LIPID* EXCRETION.

III. FURTHER STUDIES OF THE QUANTITATIVE RELATIONS OF THE FECAL LIPIDS.†

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The presence of fatty substances in the feces, under some conditions at least, has been known over a hundred years, a number of interesting observations on the subject having been recorded by Home (1) in 1813. In 1884 Müller (2), in the course of a general discussion of intestinal excretion, arrived at the conclusion that, inasmuch as fatty material is present in large amount in fasting feces and meconium, it, like other constituents, must represent some sort of an excretion. Numerous analyses of fecal lipids may be found in the literature; but practically all of them (with the exception of a few concerned with the sterols, to be discussed later) have been primarily concerned with various phases of the question of lipid absorption. Many of the authors have determined simply total, ether-extractable material excreted; others have investigated the relationships between the neutral fats, soaps, and sterols; no one appears to have made a detailed study of the fatty acids of the feces with the possibility in mind that valuable information with a bearing on the problems of lipid metabolism might thus be obtained. It seemed worth

* The term "lipid" is used in accordance with the recommendation of the International Congress of Pure and Applied Chemistry to include the fats and all substances ordinarily associated with them; *i.e.*, neutral fat (esters of fatty acids and glycerol), fatty acids, soaps, phospholipids, sterols, and esters of the sterols and fatty acids.

† The material presented in this paper is taken in greatest part from a thesis presented by the author to the faculty of the University of Rochester in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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while, therefore, to make such a detailed examination of fecal lipids, particularly of the fatty acids, in the hope of discovering the source and the purpose of the excretion. The possibility that unusual fatty acids might be found was especially attractive. The identification, for example, of fatty acids with an uneven number of carbon atoms, or with double bonds in positions where they do not ordinarily occur, or even the discovery of mixtures in which the solid and liquid acids were present in considerably different ratios from those in which they usually occur in the body, would have a very important bearing indeed upon problems of intermediary fat metabolism. From this standpoint the results so far have been negative. Though the possibility that unusual fatty acids are present has not been ruled out entirely, the data collected indicate that the fatty acids of the feces are such as may be found almost anywhere in the body and that they are present in about the same proportions as in the tissues. In a recent paper Eckstein (3), using the same methods, has obtained values for human stored fat very similar to those found for the fecal lipids.

In the previous papers of this series (4, 5) it was reported that, when moderate amounts of olive oil and coconut oil were fed, the lipids of the feces were to a large extent independent of the dietary lipids, although there was some similarity, evidenced by a comparatively high ratio of liquid to solid acids in the fecal lipids with an olive oil diet and the reverse with coconut oil. There was also a decided, though not large, increase in the total lipid excretion with diets containing lipids above that when the animals were receiving no lipids in their food. A considerably smaller excretion during fasting than with a lipid-free diet and a rather marked similarity between the lipids of the blood and feces were noted. From these results it appeared most probable that there is a basal excretion of lipid material, most nearly represented by that obtained with a lipid-free diet, and that this basal excretion is augmented by a small amount of lipids escaping absorption, or being absorbed and reexcreted, in the case of diets containing lipids. It seemed most desirable, therefore, in continuing the work, to make a more complete and careful study of the excretion with a lipid-free diet, and it is with such an investigation that the present paper is chiefly concerned.

EXPERIMENTAL.

General Procedure, Diet.—The technique of handling the dogs (used exclusively as subjects) and of collecting feces was the same as described in the preceding paper except for the use of alcohol to cover the excreted material during the collection period in the hope of reducing the oxidation of highly unsaturated acids.

The casein used in the diet was extracted with hot alcohol for 16 hours, either in the Clarke extractor (6) or a modification suggested by Dr. W. R. Bloor of this laboratory. The principal

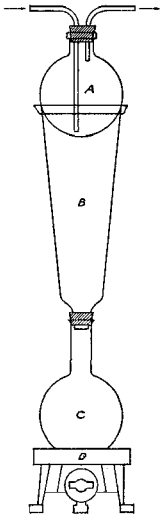

FIG. 1. Continuous extraction apparatus. *A*, round bottom flask used as condenser; *B*, percolator; *C*, flask containing solvent; *D*, electric stove.

change from the original in the latter apparatus, a diagram of which is shown in Fig. 1, is the replacement of the cumbersome funnel (employed as container for the solid being extracted in the Clarke extractor) by a percolator, thus allowing the use of a much smaller flask as condenser. The material to be extracted is supported in the percolator (*B*), either in a cloth bag or fluted filter paper (a cloth bag is used with casein), and three or four glass tubes are placed in a vertical position around the bag to allow the alcohol vapor to pass up to the condenser (*A*). The tubes are especially important when casein is being extracted

since this substance swells considerably when wet with alcohol. A small funnel may be placed in the bottom of the percolator to aid in supporting the material and prevent its stopping the neck. Either a 500 or 1000 cc. flask, depending on the size of the percolator, is used as condenser.

The completeness of extraction of lipids was determined by digesting samples of the treated casein with 25 per cent NaOH on the steam bath for about 4 hours, acidifying with H_2SO_4, extracting with ether, distilling the ether, extracting the residue left by the ether with petroleum ether, and weighing the material thus extracted after distilling the petroleum ether and drying. Negligible amounts of lipids were found by this method, but, by accident, after most of the work to be reported had been completed, a sample was allowed to digest with alkali on the steam bath for a somewhat longer period of time (about 7 hours) and here a very appreciable amount of lipid substance was obtained amounting to 0.412 per cent. Further tests were carried out at once on casein which had been extracted 16 hours and was considered to be lipid-free and it was confirmed that there still remained about 0.5 per cent of lipids, which could only be removed after completely breaking down the protein molecule through long digestion with strong alkali. Analysis indicated that this substance consisted almost entirely of fatty acids, of which the average molecular weight was about 250 to 270. It was found that, if the casein were reextracted as before with alcohol for a period of several days, it was possible to remove completely all traces of lipids as shown by 24 hour digestion with 25 to 30 per cent NaOH and extraction with ether and petroleum ether as outlined above. Later work has shown that the same end may be accomplished by two or three much shorter periods of extraction, provided that the casein is removed from the apparatus, pulverized, and dried between them, and it is thus indicated that the failure to extract the lipids completely the first time is simply due to incomplete penetration of the alcohol to all parts of the mass of casein. If this be true, however, it is difficult to understand why it is necessary to break the protein down by long treatment with strong alkali in order to free the fatty substances so they may be extracted by ether. The whole matter presents an interesting problem which is to be made the subject of a later communication.

