were the lowest in the distal ¼ and distal sections of the adult dogs' small intestine. We did not demonstrate a consistently higher lactase activity in the jejunum than the duodenum of the adult dog, as reported previously(6). The distribution of the different disaccharidases along the small intestine appears to depend on the species of the animal (10).

Summary. 1. Lactase and alkaline phosphatase activity in the small intestine of the dog sacrificed soon after birth was higher than that in adult animals. The activity of these 2 enzymes during the first few weeks after birth decreased and reached their lowest levels in the adult animals. Sucrase and maltase activity, on the other hand, were low in the young dogs and highest in the adult dogs. 2. The activity of all the enzymes measured, lactase, sucrase, maltase and alkaline phosphatase was highest in the proximal and middle sections of the adult dog small intestine and decreased strikingly toward the distal portions.

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The Fate of Dietary Wax Esters in the Rat.* (30581)

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Matsuo(1) has reviewed a number of reports describing the seborrheic and rapidly fatal effect of some wax esters. To gain some insight into this process, a C_{34}-ester, oleyl palmitate, was fed to rats as 15% of the diet.

The original intention of this study was to investigate the nature of the oil presumed to be secreted by the sebaceous glands. It soon became clear, however, that the effects of long-chain esters on the rats could be ascribed to a cause other than seborrhea, and further knowledge of the digestibility and absorption of wax esters was required.

Previous literature on the subject is scanty. Munk and Rosenstein in 1891(2) found that single small doses of spermaceti (cetyl palmitate) were well utilized. Carter and Malcolm(3) fed much larger doses of muttonbird oil (largely cetyl and oleyl oleates) to rats over periods of 8 days and found that they could absorb 3.3 g/kg/body wt/day. Cats given large amounts absorbed some but also excreted cetyl esters and cetyl alcohol in the feces. Savage(4) found that rats ingesting jojoba oil at 15% of the diet absorbed about 70% and excreted the remainder as wax esters and free alcohols. She also observed a purgative effect of this diet.

This paper describes some aspects of the metabolism of oleyl palmitate in the gut and the accumulation of wax ester and free alcohol in the liver.

Materials and methods. Oleyl palmitate.

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TABLE I. Fatty Acid Composition (Weight %) of Dietary, Fecal, and Liver Lipids of Rats Fed Oleyl Palmitate or Oleyl Alcohol.

	Basal diet			Oleyl palmitate group				Oleyl alcohol group			
				Feces		Liver		Feces		Liver	
	Total lipid	FFA*	Administered wax ester	Wax	FFA*	Wax	TG†	Wax	FFA*	Wax	TG†
14:0	1.0	1.2	.5	2.2	1.3	8.5	3.2	1.7	6.6	.3	6.2
14:1	—	—	.4	.3	.8	.4	.7	3.5	7.4	.3	.8
16:0	15.5	15.5	97.8	68.3	86.8	79.7	53.3	19.8	24.0	67.2	27.9
16:1	1.4	1.2	1.0	—	—	2.1	3.4	2.8	2.5	2.8	4.1
18:0	3.1	2.8	.4	4.2	4.0	5.0	5.9	12.0	10.5	17.9	6.1
18:1	21.2	22.7	—	14.5	4.9	4.3	19.6	36.8	25.1	7.2	28.6
18:2	54.1	51.1	—	10.6	2.2	—	10.9	18.9	20.9	4.3	20.6
18:3	3.8	5.6	—	—	—	—	3.0	4.3	3.0	—	5.6

* FFA = free fatty acids.

† TG = triglycerides.

This ester was prepared by refluxing equimolar amounts of oleyl alcohol and palmitic acid dissolved in benzene containing 0.05% p-toluenesulfonic acid. Water was removed by azeotropic distillation as the esterification proceeded to 98% completion. The catalyst was removed by washing the solution with water, following which the solvent was completely removed. The purity of the product was checked by thin-layer chromatography (TLC) infrared spectroscopy, and gas-liquid chromatography (GLC). Residual free fatty acids were methylated by reaction of the mixed product with diazomethane, and measured by GLC after addition of an internal standard.

