Digestion in the rabbit's stomach

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The stomach of the rabbit is a relatively large organ, and Carmichael, Strickland & Driver (1945) showed that even after withholding food for 24 h it remained half full. However, Elsden, Hitchcock, Marshall & Phillipson (1946) studied the volatile fatty-acid content of the different parts of the alimentary canal of various animals, and showed that, in simple-stomached herbivores, the large intestine was the site of volatile fatty-acids (V.F.A.) production, and fermentation in the stomach of these animals was thought to be negligible. The prolonged retention of food in the stomach of the rabbit and the relatively large capacity of this organ seemed favourable for fermentation, and the present work was undertaken to follow the production of lactic acid and V.F.A. in this organ.

EXPERIMENTAL AND RESULTS

Acute experiments with fed and fasted rabbits

Adult rabbits were maintained under standard conditions for at least 7 days before the beginning of an experiment. They usually received a mixed diet consisting of oat bran, hay and cabbage. A green diet consisting of cabbage only was given to some fistulated rabbits to study the effect of change of diet. Clean fresh water was always available. The guinea-pigs, examined for comparison, were kept under the same conditions. The numbers of animals used are shown in the appropriate tables.

The rabbits used in acute experiments were anaesthetized with 25 % urethane solution given intravenously. The abdomen was opened and blood samples were drawn from the portal vein (0·5 ml.) and carotid artery (3 ml.) into bottles containing potassium oxalate and sodium fluoride. The animals were killed and the stomach, small intestine, caecum and colon and the rectum were separated between the ligatures and carefully dissected out. The contents of each portion were collected in tared beakers and weighed. For determination of the dry weight a weighed portion of the thoroughly mixed sample from each part of the gastro-intestinal tract was dried to constant weight in a drying oven at a temperature of 80–100°. To determine the lactic-acid concentration and pH of different portions of the stomach the organ was carefully opened without disturbing the contents and 5 g portions were taken from the different regions. For estimation of lactic acid and V.F.A. of the gastro-intestinal contents 5 g of the thoroughly mixed sample were diluted with distilled water to 10 ml., filtered through a gauze and a portion was taken.

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Acute experiments to study the absorption of lactate

Acute experiments were performed to study the absorption of lactate from the stomach and small intestines. The abdomen was opened and through a small incision in the stomach all the contents were removed and the organ was washed out with warm normal saline after ligation of both the pyloric and cardiac openings, and a cannula was fitted in the stomach. Warmed, isotonic, calcium-lactate solution (25 ml.) was introduced and the stomach and abdomen were closed. Samples of gastric contents (3 ml.) were withdrawn through the cannula at half-hourly intervals and the lactic-acid content was determined. After 2 h the abdomen was opened and the first 10–15 cm of the small intestine were isolated between ligatures and the contents were removed. The intestine was washed with normal saline and isotonic lactate introduced. The abdomen was closed and samples were taken from the intestine every half-hour by syringe for 2 h. Samples of portal blood were drawn before introduction of lactate into the stomach and intestines and at the end of the experiment. A sample of carotid blood was also taken before and at the end of the experiment.

Experiments to study the production of lactate by
stomach contents in vitro

In vitro fermentation experiments were conducted with both mixed food and green food homogenates inoculated with gastric contents from rabbits with a permanent gastric fistula, described below, kept on a mixed diet and green diet respectively. McIlvaine's (Hodgman, 1952) citric-acid phosphate buffer at pH 2·2, 3·2, 4·2 and 5·2 was used as diluent. Equal volumes of gastric contents, food homogenates and the buffer were taken in test tubes fitted with Bunsen valves and incubated at 37°. Hourly samples were taken for 5 h. In every experiment a control was kept which contained the food homogenate, buffer and gastric inoculum with sodium fluoride.

