with oleyl alcohol and a small amount of cholesterol, Carter and Malcolm [1927] found that when they administered the oil direct, 3 adult rats were able to absorb almost all of the 12.33 g. which they received in 8 days. This amount of oil contained 4.7 g. of unsaponifiable matter, *i.e.* the absorption of the unsaponifiable fraction was at the rate of 0.19 g. per day per rat, whereas when they administered cetyl alcohol as a suspension in olive oil only 0.056 g. per day was absorbed. In Exps. 6 and 7, in which cetyl alcohol constituted 1 % of the diet, the alcohol and the butter of the diet were melted up together before incorporation with the other constituents, and an absorption of 0.09 g. per rat per day resulted. The results obtained with cetyl alcohol confirm the observations of Thomas and Flaschenträger [1923] who found that a dog would absorb 4 to 6 g. of cetyl alcohol per day.

Cholesterol on the other hand seems to be absorbed to a much smaller

extent, namely at the rate of 0.016 g. per day. It seems safe to conclude, therefore, that of the alcohols used in these experiments, phytol is most readily absorbed; oleyl alcohol is next, and is followed by cetyl alcohol: cholesterol is absorbed to the least degree. It will be observed that the first two alcohols are liquid, whereas cetyl alcohol and cholesterol have m.p. 51° and 148.5° respectively.

Other considerations pointed to the fact that partial absorption of some of these substances might occur, for it is to be remembered that the animal depends for its existence on its ability to absorb vitamin A, which is an unsaponifiable substance, and probably a higher alcohol [Drummond, Channon and Coward, 1925]. Further, the animal can absorb vitamin D, which is again an unsaponifiable substance. The production of vitamin D by the action of ultra-violet light on ergosterol [Rosenheim and Webster, 1927] makes it appear possible that vitamin D is an unsaturated alcohol also.

Further, reference may be made to the lipochrome pigments carotene, $C_{40}H_{56}$, and xanthophyll, $C_{40}H_{56}O_2$. Of the earlier workers, Sauer mann [1889] showed that plant carotinoids can be transferred to the feathers and egg-yolks of birds, and Willstätter and Escher [1912] demonstrated that certain of the animal lipochromes are identical with plant carotinoids. More recently Palmer [1922] and his colleagues have shown that the carotene of the butter-fat, adipose tissue and blood-serum of cattle is derived from the food, as is the xanthophyll of the tissues and egg-yolks of hens. These results have been confirmed by other workers and sufficient evidence seems available to make it certain that the lipochrome pigments present in animal tissues are derived solely from the diet, so that here again we have further examples of the absorption of a hydrocarbon of high molecular weight and its related oxygen compound, both of which are common constituents of plant material.

The unsaponifiable fraction of the liver.

The results previously recorded [Channon, 1926] showed that after absorption, squalene could be detected in the liver, and that the unsaponifiable fraction of that organ was increased to two or three times the control value. In Table I there are given the weights of unsaponifiable matter and of cholesterol calculated per 100 g. of liver from the foregoing results.

Table I. *Weights of unsaponifiable matter and cholesterol contained in 100 g. of liver.*

Experi- ment	Control	Unsap. matter per 100 g. liver g.	Cholesterol per 100 g. liver g.		Unsap. matter per 100 g. liver g.	Cholesterol per 100 g. liver g.
1	"	0.3883	0.3359	Control+	0.5087	0.3666
2	"	0.5250	0.2777	oleyl alcohol	0.5750	0.3737
3	"	0.5083	0.2912	phytol	0.5555	0.3651
4	"	0.3717	0.2836	"	0.6511	0.4528
5	"	0.4335	0.3336	cetyl alcohol	0.4101	0.2799
6	"	0.4119	—	"	0.4925	—
7	"	0.3445	0.2287	"	0.4691	0.2540
8	"	0.4335	0.3336	cholesterol	1.2510	1.1810
9	"	0.4625	0.3517	"	1.3220	1.1610

Since all the control animals were receiving the same diet, an average value of the figures for all of them calculated on the basis of 100 g. of liver may be taken, and mean values taken also for the results of the experiments with each substance. These results are recorded in Table II together with the results obtained in the experiments with squalene [Channon, 1926].

