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THE COMPOSITION OF LIPID FROM JEJUNAL CONTENTS OF THE DOG AFTER A FATTY MEAL

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It is generally accepted that lipase splits fat to some extent in the intestine, but the degree of lipolysis is still uncertain. It has been suggested (Frazer, 1946) that not more than 30% of the total fatty acid is liberated in the intestine and that no glycerol is formed: and it was also maintained that more than 60% of ingested fat is absorbed as unsplit triglyceride in the form of particles of less than 0·5μ in diameter, stabilized as an emulsion by monoglyceride, fatty acid and bile salts. The evidence adduced in support of these contentions was largely indirect, being based on *in vitro* studies of lipolysis, on the composition of fat from the intestinal contents of rats after fat administration, on *in vitro* studies of emulsification and on changes in chylomicron counts in blood during fat absorption.

In vitro studies on lipolysis have been carried out by Desnuelle who summarized (1951) the results obtained by his group. They found that at pH 8 the extent of fat hydrolysis by pancreatic extracts was low, except in the presence of calcium salts and of large amounts of bile, when hydrolysis proceeded to completion. Borgström (1952) used as a source of lipase a physiological mixture of bile and pancreatic juice obtained by cannulating the duodenal papilla of the rat and subsequently adjusted to pH 6·5. Added corn oil was rapidly hydrolysed, 50% of the fatty acid being liberated in 3 hr and 70% in 6 hr.

Many workers on fat digestion have analysed the fat obtained from intestinal contents of animals killed after having been fed with olive oil by stomach tube. Desnuelle & Constantin (1952) found 16–24% fatty acid, Borgström (1952) found 37 and 43% and Mattson, Benedict, Martin & Beck (1952) 15–25% in fat from rats killed 3 hr after feeding on olive oil. In similar experiments with dogs Desnuelle & Constantin (1952) found 17% fatty acid 3 hr after feeding and 49% after 5 hr. These analyses are all of the unabsorbed

residue of fat in the intestine and can provide little evidence as to the degree of lipolysis of absorbed material.

Blankenhorn & Ahrens (1955) used a continuous aspiration technique to obtain jejunal contents throughout the whole period of fat digestion in humans. They found that 58 and 60% of the fat isolated was fatty acid. This method of collection provides more valuable evidence as to events in the intestine than the collection of intestinal contents at a fixed time interval after fat feeding.

Several workers have fed rats on fats labelled in the fatty acid and/or glycerol part of the molecule, collected lymph and deduced the amount of lipolysis which had taken place in the intestine from distribution of labelled material in the fat isolated from lymph. Favarger, Collet & Cherbuliez (1951) deduced that the maximum hydrolysis varied between 12 and 45%. Reiser, Bryson, Carr & Kuiken (1952) claimed that 25–45% of triglyceride is completely hydrolysed to glycerol, the remaining 55–75% being hydrolysed to monoglyceride: this is equivalent to 75–80% of the fatty acid being liberated. Bernhard, Wagner & Ritzel (1952) also found that 24–53% of the glycerol of triglyceride eaten must have been liberated during digestion. Borgström (1954*a*) has shown that di- and triglycerides can be synthesized from monoglyceride during digestion, a finding which throws some doubt on the validity of deductions from this type of experiment. Bergström, Borgström & Carlsten (1945) showed, by feeding labelled fat to cats, that extensive hydrolysis of fat takes place. No direct observations have been made on the chemical composition of highly emulsified fat in intestinal contents, although Bergström & Borgström (1953), in their review, conclude that 'it seems rather unlikely that any globules of unchanged triglyceride...should pass through the mucosal cell...in the rat'.

We have tried to obtain as nearly as possible normal intestinal contents from a conscious dog and have used this material to find the amount of lipolysis which can take place *in vitro*. We have also separated the highly emulsified fat particles from the intestinal contents and report their composition.

METHODS

The general method used was to obtain intestinal (upper jejunal) contents from dogs with jejunal fistulae after feeding with meals containing olive oil. Considerable care was taken to ensure that the animals ate the food willingly so that as far as possible normal conditions of digestion and secretion obtained. The pH of the intestinal contents was determined and in most cases specimens were examined under dark ground illumination for fat particles. The intestinal contents obtained were then either acidified to stop lipolysis or shaken at 38° to promote further lipase action. The fat was extracted and analysed chemically.