Experiments on rabbits with a permanent gastric fistula

Five rabbits were prepared with a permanent gastric fistula through which gastric contents could be withdrawn at any time. The rabbits were anaesthetized with pentobarbitone and a laparotomy was performed. A portion of the greater curvature of the stomach was isolated by means of a clamp and the cannula introduced and secured by purse-string sutures. The cannulas were prepared from Alkathene (I.C.I. Ltd) (Pl. 1), and an important modification was the addition of a fenestrated flange which was secured by stitches to the serous surface of the stomach, and to the body of the cannula by heat. This flange prevented rotation of the cannula and subsequent leakage. The cannula tube was $\frac{1}{2}$ in. in external diameter and $1\frac{1}{2}$ in. long. The flanges were $1\frac{1}{4} \times \frac{7}{8}$ in. The cannula was delivered to the outside through a stab incision, thereafter the abdominal wall was sutured in the usual manner. Rabbits so treated remained in good condition for several months, the main complication arose from the animal chewing the cap of the cannula. This difficulty was overcome by using a Perspex instead of an Alkathene cap. The samples collected through the cannula were filtered through gauze,

and portions were used for different estimations. Occasionally, particularly in the later stages of an experiment, it was necessary to dilute the filtrate with an equal volume of distilled water as very little material could be obtained.

Analytical methods

For estimation of lactic acid in the different samples the method of Markus (1950) was used. Readings were taken in a Unicam S.P. 350 spectrophotometer with a 570 mμ filter. For determination of the V.F.A. content of the different samples the usual procedure of steam distillation of acidified samples and subsequent titration with 0·05 N-NaOH was used. The pH of the samples were determined in a Marconi pH meter.

Distribution of digesta

The stomach of the fed rabbit contained more digesta than the combined caecum and colon. Withholding food for 24 h decreased the stomach contents and increased their fluidity. This treatment had little effect on the caecal and colonic contents. The results of these experiments are shown in Table 1.

Table 1. *Distribution and weight of digesta in the*
alimentary tract of the rabbit

	Weight (value and standard deviation) (g)			
	Before fasting		After 24 h fasting	
Organ	Fresh	Dry	Fresh	Dry
Stomach	127·8 ± 22·9	21·1 ± 2·45 (10)	48·0 ± 12·6	4·5 ± 0·49 (6)
Small intestine	30·9 ± 8·62	2·05 ± 0·24 (10)	18·0 ± 2·7	1·1 ± 0·07 (6)
Caecum and colon	100·6 ± 16·2	23·2 ± 2·29 (10)	90·0 ± 18·6	20·5 ± 1·9 (6)
Rectum	23·1 ± 5·76	5·28 ± 0·44 (10)	11·0 ± 2·68	2·0 ± 0·14 (6)

The figures in parentheses are the number of animals.

Content of lactic acid and V.F.A. of rabbit digesta

Although the lactic-acid concentration of the gastric contents was less than that of the contents of the small intestine the total quantity of lactic acid was much greater. In contents of the caecum and colon there was very little lactic acid and in the rectal contents none. The V.F.A. content of the caecum and colon was considerable, whereas only a trace was present in the stomach. Withholding food for 24 h greatly reduced the lactic-acid content of stomach and small intestine and slightly reduced the V.F.A. in the caecum and colon. These results are given in Table 2.

Content of lactic acid and V.F.A. in the alimentary tract of the guinea-pig

The lactic-acid content of the guinea-pig's stomach was very much smaller and the V.F.A. content similar to that in the rabbit (Table 3).

Rate of lactic-acid production in the rabbit's stomach

A sample of gastric contents was taken from fistulated rabbits deprived of food overnight. Food was then given for 1 h and removed at the end of that period. Thereafter, hourly samples were taken over a 5 h period. The lactic-acid

Table 2. *pH, lactic acid, and V.F.A. in different parts of the gastro-intestinal tract in fed and fasted rabbits*

Organ	Fed (10)				After 24 h fasting (6)			
	pH Mean	pH Range	Lactic acid (mean value and standard deviation) (m-equiv./l.)	V.F.A. (mean value and standard deviation) (m-equiv./l.)	pH Mean	pH Range	Lactic acid (mean value and standard deviation) (m-equiv./l.)	V.F.A. (mean value and standard deviation) (m-equiv./l.)
Stomach	1·1	1·0–1·3	4·01 ± 1·10	33·3 ± 5·04	1·0	1·0–1·2	0·87 ± 0·02	Trace
Small intestine	7·9	7·6–8·0	8·51 ± 2·40	—	8·0	7·9–8·2	2·40 ± 0·49	—
Caecum and colon	7·6	7·4–7·9	0·28 ± 0·12	208·3 ± 10·52	7·8	7·7–7·9	None	175·0 ± 5·00
Rectum	7·6	7·1–7·7	None	—	7·7	7·5–7·8	None	—