Table II.

	Unsap. matter per 100 g. liver g.	Cholesterol per 100 g. liver g.
Controls	0.4310	0.3045
„ + oleyl alcohol	0.5419	0.3701
„ + phytol	0.6033	0.4089
„ + cetyl alcohol	0.4541	0.2669
„ + cholesterol	1.2865	1.1700
„ + squalene	1.1156	0.6437

The average figures for the nine control groups of animals are very close to those recorded previously [Channon, 1926] for an average of five groups which served as controls in the experiments with squalene, namely 0.4226 g. for the unsaponifiable fraction and 0.3179 g. for the cholesterol per 100 g. liver. There seems, therefore, some justification for considering these figures for the control groups as of significance, although the variations from them in three cases are greater than desirable. Compared with the average of 0.4310 g. for the unsaponifiable fraction of the control groups, the figures for the oleyl alcohol and phytol experiments, namely 0.5419 and 0.6033 g., seem definitely raised outside the variation obtained in the figures for the control animals. The results with cetyl alcohol, however, are inconclusive. The cholesterol experiments show that the unsaponifiable matter for 100 g. of liver has been raised to about four times the control value, and the increase is wholly accounted for by the rise in the amount of cholesterol in this fraction.

It seems justifiable, therefore, to conclude that the amount of the unsaponifiable fraction of the liver is increased when unsaponifiable substances are included in the diet. In the phytol experiment in which the amount of unsaponifiable fraction has increased, there appears to be a definite increase in the amount of cholesterol. The cholesterol figure in the oleyl alcohol experiment shows a similar, although smaller, increase. These results, however, may help to explain those obtained by administering squalene, when the figure for cholesterol rose to 0.6437 g., while the amount of the unsaponifiable fraction increased to 1.1156 g. They suggest possibly that when the amount of the unsaponifiable fraction of the liver is increased, the amount of cholesterol in that fraction shows a similar tendency to increase, rather than that squalene is a precursor in cholesterol synthesis in the animal body. Further experiments are being undertaken with a view to procuring additional evidence on this point, but it is to be observed that such a relationship would be the reverse of that which has been demonstrated in certain fish-liver oils [Channon, 1928].

THE PROCESS OF ABSORPTION.

We may now consider the possible methods by which these unsaponifiable substances are absorbed. Moore and Parker [1901] extended the early observations that bile would dissolve fatty acids by showing that the solubilities of such acids in bile salt solutions which contained lecithin were very much greater than in bile salt solution alone, and these results coupled with those of other workers have given rise to the modern view that fat is first hydrolysed by the pancreatic lipase, and that the resulting fatty acids are absorbed chiefly as a solution in bile. This suggested the desirability of determining the solubility of these unsaponifiable substances in solutions of bile salts. Commercial bile salts were purified by repeated solution in alcohol and crystallisation at 0° after the addition of ether. A 5 % solution was then shaken for 12 hours at 37° with excess of the different substances. The mixtures were then filtered through Whatman No. 3 paper at 37° to give clear golden brown solutions, 25 cc. portions of which were exhaustively extracted with ether, and the ether extracts, after washing with water, were evaporated to dryness *in vacuo* at 100°, taken up in ether, filtered, and weighed after removing the solvent. The mean values are given in Table III.

