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Studies on the site of fat absorption

1. The sites of absorption of increasing doses of ^{131}I-labelled triolein in the rat

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SYNOPSIS These studies on fat absorption in rats show that a small dose was relatively rapidly absorbed in the jejunum. With larger doses it was also absorbed in the ileum. When larger doses were given the jejunum responded to the increased load with an increased absorption, and fat was also absorbed in the ileum.

Absorption of different substances does not occur uniformly in the small intestine. Glucose, for instance, is absorbed rapidly from the jejunum but more slowly in the distal small intestine (Cummins and Jussila, 1955; Verzár and McDougall, 1936) and under physiological conditions vitamin B_{12} is predominantly absorbed from the ileum (Booth, 1958; Booth and Mollin, 1959).

Although the site of fat absorption has been studied by several workers, the relative importance of the proximal and distal small intestine in the absorption of fat is uncertain. Kremen, Linner, and Nelson (1954), from observations in dogs subjected to intestinal resection, have concluded that fat is primarily absorbed in the ileum, and the upper ileum is said to play a major rôle in the absorption of ^{131}I-labelled olive oil in the rat (Benson, Chandler, Vansteenhuyse, and Gagnon, 1956). On the other hand, Turner (1958) considers that the jejunum is the main site of absorption of fat in the dog, and Borgström, Dahlqvist, Lundh, and Sjövall (1957) have suggested that in man fat is absorbed in the proximal 100 cm. of the jejunum.

In view of these conflicting reports, we have made a further study of the site of fat absorption in the rat, using ^{131}I-labelled triolein. Animals were killed at frequent intervals during absorption of different doses of labelled fat and the amount of radioactivity in the various parts of the small intestine was measured. The use of ^{131}I-labelled triolein provides a simple method for this purpose. Digestion of the labelled material does not release significant amounts of free iodide (Balint, Pendower, and Ramsey, 1960) and ^{131}I therefore remains bound to triolein during absorption at least until it has reached the intestinal mucosal cell. Studies of thoracic duct lymph after oral administration of ^{131}I-labelled triolein suggest that it may be partially de-iodinated during absorption (Van Handel and Zilversmit 1958), but this probably occurs within the intestinal mucosal cells since free iodide may be released from ^{131}I-labelled triolein by incubation with freshly prepared extracts of intestinal mucosa (Balint *et al.*, 1960).

This paper is concerned with the detection of labelled triolein only during its passage along the intestinal tract and entry into the mucosal cell and the measurement of the total radioactivity in the various parts of the gut is therefore a satisfactory method of tracing the absorption of ^{131}I-labelled triolein. The techniques used were similar to those reported by Parkins, Dimitriadou, and Booth (1960).

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS Laboratory-bred male or female white rats weighing approximately 200 g. were used throughout the experiments.

^{131}I-LABELLED TRIOLEIN Triolein was iodinated with ^{131}I using a modification of the method described by Lubran and Pearson (1958). The ^{131}I-labelled triolein was purified first by dialysis and then by passage through an ion-exchange resin to remove free iodide. The resulting material usually contained a concentration of 30 to 40 mg. of triolein per ml., of specific activity 0·8 to 0·9 μc. per mg. It was tested for purity by ascension chromatography in a butanol/acetic acid/water medium, the distribution of radioactivity in the chromatograms being determined by autoradiography. Chromatograms were also made after incubation of the ^{131}I-labelled triolein with pan-

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creatic extracts or with rat intestinal juice for 24 hours. As reported by Balint and his colleagues (1960), digestion failed to release significant amounts of ^{131}I from the material.

Test doses of 10, 50, 100, and 500 mg. of ^{131}I-labelled triolein in 1-ml. volume were prepared by dilution and the addition of suitable amounts of unlabelled triolein, the radioactivity of the test doses being adjusted to approximately 2 μc. of ^{131}I.

EXPERIMENTAL PROCEDURE

ABSORPTION OF ^{131}I-LABELLED TRIOLEIN BY INTACT ANIMALS Successive doses of 10, 50, 100, and 500 mg. of ^{131}I-labelled triolein were given to a group of six rats.

The animals were starved for 12 hours beforehand and the labelled fat was fed from a graduated syringe through a fine polythene tube passed into the stomach. Each animal was then placed in an individual metabolism cage and the faeces were collected for 48 hours, urine being collected separately. The excretion of radioactivity in the faeces of these animals was measured and absorption was calculated by assuming that the radioactive material not recovered in the faeces had been absorbed.

Measurement of radioactivity The faecal specimens were collected in waxed cartons and counted by positioning each carton between two scintillation counters, according to the method described by Lewis and Porter (1960). This counting arrangement made it possible to count the radioactivity without homogenization. The sensitivity of the counter was approximately 60,000 counts per minute per μc of ^{131}I. Background counting rates ranged from 400 to 600 per minute.

