EXPERIMENTAL OESOPHAGITIS IN RATS.

K. V. LODGE.*

From the Department of Pathology, University of Manchester.

Received for publication December 9, 1954.

In a recent study of non-specific oesophagitis at autopsy (Lodge, 1955) it was found that the lesion occurred much more frequently than is generally recognised. It appeared that conditions which led to excessive contact between gastric contents and the oesophageal epithelium predisposed to the development of oesophagitis. The present paper reports experiments which were designed to test and clarify these conclusions.

Previous workers on gastric ulcer (Buchner, Siebert and Molloy, 1928 ; Hoelzel and Da Costa, 1937) noted that ulcers developed in the proventriculi of rats when they were starved. It was suggested that deficiency of some factor in the diet, such as protein, was responsible for the ulceration. These workers had not, however, appreciated that the proventriculus, which is lined by squamous epithelium, and acts as a food reservoir, is essentially a dilated portion of the oesophagus, as was suggested by Kammeraad (1942) in his study of the development of the gastro-intestinal tract of the rat. The rat, therefore, seemed suitable for the investigation of oesophagitis. In addition it was sufficiently economical in feeding and housing to permit adequate numbers to be used in each experiment, but large enough and with a satisfactory resistance to shock to permit fairly intricate surgical procedures. It was known from previous work on adrenal function (Langley, Lodge and Woodcock, unpublished observations) that rats could be maintained in good condition for up to three weeks on a diet of glucose and sodium chloride solutions without solid food. It seemed reasonable, therefore, to use this diet, which was deficient in protein, to produce partial starvation in rats. An initial experiment was therefore designed to study the formation of ulcers on this diet, and to compare the pH of the gastric contents with that of a control group of rats on normal diet.

EXPERIMENTAL.

Experiment 1.—*Solution Feeding.*

Pure bred Wistar rats of both sexes were used. They were kept in cages with two feeding bottles, one containing a solution of 25 per cent glucose in water, the other 25 per cent glucose solution with 0·5 per cent sodium chloride added. Sixty rats were used in the experiment. In addition, a group of 36 litter mates of the experimental animals were used as controls, and were fed on diet No. 41 of the Associated London Flour Millers Ltd. Of the 60 test animals, 5 were killed after 2 days of solution feeding, and further groups of 5 rats were killed on alternate days until the completion of the experiment on the 24th day. The control rats were killed at similar 2-day intervals. The oesophagus, proventriculus and stomach of each animal were opened and any fluid present carefully transferred to an agglutination tube. The pH of the supernatant fluid was estimated using a B.D.H. capillator apparatus. The mucosa was examined and in all cases blocks of oesophagus, proventriculus and stomach were taken for microscopy.

* Present address : Wythenshawe Hospital, Wythenshawe, Manchester.

The results are shown in Table I. It can be seen that of the 60 rats used, 52 (87 per cent) had ulcers, and that from the 6th day onwards all the rats on solution feeding had ulcers when killed. All 52 had ulceration of the proventriculus and 14 also had ulcers in the oesophagus. The average number of ulcers per rat increased the longer the animals were kept on the diet. No ulcers were present in any of the control rats. The mean pH of the gastric contents of the rats on solution feeding was 1·94 (S.D. 0·291) compared with the much higher pH of 3·64 (S.D. 0·277) in the normal animals.

TABLE I.—*Production of Oesophageal and Proventricular Ulcers in Rats after a Diet of Glucose and Sodium Chloride.*

Number of days on diet.	Number of rats killed.	Number of rats with ulcers.	Average number of ulcers per rat.
2	5	0	0
4	5	2	1·6
6	5	5	5·8
8	5	5	7
10	5	5	9·4
12	5	5	10
14	5	5	17
16	5	5	22
18	5	5	42·6
20	5	5	47·2
22	5	5	37
24	5	5	42
	60	52	

Description of ulcers.

Distribution.—In some animals ulcers were numerous throughout the proventriculus, but they were most frequently present in its upper part and at its junction with the oesophagus. Ulcers occurring in the oesophagus were of similar appearance to those in the proventriculus; they were limited to the lower third and were most frequent just above the proventriculo-oesophageal junction. Rarely a few ulcers were seen in the glandular epithelium of the stomach.