As far as the present paper goes we are principally concerned with the effect which these overlooked lipids may have had on the results of the experiments to be reported. In the first place one would hardly expect that any of such small amounts of lipids, amounting to an average of about 0.25 gm. per dog per day, would escape absorption; but in order to be sure that the lipids found in the feces with lipid-free diets, in which casein containing these small amounts of lipids was used, did not come from unabsorbed casein lipids, two of the dogs employed in these experiments were fed with the same diet used before, but with strictly lipid-free casein, for a period of 2 weeks and the feces were divided into weekly periods by charcoal demarcation and analyzed according to the methods described below. The results, the average values of which will be found in Table I (Lipid-free 5), were in general about the same as those obtained with casein which was not strictly lipid-free. Unfortunately there was a considerable fall in the total lipid excretion in the case of one of the dogs during the 2nd week, the amount excreted being somewhat less than that usually obtained. There was also a decrease in the proportional amount of solid acids in the 2nd week with both animals. It is thought, however, that these changes are due to normal variation in the animals and in view of the general similarity of the results to those obtained with casein, which was not strictly lipid-free, it seems unlikely that the small amounts of lipids present could have had any effect on the results and it will be assumed that the diet was to all intents and purposes strictly lipid-free.[1]

The diets were computed on the basis of 80 calories per kilo but it was found necessary to multiply the amounts by $\frac{9}{8}$ in the case of 5 to 7 kilo dogs and by $\frac{8.5}{8}$ in the case of 7 to 9 kilo dogs.

[1] In another series, which was continued for a period of 3 weeks and in which strictly lipid-free casein was used, three dogs continued to excrete lipids up to the end of the experiment without any appreciable diminution in the amount. The average total excretion was about 2 gm. per week and the composition was similar to that of the material excreted when the casein of the diet was not strictly lipid-free. This experiment was carried out as a control in work with bile fistula animals and will be published with the data from that investigation.

TABLE I.

Average Composition of Fecal Lipids.

Experiment.	No. of dogs used.	Weight of dogs.	Total lipids.	Unsaponifiable.		Non-volatile fatty acids.			Solid fatty acids.				Liquid fatty acids.			Ratio of liquid to solid fatty acids.
				Weight.	In total lipids.	Weight.	In total lipids.	Melting point.	Weight.	In total lipids.	In non-volatile fatty acids.	Melting point.	Weight.	In total lipids.	In non-volatile fatty acids.	
			A	B	$\frac{100B}{A}$	C	$\frac{100C}{A}$		D	$\frac{100D}{A}$	$\frac{100D}{C}$		E	$\frac{100E}{A}$	$\frac{100E}{C}$	$\frac{E}{D}$
		kg.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	*°C.*	*gm.*	*per cent*	*per cent*	*°C.*	*gm.*	*per cent*	*per cent*	
Low		5.4	3.636	1.464	36.9	2.046	56.5	37	0.408	10.7	17.2	49	1.476	38.4	61.0	3.20
Olive oil 1	3	8.8	3.840	1.498	39.1	2.302	59.8	38	0.438	11.4	19.2	50	1.516	39.5	66.3	3.40
High		13.8	4.074	1.526	42.0	2.566	63.0	40	0.465	12.8	22.7	50	1.561	40.5	72.0	3.70
Low		5.4	2.181	0.902	38.6	1.207	47.5	27	0.181	7.0	13.1	47	0.826	32.3	65.9	3.22
Olive oil 2	6	8.8	3.309	1.437	43.4	1.693	51.2	28	0.300	9.0	17.6	50.2	1.215	36.6	71.4	4.23
High		12.9	4.298	1.851	46.6	2.271	55.2	29	0.464	10.8	21.2	52	1.602	43.6	81.0	6.20
Low		5.1	0.552	0.242	43.8	0.284	35.2	36	0.025	4.5	8.3	48	0.241	29.8	70.0	2.60
Fasting	5	9.2	0.948	0.503	52.9	0.436	46.2	36.5	0.096	9.1	20.0	48.5	0.350	38.5	82.7	5.26
High		13.2	1.608	0.834	65.0	0.762	54.7	37	0.213	13.3	28.0	49	0.545	54.0	98.5	11.90
Low		4.8	1.955	0.643	24.9	1.290	66.0	34	0.567	23.6	32.4	45	0.683	32.8	46.7	0.88
Coconut oil	6	8.1	2.859	0.860	30.3	2.019	70.3	37	0.868	30.5	43.5	47	1.037	36.1	51.4	1.21
High		12.1	4.217	1.197	34.6	2.937	76.1	38	1.273	37.2	53.0	48	1.534	39.5	54.4	1.67
Low		5.9	1.515	0.582	31.4	0.851	56.0	34	0.249	13.8	23.0	50	0.555	36.6	62.5	2.19
Lipid-free 1	4	9.5	2.174	0.793	36.9	1.263	56.5	37	0.340	15.9	27.4	50.5	0.829	37.8	65.2	2.40
High		13.6	2.852	1.012	40.5	1.706	59.7	40	0.406	17.1	29.2	51	1.147	40.1	67.1	2.92

