LXX. OBSERVATIONS ON THE BIOCHEMISTRY OF "MUTTON BIRD" OIL¹.

BY CHARLIE LYONS CARTER AND JOHN MALCOLM.

From the Departments of Chemistry and Physiology, University of Otago, Dunedin, N.Z.

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MANY seabirds, especially those of the petrel family, express their annoyance at the approach of an intruder by ejecting the contents of their stomach. This material is usually of an oily nature and in several different parts of the world advantage has been taken of this to obtain an oil that can be put to various uses. Thus the inhabitants of St Kilda collect the oil of the fulmar petrel and on the coasts of Tasmania and New Zealand a "stomach" oil is obtained from birds of the same family.

"Mutton bird" oil, the subject of this paper, is a mobile, reddish brown oil obtained in New Zealand from the petrel, *Aestrelata lessoni*, which nests on certain small islands off the south coast. When about to leave the nest, the young birds are killed for food by the Maoris, and if suspended head downwards a small quantity of the oil mixed with watery fluid can be collected as it flows from the beak. The oil can also be obtained in the same way from the parent birds, and the opinion is widely held that it is a nutrient fluid secreted by the parent birds and fed to the young. It occurred to us that another explanation might be that the oil represents an indigestible residue of the fatty fish (sprats, shell-fish, etc.), on which the petrel is said to feed during the nesting period.

To test this we were led to study the digestibility of the oil and from that we proceeded to trace as far as we could the fate of the oil during absorption and its behaviour when introduced parenterally. As yet we have not succeeded in unravelling the problems that presented themselves but we think it advisable to publish what we have done.

One of us (C. L. C.) has already published [1921] some work on the chemistry of the oil, and has shown that it has a close resemblance to sperm oil and consists largely of cetyl oleate together with esters of related alcohols and acids. It contains only traces of glycerides and a small amount of cholesterol. When our work began, we were not aware of any paper on the fate of cetyl esters in the body beyond the well-known work of Munk [1891],

¹ This paper was despatched from New Zealand before the receipt in that country of Rosenheim and Webster's paper on the stomach oil of the fulmar petrel [*Biochem. J.* (1927), **21**, 111].—EDITOR.

who showed that spermaceti (cetyl palmitate) is absorbed and appears in the thoracic duct as palmitin. According to Hutchison [1922] spermaceti was at one time used medicinally as an aid to fattening and was said to be well borne and not difficult to absorb. Purdy [1918] reports that in Tasmania an emulsion of mutton bird oil and almond oil had gained at one time a considerable reputation in the treatment of phthisis.

Thomas and Flaschenberger [1923], in work on the absorption of cetyl alcohol and cetyl acetate in dogs, found that a dog could absorb 4 to 6 g. of cetyl alcohol per day on a daily intake of 20 g. and about the same amount of the ester. They call attention to the difficulty in understanding how the cetyl alcohol, a substance insoluble in water and having such a high melting point (50°), can be absorbed, and they suggest that it may be due to combination with the desoxycholic acid of the bile, which Wieland and Sorge [1916] have shown can unite chemically with various similar substances to form compounds that are water-soluble.

A recent paper by Channon [1926] on the absorption and fate of the unsaturated hydrocarbon "squalene" (the "spinacene" of Chapman) is also of interest in this connection, for with both squalene and cetyl alcohol there is the same difficulty of understanding how absorption can occur.

DIGESTION *IN VITRO*.