DISTRIBUTION OF RADIOACTIVITY IN GASTROINTESTINAL TRACT DURING ABSORPTION Four groups of 21 animals were used. The animals were starved for 12 hours and each group of rats received intragastric test doses of either 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein. Three animals from each group were then killed at each of the following times after the test doses were given: quarter, half, one, one and a half, two, three, and four hours. The gastro-oesophageal junction, pylorus, and rectum were clamped and the entire gastrointestinal tract was removed. The small intestine was divided into four segments of equal length as follows: (1) duodenum and upper jejunum, (2) lower jejunum, (3) upper ileum, (4) lower ileum. The stomach and different segments of the small intestine and colon were then placed in separate containers and dissolved in 20 ml. of a solution containing equal quantities of 40% sodium hydroxide and absolute alcohol, the mixture being incubated overnight at 56°C.

Measurement of radioactivity The radioactivity of the stomach and intestinal specimens was measured in a coronet-type counter similar to that described by Veall and Baptista (1954). With this counter, 1 μc of ^{131}I recorded approximately 9,000 counts per minute. Background counting rates on this counter ranged from 25 to 45 per minute.

RESULTS

ABSORPTION OF TEST DOSES OF ^{131}I-LABELLED TRIOLEIN BY INTACT RATS The amounts of radioactivity recovered from the faeces of the six animals which received test doses of 10, 50, 100, and 500 mg. of ^{131}I-labelled triolein are given in Table I; the mean excretion of radioactivity after each dose is shown in Fig. 1. The actual amounts of ^{131}I-labelled fat absorbed from the different doses, calculated by assuming that the radioactive material not recovered in the faeces was absorbed, are given in Table II; the mean absorption at each dose level is illustrated in Fig. 2.

TABLE I

FAECAL EXCRETION OF ^{131}I-LABELLED TRIOLEIN (% OF DOSE) AFTER INTRAGASTRIC DOSES OF 10 TO 500 MG.

Rat No.	*Amount Fed (mg.)*			
	10	*50*	*100*	*500*
1	11·0	8·3	14·8	30·3
2	11·0	11·6	13·9	22·8
3	15·6	9·6	14·3	23·0
4	10·5	14·6	14·8	23·8
5	13·5	12·5	20·6	22·4
6	9·2	9·4	18·3	23·1
Mean ± S.D. =	11·8±2·33	11·0±2·34	16·0±2·7	24·2±3·01

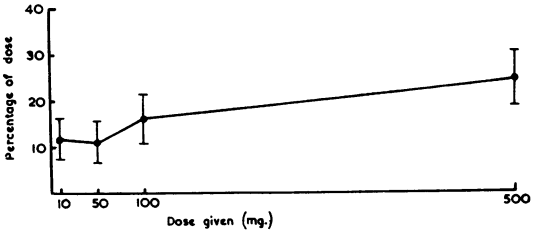

FIG. 1. *Mean percentage of administered dose (± 2 S.D.) excreted in the faeces of six rats given 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein.*

When 10 mg. of ^{131}I-labelled triolein was given, the percentage of this dose excreted in the faeces ranged from 9·2 to 15·6 (mean 11·8%). Similar amounts were excreted after doses of 50 mg., but the percentage excretion of the dose increased when doses of 100 or 500 mg. were fed, the faecal excretion ranging from 13·9 to 20·6 (mean 16·0%) when 100 mg. was given, and from 22·4 to 30·3 (mean 24·2%) after the 500 mg. dose. Although the percentage of the dose excreted in the faeces was greater when the larger doses were given, there was a progressive and linear increase in the actual amount of ^{131}I-labelled fat which was absorbed when the concentration of the dose was increased (Table II, Fig. 2).

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TABLE II

CALCULATED ABSORPTION[1] OF ^{131}I-LABELLED TRIOLEIN (MG.) FROM INTRAGASTRIC DOSES OF 10 TO 500 MG.

Rat No.	*Amount Fed (mg.)*			
	10	*50*	*100*	*500*
1	8·9	45·8	85·2	348·0
2	8·9	44·2	86·1	386·0
3	8·4	45·2	85·7	385·0
4	8·9	42·7	85·2	381·0
5	8·6	43·7	79·4	388·0
6	9·1	45·3	81·7	384·0
Mean =	8·8	44·5	83·9	379·0

[1]The absorption of ^{131}I-labelled triolein has been calculated by assuming that the radioactivity not recovered in the faeces (Table I) was absorbed.

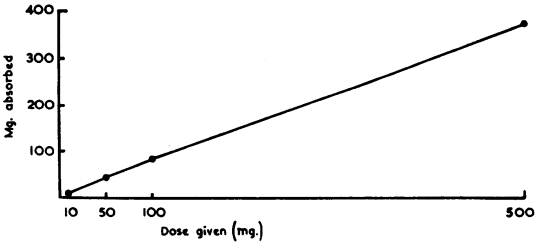

FIG. 2. *Mean absorption of labelled fat by six rats given doses of 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein.*

DISTRIBUTION OF RADIOACTIVITY IN STOMACH AND INTESTINES DURING ABSORPTION The percentages of the different doses found in the stomach and different segments of the small intestine and the colon of the four groups of rats killed at varying time intervals after receiving 10 to 500 mg. of ^{131}I-labelled triolein are given in Tables III (10 mg.), IV (50 mg.), V (100 mg.), and VI (500 mg.). From these results the percentage gastric emptying and percentage absorption of the test doses have been calculated for each animal.