The number of animals is shown in parentheses.

concentration was found to increase. The increase was more marked in rabbits on a diet of cabbage than in those on a mixed diet. The results shown in Table 4 are compiled from experiments in which the sampling was performed at several different times. The distribution of lactic acid in the different regions of the stomach was determined in acute experiments, but no great difference was found (Table 5).

Table 3. *Lactic acid and V.F.A. in the gastro-intestinal*
tract of the guinea-pig

No. of animals	Part of gastro-intestinal tract	Lactic acid (mean value and standard deviation) (m-equiv./l.)	V.F.A. (mean value and standard deviation) (m-equiv./l.)
8	Stomach	0·66 ± 0·09	38·33 ± 3·33
	Caecum and colon	0·24 ± 0·06	113·3 ± 8·33

Table 4. *Concentration of lactic acid in the stomach of rabbits*
with a permanent gastric fistula

	Mixed diet			Green diet		
Time after feeding (h)	Lactic acid (mean value and standard deviation) (m-equiv./l.)	pH Mean	pH Range	Lactic acid (mean value and standard deviation) (m-equiv./l.)	pH Mean	pH Range
16	1·84 ± 0·02	1·1	1·0–1·2 (12)	0·92 ± 0·27	1·0	1·0 (6)
0	2·88 ± 0·68	2·5	1·2–2·8 (10)	3·75 ± 0·55	4·8	4·3–5·1 (6)
1	3·64 ± 0·66	3·4	2·1–3·8 (5)	5·53 ± 0·89	4·6	4·5–4·7 (3)
2	4·16 ± 0·74	4·1	4·0–4·2 (6)	6·53 ± 0·71	3·2	3·1–3·2 (3)
3	5·48 ± 0·46	4·2	4·1–4·3 (4)	6·27 ± 0·97	2·6	2·3–2·7 (3)
4	6·06 ± 0·74	3·2	3·0–4·2 (5)	4·93 ± 0·45	1·5	1·4–1·6 (4)
5	5·67 ± 0·48	1·5	1·4–2·4 (5)	—		—
24	1·17 ± 0·35	1·0	1·0–1·3 (6)	0·91 ± 0·22	1·0	1·0 (6)

The figures in parentheses are the number of observations carried out on a group of five rabbits.

Table 5. *pH and lactic-acid content of different portions*
of stomach contents of four rabbits

Region of the stomach	Portion of the contents	Lactic acid (m-equiv./l.) Mean	Lactic acid (m-equiv./l.) Range	pH Mean	pH Range
Cardiac	Peripheral	2·55	1·55–3·11	2·3	1·8–2·6
	Central	3·51	2·44–4·01	2·5	2·3–2·9
Fundic	Peripheral	5·11	3·11–5·76	1·5	1·0–2·3
	Central	4·00	3·00–5·65	1·6	1·2–2·2
Pyloric	Peripheral	3·28	1·95–4·22	1·7	1·2–1·8
	Central	4·11	3·50–4·82	1·2	1·2–1·8

Effect of change of diet

Rabbits fed on a mixed diet were deprived of food overnight, and then offered cabbage for 1 h on the next morning. Thereafter, samples of gastric contents were taken over a period of 4 h. These animals were subsequently fed on a diet of cabbage

only, and the experiment was repeated every other day for 9 days. During the first few days lactic-acid production was irregular. A group of three rabbits was used and the result of a typical experiment is shown in Table 6.

Lactic-acid production in vitro

The food was free from lactic acid. Appreciable quantities of lactic acid were produced at pH 2·2–5·2. More was produced from cabbage homogenate than from the mixed diet. Six experiments were done and typical results are shown in Table 7. Incubation of buffered food homogenate with gastric contents after addition of sodium fluoride did not result in the production of lactic acid.