Low.....		5.3	1.329	0.565	36.8	0.674	47.0	33	0.225	14.1	25.7	49	0.422	28.6	57.1	1.75
Lipid-free 2 A.....	5	7.5	2.077	0.853	41.3	1.088	51.9	36	0.325	15.7	30.1	51	0.672	32.4	61.7	2.08
High.....		10.9	3.177	1.170	45.0	1.850	58.2	42	0.501	17.2	34.7	52	1.112	36.7	67.8	2.44
Low.....		5.3	1.807	0.708	39.2	0.857	38.2	*	0.202	9.0	20.8	48	0.536	23.8	59.3	2.34
Lipid-free 2 B.....	4	6.7	1.971	1.022	50.9	0.964	49.7	*	0.220	11.3	23.1	50	0.525	29.9	60.6	2.64
High.....		9.0	2.243	1.444	64.3	1.096	60.6	*	0.228	12.6	26.0	51	0.654	36.2	62.6	2.86
Low.....		5.5	1.176	0.597	36.6	0.525	44.6	*	0.140	9.6	19.0	47	0.369	25.2	46.2	2.16
Lipid-free 2 C.....	4	7.9	1.599	0.709	45.7	0.795	48.8	*	0.181	11.5	23.9	49	0.489	31.2	64.4	2.79
High.....		10.8	2.193	0.801	51.8	1.198	54.5	*	0.233	13.8	30.5	50	0.595	38.6	75.6	4.00
Low.....		7.0	1.280	0.377	28.2	0.815	58.0	32	0.242	18.9	29.7	46	0.505	32.3	55.6	1.42
Lipid-free 3 A.....	5	9.5	1.974	0.596	30.0	1.255	63.6	35	0.348	20.5	32.6	47	0.624	37.5	59.3	1.85
High.....		12.9	3.152	0.973	33.0	2.044	66.5	39	0.473	22.8	39.4	50	0.669	40.3	62.0	2.09
Low.....		6.6	1.431	0.497	29.6	0.867	55.5	38	0.284	19.2	31.1	46	0.493	25.6	41.5	1.33
Lipid-free 3 B.....	5	9.1	1.872	0.598	32.3	1.127	60.0	39	0.378	20.3	34.0	47.4	0.594	32.0	53.4	1.57
High.....		12.5	2.411	0.716	35.3	1.505	62.5	41.5	0.468	22.8	38.2	49	0.842	35.3	59.0	1.80
Low.....		6.2	1.033	0.405	28.4	0.648	57.0	36	0.218	21.1	33.6	49	0.333	27.2	47.4	1.20
Lipid-free 3 C.....	4	9.1	1.594	0.555	35.3	1.029	64.1	37	0.372	23.0	36.1	50	0.513	32.2	50.1	1.40
High.....		12.0	2.003	0.670	40.4	1.369	68.3	37	0.483	24.3	39.8	50	0.446	37.0	54.0	1.53
Low.....		7.2	1.316	0.413	31.4	0.871	59.1	36	0.304	21.2	34.9	47.5	0.460	29.8	48.5	1.28
Lipid-free 3 D....	4	8.8	1.940	0.676	34.1	1.185	61.6	37.5	0.439	22.6	36.6	48.6	0.615	32.1	52.1	1.43
High.....		11.1	2.622	0.978	37.2	1.553	66.4	38.5	0.594	23.3	38.2	50	0.817	35.0	54.5	1.54

TABLE I—*Concluded.*

Experiment.	No. of dogs used.	Weight of dogs.	Total lipids.	Unsaponifiable.		Non-volatile fatty acids.			Solid fatty acids.				Liquid fatty acids.			Ratio of liquid to solid fatty acids.
				Weight.	In total lipids.	Weight.	In total lipids.	Melting point.	Weight.	In total lipids.	In non-volatile fatty acids.	Melting point.	Weight.	In total lipids.	In non-volatile fatty acids.	
			A	B	$\frac{100B}{A}$	C	$\frac{100C}{A}$		D	$\frac{100D}{A}$	$\frac{100D}{C}$		E	$\frac{100E}{A}$	$\frac{100E}{C}$	$\frac{E}{D}$
		kg.	*gm.*	*gm.*	*per cent*	*gm.*	*per cent*	*°C.*	*gm.*	*per cent*	*per cent*	*°C.*	*gm.*	*per cent*	*per cent*	
Low		5.4	0.730	0.202	32.6	0.414	56.6	34	0.148	15.7	26.1	48	0.264	36.2	63.0	1.78
Lipid-free 3 E	3	6.4	1.332	0.456	35.3	0.810	60.1	36	0.245	18.8	31.4	48.2	0.516	38.3	63.7	2.06
High		6.9	1.724	0.561	40.0	1.032	63.8	38	0.318	20.6	35.8	49	0.662	40.3	64.0	2.45
Low		7.6	0.588	0.244	35.8	0.386	50.0	36.5	0.078	10.8	18.8	48	0.206	26.2	52.0	1.61
Lipid-free 4	5	10.2	1.410	0.587	42.7	0.787	57.6	37.4	0.206	13.9	24.3	49.5	0.426	31.4	54.4	2.33
High		13.1	2.600	0.931	50.0	1.392	65.5	38.5	0.448	17.2	32.2	51	0.724	35.5	57.6	3.00
Low		7.9	0.974	0.323	27.8	0.626	52.4	35	0.134	11.7	21.4	45.5	0.294	23.6	44.7	1.19
Lipid-free 5	2†	8.0	1.981	0.662	33.2	1.124	58.0	37.8	0.347	16.6	28.9	47.6	0.521	27.0	46.6	1.72
High		8.4	2.781	1.027	36.8	1.469	64.5	39	0.552	21.3	37.6	49.5	0.656	30.2	47.8	2.20

* Material too dark for melting points to be seen.

† Four weekly determinations.