Gastric emptying The percentages of gastric emptying in the four groups of animals are detailed in Tables III to VI. The mean gastric emptying in the three rats killed at each time after the different doses is shown in Fig. 3.

At quarter of an hour after feeding, there was no significant difference in the percentages of the different doses which had left the stomach, the mean percentage gastric emptying ranging from 41·2 to 50·3% (Fig. 3). By half and one hour, the percentages of gastric emptying had increased after all four

TABLE III

PERCENTAGE OF 10 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
7	¼	39·5	14·0	33·4	5·2	0·0	0·0	92·1	7·9	61·5
8	¼	45·0	25·0	13·8	3·1	0·1	0·2	87·3	12·7	55·0
9	¼	64·5	20·2	12·0	0·6	0·3	0·3	97·9	2·1	35·5
	Mean =	49·7	19·7	19·7	3·0	0·1	0·2	92·4	7·6	50·3
10	½	22·3	6·8	9·7	13·9	0·0	0·0	52·7	47·3	77·7
11	½	17·2	26·6	21·4	0·2	0·2	1·3	66·9	33·1	82·8
12	½	31·2	11·4	10·6	10·6	0·2	0·7	64·7	35·3	68·8
	Mean =	23·6	14·9	13·9	8·2	0·1	0·7	67·4	32·6	76·4
13	1	36·8	3·1	4·5	6·5	0·5	0·4	51·8	48·2	63·2
14	1	29·6	5·5	3·3	8·1	3·4	0·5	50·4	49·6	70·4
15	1	29·5	4·6	4·2	11·7	0·2	1·5	51·7	48·3	70·5
	Mean =	31·9	4·4	4·0	8·8	1·4	0·8	51·3	48·7	68·1
16	1½	28·5	5·8	3·9	10·6	0·5	0·6	49·9	50·1	71·5
17	1½	10·4	6·8	7·4	9·8	0·3	1·0	35·7	64·3	89·6
18	1½	25·5	5·9	4·7	6·0	4·6	1·2	47·9	52·1	74·5
	Mean =	21·5	6·2	5·3	8·8	1·8	0·9	44·5	55·5	78·5
19	2	20·0	4·2	2·3	7·5	1·9	0·8	34·7	65·3	80·0
20	2	16·7	3·7	2·5	5·9	2·9	4·8	37·5	62·5	83·3
21	2	17·4	4·1	7·7	4·7	1·1	0·7	35·7	64·3	82·6
	Mean =	18·0	4·0	4·2	6·0	2·0	2·1	36·3	63·7	82·0
22	3	10·6	1·6	2·2	1·3	4·8	3·9	24·0	76·0	89·4
23	3	5·4	1·0	1·2	1·2	5·7	7·7	22·2	77·8	94·6
24	3	19·4	1·1	1·0	0·6	4·4	5·2	31·7	68·3	80·6
	Mean =	11·8	1·2	1·5	0·8	4·9	5·6	25·9	74·1	88·2
25	4	17·6	0·8	1·6	0·8	7·9	3·1	31·8	68·2	82·4
26	4	15·8	0·9	1·8	1·8	8·6	5·7	34·6	65·4	84·2
27	4	9·3	1·1	2·3	0·9	4·6	5·9	24·1	75·9	90·7
	Mean =	14·3	0·9	1·9	1·2	7·0	4·9	30·2	69·8	85·7

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

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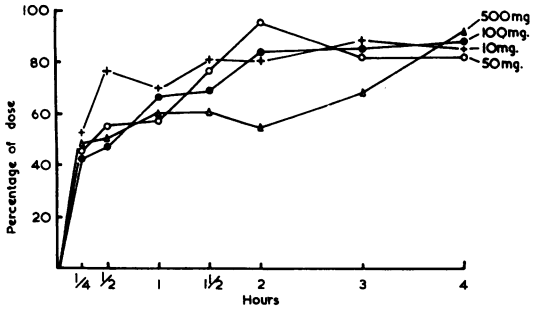

FIG. 3. *Mean gastric emptying at intervals of quarter hour to four hours after feeding doses of 10* (+——+), *50* (o——o), *100* (●——●), *or 500* (▲——▲) *mg. of* ^{131}*I-labelled triolein.*

doses. Between one and three hours, however, gastric emptying was invariably less complete after the 500 mg. dose than after doses of 10, 50, or 100 mg. of labelled fat. Although more than 80% of doses of 10 to 100 mg. of fat had left the stomach at two and three hours, for instance, the mean gastric emptying after doses of 500 mg. was only 54·2% at two hours and 69·6 at three hours (Fig. 3). By four hours, the degree of gastric emptying was similar in the four groups of animals.

Rates of absorption The total amounts of radioactivity recovered from the intestinal tracts of the four groups of rats are also given in Tables III to VI. The absorption by the different rats killed at each time interval, calculated by assuming that the radioactivity not recovered had been absorbed, is given in these Tables. The mean amounts absorbed by the three rats killed at each time after the four different doses are shown in Figs. 4 and 5.