Animals

Two mongrel terrier bitches were prepared with jejunal fistulae of the Mann-Bollman type (Mann & Bollman, 1931). In the first animal (B) (wt. 10 kg) the jejunum was divided about 10 cm below the junction with the duodenum, and the upper section was anastomosed to the lower

segment about 12 cm further down. The free end of the lower segment was then closed and sewn to the muscle of the abdominal wall: the fistula was opened 5 days later. In the second animal (A) (wt. 12 kg) a fistula was made by removing 12 cm of jejunum from a point 40 cm below the duodeno-jejunal junction and anastomosing the distal end of the segment with the jejunum 12 cm below the duodeno-jejunal junction: the proximal end was sewn to an incision in the abdominal wall. Both animals made rapid recoveries, the first maintaining its weight for 4½ months, while the second animal's weight rose from 12 to 13 kg during 3 months.

Maintenance. The animals were fed on a dried milk diet for 4 days after operation, and thereafter on a normal meat diet. Despite the direction of peristalsis being away from the fistula, leakage occurred in both animals after feeding, and it was found necessary to close the orifice with a small rubber balloon attached to rubber tubing with a 2 in. diameter rubber disk. The balloon and tubing were inserted into the fistula and the balloon inflated with 5–10 ml. water, the rubber tubing being closed with a glass rod of ¼ in. diameter. Peristalsis drew in the balloon until the rubber flange was close up to the abdominal wall. A strip of bandage was tied to the rubber tubing and then tied to a harness on the animal's back. A pad of absorbent cellulose wadding sprinkled with aluminium hydroxide powder was applied and held in position with a broad crepe bandage, the whole being covered with a canvas coat. A large pressed-fibre disk collar prevented any interference with the dressing by the animal. The dressings and 'stopper' were replaced daily. The animals were not used for experimental purposes until at least 6 weeks after the operation. Table 1 gives the post-mortem measurements of the portion of intestine operated on.

TABLE 1. Measurements* (post-mortem) of position of jejunal fistulae

	Length (cm)	
Segment of intestine	Animal B	Animal A
Pylorus to anastomosis	30	20
Pylorus to pancreatic duct	6	9
Abdominal surface to anastomosis	12	10

* These measurements are less than those made at operation owing to post-mortem shortening.

Experimental procedure. The experiments were all carried out in the morning, the animal having been fasted 18 hr with access to water. The animal stood in bandage slings on a dog stand. Considerable care was taken to ensure that the slings were comfortable and of such a length that when the animal relaxed, some of its weight was taken by the slings.

Intestinal contents were collected by inserting a bi-lumen soft rubber tube carrying a balloon on one lumen into the fistula and inflating the balloon so as to occlude the intestine below the anastomosis. The flow of material was facilitated by cutting several ¼ in. holes in the lumen of the tube. A prostatic catheter, size 20 French gauge, was found to be convenient for the collection. With the first animal, where the intestine was continuous from the abdominal wall, no difficulty was found; but in the second animal, where the intestine from the abdominal wall to the anastomosis was not continuous with the jejunum, occasional difficulty was found in getting the tip of the catheter into the jejunum below and not above the anastomosis. That the catheter balloon was in the correct position was easily determined because it was then pulled in firmly by peristalsis until stopped by a wide rubber flange attached to the catheter. The arrangement is shown in Fig. 1. The intestinal contents were collected in 100 ml. cylinders which were held off the table and in position by a bandage tied round the animal's body.

Material from the intestine was collected for 30 min before feeding; the cylinder was then changed and the animal fed. Collection was made continuously for 1–2 hr, the cylinders being changed every 10–30 min, depending on the rate of flow. The pH was determined on the contents of each sample by a Cambridge pH meter using a glass electrode. Most specimens were examined under dark ground illumination to determine the degree of fat dispersion. The specimens were either pooled and kept until the end of the experiment or acidified (8 ml. glacial acetic acid per

100 ml. intestinal contents; the resultant pH was about 3) soon after the end of collection and then bulked and extracted for fat.