The composition of the diets on the basis of 80 calories per kilo, expressed in gm. per kilo per day, was as follows:

	Lipid-free 2.	Lipid-free 3.	Lipid-free 4.	Lipid-free 5.
Casein	6.4	6.4	6.4	6.4
Sugar	13.6	13.6	13.6	13.6
Bone ash	0.4	0.4	0.4	0.4
Meat extract	0.5		0.5	0.5
Lettuce		3.0		
Water	1.75	2.0	1.75	1.75

In order to eliminate any possible influence of stagnation or incomplete demarcation of the feces it seemed desirable to carry out experiments of longer duration than the single week used heretofore and two such series have been conducted with lipid-free diets, one 4 weeks and the other 5 weeks in length. The feces were collected in weekly periods, charcoal being used for demarcation. The first of these series (Lipid-free 2) was marred by the abnormal condition of three of the dogs, one showing pathological symptoms during the 3rd week and the others at the beginning of the 4th week of the series. They appeared to be suffering from distemper, of which there was an epidemic in the kennels at the time, accentuated perhaps by dietary deficiency. It had been intended at the start to continue the experiment for 2 weeks only and was, therefore, thought unnecessary to add accessory factors to the diet; but as the animals appeared to be normal and were eating well at the end of that time, it was decided to carry it on for a longer period.

In the second series (Lipid-free 3), with the exceptions noted below, the animals were normal throughout, no pathological or unusual symptoms, aside from loss of weight, having been observed. One dog, not being accustomed to experimental diets, ate rather poorly from the beginning and refused to eat at all after the 4th week. No data were obtained from this dog during the 5th week. Another dog was pregnant and aborted during the 4th week. Though the values obtained with this animal were for the most part in close agreement with the averages from the others, they are not included in the averages because of the possible effect of the abnormality of the dog. Lettuce was included

in the diet because it offered the advantage of supplying vitamins with a minimum of fat. It has been found in this laboratory to maintain dogs in health for a period of at least 4 weeks.

Analytical Methods.—The analytical procedure was the same as that described in the preceding paper with a few minor changes, one of which had as its object the more complete recovery of the volatile acids and consisted in distilling the filtrate from the acidified digestion mixture of the feces at once before extraction. After removal of volatile acids the solids from the filtration and the residual liquors from distillation were separately extracted with ether; the ether solutions were combined, washed with water, concentrated, and finally dried on the water bath. The residue was extracted with petroleum ether to obtain the non-volatile lipids.

Two slight modifications were also made in the separation of solid from liquid acids. Ether was substituted for petroleum ether as extracting agent because it seemed to show less tendency to cause emulsions, and HNO_3 (2:50) was substituted for HCl in decomposing the lead soaps of the liquid acids because of the trouble caused by insoluble $PbCl_2$. In order to determine the possible effect of HNO_3 in oxidizing unsaturated acids, the following experiments were performed. Three equal portions of linseed oil were carried through the procedure used in the separation of the fecal lipids, all being treated in the same manner except for the use of HNO_3 (2:50), HCl (2:50), and acetic acid (2:50) respectively in setting free the liquid acids from their lead soaps. The ether solutions of the fatty acids were allowed to stand in contact with the acid solutions for 24 hours—a longer period than was ever used in the analysis of the fecal lipids. The iodine numbers of the acids thus obtained were as follows:

Acid used.	Iodine No.
HNO_3	126
HCl	116
CH_3COOH	118

These results indicate that HNO_3 in the concentration used has no more oxidizing effect than the other acids. The low iodine numbers show incidentally that there is much opportunity for oxidation in the method and that, therefore, in all probability

only a portion of the highly unsaturated acids of the feces are recovered.

In another experiment three equal portions of linseed oil were dissolved in equal volumes of ether without preliminary treatment and shaken in separatory funnels with equal volumes of the acid solutions used before. The mixtures were allowed to stand 4 days, after which the iodine numbers of the three samples were determined. Great care was exercised in giving all the samples as nearly the same treatment as possible throughout. The following iodine numbers were obtained:

Acid used.	Iodine No.
HNO_3	168
HCl	167
CH_3COOH	178

These experiments taken together seem to demonstrate that HNO_3 in the concentration employed has no appreciable oxidizing influence and, in fact, no effect was seen in the recovery of highly unsaturated acids.

Computation of Data and Explanation of Table I.—It would perhaps seem most reasonable to compute the results in terms of excretion per unit body weight; but the variations between animals and in the same animal during different weeks, so far as total excretion goes, have been found to be so great in all of this work that there seemed to be no point in the use of such a method. The data have been computed, therefore, in terms of total weekly excretion per animal. Inasmuch as the same dogs were used in most of the experiments, this method of treatment gives quite comparable results.

Because of lack of space it is not possible to include the values obtained with individual animals. The results of each weekly experiment have been averaged and the averages together with the maximum and minimum values are given in Table I. The letters are used in connection with the long series to designate the week of the experiment; *e.g.*, Lipid-free 2 B includes the data obtained in the 2nd week of the series called Lipid-free 2.

Because of the failure to show any relationship between the volatile fatty acids and non-volatile lipids, the loss of volatile fatty acids during acidification of the fecal digestion mixtures,

and the necessity of analyzing the volatile fraction for each animal separately when it is to be added in with the non-volatile lipids, it has seemed more and more desirable as the work has progressed to confine the attention to the non-volatile lipids. No attempt has been made, therefore, to include values for the volatile acids in the "total lipid" fraction as was done in the preceding paper (see discussion of volatile acids below) and "total lipids" include only total non-volatile lipids. The data from some of the experiments reported in the preceding paper have been recomputed with the omission of volatile acids. The results thus obtained are much more concordant and the average values are included in Table I for comparison.

In Table I are also included the results from an experiment with a diet containing olive oil (Olive oil 2) carried out in the same manner as the one (Olive oil 1) described in the preceding paper, in order to confirm if possible the results of that work. In general the data are in complete agreement with those obtained in the earlier experiment.

Alcohol Extraction Method. Lipid-Free 4.—Continuing the idea of making a detailed examination of the basal excretion with lipid-free diets, an experiment was carried out in which the feces excreted by dogs during a week on such a diet were extracted in the modified Clarke apparatus described above with hot alcohol for 3 hours without preliminary saponification, after which they were removed, pulverized, and extracted 34 hours more. The method of analysis consisted briefly in separating the neutral and fatty acid portions by extraction from alkaline and acid solutions respectively with petroleum ether, saponification of the neutral fraction with alcoholic KOH, separation of the saponified neutral fraction into unsaponifiable and fatty acid portions by the usual technique, distillation of volatile fatty acids from the free fatty acid fraction, and separation of both the free fatty acids and the acids from the neutral portion into solid and liquid fractions.