The rates of absorption of the different test doses varied according to the amount of triolein which was given. In terms of the percentage of each dose absorbed, absorption was most rapid after feeding the 10 mg. dose and became progressively slower as the amount of fat in the test dose was increased (Fig. 4). If these results are expressed as the absolute amount of fat absorbed, on the other hand, it can be seen that the more fat was given, the greater was the actual amount of fat absorbed in a given time (Fig. 5).

Distribution of radioactivity in small intestine The percentages of the administered radioactivity found in the four segments of the small intestine of each rat are given in Tables III to VI. The mean amounts in the different segments of the three rats killed at each time after the various doses are shown in Fig. 6.

TABLE IV

PERCENTAGE OF 50 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
28	¼	25·7	26·8	39·6	5·3	0·0	1·2	98·6	1·4	74·3
29	¼	73·7	7·2	11·9	0·0	0·0	0·0	92·8	7·2	26·3
30	¼	62·7	10·2	25·2	0·0	0·0	0·0	98·1	1·9	37·3
	Mean =	54·0	14·7	25·6	1·8	0·0	0·4	96·5	3·5	46·0
31	½	52·3	16·0	15·2	0·9	0·2	0·8	85·4	14·6	47·7
32	½	37·1	6·3	9·4	19·2	0·3	0·8	73·1	26·9	62·9
33	½	46·7	3·0	32·2	1·7	0·0	0·0	83·6	16·4	53·3
	Mean =	45·4	8·4	18·9	7·3	0·2	0·5	80·7	19·3	54·6
34	1	35·4	8·7	5·1	6·4	15·0	0·5	71·1	28·9	64·6
35	1	30·6	5·9	7·3	12·2	0·0	0·2	56·2	43·8	69·4
36	1	61·5	7·1	2·1	13·4	0·5	0·2	84·8	15·2	38·5
	Mean =	42·5	7·2	4·8	10·7	5·2	0·3	70·7	29·3	57·5
37	1½	35·0	7·1	4·3	4·5	12·2	1·2	64·3	35·7	65·0
38	1½	19·5	2·3	2·9	21·8	0·3	0·6	47·4	52·6	80·5
39	1½	14·2	4·3	16·4	22·3	0·3	0·5	58·0	42·0	85·8
	Mean =	22·9	4·6	7·9	16·2	4·3	0·8	56·6	43·4	77·1
40	2	15·6	7·7	13·3	8·3	0·5	0·9	46·3	53·7	84·4
41	2	8·3	1·4	3·4	8·2	11·8	0·6	39·7	66·3	91·7
42	2	18·6	2·9	12·2	12·9	0·4	0·5	47·5	52·5	81·4
	Mean =	14·2	4·0	9·6	9·8	4·3	0·7	42·5	57·5	95·8
43	3	24·5	2·6	1·9	1·6	2·0	8·8	41·6	58·4	76·5
44	3	11·8	2·8	2·0	4·4	3·0	11·0	35·0	65·0	88·2
45	3	13·2	2·1	1·7	1·0	8·4	2·6	29·0	71·0	86·8
	Mean =	16·5	2·7	2·9	2·3	4·5	7·5	35·2	64·8	83·5
46	4	6·8	4·5	4·3	0·6	1·5	8·6	26·3	73·7	93·2
47	4	13·6	1·4	1·4	1·4	1·5	7·9	27·2	72·8	86·4
48	4	27·9	2·2	2·2	1·1	2·5	8·9	34·8	63·2	72·1
	Mean =	16·1	2·7	2·5	1·0	2·8	8·5	30·1	69·9	83·9

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

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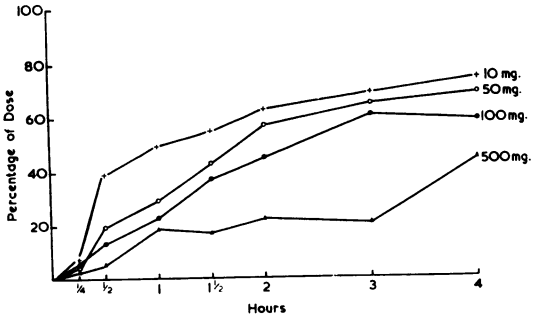

FIG. 4. *Mean percentage absorption of doses of 10* (+——+), *50* (o——o), *100* (●——●), *or 500* (▲——▲) *mg. of fat at intervals of quarter of an hour to four hours after feeding.*

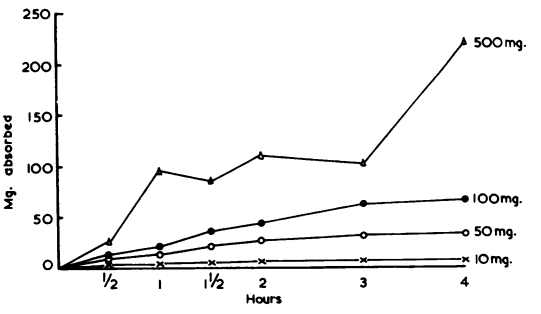

FIG. 5. *Mean amounts of fat absorbed (mg.) at intervals of quarter hour to four hours after feeding doses of 10* (x——x), *50* (o——o), *100* (●——●), *or 500* (▲——▲) *mg. of* ^{131}*I-labelled triolein.*