The oil being of the nature of a fluid wax is not easily emulsified. The best results were obtained with gum acacia as recommended by Lueders and Bergeim [1923]. Toluene was added to inhibit bacterial action. The lipase preparation used was a fresh pig's pancreas, minced and pounded in a mortar with a small quantity of bile, then strained through cheese cloth. When 2 g. of this was added to 10 cc. of mutton bird oil emulsion made slightly alkaline to litmus, the mixture became distinctly acid in 17 hours, and on being made alkaline again developed an acid reaction. After 42 hours, it was made alkaline and extracted with ether. On comparing the weight of this extract with that of the fatty acids obtained from the alkaline residue it was found that 42 % of the original oil had been digested, while a control with olive oil emulsion and the same amount of lipase showed 83 % digestion. In another experiment, at the suggestion of Professor Inglis, we added lithium carbonate to the prepared tubes so as to provide a continuous supply of base to neutralise the fatty acids. In 3 days there were formed 3.416 g. fatty acid from 11.0 g. mutton bird oil, while a control with boiled ferment yielded 0.1 g. fatty acid from 10.5 g. of the same oil. This represents 50 % digested.

We could quote several other experiments but these are enough to show that the mammalian pancreatic lipase can hydrolyse the cetyl esters found in this oil, although the action appears to be slow as compared with that on glycerides. We have not had the opportunity to test the lipase of the bird itself under satisfactory conditions.

ABSORPTION.

Ducks. Unsuccessful attempts, lasting over a week, were made to induce two ducks to feed on a paste made of pollard containing about 5 % of mutton bird oil.

Cats. A uniform diet of minced meat, porridge and milk was used and the oil was administered as an emulsion by stomach tube under light anaesthesia. The faeces were collected in 2 to 3 day periods, and extracted with ether after being finely broken up in water. The ether-soluble material was then saponified and the unsaponifiable fraction extracted with ether. In the second experiment the residue of faeces after the first ether extraction was saponified and again extracted so that we could be tolerably certain of having obtained all the cetyl alcohol excreted. The results are shown in Table I.

Table I. *Faeces (cat).*

	No.	Weight g.	Total ether soluble g.	Total unsaponi- fiable g.	Remarks
<i>First experiment:</i>					
Preliminary, 4 days	I	38	—	1.55	—
Experimental, 2 „	II	30	7.345	5.435	About 22 g. M.B. oil given
Subsequent, 4 „	III	?	1.945	1.415	—
<i>Second experiment:</i>					
Preliminary, 2 days	I a	23	2.69	1.58	—
Experimental, 2 „	II a	51	8.11	6.14	About 19 g. M.B. oil given
Subsequent, 3 „	III a	39	1.21	0.825	—

It will be noticed that in each experiment there was a large increase in the output of unsaponifiable matter, but the increase is less than the total unsaponifiable fraction (cetyl and other alcohols) of the oil given.

Thus in faeces II, if we subtract from the total unsaponifiable part (5.435 g.) the amount (= 0.77 g.) of such material that would normally be excreted on the diet given during the same time, there remain 4.7 g., whereas the unsaponifiable alcohols in the 22 g. of oil administered amount to 8.4 g.

Similarly; in the more satisfactory second experiment, II a, the gross output is 6.14 g. unsaponifiable matter, from which we may subtract 0.8 g. as the normal output, leaving only 5.34 g. as compared to 7.22 g. of unsaponifiable alcohols in the 19 g. of oil given.

In addition, we were convinced at the time that much of the unsaponifiable matter of the experimental periods consisted of cholesterol but unfortunately we were unable, owing to other work, to differentiate between the cholesterol and the alcohols of the mutton bird oil.

Although these two experiments were faulty because of the unnecessarily large doses given, the anaesthesia, and the incomplete analysis, they showed that the oil had no serious effect on the health of the cat and that a certain amount of the cetyl and other alcohols had been absorbed.

Rats. I. Three young rats, kept on a fairly uniform mixed diet, received daily doses of approximately 0.08 g. mutton bird oil administered by a pipette as in vitamin experiments. Three others of the same litter, similarly fed, were given the same amount of olive oil. During 16 days they received in all 3.12 g. of the oils. A sample of 25 g. of faeces from each group was boiled with acid alcohol to break up possible soaps; the alcohol was driven off and the residue extracted with ether. The resulting ether-soluble substances weighed in the mutton bird oil group 2.49 g., in the olive oil group 2.89 g. This material was then saponified and extracted with ether—the unsaponifiable fraction weighed 0.45 g. in the former, and 0.43 g. in the latter, and consisted only of sterols.