Table 6. *Lactic acid (m-equiv./l.) in the stomach of rabbits after a change from a mixed to a green diet (see p.* 69)

Day	Time after feeding (h)					
	16	0	1	2	3	4
1	1·02	2·12	1·80	2·44	2·90	2·57
2	1·24	1·70	2·02	3·12	3·22	2·73
3	1·02	2·03	3·01	3·94	3·55	2·55
4	1·51	3·17	4·32	4·89	3·44	2·84
5	1·71	3·00	4·66	6·02	5·77	5·06

Table 7. *Lactic-acid production in vitro from food homogenates inoculated with rabbit stomach contents*

Type of food homogenate	Period of incubation (h)	Lactic acid (m-equiv./l.)			
		pH 2·2	pH 3·2	pH 4·2	pH 5·2
Mixed diet	0	0·05	0·05	0·05	0·05
	1	1·33	0·95	1·51	0·81
	2	2·04	1·77	2·51	1·86
	3	3·75	3·25	4·36	3·01
	4	4·73	4·55	5·78	3·88
	5	4·71	4·95	5·82	3·84
Green diet	0	0·00	0·00	0·00	0·00
	1	2·70	2·22	2·77	2·25
	2	4·73	4·26	4·66	4·07
	3	6·00	5·57	6·79	5·33
	4	5·73	6·11	6·00	5·73
	5	4·92	5·43	5·57	4·63

Effect of antibiotics on in vitro fermentation

The irregularity of lactic-acid production after a change of diet and the in vitro production of lactate after inoculating food homogenate with gastric contents, suggested that the lactic-acid production was due to microbial activity. Fermentation experiments were carried out as before excepting that penicillin (0·35 mg/ml.) and chloramphenicol (2 mg/ml.) were added to some of the incubating tubes. These antibiotics suppressed lactic-acid production. Moreover, incubation of food homogenate with gastric mucous membrane with or without penicillin did not result in lactic-acid production. Results of a typical experiment are shown in Table 8.

Table 8. *Effect of antibiotics on the production of lactic acid in vitro from food homogenates inoculated with rabbit stomach contents*

Substrate	Inoculum	Drug	pH	Lactic acid (m-equiv./l.)		
				0 h	2 h	4 h
Homogenized cabbage	Gastric contents	None	0·72	0·72	5·54	4·80
		None	4·2	0·72	5·24	4·45
		Penicillin	2·2	0·72	Trace	Trace
		(0·35 mg/ml.)	4·2	0·72	Trace	Trace
		Chloramphenicol	2·2	0·72	None	None
		(2 mg/ml.)	4·2	0·72	3·3	None
	Gastric mucosa	None	2·2	0·13	Trace	Trace
		None	4·2	0·14	Trace	Trace
		Penicillin	2·2	0·11	Trace	Trace
		(0·35 mg/ml.)	4·2	0·13	Trace	Trace

Absorption of lactate

The difference in lactic-acid concentration between the arterial and venous blood was almost three times greater in fed as compared with fasted rabbits (Table 9). Moreover, estimations of the lactic-acid concentration in the normal contents of the small intestine showed a progressive reduction in an aboral direction (Table 10).

Table 9. *Lactic acid (m-equiv./l.) in the portal and carotid blood of rabbits*

Treatment	No. of animals	Portal blood (mean value and standard deviation)	Carotid blood (mean value and standard deviation)
Fed	15	11·05 ± 1·55	3·94 ± 0·70
Food withheld for 24 h	12	5·91 ± 0·70	3·18 ± 0·62

Table 10. *Lactic acid (m-equiv./l.) in different portions of the normal contents of the small intestine of rabbits*

Rabbit no.	Part of small intestine		
	Proximal	Middle	Distal
1	11·83	3·12	0·05
2	11·08	3·72	0·0
3	11·28	3·55	0·01
4	9·45	2·66	0·0