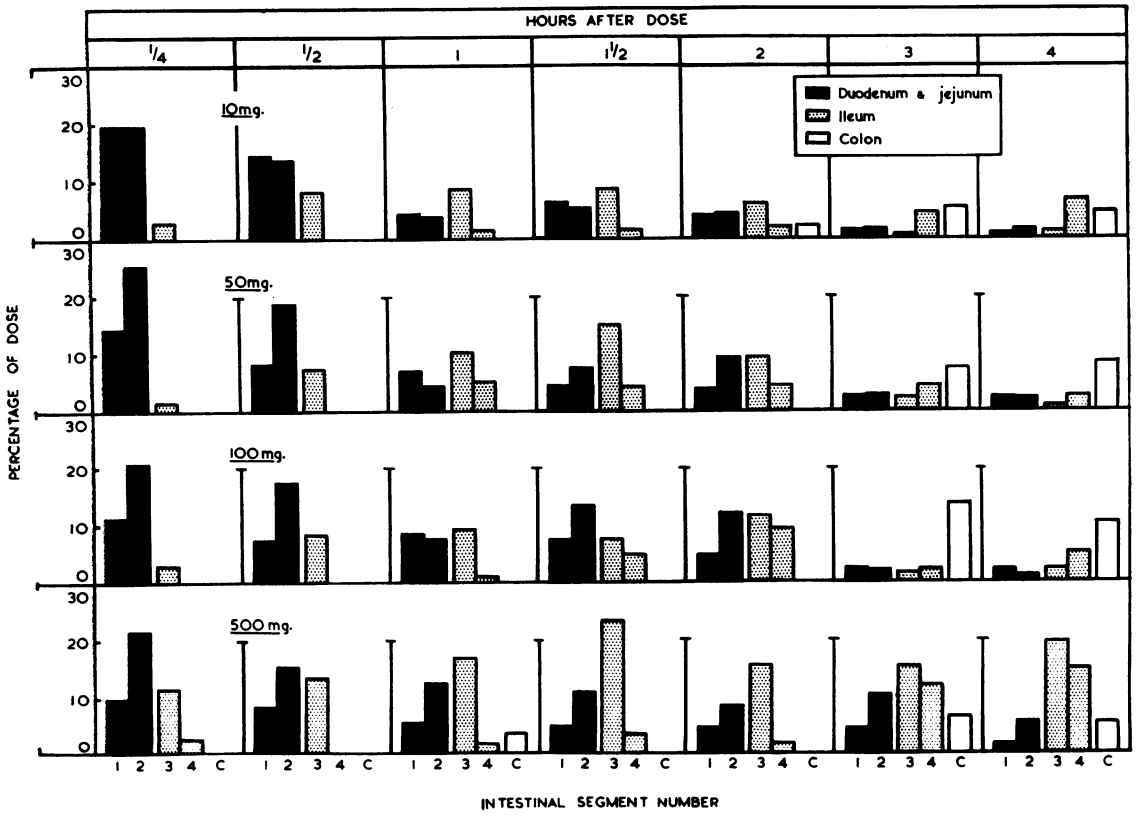

FIG. 6. *Distribution of radioactive material in the different segments of the small intestine and in the colon of rats killed at intervals of quarter of an hour to four hours after feeding doses of 10, 50, 100, or 150 mg. of* ^{131}*I-labelled triolein. The intestinal segments were numbered as follows: (1) Duodenum and upper jejunum, (2) lower jejunum, (3) upper ileum, (4) lower ileum. C indicates the colon.*

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When 10 mg. of ^{131}I-labelled triolein was given, the mean percentage of the dose found in each of the proximal two segments at quarter of an hour was 19·7% (Fig. 6, Table III). The radioactivity in these segments fell gradually during the next one to three hours as absorption took place (Fig. 4), so that at three and four hours no more than 1·9% of the administered radioactivity was present.

At the same time, the mean radioactivity in the third segment rose from 3·0% at quarter of an hour to 8·8% at one and one and a half hours. It then fell to 0·8 and 1·2% at three and four hours. The fourth segment contained only small amounts of radioactivity until three and four hours, at which times the mean radioactivity in this segment was 4·9 and 7·0% respectively (Fig. 6, Table III).

When the dose was increased to 50 mg., the mean percentage of the radioactivity present in the first segment at quarter of an hour was less than after 10 mg., being 14·7%. At this time, more radioactivity (25·6%) was found in the second segment. The radioactivity in these two segments fell more slowly than when 10 mg. was given, 9·6% of the dose being still present in the second segment at two hours (Fig. 6, Table IV). By three and four hours, as absorption proceeded, less than 2·9% was present in either of segments 1 and 2.

The mean radioactivity in the third segment was only 1·8% at quarter of an hour but rose to a peak of 16·2% at one and a half hours. It then fell gradually to 2·3% at three hours and 1% at four hours (Fig. 6, Table IV).