II. In this second experiment a similar procedure was adopted but the dose was increased so that in 20 days the same three rats received 11.47 g. mutton bird oil, which should have yielded 4.36 g. cetyl and other alcohols. The total faeces were collected and the unsaponifiable portion carefully examined. It weighed 1.523 g. and the first, second, and third crystallisations yielded crystals melting at 137° and in all probability consisted of cholesterol. No evidence of cetyl alcohol was obtained. The fatty acids of the faeces gave an iodine number of 77.8 as compared to 178 for mutton bird oil acids.

III. From the foregoing results we concluded that a rat is able to dispose of at least 0.2 g. mutton bird oil per day, and a further experiment was made in which the dose was increased to 0.5 g. per day. To three adult rats, a total of 12.33 g. of the oil was given over a period of 8 days. The unsaponifiable matter in this amount of oil would be about 4.7 g., while the unsaponifiable matter in the faeces collected during the 8 days weighed only 0.66 g. and fractional crystallisation and melting point determinations again showed that the bulk of it consisted of sterols. A trace of cetyl alcohol was found, enough to be identified by its melting point but not enough to weigh satisfactorily. The iodine value of the fatty acids of the faeces was 67.4 (Wijs).

In these experiments on rats the practical absence of cetyl alcohol and the low iodine value of the fatty acids indicate a very complete absorption of both components of the mutton bird oil up to approximately 3.3 g. per kg. body weight.

Cetyl alcohol experiment. It occurred to us that it would be interesting to test the absorption of cetyl alcohol by itself under similar conditions. A weighed amount of pure cetyl alcohol was mixed with olive oil to the extent of 15.74 %. When slightly warmed this mixture was sufficiently fluid to be administered by pipette as in the foregoing experiments. Two of the same rats as were used for Exp. III were given daily doses of the mixture over a period of nearly 3 weeks, during which they received in all 2.36 g. cetyl alcohol. The total faeces, collected and treated in the same way as for Exp. III, yielded 0.46 g. unsaponifiable matter from which 0.192 g. cetyl alcohol was recovered by crystallisation and recognised by its melting point, so that 91 % was absorbed, or at least disappeared. As compared to Exp. III, however, the absorption

of cetyl alcohol is not so good as that of the mixed alcohols of mutton bird oil, for in Exp. III the alcohols present in the daily dose of oil amounted to 0.19 g. (38 % of 0.5 g.) and this was almost completely absorbed—while, in the cetyl alcohol experiment, the daily dose was only one-third as much (0.062 g. per rat per day) and yet about 9 % remained unabsorbed.

FATE OF THE MUTTON BIRD OIL DURING AND AFTER ABSORPTION.

Having satisfied ourselves that the oil can be digested and absorbed, we next proceeded to investigate in what tissues or organs the simple chemical change of cetyl or other higher alcohol into fatty acid or other substance occurs. That it takes place naturally in the petrel cannot be doubted for these alcohols are absent from the body-fat of the bird.

To avoid repetition it may be stated here that the method used in estimating the alcohols was to saponify carefully and completely. The unsaponifiable fraction was then separated from the soaps by shaking with ether in a separating funnel and the constituents estimated by fractional crystallisation, weighing and determination of melting points. A certain amount of loss no doubt occurred, for from a mutton bird oil emulsion of known strength in one test case we recovered only 85 % of the alcohols.

(a) *Intestinal epithelium.* From the investigations of Munk on fat absorption it has long been known that cetyl alcohol does not appear in the chyle of the thoracic duct while spermaceti is being absorbed, and the intestinal epithelium has commonly been credited with the ability to transform this and other unusual fats into forms natural to the animal. In our experiments the small intestine of the rabbit with about half an inch of attached mesentery was excised, and, after washing out with Locke's fluid, filled with an emulsion of mutton bird oil of known strength to which had been added a strained suspension of the animal's own mashed pancreas and gall bladder bile. The caecal end of the intestine was then joined to the duodenal end by a very short piece of glass tubing, and the whole was immersed in a bath of warm oxygenated Locke's fluid. For from 4 to 6 hours the intestine continued to show movements, and no doubt a certain amount of circulation of the intestinal contents took place. A control of the same amount of emulsion plus pancreas and bile was kept in a beaker in the same bath.