The average values of the data, arranged to show the relationships brought out by the method of analysis, will be found in Table II. The data have also been computed for comparison with the results of the other experiments and the average values will be found in Table I.

TABLE II.

Lipid-Free 4 (Alcohol Extraction Method).

	Average.	Low.	High.
Weight of total lipids.....A	1.112	0.588	1.425
" " neutral fraction.....B	0.664	0.304	1.025
Percentage of neutral fraction in total lipids.....$\frac{100B}{A}$	58.1	49.9	72.0
Weight of unsaponifiable fraction.....C	0.501	0.244	0.711
Percentage of unsaponifiable fraction in neutral fraction.....$\frac{100C}{B}$	77.1	69.5	83.0
Weight of non-volatile fatty acids in neutral fraction.....D	0.194	0.111	0.321
Percentage of non-volatile fatty acids in neutral fraction.....$\frac{100D}{B}$	29.8	23.3	36.6
Weight of solid fatty acids in neutral fraction.....E	0.051	0.021	0.096
Melting point of solid fatty acids in neutral fraction.....	48.4	48	50
Weight of liquid fatty acids in neutral fraction.....F	0.101	0.056	0.157
Ratio of liquid to solid fatty acids in neutral fraction.....$\frac{F}{E}$	2.17	1.64	2.66
Weight of free fatty acids.....G	0.442	0.275	0.606
Percentage of free fatty acids in total lipids.....$\frac{100G}{A}$	41.1	27.2	50.0
Weight of solid fatty acids in free fatty acid fraction.....H	0.094	0.057	0.163
Melting point of solid fatty acids in free acid fraction.....	50.2	47.8	52.5
Weight of liquid fatty acids in free acid fraction.....I	0.251	0.150	0.355
Ratio of liquid to solid fatty acids in free fatty acid fraction.....$\frac{I}{H}$	2.78	2.18	3.32

Volatile Acids.—In previous work analysis of the volatile fatty acids by the method of Dyer (7) indicated that butyric was the principal one present and the weight of this fraction was computed on the assumption that it was made up entirely of butyric,

so that it might be added with the non-volatile lipids to give a value for total lipids. It was felt that, if such an addition were to be made, the volatile acids should be more accurately analyzed than was possible with the Dyer method, which does not pretend to give quantitative data when more than one acid is present, and the relatively accurate but tedious modification of the Duclaux method, proposed by Gillespie and Walters (8), was adopted. The samples for analysis were obtained by concentrating the volatile acid distillates after titration in alkaline solution, acidifying with H_2SO_4, and distilling almost to dryness. The principal difficulty in the method, aside from the laborious computations, is the necessity of discovering what acids are present by a cut and try procedure, it being fairly certain that the correct ones have been chosen when consistently positive results are obtained. Brieger (9) studied the volatile acids of the feces by means of their silver and guanidine salts and concluded that they contained 75 per cent acetic. He also demonstrated the presence of butyric and, as he thought, valerianic; but his evidence can be just as well interpreted as showing caproic. Edelstein and Csonka (10), using vacuum steam distillation to separate the volatile acids and fractionation of the silver salts to determine them quantitatively, found that in the feces of normal and dyspeptic infants they consisted in largest part of acetic with small amounts of butyric, caprylic, and formic present. The experiments with the Dyer method indicated the presence of butryic and small amounts of caproic. On the basis of the foregoing evidence it seemed reasonable to compute the results assuming that the volatile acids consisted of acetic, butyric, and caproic, and that this guess was correct is strongly indicated by the uniformly positive results obtained. In fourteen determinations upon feces from dogs on a lipid-free diet the following average values were obtained.

	Acetic.	Butyric.	Caproic.
	per cent	*per cent*	*per cent*
Low	44.30	9.89	3.64
Average	65.35	23.33	11.31
High	77.81	35.74	25.20

Though there is considerable variation in the results, they show perhaps as much uniformity as can be expected with an indirect method involving complex computations such as this. They are in general agreement with the work mentioned before and with the results recently reported by Cecchini (11). In studying fecal volatile acids in connection with their bearing on intestinal putrefaction he used the Duclaux method but did not apply any arithmetical treatment such as that of Gillespie and Walters. He simply placed the curve of the unknown showing speed of distillation between the two nearest curves of pure acids and assumed only these two acids to be present in inverse ratio to the distances from the curve of the unknown to the curves of the pure acids. In most cases he found these to be acetic and

TABLE III.

Mixture of acids from:	Palmitic.	Stearic.
	per cent	*per cent*
Lipid-free 3 A	49.6	52.5
" " 3 B	49.5	47.6
" " 3 C	41.5	62.5
" " 3 D	44.0	40.2
" " 3 E	37.2	53.0
" " 4 (free acids)	48.4	63.2
" " 4 (combined acids)	41.5	45.5
Average	44.5	52.1

butyric, the unknown curve lying about half way between the curves of these acids; but he could have explained his results as easily and probably more accurately by assuming that some caproic and less butyric was present.

Solid Acids.—The saturated fatty acids from some of the experiments were analyzed by the melting point method of Twitchell (12), using pure lauric, myristic, palmitic, and stearic acids manufactured by the Eastman Kodak Company for reference. No indication of the presence of lauric or myristic acids was found in any of the samples analyzed, the lowering of the melting points of these acids being a little greater in most cases with the fatty acids from the feces than with the same amount of pure palmitic. The results with palmitic and stearic are given in Table III.

The results are not as uniform as might be desired and in two cases total over 100 per cent, but this may be partially explained by the difficulty which was encountered in taking comparable melting points. The variation in reading was probably 0.2–0.3°, equivalent to an error of 5 to 10 per cent. It seems fairly certain that the solid fatty acids of the feces consist almost, if not entirely, of palmitic and stearic acids with the stearic present in slightly larger amount.