The fourth segment contained little or no radioactivity at quarter or half an hour but between 4 and 5% of the administered radioactivity was found in this segment between one and three hours.

After the dose of 100 mg. the mean percentage radioactivity in the different segments was essentially similar to that after a dose of 50 mg. However, a greater proportion of the administered radioactivity was present in segments 3 and 4 at two hours than when either 10 or 50 mg. was given (Fig. 6, Table V).

When a dose of 500 mg. was given, the percentage of the radioactivity recovered from segments 1 and 2 between a quarter of an hour and two hours was similar to that after 50 or 100 mg. However, the radioactivity in these segments fell more slowly than after the smaller doses, for segment 1 still contained as much as 4·6% and segment 2, 10·4% at three hours, at which time the mean radioactivity in these segments was invariably less than 2 to 3% after doses of 10 to 100 mg. (Fig. 6, Table VI).

The mean radioactivity in the third segment was

TABLE V

PERCENTAGE OF 100 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
49	¼	71·0	9·0	11·2	7·6	0·0	0·1	98·9	1·1	29·0
50	¼	56·0	83·0	23·3	1·5	0·0	0·4	94·2	5·8	44·0
51	¼	49·3	13·2	28·6	0·0	0·0	0·0	91·1	8·9	50·7
		Mean=58·8	11·7	21·0	3·0	0·0	0·2	94·7	5·3	41·2
52	½	71·7	4·5	6·1	13·7	0·5	0·7	97·2	2·8	28·3
53	½	44·5	7·3	22·6	9·3	0·0	0·6	84·3	15·7	55·5
54	½	40·2	10·3	24·6	1·0	0·3	0·4	76·8	23·2	59·8
		Mean=52·1	7·4	17·8	8·3	0·3	0·6	86·1	13·9	47·9
55	1	59·5	3·1	6·9	9·1	1·9	1·1	79·6	20·4	40·5
56	1	37·2	11·7	7·4	9·2	1·0	0·0	66·5	33·5	62·8
57	1	57·0	11·0	8·7	9·9	0·0	0·3	86·9	13·1	63·0
		Mean=51·2	8·6	7·7	9·4	1·0	0·5	77·7	22·3	68·8
58	1½	48·9	1·9	3·4	9·0	9·8	0·4	73·4	26·6	51·1
59	1½	28·2	10·8	20·4	7·3	0·3	0·7	67·7	32·3	71·8
60	1½	16·0	10·2	17·0	7·2	4·9	0·9	56·2	43·8	84·0
		Mean=31·0	7·6	13·6	7·8	5·0	0·7	62·4	37·6	69·0
61	2	7·4	1·5	8·3	18·8	22·3	0·6	59·9	40·1	92·6
62	2	12·9	6·9	19·1	7·0	1·9	1·3	49·1	50·9	87·1
63	2	28·8	6·7	8·8	9·5	3·5	0·9	58·2	41·8	71·2
		Mean=16·4	5·0	12·1	11·8	9·3	0·9	55·7	44·3	83·6
64	3	7·3	1·9	3·0	1·5	2·5	11·4	27·6	72·4	92·7
65	3	23·5	3·9	2·3	2·5	3·1	13·2	48·5	51·5	76·5
66	3	16·2	1·6	1·6	1·8	1·7	17·4	40·3	59·7	83·8
		Mean=15·7	2·5	2·3	1·9	2·4	14·0	48·8	61·2	84·3
67	4	5·6	2·3	1·4	2·4	2·3	13·8	27·8	72·2	94·4
68	4	16·6	1·7	1·1	2·8	8·6	6·8	37·6	62·4	83·4
69	4	12·4	2·3	1·1	1·8	5·2	11·8	34·6	65·4	87·6
		Mean=11·5	2·1	1·2	2·3	5·4	10·8	33·3	66·7	88·5

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

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TABLE VI

PERCENTAGE OF 500 MG. DOSE OF ^{131}I-LABELLED TRIOLEIN IN STOMACH AND INTESTINES OF RATS KILLED AT INTERVALS OF QUARTER OF AN HOUR TO FOUR HOURS