On examining the intestinal contents and the control we recovered the cetyl alcohol to about the same extent in each, and in one case the ratio of the alcohol to the fatty acid was also examined and found to be unchanged. Four such experiments were done and in no case did we find any evidence that the surviving intestinal epithelium had affected the cetyl alcohol. It may be noted, however, that the amount of digestion may have been small, the leucocytes in the wall would be diminished, and the pumping action of the villi impaired owing to there being no pressure of blood in the blood vessels, so that the experiments did not exactly imitate the natural conditions

of absorption. The method used here was based on that used by Cohnheim [1911] in his experiments on intestinal absorption in fishes.

(b) *Parenteral administration.* This was tried both subcutaneously and intraperitoneally. A pregnant cat received 16 g. pure mutton bird oil under the skin of the back in two doses, one 14 days after the other, and both as nearly as possible in the same area. Seven days after the second dose the animal was killed.

At the site of the injection the effect was easily recognised by the presence of a deposit of canary-yellow fatty matter. The subcutaneous tissues were matted together so that the skin did not separate easily. The yellow colour could be traced for a considerable distance headwards and ventrally so that it infiltrated the mammary gland on the side of the injection. Wherever it had penetrated it seemed to have caused increased vascularity, probably inflammatory. The uterus contained five embryo kittens, and in each case there were streaks of the same yellow colour in the foetal membranes. Three samples of fat and subcutaneous tissue were taken for analysis, (I) from the actual site of injection, (II) from coloured fatty tissue near the mammary gland, and (III) normal fat from the opposite side of the body. The results of examining these are given in Table II.

Table II.

	Ether-soluble substance g.	Unsaponifiable matter g.	Fatty acids g.	Iodine value of fatty acids
I	13.234	1.708	10.222	91.4
II	14.560	1.438	8.326	90.5
III	12.280	Traces only	Not taken	63.0

The unsaponifiable matter found in these samples consisted of practically pure cetyl alcohol and the figures indicate that the injected oil that was recovered had undergone little or no change. It had merely become mixed with the fatty tissue. Thus in samples (I) and (II), if a calculation is made of the quantity of fatty acid corresponding to the unsaponified alcohols found (in ratio of 60 : 38, which is the ratio in mutton bird oil), and if the iodine value of the fatty acids of the oil (178) and of the cat's normal fatty acids (63) be applied to these respective amounts, the iodine value of the mixture should be 93 to 94, which corresponds fairly well with the figures found, viz. 90.5 and 91.4. We did not attempt to recover all the oil injected because it had spread so widely. The general distribution of the streaks of yellow oil indicated transportation along the lymphatics. We have no explanation to offer of the well-marked presence of the same material in the foetal membranes.

For intraperitoneal injection we first used a cat. Nine g. of neutralised and sterilised oil were injected, and the animal killed 3 days later. The surfaces of the abdominal organs as well as the omentum and mesentery were found covered irregularly with a greasy, yellow, fibrinous deposit. As far as possible

this was washed out with alcohol followed by ether without extracting the deeper layer of colourless fat such as that surrounding the kidneys. The unsaponified alcohols (cetyl, etc.) recovered corresponded to that present in only 3.7 g. of the oil as against 9.0 g. injected. The iodine value of the fatty acids of the ethereal and alcoholic washings showed a rise as compared to normal.

On opening the thorax two small lymph glands were found in the anterior mediastinum. They were stained yellow like the abdominal organs and this also indicates lymphatic transportation.