Two types of fat meal were used. The first consisted of mashed potato (200 g) with olive oil (40 ml.) incorporated and flavoured with 'Oxo'. This was used for the first four experiments with animal B but was not readily eaten. The second diet consisted of ice-cream made with olive oil and was used for five experiments with the first animal and nine with the second. The ice-cream was prepared by dissolving 28 g sucrose and 2 g gelatin in one-third pint fat-free milk and then emulsifying 40 ml. olive oil with this mixture by passing through a domestic cream-maker. The emulsion was kept in the refrigerator for 1 hr, stirred thoroughly and then stored in the refrigerator until required. The fat content was approximately 16·7 g/100 g food. The food was brought into the laboratory when the animal was on the stand and left within its field of vision. Both animals ate the ice-cream with avidity and showed marked signs of appreciation. They usually stood quietly for an hour or more after their meal, one animal developing the habit of relaxing into the bandages and dozing.

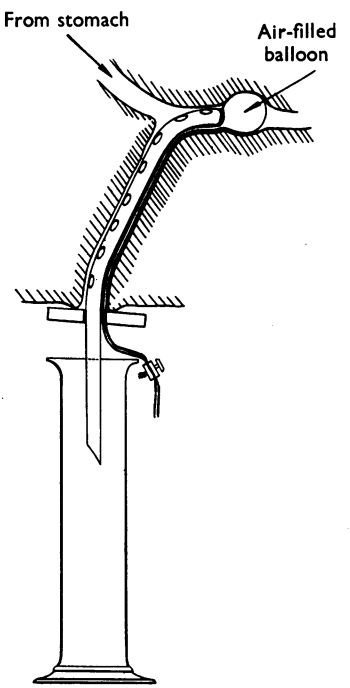

Fig. 1. Position of catheter during the collection of jejunal contents.

Chemical methods

Extraction of lipid. Each 100 ml. of intestinal contents was acidified by the addition of 8 ml. glacial acetic acid, mixed with 75 ml. 95% (v/v) ethanol and shaken with 120 ml. di-ethyl ether. After centrifuging and removing the ether, the aqueous residue was extracted three times with 60 ml. ether. The ether solutions were washed four times with 100 ml. portions of water. The ether was then removed from the washed ethereal solution of lipid by evaporation under reduced pressure in an atmosphere of N_2.

The acetic acid was removed by repeatedly adding water and distilling under reduced pressure in an atmosphere of N_2, the temperature of the water-bath being less than 45° C. This process was repeated until the distillate needed $<0{\cdot}5$ ml. 0·01 N alkali for neutralization. Water was then

removed by adding absolute ethanol and distilling under reduced pressure, the traces of alcohol being removed by adding and distilling off ether. The fat was then dissolved in dry ether, which was filtered, the ether evaporated and the fat weighed. Any phospholipid was removed by dissolving in acetone, filtering, and removing the acetone. Only traces of acetone-insoluble material were found when the potato diet was used and none with the ice-cream diet. The fat was transferred to specimen tubes and subsequently analysed.

Fatty acid was estimated by titration with 0·02 N alcoholic potash (CO_2-free) in a stream of N_2, the fat being dissolved in chloroform (3 ml.) with 10 ml. benzene added, using thymol blue as indicator. The results were calculated as oleic acid.

1-*Monoglyceride* was estimated by oxidation with periodic acid for 30 min then distilling and estimating the formaldehyde formed, using chromotropic acid (Dowse & Saunders, 1956). Values were calculated as 1-mono-olein.

TABLE 2

		Intestinal contents collected		
			Fat recovered	
Expt.	Food eaten (g)	Total (ml.)	(g)	% fat eaten
	(Potato mixture)			
B2	114	124	5·0	27
B3	162	153	4·2	14
B5	115	161	3·8	23
B6	147	127	1·6	7
Mean ± S.E.		141 ± 9		
	(Ice cream)			
B8	180	315	13·8	46
B9	88	203	7·4	50
B10	150	158	2·7	11
B12	117	138	3·0	15
Mean ± S.E.		204 ± 40		
All B		172 ± 22		
	(Ice cream)			
A3	163	288	23·1	77
A4	208	309	27·5	88
A5	209	344	31·5	82
A6	232	370	32·6	82
A7	215	346	32·1	82
A8	104	247	10·6	66
A9	170	270	18·4	66
Mean ± S.E.		311 ± 32		

RESULTS

Intestinal contents collected

The collection of intestinal contents after feeding was continued until the rate fell to a low figure (< 1 ml./min). With the second dog there was a rapid flow which fell off rapidly, but with the first dog the rate of flow was much less and fell off more slowly. The total period of collection varied between 60 and 100 min after feeding for the second animal and between 100 and 150 min with the first. Table 2 gives the figures for the total volume for fifteen experiments where the collection was satisfactory. The marked difference between the two animals in the percentage of fat eaten which was recovered, and between the

time patterns of collection, can best be interpreted as being due to a slow passage of the stomach contents into the duodenum in animal B and a very rapid passage in animal A.