Esters were methanolysed in borontrifluoride-methanol(5) and the methyl esters purified by preparative TLC. In the case of the wax esters, which were completely methanolysed within 90 minutes under the conditions recommended for triglycerides, the alcohol fraction was also recovered and reacted with trifluoroacetic anhydride before GLC. The fatty acid composition of the oleyl palmitate preparation is shown in Table I. The oleyl alcohol was found to contain approximately 20% of lower homologues.

Gas-liquid chromatography. GLC was performed with a Barber-Coleman Model 10 chromatograph, using the hydrogen flame detector. A 41 in. × 4 mm I.D. glass U-tube column packed with 12.2% ethylene glycol succinate polyester (EGS) coated on dimethyldichlorosilane-treated HCl-washed chromosorb G was used at 178-186° and 25 p.s.i. argon. The performance of the instrument was checked routinely with standard samples.

Feeding Experiment 1. Five weanling male Sprague-Dawley rats were placed in each of 2 cages. One group was given *ad lib* a diet of crushed Wayne Lab-Blox§ mixed with oleyl palmitate, 85:15 (w/w). The control group received a mixture of Lab-Blox and corn oil in the same proportion. Both groups were killed at the end of 4 weeks.

Feeding Exp. 2. Eight weanling male rats were placed in separate cages. For the first 5 days each rat was given Lab-Blox only, after which 4 received the oleyl palmitate diet described above. The other 4 were fed a mixture of Lab-Blox and oleyl alcohol, 96:4 (w/w). All of these animals were killed 2 weeks after the commencement of the experimental diet.

Removal of organs for analysis. Before killing, a number of the animals were lightly anesthetized with ether and the whole of the liver and intestine removed quickly, washed with 0.9% NaCl, and frozen in dry ice. The animals were then killed by exsanguination.

Lipid extracts. Samples of diet or feces, or entire excised organs were homogenized mechanically under nitrogen with approximately 20 vol. chloroform-methanol (2:1, v/v). The mixture was filtered and the solvent removed under a stream of nitrogen. The dry lipid was taken up in chloroform and kept refrigerated under nitrogen.

§ Allied Mills, Inc., Chicago, Ill. Lab-Blox is a highly nutritive preparation containing 24% protein and 4% fat. TLC of the lipid revealed that an appreciable quantity of free fatty acid was present in addition to triglyceride. The fatty acid compositions of the total lipids and of the free acid fraction are shown in Table I.

Thin-layer chromatography. For most purposes Silica Gel G (Merck) plates were developed in a saturated atmosphere with either (A) pentane-ethyl ether-acetic acid, 100:10:1 (v/v)(6), or (B) hexane-ether-methanol-acetic acid, 100:20:5:10 (v/v)(7). System (A) failed to separate wax esters from cholesterol esters, while system (B) did not separate wax esters from triglycerides. Where these separations were desired the chromatograms were developed in an unsaturated atmosphere with hexane benzene-acetic acid, 70:30:1 (v/v). The lipids were located by brief exposure of the chromatogram to iodine vapor.

Pancreatic lipase action in vitro. For these experiments, porcine pancreatic lipase (Calbiochem, activity 1000 units/g) was used. In the study of the hydrolytic reaction, 10 mg chromatographically pure oleyl palmitate and 1 mg monostearin were emulsified in 1.9 ml 0.1M Tris buffer, pH 7.0 or 8.2, containing 2 mg Na taurocholate. After 8 μl of the mixture had been withdrawn and applied to the starting line of a TLC plate, 0.5-2.5 mg enzyme suspended in 0.5 ml Tris buffer was added to the reaction vessel. The mixture was shaken at 40°C for 60 minutes, 10 μl samples being taken at intervals and placed on the TLC plate. After the last sample had been taken, the mixture was extracted with ether and the total lipid extract subjected to preparative TLC. Both plates were developed in TLC system (A). The lipid fractions from preparative TLC were eluted and weighed. Semiquantitative estimates of the lipids present in the samples at zero time and after intervals up to 60 minutes were made on the basis of relative spot areas after charring with sulphuric acid-dichromate(6).