In a later experiment we used a litter of young rats, and injected into the peritoneal cavity of each 2 cc. mutton bird oil representing 0.67 g. cetyl and other alcohols. One as a control was killed within a few minutes, and the whole carcass was minced and examined for the alcohols in the usual way (saponification, extraction with ether, etc.). The amount recovered from the control was only 0.35 g. In the two others similarly injected and examined at the end of 4 weeks and 5 weeks we recovered only 0.13 g. and 0.14 g. respectively. This probably indicates that some part of the unsaponifiable material (oleyl alcohol?) underwent metabolic change more readily than the cetyl alcohol which accordingly persisted longer in the body of the rat.

The fate of parenterally introduced fat in general was too large a subject for us to investigate, but our results agree with those of Binet and Verni [1925], for, before we had read the abstract of their paper, we had come to the conclusion that the best explanation of our experimental results was that the oil had excited irritative and mildly inflammatory changes which had led to ingestion of the oil by leucocytes and so to its removal from the site of injection; further, that the oil then underwent changes which led to loss of the characteristics of both parts of the ester. The first (subcutaneous) experiment shows that the esters are removed or changed without dissociation of their components—unlike what may occur in the case of cholesteryl oleate in the alimentary tract [Faust and Tallquist; quoted by Leathes, 1910].

Influence of the liver. In the hope of finding some fermentative action on the part of the liver which might change the alcohols, we made one experiment on freshly killed liver incubated with an emulsion of cetyl alcohol. The alcohol was almost quantitatively recovered from this and from the control of boiled liver.

VITAMIN A CONTENT.

The widely held opinion that the "stomach oil" plays a special part in the nourishment of the young bird led one of us (J. M.) to test its vitamin A activity, and some of the results have been already published [1926].

In the first set of experiments it was found that 0.04 g. and in some cases 0.02 g. daily was sufficient to produce rapid growth and to cure eye trouble in rats on a basal diet lacking in vitamin A. Later experiments on other samples have not always given the same result. Thus a quantity of oil pro-

cured in May 1925 gave maintenance only in doses of 0.06 g. A sample obtained in December 1925 gave good growth when administered at the same dosage. A sample examined in 1926 gave little or no growth. Such variations in activity are of course common in other cases and may have been due to diet, but we have reason to believe that one of the samples may have been subjected to some "refining" treatment by the Maoris before being delivered to us.

An investigation of the alcohols of mutton bird oil on lines suggested by the work of Drummond and Watson [1922], Fearon [1925], and Drummond, Channon and Coward [1925] has been commenced by one of us (C. L. C.) and his results will be published separately. It was found in general that those samples of oil which gave good results on growth also gave the sulphuric acid (purple) test, the trichloroacetic acid test with the oil in acetic anhydride, and the phosphorus pentoxide test.

On distillation of the unsaponifiable fraction of the oil at low pressure (4 mm.) and a temperature of 180°–200° a fraction consisting of practically pure oleyl alcohol came over, which gave the above colour reactions and which also caused a fair amount of growth in three rats which were being fed on basal diet containing oxidised cod-liver oil (20 hours) as source of vitamin D. These preliminary experiments showed that something came over with the oleyl alcohol, which promotes growth, though in two of the rats the eye symptoms persisted. Thus in a doe rat (*Ag* II, 3) 0.2 g. distillate (oleyl alcohol) daily for 4 days and after an interval of a week, during which the weight began to rise, the same dose repeated daily for 5 days caused ultimately a rise to 115 g. from 92 g., the weight when treatment began.

In another doe (*Ah* IV, 1) treated similarly in the same cage the weight rose from 66 g. to 87 g. and in a third (*Ad* V, 2) from 90 g. to 104 g. with cure of eye symptoms. In the two rats first mentioned the eye symptoms remained stationary.

ORIGIN OF THE OIL.