Acidity. The pH of intestinal contents after feeding on either diet was very constant, 89 values on 17 different days being between 6·0 and 6·5. One value each of 5·7, 6·6 and 6·7 was obtained. In one experiment five values ranging between 6·7 and 7·0 were obtained, but the degree of lipolysis was similar to that with more acid intestinal contents.

TABLE 3. Degree of lipolysis of lipid in jejunal contents

Expt.	Lipid in intestinal contents (mg/ml.)	Fatty acid (g/100 g lipid)	Time after leaving catheter until reaction stopped Mean (range) (min)
A3	80·2	17·6	9·6 (5–15)
A2	50·5	20·2	13 (7–19)
B53	24·6	22·4	13 (6–21)
A13	32·8	33·6	22 (10–45)
B52	22·9	26·4	30 (10–63)
B63	11·7	36·6	33 (15–50)
B62	12·7	40·6	40 (20–60)

Degree of lipolysis. Presumably lipase acts on the fat from the moment of mixing of pancreatic juice with the duodenal contents; and in our experiments lipolytic activity would continue while the intestinal contents traversed at least 11 cm of intestine (animal A) and then for the time that the material stood during collection and before acidification. In Table 3 are recorded the values for the mean time during which lipolysis occurred and the concentration of fatty acid in the fat isolated, showing that the fatty acid increased from 18 to 41 % when the mean time before stopping lipolysis increased from 10 to 40 min.

Maximum amount of lipolysis. Nine experiments were carried out in which the intestinal contents were collected (but not acidified) until the animal yielded no further material. A sample of the collected intestinal contents was then removed for analysis: the remainder was shaken at 37° for further periods up to 3 hr and the fat then extracted. The results, given in Table 4, show that the maximum degree of hydrolysis achieved after 3 hr shaking at 37° varied from 41 to 76 % (mean 54 %).

1-*Monoglyceride content.* 1-Monoglyceride determinations were done on eighteen specimens of lipid from intestinal contents by the chemical method developed by Dowse & Saunders (1956), the values ranging from 3·2 to 8·1 g/100 g lipid. These figures are given in two groups in Table 5 divided into samples of intestinal contents which had been incubated with shaking for 1–3 hr and those which had not been so treated.

The mean value ± S.E. (no. of samples) for fatty acid was 44·5 g/100 g lipid ± 1·3 (9) for material incubated at 37° with shaking for 1–3 hr; and

26·8 g/100 g. lipid ± 1·75 (9) for lipid from material not incubated, i.e. acidified to stop lipolysis. The figures for 1-monoglyceride are 6·5 g/100 g lipid ± 0·3 (9) for incubated material and 4·6 g/100 g lipid ± 0·4 (9) for material not incubated. The difference between the means is highly significant, both for fatty acid ($P \ll 0{\cdot}001$) and for 1-monoglyceride ($P < 0{\cdot}001$).

TABLE 4. The amount of lipolysis in jejunal contents after incubation (with shaking) at 37° C

Expt.	Wt. of lipid in intestinal contents (mg/ml.)	Treatment after collection (hr)*	Fatty acid (g/100 g lipid) Time of shaking (hr) 0	1	2	3
B10	17	½–2¼ at 18° C	34·5 (6·2)	—	60·2 (6·0)	—
B11	21	½–2¾ at 18° C	50·0 (6·1)	—	—	76·4 (5·7)
B12	22	½–2¼ at 18° C	38·4 (6·0)	—	—	71·5 (5·5)
A4	90	½–2 at 18° C	30·9 (5·8)	—	—	41·1 (—)
A5	91	½–2 in ice water	26·2 (5·8)	41·0 (5·8)	46·0 (5·8)	48·3 (5·8)
A6	95	¼–1½ in ice water	24·4 (5·8)	40·9 (5·7)	43·4 (5·7)	45·8 (5·8)
A7	93	1–2½ in ice water	28·5 (5·8)	—	—	52·0 (—)
A8	43	1–2¼ in ice water	33·4 (6·0)	—	—	44·6 (—)
A9	68	1–2 in solid CO_2/$CHCl_3$	29·2 (6·0)	—	—	52·7 (5·9)