The synthetic reaction was studied under identical conditions except that oleyl palmitate was replaced by 5 mg oleyl alcohol + 5 mg oleic acid.

Results. Growth response to the oleyl palmitate and oleyl alcohol diets was poor compared with controls, partly due to unpalatability. By Day 7 of Exp. 1, the skin and fur of all the animals receiving oleyl palmitate appeared very oily, as described by some earlier authors(1). All had some degree of diarrhea, and their behavior suggested that each was spreading fecal material on the fur of the others. This feces contained much unabsorbed lipid since the absorption of oleyl palmitate was only about 50%, as found by Savage(4) for jojoba oil. On Day 14, one of the rats was lightly anesthetized with ether and the fur oil obtained by washing the anterior part of the animal with pentane, taking care not to contaminate the sample with the animal's own fecal material. The lipids obtained were analysed by TLC together with a lipid extract of feces collected from the group, and the TLC patterns were found to be identical. The pentane-washed rat was placed in a separate cage on the same diet and its fur remained free of oil for the remainder of the experiment.

TABLE II. Composition of Fecal Lipids.*

	Oleyl palmitate group (g %)	Oleyl alcohol group (g %)
Wax ester	51	46
Free fatty acid	17	6
" " alcohol	28	27
Diglyceride	—	5
Monoglyceride†	4	16
	(g/24 hr)	(g/24 hr)
Total fecal lipid	.62	.38

* Determined by weighing fractions after preparative TLC in system (A).
† Includes small amounts of more polar material (R_f 0.00 in all 3 TLC solvent mixtures).

In Exp. 2, none of the animals developed oily fur, although those given oleyl palmitate exhibited diarrhea by Day 7. None of the rats given oleyl alcohol developed diarrhea, yet the fecal lipid composition was similar to that of the rats fed oleyl palmitate (see below). This seems to indicate that the purgative action of the wax ester was directly related to the amount of unabsorbed lipid present in the intestine(8) and possibly to the higher concentration of unabsorbed free fatty acid in this group(9).

Composition of fecal lipid. A 24-hour collection of feces was made on Day 11 of Exp. 1 from the oleyl palmitate group, and on Day 9 of Exp. 2 from the oleyl alcohol group. The quantity, and the composition according to lipid class, of the lipids present are shown in Table II. It is evident that hydrolysis of oleyl palmitate occurred in the gut, but whether in the small or large intestine is not

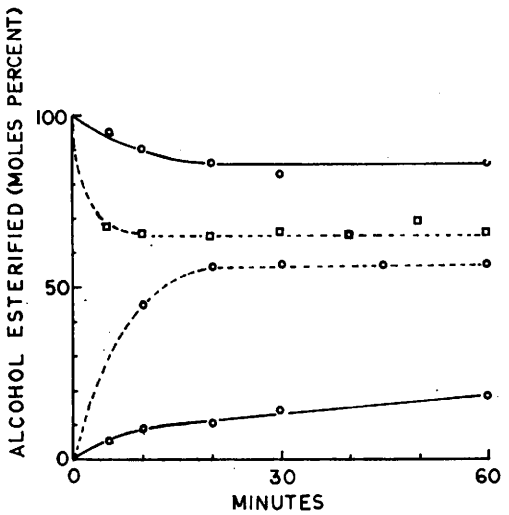

FIG. 1. Interconversion between oleyl alcohol and its esters in presence of pancreatic lipase. O—O at pH 7.0, .5 mg lipase; O- -O at pH 7.0, 2.5 mg lipase; □- -□ at pH 8.2, 2.5 mg lipase. 10 mg oleyl palmitate, or 5 mg oleyl alcohol + 5 mg oleic acid, was present in a total volume of 1.7 ml. The values at 60 min were obtained by measuring wts of fractions isolated by preparative TLC. Intermediate values are approximations based on a comparison of spot areas after TLC.

clear. The fact that the fatty acid concentration was less than the free alcohol concentration indicates greater absorption of the former.