As stated above (p. 484) we at first thought it possible that the oil might be an indigestible residue of the bird's food. During the nesting period the petrels are believed by some to feed chiefly on the shoals of sprats and other members of the herring family that occur in these waters, by others they are believed to feed chiefly on shell-fish. In either case a residue of fatty material might result from gastric digestion of the food, and it has been pointed out by others that birds in general have a poor supply of lipases in their digestive secretions. Against this view, however, there is the evidence of a great degree of uniformity in the composition of the oil, and the low concentration of cholesterol.

During the course of investigations on the food value of New Zealand fishes we took the opportunity to examine the unsaponifiable part of many of the fish fats, liver oils, and shell-fish fats, on the chance of discovering a

clue to the source of the cetyl alcohol—but without success. The chief unsaponifiable alcohol in these oils is cholesterol.

Another view widely held is that the oil is secreted by the parent birds for the nourishment of the young. Such secretion is, we think, very unlikely. The petrel has no crop such as the pigeon has, and the oil is not accompanied by the protein that one would expect if this were a nutritive fluid like pigeon's milk.

As the research proceeded, a third view has gradually impressed itself on us as more likely than either of these, and that is, that the oil is the excess of tail gland secretion swallowed by the parent bird and more or less accidentally included in the material fed to the young. Possibly, also, the young bird may produce and swallow some of its own tail gland secretion. In support of this we may call attention to the fact that cetyl and octadecyl alcohols have been found to occur up to 40 % in the "fat" extracted from the tail gland of geese [Metzner, 1907]. Being unable at present to obtain adult mutton birds, we have investigated the tail gland of ducks. In these we find it easy by mechanical stimulation to cause oily drops to appear on the feathered apex of the nipple-like projection which forms the outlet for the gland. We procured a number of duck tail glands from a local poultry dealer and tested the ethereal extract of these for vitamin A. Doses of 0.1 g. gave no evidence of its presence, but this result is not conclusive, for all would depend on the food of the ducks. That skin secretions may contain vitamin A has already been shown by Gudjonsson [1926]. During the coming winter we hope to secure evidence on this point from the mutton bird itself.

SUMMARY AND CONCLUSIONS.

The oil obtained from the stomach of the mutton bird is capable of being digested, absorbed and utilised by mammals. It may contain vitamin A in high concentration but the amount present is variable. The cetyl, oleyl and other alcohols of the oil are changed during or after absorption so that they can no longer be detected, but experiments on the action of surviving intestinal wall and liver cells failed to show where the change occurs.

On parenteral injection, subcutaneously and intraperitoneally, there is evidence that no local dissociation of the esters occurs, and the oil appears to be taken up by leucocytes and to travel by the lymphatics.

The view is put forward that the stomach oil of the chick is the tail gland secretion more or less accidentally swallowed.

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REFERENCES.

- Binet et Verni (1925). *Compt. Rend. Soc. Biol.* 83. [*Physiol. Abstr.* 10, 473.]
Carter (1921). *J. Soc. Chem. Ind.* 40, 220 T.
Channon (1926). *Biochem. J.* 20, 400.
Cohnheim (1911). *Z. physiol. Chem.* 76, 293.
Drummond, Channon and Coward (1925). *Biochem. J.* 19, 1047.
Drummond and Watson (1922). *Analyst*, 47, 314.
Fearon (1925). *Biochem. J.* 19, 888.
Gudjonsson (1926). *Amer. J. Physiol.* 75, 533.
Hutchison (1922). Food and dietetics, 564.
Leathes (1910). The fats, 120 (London).
Lueders and Bergeim (1923). *Amer. J. Physiol.* 66, 297.
Malcolm (1926). *Trans. New Zealand Inst.* 56, 650.
Metzner (1907). *Nagel's Handb. Physiol.* 2, 388.
Munk (1891). *Virchow's Arch.* 123, 230 and 486.
Purdy (1918). *Brit. Med. J.* i, 174.
Thomas and Flaschenberger (1923). *Skand. Arch. Physiol.* 43, 1.
Wieland and Sorge (1916). *Z. physiol. Chem.* 97, 1.