* Shortest and longest periods during which any portion of intestinal contents stood from the moment of leaving the catheter to the start of the incubation at 37°.

Figures in brackets show pH of intestinal contents.

TABLE 5. The effect of incubation with shaking at 37° on the composition of lipid from jejunal contents (mean values ±S.E.)

Material	No. of expts.	Fatty acid (g/100 g lipid)	1-mono-glyceride (g/100 g lipid)	Glycerol liberated (calculated) as % of total glycerol if residue is assumed to be: Diglyceride	Triglyceride
As collected	9	26·8±1·75	4·6±0·4	—	20·4±1·7
Incubated at 37° for 1–3 hr	9	44·9±1·30	6·5±0·3	13·3±2·6	35·9±1·5

Degree of dispersion of fat. In many specimens visible fat rose to the surface of the cylinder. Microscopic examination under ordinary illumination ($\frac{1}{6}$ in. objective) and under dark ground illumination ($\frac{1}{12}$ in. objective) showed fat in all degrees of dispersion in all specimens, from very small particles ('microns') of probably $< 0{\cdot}5\mu$, which were exhibiting violent Brownian movement, to medium (*ca.* 5μ) droplets and large drops ($> 10\mu$). The ice-cream on which the animals fed contained mainly medium-sized droplets. The only conclusion we came to was that in our experiments the olive oil was never completely emulsified.

Composition of emulsified fat

Three experiments were done to try to find the composition of fat in a stable emulsion in the intestinal contents. After completing collection, the jejunal contents were stored in ice-water or freezing mixture. Each collection was divided into two parts: the first half was centrifuged at 1000 *g* for 15 min, while the second half was shaken at 37° C for 3 hr and then centrifuged at

1000 *g* for 15 min. This procedure produced a lower watery layer containing highly emulsified lipid and a supernatant oily layer. In each case the lower watery layer was siphoned off from below the oily layer, great care being taken to avoid removing any of the oily layer. The lower watery layers showed very large numbers of 'microns' and only an occasional droplet. The upper oily layers still contained traces of the emulsified layer. The lipid was extracted from each layer. The analytical figures are given in Table 6.

TABLE 6. Jejunal contents separated by centrifugation

	Expt.	Vol. separated layer (ml.)	Lipid isolated (g)	Lipid isolated (mg/ml.)	Fatty acid (g/100 g lipid)	1-Monoglyceride (g/100 g lipid)
Upper (fat) layer						
Before shaking	7	44	14·48	—	26·2	4·0
	8	50	4·60	—	30·7	5·6
	9	44	7·90	—	26·9	4·6
After 3 hr shaking at 37°	7	38	(7·47)*	—	51·3	4·2
	8	30	3·13	—	40·8	5·6
	9	32	7·54	—	52·0	4·9
Lower emulsified layer						
Before shaking	7	126	1·56	12·4	50·0	16·5
	8	75	0·87	11·6	48·0	—
	9	84	1·41	16·8	42·3	—
After 3 hr shaking at 37°	7	132	2·21	16·7	59·1	5·1
	8	96	2·04	21·2	50·3	9·4
	9	96	1·55	16·1	56·4	8·3

* Some fat lost.

The mean of the fatty acid concentration in the upper layers before shaking is significantly less than the mean of all the other fatty acid concentrations ($P < 0{\cdot}001$), but any differences in the means of the other groups of fatty acid concentrations and between any 1-monoglyceride values are not significant. The average composition of the emulsified fat is $51 \pm 2{\cdot}5\%$ oleic acid and $9{\cdot}8 \pm 2{\cdot}3\%$ 1-monoglyceride.