Liquid Acids.—The combined liquid acids from some of these experiments were separated by the barium soap-benzene method of Farnsteiner (13) to remove small amounts of solid acids which might have escaped removal before. The method was said by its author to separate oleic from more highly unsaturated acids; but according to Lewkowitsch (14) the separation is not nearly complete when an excess of oleic is present. The portion soluble in benzene, containing part of the oleic and more highly unsaturated acids, was brominated and separated into ether-soluble and ether-insoluble portions, which were analyzed for bromine, and in some cases carbon, by combustion in the Parr bomb. Where carbon was determined a weighed amount of benzoic acid was used as accelerator and the CO_2 formed was measured in the Parr (15) total carbon apparatus. Concentrated HNO_3 was used to generate the CO_2 instead of the HCl ordinarily employed in order that by simply adding ferric alum indicator bromine might be determined by the regular Volhard titration in the same sample. To check the apparatus and the method a sample of benzoic acid resublimed in the laboratory and another obtained from the Eastman Kodak Company were analyzed and gave the following results:

	Resublimed.	Eastman.	Theory.
Carbon, *per cent*	68.2	68.4	68.8

p-Bromoacetanilide also from the Eastman Kodak Company gave the following results:

	Found.	Theory.
Carbon, *per cent*	44.5	44.7
Bromine, " "	37.21	37.28

Melting points of the ether-insoluble bromides were determined in some cases, the figures given indicating the point at which the material blackened and fused together. It was found in qualitative tests that the ether-insoluble substance was also practically insoluble in chloroform and benzene.

The data are collected in Table IV together with some results from previous work and the composition and melting points of some common bromo acids are included for comparison. The evident lack of uniformity is probably to have been expected when it is considered that the syrupy nature of the ether-soluble and the small amount and insolubility of the ether-insoluble bromides precluded any attempt at purification. Moreover there was strong possibility of incomplete combustion of ether-soluble bromides due to the difficulty of mixing the syrupy material with the peroxide and accelerator in the bomb and also the probability of error in the analysis of the ether-insoluble bromides was great because of the indirect method necessitated by the small amounts available.

Though most of them are low, the bromine analyses appear to indicate that the ether-soluble fraction is made up mainly of dibromostearic acid and the syrupy nature of the substance leads to the same conclusion. On the other hand the carbon analyses seem to point to the presence of dibromopalmitic although the variations are so great that conclusions are difficult to draw. Though there is no evidence to show it, the possible presence of tetrabromo acids is by no means excluded.

In the previous paper it was stated that the ether-insoluble portion appeared from the analysis to consist of the hexabromide of an acid containing 18 carbon atoms. Its insolubility in chloroform and benzene and its high charring point force one, however, to the view that it is an octabromide and very probably the octabromide of arachidic acid. It is not difficult to correlate the other data with the assumption that this acid is present. There is not a great deal of difference, in comparison with the accuracy of the analysis, in the percentages of bromine and carbon in hexabromostearic and octabromoarachidic acids and, moreover, several of the bromine values are high for hexabromostearic while, due to the small samples used and the probable presence of impurities, we would expect low results. The carbon analyses prob-

TABLE IV.

Analysis of Liquid Acids.

Experiment.	Amount analyzed.	Fraction from insoluble barium soaps.		Fraction from soluble barium soaps.	Ether-soluble bromo acids.			Ether-insoluble bromo acids.			
		Weight.	Iodine No.		Weight.	Bromine content.	Carbon content.	Weight.	Charring point.	Bromine content.	Carbon content.
	gm.	*gm.*		*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*°C.*	*per cent*	*per cent*
Olive oil 1	5.167	1.257	62.5	3.324	4.287	36.02		0.1756		63.59	
Coconut oil	6.223	2.109	39.8	2.933	4.286	32.70		0.1762		62.74	
Lipid-free 1	2.564	0.311	46.5	1.322	1.807	34.46		0.1506		64.94	
Olive oil 2	6.875	1.977	62.5	4.633	6.617	28.75		0.0986	217	61.60	
Lipid-free 2 A	3.358	0.536	50.8	1.724	2.493	33.75	45.0	0.1418	214	61.60	28.8
" " 2 B	1.792	0.648	49.0	1.125	1.789			0.0633			
" " 2 C	1.558	0.857	56.4	0.671	1.043	35.40	43.3	0.0461		64.60	23.6
" " 2 D	1.003	0.269	78.6	0.591	0.920			0.0579			
" " 3 A	4.533	1.946	47.0	2.488	3.285	28.40	50.6	0.0393	215		
" " 3 B	3.192	0.758	46.5	2.221	3.188	36.10	46.7	0.1911	210	62.20	30.0
" " 3 C	1.777	0.882	66.6	0.866	1.379	35.0	42.0	0.0208			
" " 3 D	2.371	1.736	80.4	0.309	0.601	35.9	37.6				
" " 3 E	1.260			1.119	1.550	27.0	44.2	0.0265			
" " 4 (free acids)	1.775	0.476	68.0	1.005	1.523			0.0817	215		
" " 4 (combined acids)	1.035	0.243	71.0	0.712	1.008						

Composition of various bromo acids.

Acid.	Bromine.	Carbon.	Melting point.	Acid.	Bromine.	Carbon.	Melting point.
	per cent	*per cent*	°*C.*		*per cent*	*per cent*	°*C.*
Dibromostearic	36.18	49.00	Oil.	Hexabromostearic	63.32	28.47	180–181
Dibromopalmitic	38.66	46.50	29	Hexabromopalmitic	65.75	26.30	
Tetrabromostearic	53.33	36.00	113–114	Octabromostearic	69.80	23.60	Chars 200
Tetrabromopalmitic	55.94	33.60		Octabromoarachidic	67.87	25.41	" 220

ably do not signify a great deal for reasons mentioned except to show the general order of the carbon content, and the best of these, run on a fairly large sample of the combined bromo acids of three experiments, showed a result much too low for hexabromostearic but not far from that of octabromoarachidic.

Taking these data as a whole they seem to indicate that the liquid acid fraction is made up very largely of an acid or acids with one double bond—probably oleic—together with a small amount of highly unsaturated portion which consists almost entirely or arachidonic acid.