It seems remarkable that dietary oleyl alcohol and oleyl palmitate should yield fecal lipids of similar composition. This must mean that wax esters were synthesized in the gut by esterification of the alcohol with fatty acids originating either from the chow diet lipids or as oxidation products of oleyl alcohol. Esterification might have been brought about by pancreatic or intestinal enzymes or by the bacteria of the large intestine.

Table I shows further evidence for synthesis of wax esters in the intestine of both groups of rats, by demonstrating that fatty acids other than those in the dietary oleyl palmitate were incorporated into the wax ester fraction. A simple estimation also reveals that the disappearance rate of linoleic acid was very much greater than that of palmitic or oleic acids(10) and that in each group slightly more stearic acid was excreted than ingested. It may be significant that the fatty acid composition of fecal wax ester resembled, though not exactly, that of the free fatty acids which failed to be absorbed.

Wax ester hydrolysis and synthesis in vitro. It seemed logical to attempt a brief investigation of the action of pancreatic enzymes on oleyl palmitate and on a mixture of fatty alcohol and fatty acid. The conditions used for this reaction, described in the Experimental section, were similar to those used for hydrolysis of triglycerides(7). The small amount of added monoglyceride facilitated emulsification and would certainly have been present *in vivo*. No diglyceride, which might have resulted by transesterification, could be detected. Calcium ions interfered with emulsification and were therefore not added. Hydrolysis of the wax ester was slower than hydrolysis of triglyceride but by increasing the enzyme concentration an equilibrium between hydrolysis and esterification appeared to be reached when approximately 60% of the alcohol was esterified (Fig. 1). In the intestine, selective absorption of free fatty acids may displace the equilibrium and thus account for the lower ratio of esterified to free alcohol.

Deposition in the liver. The lipid content of the livers and intestines of rats on each of the diets used is shown in Table III. The lipid extracts were subjected to TLC which revealed a significant amount of wax ester and free alcohol in the liver lipid from the

TABLE III. Lipid Content of Livers and Intestines.

	Period on diet (wk)	Liver		Intestine	
		Avg organ wt (g)	Avg lipid wt (g)	Avg organ wt (g)	Avg lipid wt (g)
Corn oil group	4	10.0 (2)*	.60	6.7 (3)	.19
Oleyl palmitate group	4	5.5 (2)	.24	6.7 (3)	†
" alcohol "	2	6.0 (2)	.26	4.7 (3)	.08

* No. of organs pooled given in parentheses.

† Part of sample lost.

TABLE IV. Composition of Liver Lipids.*

	Oleyl palmitate group	Oleyl alcohol group
Wax ester	14	7
Triglyceride	15	11
Free fatty acid	3	3
Free fatty alcohol (+ cholesterol)	12	6
Phospholipid	56	72

* Determined by weighing fractions after preparative TLC in system (c).

oleyl palmitate and oleyl alcohol groups. Neither substance could be detected in the intestinal lipids. The liver lipids of these two groups were therefore fractionated by preparative TLC with the results shown in Table IV. The fatty acid composition of the wax ester fractions (Table I) was characterized by a striking preponderance of palmitate in both cases and bore little resemblance to the composition of the liver triglycerides. The proportion of stearate in the oleyl alcohol group also seemed quite high in view of the amount of stearate excreted, as pointed out above. Possibly stearate was formed from oleyl alcohol.

There was no evidence that oleyl alcohol was converted to other fatty alcohols. The free and esterified fatty alcohols present in the liver and feces of rats fed oleyl palmitate and those fed oleyl alcohol were analysed by TLC in the 3 solvent systems described, by GLC of their trifluoracetates, and by infrared spectroscopy. In each case the alcohol isolated was identical with that fed.