Macroscopic appearances.—In the earliest stage there is loss of the normal glistening, translucent appearance of the mucosa and white oedematous patches 1 to 2 mm. in diameter are found. Later irregular ulcers appear in these patches (Fig. 1). The ulcers have a white

DESCRIPTION OF PLATES.

FIG. 1.—The proventriculus and oesophagus in the specimen on the left contain well defined ulcers. The specimen is from a rat fed on glucose and salt solutions for 10 days; the control specimen, right, without ulcers, is from a litter mate fed on normal diet. × 1½.

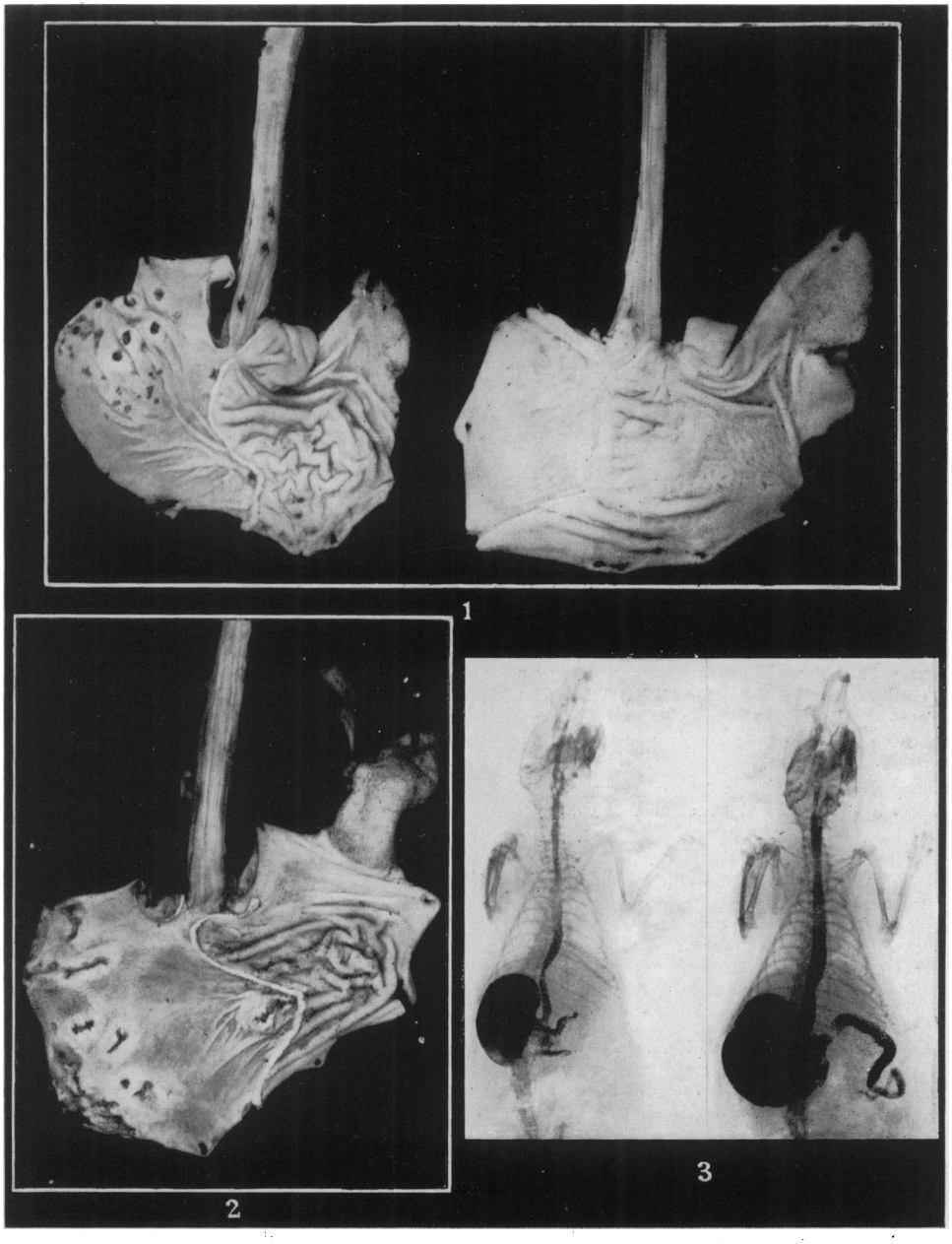
FIG. 2.—The ulcers in the proventriculus are tending to coalesce so that they become oval. In the extreme lower left portion of the specimen this had led to the formation of a large area of ulceration with a ragged margin. The animal was fed on glucose and salt solutions for 16 days. × 1½.