DISCUSSION.

One of the principal purposes of this investigation was that of determining the average composition of the lipids excreted with a lipid-free diet. Though there is a marked uniformity in composition exhibited in the case of the different animals in Lipid-free Series 2 and 3, a distinct difference between the series themselves is noticeable, the ratios of unsaponifiable matter to fatty acids and of liquid to solid acids being higher in Series 2 than in Series 3. In searching for a reason for the discrepancy one thinks of the pathological condition of some of the animals near the end of Series 2; but it is not likely that it could have affected the results of the first 2 weeks when the dogs were all normal. It seems more likely that the difference is due to the previous diet and condition of the dogs, for, though the normal laboratory diet, which preceded both of the series, is quite constant in composition, they were in considerably better condition at the start, as evidenced by comparative weights of dogs used in both series, in Series 3 than in Series 2. If this be true, however, it is difficult to see why the composition of the lipids excreted in Series 3 should not have approached that of Series 2 as the experiment went on and the animals lost weight. There appear to be other factors involved and it is deemed best, for the present at least, to strike a rough average between the two series, such as is shown by the results of Lipid-free 1, and consider it as the composition of the normal, basal lipid excretion.

The data obtained in these experiments present additional evidence in favor of the old view of Müller that a large proportion at least of the fecal lipids come from some other source than

unabsorbed food. The continued excretion of these substances over long periods of time by animals receiving no lipids in their food leaves little doubt concerning this point. When lipids are present in the food, they seem, however, to have an effect on the fecal lipids as shown by a larger total excretion and an increase in the proportion of liquid acids of the feces with an olive oil and in the solid acids with a coconut oil diet. The increase in unsaponifiable excretion with an olive oil diet is also traceable in all probability to the unsaponifiable matter of the olive oil. Such results suggest at once that we are dealing here with a basal excretion plus a certain amount of diet lipids which either have escaped absorption or have been absorbed and reexcreted as the result of a plethora such as might be expected if the fecal lipids represent some sort of leakage from the blood. In either case we would expect the lipids in excess of the basal excretion to be similar in composition to those of the diet and, in fact, when the values for the basal excretion, which we have assumed to be represented by those from Lipid-free 1, are subtracted from the corresponding values obtained in the olive oil experiments, the solid fraction cancels out leaving unsaponifiable and liquid acids in about equal proportions. It seems reasonable to assume that a large proportion of the unsaponifiable material has escaped absorption or, more probably, has been absorbed and excreted again, while a much smaller proportion of the liquid acids has suffered the same fate. When the same procedure is applied to the coconut oil experiment, there is found a small excess of unsaponifiable over the basal, a decided excess of solid acids, and also a smaller but definite excess of liquid acids. This last is difficult to understand on the assumption of a basal excretion plus some diet lipids unless we incline to the second view expressed above (that the increased excretion with lipids in the diet is due to a plethora) and explain it as being brought about by partial desaturation of the solid acids of the coconut oil.

There are several possible sources of the fecal lipids. The finding of lipids in the intestinal mucosa by Schmidt (16) suggests that they may come from desquamation of intestinal epithelial cells; but it may be that the lipids he found were simply in transit into the gut. The finding of large amounts of lipids in isolated intestinal loops where there could have been relatively little

desquamation of cells indicates that this is not the source. It is hoped that some light on this question may be obtained by experiments, which are being planned, involving the examination of the mucosa of the intestine for lipids by the methods used in this investigation. It is realized, however, that even if lipids are found, of the same composition as those of the feces, it will be difficult to say whether they formed a constituent of the cells or were merely on their way from the blood into the lumen of the intestine.

Another possible source is the intestinal bacteria, concerning the lipid content of which there is an unfortunately small amount of information available. Nencki and Schaffer (17) found a lipid content of dried putrefactive bacteria of 6.04 per cent while Kappes (quoted from Kendall (18)) found 4.8 per cent in *Bacillus prodigiosus*. Nicolle and Alilaire (19) found somewhat higher percentages using acetone and chloroform as solvents for extraction in a large variety of bacteria; but the recent work of Larson and Larson (20), who found even larger amounts of acetone extract, of which, however, only a small portion in most cases was soluble in petroleum ether, indicates that these results were in error. The last named authors computed as lipids the sum of the petroleum ether and ethyl ether extracts and found the percentage amounts on a dry basis to lie between 3.06 and 6.80 with one exception where a comparatively very small sample was used. These significant results were obtained with *Staphylococcus albus*, *Bacillus mucosus*, *Bacillus coli communis*, and *Bacillus coli communior*, all of which are normal inhabitants of the gut. From what evidence there is, it appears that the average lipid content of bacteria native to the intestine lies between 4 and 5 per cent. Kendall estimates that an adult human excretes about 5 gm. of dried bacteria per day, from which it hardly seems probable that the small dogs used in these experiments would excrete more than 1 gm. per day equivalent to 0.3 to 0.35 gm. of lipids per week at the maximum.

Further evidence that bacterial lipids form at most a small part of fecal lipids is presented by the fact that the sterile meconium contains much lipid material and by the finding of lipids in large amounts in isolated intestinal loops where bacterial life must have been reduced to a minimum. Kobert and Koch (21) ob-

tained lipids from a loop of the large intestine after they had sterilized it with antiseptic substances.