Discussion. In contrast to the few reports on absorption of wax esters already cited, the absorption of fatty alcohols is much better documented. Small amounts of cetyl alcohol (3,11-14), stearyl alcohol(13,15), oleyl alcohol, and phytol(12) appear to be well absorbed. Conversion to the corresponding fatty acid or an acid with two additional methylene groups occurs(13,15), apparently within the intestinal mucosa(14). Some, at least, escapes oxidation and appears in the lymph (14) and the liver(12). It has also been suggested that cetyl alcohol is a normal constituent of mammalian feces(15,16).

The fact that dietary wax ester is partly absorbed and partly excreted as a mixture of altered wax ester and free alcohol leads to speculation on whether hydrolysis of the ester precedes absorption. The literature lacks reliable data on the hydrolysis of wax esters in the small intestine. Carter and Malcolm (3) reported 42% hydrolysis of mutton-bird wax ester in 42 hours, using a preparation of pig pancreas, while Savage(4) obtained only 10% hydrolysis of jojoba oil esters in 24 hours at 28°. However, from the report that pancreatic lipase can attack emulsified esters such as *p*-nitrophenyl laurate(17), it seems likely that waxes should also be susceptible to hydrolysis by this enzyme. The preparation used in the present work was active in catalysing not only hydrolysis but also synthesis of wax esters. However, it was totally inactive against cholesterol esters. Apparently the small amount of monostearin used for emulsification did not take part in a transesterification reaction sequence, as no diglyceride could be detected. The attainment of an equilibrium between fatty acid, alcohol, and ester may be analogous to that catalysed by pancreatic lipase in the glyceride system (18). It is interesting to recall that a preparation of rat pancreas has been found to esterify vitamin A (a poly-unsaturated fatty alcohol) nonspecifically with fatty acids of chain length C_{12} or more(19).

Nevertheless, it is not possible from the present data to be sure whether hydrolysis, esterification, or both occurred in the small intestine or in which form, free or esterified, oleyl alcohol was absorbed. One reason for this is that the lipids deposited in the liver were almost the same whether oleyl palmitate or oleyl alcohol had been fed. It is possible that the liver accumulates oleyl palmitate selectively. This would be analogous to selection of the palmitate as the major ester of vit A(20), and selection of the oleate as the predominant ester of cholesterol(21), to be deposited in the liver. The fatty acid composition of oleyl esters found in the livers of rats fed oleyl alcohol corresponds strikingly with that of vit A esters recently reported(22), suggesting that the deposition of these substances may involve the same mechanisms.

The reported toxic effects of waxes(1) were

not seen in any animals in this experiment. Since the basic diet used here contained 24% protein (compared with 9-10% in the earlier work) and large quantities of other essential growth factors, the toxic effects may have been the result of malnutrition rather than a specific effect of wax esters. It has also been shown conclusively that the apparent seborrhea produced in our animals was due to the behavior of those in which wax ester was exerting a purgative action. In this respect it is interesting that Kaneda and Sakurai(23) found that oleyl alcohol (which did not cause purgative effects in the present work) did not produce seborrhea, although all the animals lost weight and soon died. Those authors who have used relatively small doses of waxes have not recorded purgative effects. However, Savage(4) reported the purgative effect of jojoba oil, and a similar effect of Ruvettus oil(24,25,26) can be explained by the presence of waxes, largely cetyl and oleyl oleates(8,25). One of these reports attributes the effect to the alcohol moiety (26).

Summary. Oleyl palmitate fed to rats as 15% of the diet was partly absorbed and partly excreted as a mixture of wax ester, free fatty acid, and free alcohol. Because of purgative effects, animals caged together contaminated each other's fur with fecal lipid, giving them an appearance of seborrhea. Animals given oleyl alcohol as 4% of the diet did not show purgative effects but excreted lipid which was similar in composition to that excreted by rats given wax esters. Thus both hydrolysis and synthesis of waxes can occur in the intestine. Similar reactions have been carried out *in vitro* in the presence of a pancreatic enzyme. Wax ester and free oleyl alcohol were detected in the liver after feeding either compound, and in both cases the predominant ester was oleyl palmitate. The question of whether oleyl alcohol is absorbed in the free or esterified form was not satisfactorily resolved.

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