Rat No.	*Time Killed (hr.)*	*Stomach*	*Small Intestine*				*Colon*	*Total*	*%[1] Absorbed*	*Gastric[2] Emptying*
			1	*2*	*3*	*4*				
70	¼	44·7	13·6	6·9	23·1	8·3	0·0	96·6	3·4	55·3
71	¼	56·8	10·3	24·8	7·1	0·0	0·0	98·0	2·0	43·2
72	¼	55·6	6·0	32·8	4·6	0·0	0·0	99·0	1·0	44·4
	Mean =	52·0	10·0	21·5	11·6	2·8	0·0	97·9	2·1	48·0
73	½	52·0	11·0	12·2	16·6	0·8	0·0	92·6	7·4	48·0
74	½	60·1	5·6	13·0	11·3	0·4	0·3	91·7	8·3	39·9
75	½	42·7	8·5	24·4	12·9	0·0	0·0	98·5	1·5	57·3
	Mean =	51·6	8·4	16·5	13·6	0·4	0·1	94·3	5·7	48·4
76	1	36·0	7·3	8·6	21·4	0·8	3·6	77·7	22·3	64·0
77	1	31·5	3·8	16·7	19·2	4·6	6·9	82·7	17·3	68·5
78	1	54·5	5·5	12·8	10·5	0·0	0·0	83·3	16·7	45·5
	Mean =	40·7	5·5	12·7	17·0	1·8	3·5	82·2	18·8	59·3
79	1½	35·2	5·6	14·6	20·1	3·5	0·3	79·3	20·7	64·8
80	1½	40·2	2·7	7·6	33·0	6·6	0·6	90·7	9·3	59·8
81	1½	43·3	6·3	10·4	17·5	0·0	0·1	77·6	22·4	56·7
	Mean =	39·6	4·9	10·9	23·5	3·4	0·3	82·5	17·5	60·4
82	2	66·1	2·5	3·4	4·6	3·9	2·0	82·5	17·5	43·9
83	2	30·7	7·3	12·0	24·8	1·5	0·6	76·9	23·1	69·3
84	2	40·7	4·6	10·5	17·6	0·0	0·2	73·6	26·4	59·3
	Mean =	45·8	4·8	8·6	15·7	1·8	0·9	77·7	22·3	54·2
85	3	28·2	1·2	12·7	38·2	9·7	0·5	85·5	14·5	71·8
86	3	35·0	3·1	3·6	7·7	10·5	19·4	79·3	20·7	65·0
87	3	28·0	9·5	15·0	4·5	15·9	0·9	73·8	26·2	72·0
	Mean =	30·4	4·6	10·4	15·1	12·0	6·9	79·5	20·5	69·6
88	4	5·8	1·7	3·1	28·9	19·7	0·8	59·2	40·8	94·2
89	4	8·3	2·3	5·0	9·4	12·9	14·6	52·5	47·5	91·7
90	4	9·6	1·6	9·5	21·6	12·4	1·1	55·8	44·2	90·4
	Mean =	7·9	1·9	5·9	19·9	15·0	5·5	55·8	44·2	92·1

[1]Percentage absorption was calculated by assuming that the material not recovered had been absorbed.
[2]Percentage gastric emptying is the amount of material not recovered from the stomach.

always greater than when doses of 10, 50, or 100 mg. were given. It rose from 11·6% at a quarter of an hour to a maximum of 23·5% at one and a half hours, and remained as high as 15·7, 15·1, and 19·9% at two, three, and four hours (Fig. 6, Table VI) when less than 3% was recovered from this segment after the smaller doses.

The fourth segment contained only small amounts of radioactivity until three and four hours, at which times the mean radioactivity recovered from this segment was 12·0 and 15·0% respectively. These amounts were also greater than were found in the fourth segment after the smaller doses.

Radioactivity in the colon Insignificant amounts of radioactivity were recovered from the colon until two to three hours after the doses were given (Fig. 6, Tables III to VI). At three and four hours, the colonic radioactivity was invariably less than the amounts excreted in the faeces after similar doses were given to intact rats (Table I).

DISCUSSION

AMOUNTS OF FAT ABSORBED FROM INCREASING DOSES These results show that when increasing doses of ^{131}I-labelled fat are given to rats, there is a progressive increase in the percentage of the dose excreted in the faeces (Table I, Fig. 1). In terms of the amount of fat absorbed, however, there is a progressive increase in absorption as increasing doses of ^{131}I-labelled triolein are given (Table II, Fig. 2).

SITES OF FAT ABSORPTION The sites of absorption of the various doses varied according to the amount fed. Since absorption invariably began soon after feeding (Fig. 4), it seemed likely that the labelled material was absorbed first from the upper intestine regardless of the amount of fat given. This was confirmed by the studies of the distribution of radioactivity in the different segments of the small intestine during absorption (Fig. 6). Since much of the radioactivity in the proximal two segments disappeared during the first hour after feeding without subsequently appearing in similar amounts in the more distal segments, it is evident that the jejunum absorbed considerable amounts of fat. The importance of the ileum (segments 3 and 4) in fat absorption depended on the amount of fat given, for the radioactivity reaching the distal two segments was proportional to the amount of labelled fat given, the greatest amounts being recovered from the ileum after the largest doses (Fig. 6). However, the

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recovery of radioactive material from the ileum did not necessarily indicate that absorption occurred there. Although as much as 10% of a dose of 10 mg. of ^{131}I-labelled triolein reached the ileum (segments 3 and 4, Table III, Fig. 6), it could not be assumed that this material was absorbed from that area. The mean faecal excretion after a dose of 10 mg. of fat by an intact rat was 11·8% (Table I, Fig. 1), and the 10% recovered from the ileum after a dose of 10 mg. was therefore likely to be merely in transit to the colon for subsequent elimination in the faeces. When larger amounts of fat were fed, however, the percentages which reached the distal intestine were greater than were excreted in the faeces of intact rats given similar doses (Figs. 1 and 6), indicating that part of these larger doses must have been absorbed in the ileum.