Table III. *Solubilities in 5 % bile salt solution at 37°.*

Phytol %	Oleyl alcohol %	Cetyl alcohol %	Cholesterol %	Squalene %	Paraffin %
>2.00	0.490	0.176	0.029	0.024	0.00

Not too great a reliance may be placed on these figures, for considerable difficulties arise in the determination of solubilities in bile salt solutions. Firstly, the solubilities and consequently the amounts weighed were very low and, secondly, the emulsions which arise when the ether solutions are washed with water render the values obtained somewhat uncertain. Results of the same order were obtained when the solubilities were determined in a solution containing 5 % of bile salt and 0.25 % of lecithin prepared by Levene's method [1927], which had N, 1.92 %; P, 4.06 %, the presence of the lecithin making no significant difference to the solubilities. It is of interest to observe from the above table that the degrees to which these substances are absorbed, as reported earlier in the paper, run parallel with their solubilities in bile salt solutions, save that squalene appears to be absorbed to a somewhat greater extent than cholesterol, for it was found in Exp. 1 of a previous paper [Chan- non, 1926] to be absorbed to the extent of 0.024 g. per day as against 0.017 g. per day for cholesterol. These results, therefore, would fit in well with the theory of the absorption of fatty acids—that solution in bile is the means whereby such substances are absorbed from the intestine. In this connection, the experiments of Mellanby [1927, 1] are of interest. Mellanby inserted cannulae into the gall bladder, common bile duct and pancreatic duct of a cat and introduced various fat emulsions into the duodenum. Fat absorption was

determined by inspection of the lacteals in the mesentery, and it was found that emulsified fat to which bile had been added was rapidly absorbed and 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 caused no secretion of pancreatic juice and no increase in the bile secretion. According to these results, therefore, emulsified fat in bile is absorbed by the cat without previous hydrolysis by lipase. Continuing these experiments Mellanby [1927, 2] reported that in a similar experiment in which an emulsion of paraffin and bile was injected into the duodenum of a cat there was neither absorption of the hydrocarbon nor secretion of pancreatic juice nor an increase in the amount of bile secreted. The author concludes from this result that the hydrocarbon had prevented the absorption of the bile salts by the intestinal mucosa. In view of this behaviour of liquid paraffin, Prof. Mellanby very kindly undertook to carry out a similar experiment with squalene. He injected squalene emulsified with diluted bile (2 cc. of squalene, 2 cc. bile, 5 cc. Ringer's solution, 1 cc. 1.5 % NaHCO_3) into the duodenum, and found by inspection of the lymphatics that it was absorbed to a small extent in the duodenum and upper part of the jejunum. Contrary to the behaviour of liquid paraffin, there was no interference with the absorption of bile as evidenced by the large secretion of pancreatic juice and bile following the injection. It seems clear from Mellanby's experiments both with emulsified fat and with squalene that particulate absorption of fatty substances may occur in the small intestine. Determinations of the solubility of olive oil in 5 % bile salt solution gave a figure of the order of that given by squalene. Although it is almost certain that the solubilities of these substances in bile itself will be considerably greater [Moore and Rockwood, 1897], Mellanby's results can only be interpreted as meaning that particulate absorption may occur. His failure to observe absorption of emulsified fat in the absence of bile suggests either that a sufficiently fine emulsion of the fat is produced only when bile is present, or, alternatively, that the bile salts may have a direct effect on the permeability of the mucosa of the small intestine. The latter view seems to us the more probable. The contrast in the behaviour of liquid paraffin and squalene when injected into the duodenum suggests that liquid paraffin is not absorbed on account of its lack of reactive groups. On the other hand, although the possession of such groups by a substance is a factor concerned in its ability to be absorbed, it is clear that there must be other factors, for otherwise these unsaponifiable substances should be absorbed to a much greater degree.

SUMMARY.

1. The ability of the rat to absorb phytol, cholesterol, oleyl and cetyl alcohols from the alimentary tract has been studied.
2. The degree to which these substances are absorbed is in the order phytol, oleyl alcohol, cetyl alcohol, cholesterol, the most easily absorbed being placed first.
3. The solubilities of these substances in bile salt solutions fall in the same order as the degrees to which they are absorbed by the rat.
4. The presence of unsaponifiable substances in the diet increases the unsaponifiable fraction of the liver.
5. Where such increase occurs, the cholesterol in that fraction tends to increase also.
6. The possibility of particulate absorption occurring in the small intestine is briefly discussed.

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