Table 11. *Appearance of lactate (m-equiv./l.) in the portal and carotid blood of rabbits after introduction of lactate into the stomach and small intestine*

No. of observations	Time after introduction of lactate (h)	Portal blood (mean value and standard deviation)	Carotid blood (mean value and standard devitation)
		Stomach	
4	0	6·00 ± 0·58	3·11 ± 0·37
4	2	9·55 ± 0·82	— —
		Small intestine	
4	2	18·44 ± 1·00	4·33 ± 0·75

Experiments in which isotonic solutions of lactate were introduced into the washed stomach after ligation of the oesophagus and pylorus showed that the concentration in the stomach fell whilst that of the portal blood rose. Similar experiments with a loop of small intestine showed a more rapid disappearance and a greater rise in the portal blood than in the previous experiments (Table 11). Lactic acid had almost disappeared from the small intestine 2 h after ligation of the pylorus. The disappearance of lactate was not accompanied by the production of V.F.A.

<div align="center">DISCUSSION</div>

The digestion of cellulose and related substances in mammals is accomplished by micro-organisms whose fermentative reactions take place in a part of the alimentary tract modified for this purpose. This modification takes the form of a dilatation of the stomach or of the caecum and colon. The rabbit seems to be a herbivore in which development of both stomach and large intestine has taken place. Since the stomach epithelium secretes an acid juice it did not seem likely that the interior of the organ would be a suitable microbial fermentation chamber. Moreover, Elsden *et al.* (1946) found very little volatile fatty acid in the rabbit's stomach compared to that contained in the caecum. However, the experiments of Stern, Hukovic & Fukarek (1955) which showed that the digestion of starch by rabbits depended on the activities of micro-organisms, and those of Herndon & Hove (1955) who found the digestion of cellulose in normal and caecectomized rabbits was the same although the colons of the latter animals were enlarged, suggested that some fermentative digestion might take place in the stomach. Furthermore, Phillipson's (1952) observation that large quantities of lactate were produced in the rumen of sheep on a diet rich in starch provided some analogy with the findings described here. It was, nevertheless, surprising to find that lactic acid was produced at such a low pH. The in vitro fermentation experiments clearly showed it possible, although in the experiments in which the lactic acid and pH of stomach contents were measured at intervals after feeding, the rise in lactic-acid concentration in the stomach was accompanied by a rise in pH and vice versa.

The irregularity of lactic-acid production after a change of diet and the finding that the production of lactic acid in vitro was prevented by penicillin and chloramphenicol was evidence that the lactic acid was produced by microbial activity. Moreover, the failure to produce lactic acid by incubating food homogenate with gastric mucosa made it unlikely that the lactic acid was produced by an enzyme secreted by the mucosa.