FIG. 3.—There is marked patchy oedema of the lamina propria with thinning and arching of the still intact squamous epithelium. Inflammatory cells infiltrate the lamina propria and extend through the muscularis mucosae into the submucosa. The specimen is from a rat fed on glucose and salt solutions for 4 days. H. and E., × 92.

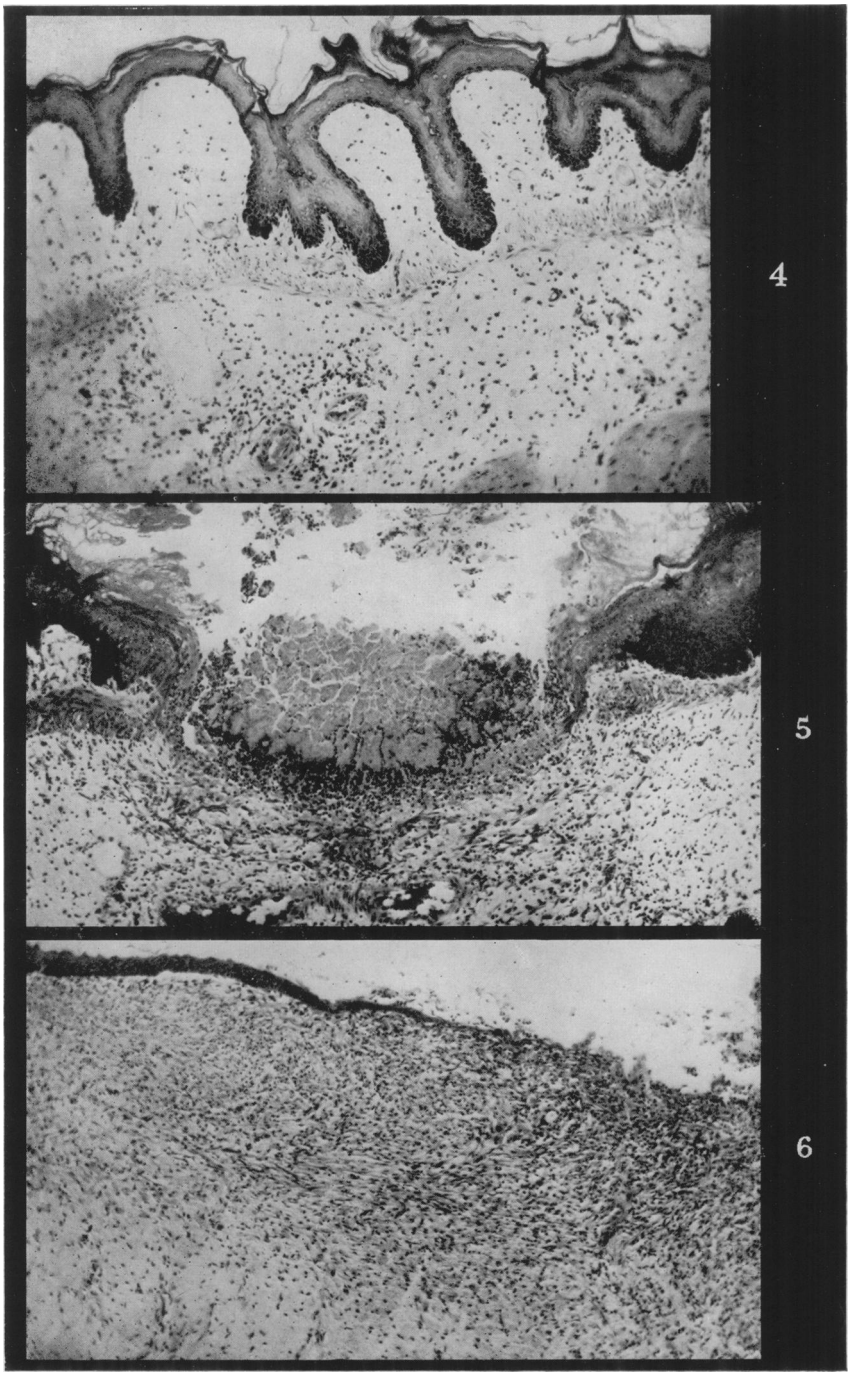
FIG. 4.—An area of ulceration extends through the muscularis mucosae, with underlying congestion and inflammatory cell infiltration. From an animal fed on glucose and salt solutions for 10 days. H. and E., × 73.