An approximate estimate of the amount of each dose which was absorbed in the ileum has been obtained by subtracting the percentage of the dose excreted in the faeces by intact rats given the various doses (Table I, Fig. 1) from the maximum percentages of each dose recovered from the ileum together with the colon at any one time (Tables III to VI, Fig. 6). If the amount of fat absorbed in the ileum is then subtracted from the amount shown to be absorbed from the equivalent doses (Table II, Fig. 2), a similar approximation of jejunal absorption may be derived. The results of these estimations, set out in Table VII, indicate that when 10 mg. of ^{131}I-labelled fat was given absorption was almost entirely in the jejunum (Table VII). Larger doses of fat were also absorbed to a great extent in the jejunum, which responded to an increased dietary load by an increased absorption, but at the same time increasing amounts of fat escaped absorption in the proximal segments and therefore passed into the ileum; an increasing proportion was then absorbed there (Table VII). The ileum of the rat, however, appears to be less efficient at absorbing fat than the jejunum, for much of the labelled material which reached the ileum passed on into the colon and was excreted in the faeces (Tables I and VII). Dawson and Isselbacher (1960), studying the esterification of palmitate-1-C^{14} by rat intestinal mucosa *in vitro*, have shown that cell-free homogenates prepared from the ileum are a quarter to a fifth as active as those from the jejunum. The results given in this paper demonstrate an interesting parallel *in vivo* to their observations.

TABLE VII

CALCULATED ABSORPTION OF ^{131}I-LABELLED TRIOLEIN FROM JEJUNUM AND ILEUM

		Amount Fed (mg.)			
		10	*50*	*100*	*500*
Mean faecal excretion (% of dose)[1]		11·8	11·0	16·0	24·2
Maximum mean radioactivity in ileum plus colon (% of dose)[2]		13·1	21·3	22·1	40·5
Calculated absorption from ileum	% of dose	1·3	10·3	6·1	16·3
	mg.	0·1	5·1	6·1	81·0
Calculated absorption from jejunum	% of dose	86·9	78·7	77·9	59·6
	mg.	8·7	39·4	77·9	298·0

[1]Data from Table I.
[2]Data from Tables III, IV, V, and VI.

The observation that fat may be absorbed in increasing amounts from both the jejunum and the ileum when the dietary load is increased provides a rational explanation for the unlimited capacity of the small intestine to absorb fat (Fig. 2). The absorption of the largest doses may also be facilitated by slower gastric emptying after such doses (Fig. 3), which serves to reduce the amount of fat delivered to the intestine at any one time.

RELATIONSHIP BETWEEN RATES AND SITES OF ABSORPTION If absorption of a substance begins in the upper jejunum, the site of its absorption depends on the relationship between its absorption rate and the upper intestinal motility (Parkins *et al.*, 1960). Glucose or iodide, for instance, are absorbed rapidly and their absorption occurs in the proximal half of the small intestine of the rat (Reynell and Spray, 1956; Parkins *et al.*, 1960). ^{131}I-labelled albumin, however, is absorbed more slowly; the rapid motility of the upper small intestine propels it more distally before absorption is complete and some absorption therefore also occurs in the ileum (Parkins *et al.*, 1960).

There appears to be a similar relationship between absorption rate and the site of absorption of different doses of fat. The 10 mg. dose of fat, for instance, was absorbed relatively rapidly (Fig. 4) and absorption therefore occurred in the jejunum (Table VII). A 500 mg. dose, however, was absorbed more slowly (Fig. 4) and a greater proportion was therefore propelled as far as the ileum before absorption was complete.

PREVIOUS OBSERVATIONS ON SITE OF FAT ABSORPTION The results given in this paper are in keeping with previous observations which have suggested that the jejunum plays a major part in fat absorption (Borgström *et al.*, 1957; Favarger and Gerlach, 1953; Frazer, 1943; Turner, 1958). Other workers have claimed that in the rat maximal absorption of ^{131}I-labelled olive oil occurs in the third quarter of the small intestine, an area said to be specifically adapted for this purpose (Benson *et al.*, 1956). Fig. 6

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illustrates a possible reason for this conclusion. These workers killed their rats only at two hours and later after the administration of labelled fat, and measured the radioactivity in the mucosa of different segments of the small intestine. When rats are killed at two hours and later after the test doses, most of the radioactive material is likely to be recovered from the ileum, for most of the jejunal fat has already been absorbed in this time (Fig. 6).

SUMMARY

The faecal excretion of radioactivity was measured in rats given doses of 10, 50, 100, or 500 mg. of ^{131}I-labelled triolein. As the dose was increased, the percentage of the dose excreted in the faeces became greater but there was a progressive increase in the amount of fat which was absorbed.

The sites of absorption of the ^{131}I-labelled triolein varied according to the dose given. A dose of 10 mg. was absorbed relatively rapidly and absorption occurred in the jejunum. Larger doses, however, were absorbed more slowly and the motility of the upper intestine propelled the fat more distally before absorption was complete; absorption therefore also occurred in the ileum when the larger doses were given.

The increase in absorption of the labelled triolein which occurred when increasing doses were given was achieved first by an enhanced jejunal absorption in response to an increased dietary load, and secondly by an increased absorption in the ileum.

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