The lactic acid produced in the stomach appeared in part to be absorbed through the gastric epithelium, and also to pass through the pylorus to be absorbed in the first part of the small intestine, which was shown by the presence of a high concentration of lactic acid in the first part of the small intestine. The rapid absorption of lactate was clearly shown, both in the acute experiments and by the difference between the lactic-acid concentration of the portal and carotid blood which increased on feeding and when lactate was present in the stomach or intestine. Further, it was shown by Drury & Wick (1956) that rabbits utilize lactate as a fuel. It was interesting to find that the guinea-pig, an animal with an anatomically similar digestive tract, and eating similar food, did not produce lactate.
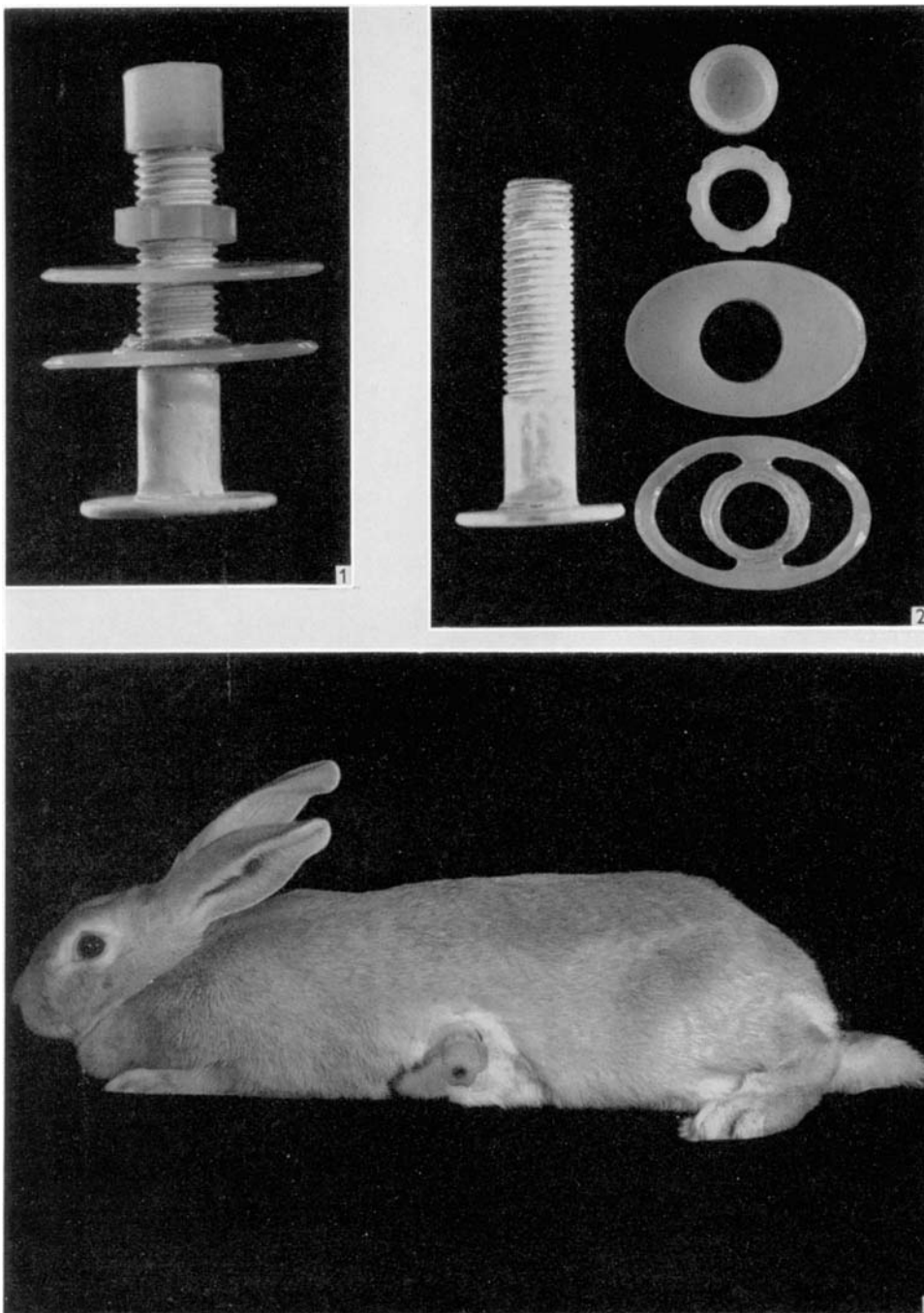
SUMMARY

1. The lactic-acid and volatile fatty-acid content of the rabbit's stomach was studied in acute experiments and with rabbits having 'permanent' gastric fistulas. The method of preparing these is described.

2. The weight of the rabbit's stomach contents was as great as of those of the caecum and colon combined.

3. The stomach contained an appreciable amount of lactic acid which was reduced by withholding food and increased with feeding.

4. Lactic acid was produced by incubating food homogenate with gastric contents. The production was prevented by penicillin and chloramphenicol.

5. The difference between the lactic-acid concentration of portal and carotid blood decreased when food was withheld and increased on feeding or introducing lactate into the stomach or intestine. Further evidence of the absorption of lactate from the stomach and intestine was obtained.

6. The guinea-pig's stomach contained very little lactic acid.

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EXPLANATION OF PLATE

Cannula for gastric fistulation in the rabbit.
1. Cannula and flanges assembled.
2. Components of cannula.
3. Rabbit with gastric cannula in position.