FIG. 5.—Left, dilatation of oesophagus and proventriculus and to a lesser degree of stomach and duodenum after vagotomy with muscle resection. Right, control litter mate showing normal calibre of oesophagus and proventriculus. The X-rays were taken after giving barium sulphate paste through a tube passed into the upper oesophagus.

FIG. 6.—Healing of an oesophageal ulcer by a thin tapering layer of squamous epithelium extending over granulation tissue. The specimen is from a rat which was given glucose and salt solutions for 10 days, followed by a diet of glucose and salt solutions with added 1 per cent sodium bicarbonate for a further 6 days. H. and E., × 95.



Lodge.



Lodge.

or yellow floor and a firm raised margin. There may be congested blood vessels seen as pin point pink spots round the periphery of the lesion. The lesions tend to coalesce and produce elongated ulcerated areas measuring up to 5 mm. in diameter (Fig. 2).

Microscopic appearances.—The earliest change is a patchy thickening of the lamina propria due to oedema. The overlying epithelium is thinned and bulges to produce the raised white areas noted macroscopically (Fig. 3). There is diffuse infiltration of the lamina propria by polymorphonuclear leucocytes and lymphocytes, similar to that seen in the early stages of oesophagitis in man. As the lesion progresses the inflammatory cells become localised, and the overlying epithelium breaks away to form an ulcer, with a floor composed of granulation tissue (Fig. 4). As the ulcers increase in size the inflammatory cells infiltrate more widely and may extend deeply into the muscularis externa.

The lesion of the rat oesophagus produced by this diet was associated with lowering of the pH of the gastric contents and possibly resulted from it. To test this suggestion a further experiment was devised to attempt to prevent ulceration by raising the pH of the stomach contents.

Experiment 2.—*Solution Feeding with the Addition of Sodium Bicarbonate.*

The rats were fed on similar solutions to those used in the previous experiment, but with the addition of 1 per cent sodium bicarbonate to each solution. Groups of 5 rats were killed at 2-day intervals as previously. The results are shown in Table II. Of 60 rats only 8 (13·3

TABLE II.—*Production of Oesophageal and Proventricular Ulcers in Rats after a Diet of Glucose, Sodium Chloride and Sodium Bicarbonate.*

Number of days on diet.	Number of rats killed.	Number of rats with ulcers.	Average number of ulcers per rat.
2	5	0	0
4	5	0	0
6	5	0	0
8	5	0	0
10	5	0	0
12	5	0	0
14	5	0	0
16	5	3	0·8
18	5	0	0
20	5	0	0
22	5	3	2·6
24	5	2	2·4
	60	8	

per cent) developed any ulcers, all of which were in the proventriculus, and the average number of ulcers per rat was much smaller than in the previous experiment. The mean pH of the gastric contents of these animals was raised to 5·94 (S.D. 1·9). This is a higher pH than that of 3·64 found in the group of normal rats and confirms the supposition that the occurrence of the ulcers was determined by the acidity of the gastric juice in contact with the proventricular and oesophageal epithelium.

The more frequent formation of ulcers in the proventriculus than in the oesophagus in Exp. 1 further supports the theory that the ulcers were due to the effects of gastric juice, for the proventriculo-oesophageal junction is narrow, whereas the junction between proventriculus and stomach is wide, and thus gastric juice can more easily regurgitate into the proventriculus than into the oesophagus. It appeared that an increased flow of gastric contents back through the proventriculus into the oesophagus should increase the extent of oesophageal ulceration. A special operation was devised for this purpose, as it was found that vagotomy alone did not produce sufficient dilatation of the proventriculo-oesophageal junction.

Experiment 3.—*Vagotomy with Muscle Resection.*

The rats were anaesthetised with intraperitoneal Nembutal (pentobarbitone sodium) and ether by inhalation. Using an aseptic technique the oesophagus and proventriculus were

exposed through a mid-line incision. Bilateral vagotomy was performed. A segment of muscle 1 mm. in width was then resected from the whole circumference of the oesophagus immediately above the proventriculo-oesophageal junction. Care was taken not to incise the mucosa. After operation it was noted that many rats, although taking a normal diet well, continually regurgitated fluid containing particles of food, so that the fur round their mouths and necks became matted and discoloured. X-ray examinations were made post-operatively after giving barium sulphate paste by stomach tube. They showed very marked dilatation of the proventriculus and the oesophagus throughout its length (Fig. 5). The oesophagus also appeared elongated, indicating a relaxation of all the muscle elements, and in some animals there was dilatation of the stomach and duodenum.