DISCUSSION

Extent of lipolysis

Fatty acid liberation. The results given in Tables 3 and 4 show that the fatty acid is liberated rapidly at first from fat in intestinal contents. The smallest concentration of fatty acid found (17·6%; Expt. A 3, Table 3) was after 10 min collection periods, each portion collected being acidified 5 min later. The total time of lipase action in this case was probably only 20–30 min, allowing 5–15 min for passage down the 21 cm of intestine from the pancreatic duct to the body surface. In the four experiments (A 5–A 8, Table 4) when collection periods of 10–15 min were used and the material subsequently stored in ice-water, the minimum of fatty acid found in the fat was 24·4% and even storage in freezing mixture produced 29·2%.

Incubation of the collected material, with shaking, at 37° produced further fatty acid liberation in all cases, with widely different fatty acid contents (41–76%). In the two experiments where samples were analysed at hourly intervals (A5, A6, Table 4) fatty acid liberation had practically ceased after only 1 hr at 37°, indicating that in these experiments the amount of fatty acid had reached a maximum. The stoppage of lipase action *in vitro* is usually attributed to an accumulation of fatty acid at the oil-water interface preventing contact between water-soluble lipase and triglyceride. This may be the explanation in our experiments, although if 41% of fatty acid in the fat virtually inhibits lipase action (Expts. A5, A6), it is difficult to imagine why 50 and 38% fatty acid allow very marked subsequent lipolysis (Expts. B11, B12).

The optimum pH for lipase activity *in vitro* is usually given as pH 7–8 and it is at this pH that the accumulation of fatty acid inhibits further lipase action (Desnuelle, Naudet & Rouzier, 1948). Borgström (1954*b*), however, finds that in the presence of bile salt lipase activity is high at pH 6·3 but falls off in more acid solutions. The pH of the intestinal contents in our experiments was between 6·0 and 6·5 and the final pH after incubation (Table 4) between 5·5 and 6·0. It seems likely that the pH of the solution is a limiting factor for the continuance of lipase activity. In the intestine the development of a pH of less than 6·0 may be prevented by the addition of intestinal secretions, which are weakly alkaline (Florey, Wright & Jennings, 1941) or by the preferential absorption of fatty acid which Borgström (1954*a*) has shown to occur in the rat. Both these factors would produce greater lipolysis *in vivo* than *in vitro*.

Monoglyceride formation. The amount of 1-monoglyceride found in fat from intestinal contents varied between 3·2 and 8·1%.

We tested eight of our fat specimens for the presence of 2-monoglyceride by the method of Mattson *et al.* (1952) or that of Borgström (1952). No 2-monoglyceride was found. The above figures therefore represent the total monoglyceride in our specimens. These figures are similar to those found by previous workers for the monoglyceride content of the fat from intestinal contents of rats and of dogs (Mattson *et al.* 1952; Desnuelle & Constantin, 1952; Borgström, 1954*a*).

Blankenhorn & Ahrens (1955) found 13 and 17% of the lipids from human jejunal contents to be monoglycerides while Kuhrt, Welch, Blum, Perry, Weber & Nasset (1952) in a similar type of experiment found 37–50% of the fat to be monoglyceride. The value given by the latter workers is very much higher than those reported by any other workers on fat digestion either *in vivo* or *in vitro*.

Glycerol formation. It has been claimed (Frazer, 1946) that no glycerol is formed during the digestion of fat. No direct estimations of glycerol were made in our experiments, but it is possible to deduce its liberation on the basis of the

following argument. Digestion of triglyceride can give rise to diglyceride, monoglyceride, glycerol and fatty acid. The glycerides and fatty acid are isolated as lipid and the fatty acid concentration is known. The remainder may be mono-, di- or tri-glyceride or any mixture. If no glycerol is formed, then the maximum fatty acid content of the isolated lipid would occur when the lipid is entirely fatty acid and monoglyceride.

If the fatty acid content in g/100 g lipid = F, then the monoglyceride content = (100 − F) %. As two molecules of fatty acid and one of monoglyceride are formed on splitting triglyceride, then $F/282 = 2 \times (100 - F)/356$: (the molecular weight of oleic acid is 282 and of mono-olein 356). From this equation F = 61·3, which means that if the fatty acid in the isolated lipid is more than 61·3 %, glycerol must have been liberated. We isolated two specimens of lipid, both from intestinal contents which had been shaken for 3 hr at 37°, which contained 71·5 and 76·4 % fatty acid. Therefore even with these unfavourable assumptions, glycerol must have been formed in these two cases.