It seems to be the general opinion of those who have studied the fecal sterols that they reach the intestine either through the food or the bile. Gardner and coworkers (22), who have done a great deal of work with sterol balance, determining these substances in the food and the feces, were long of the opinion that cholesterol was not synthesized in the body; but they have recently reversed their opinion because of the finding in many cases of greater amounts of cholesterol in the feces than in the food. They recognized early in their work that a much larger amount of cholesterol was poured into the intestine through the bile than was excreted through the feces and they concluded that the cholesterol of the bile came from breakdown of red blood cells in the liver and was reabsorbed for use again elsewhere in the body, thereby forming a sort of closed circuit, while the sterols of the feces came entirely from the food sterols, which might or might not have been absorbed and reexcreted. They now believe that some of the bile cholesterol escapes reabsorption, perhaps as difficultly hydrolyzable esters, and that more is excreted than is taken in the food. The results of the present investigation support the view that unsaponifiable matter is synthesized in the body, because it continued to be excreted though none was in the food; but the data also present some phases which cause us to question whether it reaches the intestine by the way of the bile. Because of the constant relationships between unsaponifiable material and fatty acids shown throughout the work, it seems improbable that they come from different sources; but what evidence has been obtained so far indicates that the fecal fatty acids are ordinary, usable fatty acids, which, if excreted by way of the bile, would be reabsorbed. It is true that normal oleic acid has not been definitely identified and it is possible that the liquid fraction is made up of isomeric acids with the unsaturated groups in such positions that the acids cannot be used by the organism; but, in view of the fact that the body seems able to handle any monobasic straight chain acid, even with an odd number of carbon atoms, this does not seem very possible. It seems quite definite that the solid acids consist at least largely, if not entirely, of usable stearic and palmitic. The possibility

that the fatty acids are bound to the sterols as esters and thus escape absorption is largely ruled out by the experiment in which the distribution of the lipids was studied (Lipid-free 4), where it was shown that approximately 70 per cent of the fatty acids were present in the form of free acids or soaps and that there was little, if any, difference in the composition of the free and combined acids. The same thing is of course shown in a general way by the finding of ratios of unsaponifiable to fatty acids of about 0.45 throughout the work while in cholesterol esters the ratio is about 1.5. It has seemed possible that the bile might be either eliminated or designated as the source of lipid excretion by experiments similar to those above in which animals with bile fistulas were fed a lipid-free diet. Such experiments are now being carried out and, though not enough data have been collected to warrant the drawing of conclusions, it may be stated that the results so far indicate that more lipids are excreted by dogs under the conditions described than normally. It seems rather probable, therefore, that the lipids making up the basal excretion with lipid-free diets reach the intestine through its walls below the absorbing portion.

The purpose of the excretion is as yet unknown. As pointed out in the preceding paper there is a marked similarity in the composition of the fecal lipids and those of blood plasma. In only one point is there a great difference noted, *i.e.* the liquid acids of the plasma are considerably more unsaturated than those of the feces; but this may be explained as being due to the known reducing action of the intestine. This similarity suggests that there is some sort of leakage of plasma lipids into the intestine, though why such a leakage should occur in view of the general absence of such phenomena is difficult to see. If such a leakage occurred, it might explain, however, the increased excretion when lipids were included in the food and the similarity between the lipids making up the increase and the diet lipids, assuming, as mentioned above, a plethora of lipids similar to those of the diet. The possibility has been suggested that this may be a leakage designed to lubricate the interior surface of the intestine.

There is still another possibility, which was suggested by recent work of Gardner (23) in which he separated the unsaponifiable matter of human feces and found that little over half formed

insoluble digitonides, the remainder being made up of substances marked by great stability, containing oxygen in a not very reactive form, and having a molecular composition of much the same order as cholesterol. It has been noted in this investigation that the unsaponifiable fraction was usually obtained as a glassy, non-crystalline material answering the description of some of the fractions obtained by Gardner and it is suggested that these substances may be waste products of cholesterol metabolism and the lipid excretion concerned with removing them from the body, the fatty acids and neutral fats acting in some manner, perhaps as solvent, to carry them across the intestinal wall. In favor of such a view is the tendency toward a constant excretion of unsaponifiable material, shown particularly by the high proportional excretion of this fraction during fasting, where the amounts excreted were of the same order as in Lipid-free 3 though the total lipid excretion was considerably smaller. It is noticeable also that the ratio of liquid to solid acids is much higher in the case of the fasting animals than with a lipid-free diet and it is suggested as a working hypothesis that there may be a certain optimum mixture of fats for carrying the undesirable sterols across the intestinal wall in solution; but that during fasting, when the body is attempting to conserve all of its resources, it is possible for it to carry on this process of excretion with less expenditure of useful fat by employing a higher proportion of liquid acids.

SUMMARY.

1. Dogs between 5 and 13 kilos in weight excrete an average of 1.5 to 2.0 gm. of non-volatile lipids per week on a lipid-free diet, this basal excretion being made up of 35 to 40 per cent unsaponifiable material and 55 to 60 per cent non-volatile acids, which contain about 30 per cent solid and 60 per cent liquid acids. The solid fraction is composed of palmitic and stearic acids with the stearic present in slightly larger amount, while the liquid fraction consists almost entirely of an acid with one double bond, probably oleic, together with small amounts of an acid with four double bonds, most likely arachidonic.

2. Lipids of the composition given above continue to be excreted after 5 weeks without lipids in the diet, though there is a slightly smaller amount excreted at the end than at the beginning of the experiment.

3. In one experiment with a lipid-free diet, where the feces were extracted without preliminary saponification, it was found that the fecal lipids consisted of about 40 per cent free fatty acids (including soaps) and 60 per cent neutral material. About 75 per cent of the neutral fraction was unsaponifiable while the remaining 25 per cent consisted of fatty acids. Both the free and the combined fatty acids were of essentially the same composition as described above.

4. Volatile acids have been determined by the Gillespie and Walters method. The average composition has been found to be approximately 65 per cent acetic, 23 per cent butyric, and 12 per cent caproic acids.

5. The composition of the lipids excreted by different dogs under the same conditions has been found in most cases to be remarkably uniform, though there has been considerable variation in the amount.

6. The source and purpose of lipid excretion is discussed. The presence of usable fatty acids not bound to sterols makes it probable that fecal lipids enter the intestine through its walls below the absorbing portion rather than through the bile. The similarity to plasma lipids indicates that they represent some sort of a leakage and the suggestion has been made that the leakage has the purpose of lubricating the intestine. On the other hand some evidence has been presented in favor of the view that the lipid excretion is concerned with removing undesirable or excess sterols from the organism. Further work is being carried on in an attempt to settle more definitely the purpose of lipid excretion.

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