With the eighteen specimens of lipid for which the monoglyceride content is also known (Table 5), it is possible to calculate the concentration of fatty acid which corresponds to the glyceride formed, on the assumption that all the remaining lipid is either triglyceride or diglyceride. If the actual fatty acid content exceeds the value calculated on these assumptions, then glycerol must have been formed from the hydrolysis of the original tri-olein. The amount is given in Table 5 as a percentage of the total free and combined glycerol.

Assuming the residual lipid to be diglyceride, calculation from the analytical figures for nine specimens of lipid from intestinal contents which had not been incubated gave negative values for free glycerol, and assuming the residual lipid to be triglyceride, calculation gave 20 % of the glycerol set free. It seems very doubtful if any appreciable quantity of glycerol is set free under these conditions.

The liberated glycerol in the nine experiments where the intestinal contents were incubated at 37° is calculated to be 13 % if the residual lipid is assumed to be diglyceride and 36 % if it is assumed to be triglyceride. As other workers (Borgström, 1954*a*; Blankenhorn & Ahrens, 1955) have found both di- and tri-glyceride in the lipid from intestinal contents, the true amount is probably between these two sets of values.

The composition of highly emulsified lipid

An essential part of Frazer's hypothesis was that more than 60 % of ingested fat was absorbed from the intestine as triglyceride in the form of particles of less than 0·5 μ in diameter (Frazer, 1946). No analyses of such particles have hitherto been published.

The analyses in Table 6 show that the composition of lipid in 'micron' form is 42–59 % fatty acid and 5–16 % monoglyceride, the sum of the two being

60–66 % (four values). This means that even if the whole of the remaining lipid is triglyceride it amounts to 34–40 % as compared with 60–66 % of split fat. If diglyceride were present, as has been reported for lipid from intestinal contents by other workers (Borgström, 1954*a*; Blankenhorn & Ahrens, 1955) the amount of triglyceride would be correspondingly smaller. It seems unlikely therefore that unsplit fat is absorbed to any large extent in the dog. This conclusion has already been deduced by Bergström & Borgström (1953, p. 336) in their review of fat absorption.

A further point of some interest is that the amount of this highly emulsified lipid per ml. of intestinal contents varies between 11·6 and 21·2 mg and is not significantly increased ($0{\cdot}2 > P > 0{\cdot}1$) by incubation, with shaking, at 37°: and this despite the fact that the composition of the non-emulsified lipid is very similar to that which is highly emulsified. It is, of course, possible that lipid may be absorbed from particles larger and less stably emulsified than those we separated. Macheboeuf (1948) has put forward an interesting suggestion that the free border of an intestinal epithelial cell can be regarded as being composed of orientated molecules containing groups phobic for the cytoplasm. If these groups are masked by combination with molecules in the lumen contents, the complex formed may be displaced into the cell and be replaced by other molecules hitherto denied access to the surface. He suggests that cholesterol may play such a role in relation to fatty acid and ribonucleic acid in relation to partial glycerides. By means of such a mechanism surface molecules may be stripped from a 'large' lipid particle or if the lipid particle is sufficiently small it may be completely absorbed. It seems to us that this interesting suggestion may resolve many difficulties in relation to fat absorption.

SUMMARY

1. Two dogs with Mann-Bollman type jejunal fistulae were fed with olive oil and the intestinal contents collected.

2. The lipid isolated from jejunal contents contained 18–34 % fatty acid and 3·2–6·5 % 1-monoglyceride. On shaking jejunal contents for periods of up to 3 hr at 37° the oleic acid content of the lipid increased to 41–76 % and the 1-monoglyceride to 5·5–8·1 %.

3. The pH of jejunal contents, normally 6·0–6·5, decreased to 5·5–6·0 on shaking *in vitro*. It is suggested that the pH may be a factor limiting the extent of lipolysis in these conditions.

4. Highly emulsified lipid was separated from jejunal contents by centrifugation. It contained 42–59 % oleic acid and 5–16 % 1-monoglyceride.

5. It seems unlikely that unsplit triglyceride is absorbed as such to any large extent in the dog.

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