The rats were killed at intervals of 2 days to 12 months after the operation. At autopsy there was gross dilatation of the oesophagus and proventriculus and in some animals the oesophagus was filled with food and fluid to the level of the pharynx. No ulceration or inflammatory cell infiltration was seen in any of the specimens on macroscopic or microscopic examination.

It was appreciated that the cutting of the vagi involved in the operation would interfere with the secretion of acid by the stomach but it was decided to study the effects of combining the operative procedure with solution feeding.

Experiment 4.—*Vagotomy with Muscle Resection and Solution Feeding.*

The glucose and sodium chloride solutions with added bicarbonate were given to 36 rats for 2 days to empty the proventriculus and stomach of food residue. The operation was then performed as in the previous experiment, and the glucose and salt solutions without bicarbonate which had been found to produce ulcers were given post-operatively.

Groups of 3 rats were examined at intervals of 2 to 24 days after operation. No ulcers were produced in any of the rats and the average pH of the gastric contents was 3·96 (S.D. 0·397). It was clear that the operation reduced acid secretion and so prevented ulcer formation.

In the next experiment vagotomy and muscle resection to produce regurgitation were combined with the feeding of an acid and pepsin solution. This mixture was given to overcome the rise in pH caused by the vagotomy, and it was hoped that conditions might thus be produced in the oesophagus of the rat similar to those thought responsible for oesophagitis in man.

Experiment 5.—*Vagotomy with Muscle Resection and Acid and Pepsin Feeding.*

The operation was performed on 24 rats after 2 days' preoperative feeding on solutions with added bicarbonate as in the previous experiment. Post-operatively the 24 rats were divided into 2 groups. Twelve animals were given glucose and sodium chloride solutions with the addition of a mixture of dilute HCl and glycerine pepsin B.P.C.; the remainder received glucose and salt solutions with dilute HCl but no pepsin. In each case the pH of the solution was adjusted to 1·4. This was found to be the most acid solution which the animals would drink.

Rats were killed in batches of 3 on the 8th, 12th, 16th and 20th days after operation. In the HCl + pepsin group, the numbers of rats with ulcers in each batch were 0, 2, 2 and 3 respectively; in the HCl only group they were 0, 1, 3 and 3. The results were thus similar and they are therefore considered together. Of all the 24 rats 14 (58·5 per cent) were found to have ulcers; 12 had ulcers in the oesophagus and 11 had lesions in the proventriculus. In 2 rats perforation of an ulcer occurred. One of these animals was receiving acid and pepsin, the other had had acid only. In each case the ulcer was in the oesophagus at its lower end, just above the junction with the proventriculus. The average pH of the gastric contents in this experiment was 2·14 (S.D. 0·488). Thus, with reflux of acidified gastric contents the most severe lesions occurred at the lower end of the oesophagus, and HCl without pepsin appeared as effective as the acid pepsin mixture in producing ulceration.

To complete the investigations on the effect of the pH of the gastric juice in the production of these ulcers the effect of the treatment of established ulcers with alkali was studied.

Experiment 6.—*Repair of Ulcers by Raising the pH of the Gastric Contents.*

Forty rats were given the glucose and sodium chloride solutions for 10 days so that they would develop ulcers as in Exp. 1. The diet of 20 of the animals was then changed to the

glucose and salt solutions with added 1 per cent sodium bicarbonate which had been found to prevent ulceration. The remaining 20 rats continued as controls on their ulcer-producing solutions. On the 2nd, 4th, 6th and 8th days after the change of diet groups of 5 experimental and 5 control animals were killed. It can be seen from Table III that 9 of the rats

TABLE III.—*The Effect of Sodium Bicarbonate on Ulcers Produced in Rats by a Glucose and Salt Diet.*

Number of days after change to bicarbonate diet.	Number of animals on solutions with bicarbonate showing ulcers (5 killed each day).	Average number of ulcers in rats on bicarbonate.	Number of animals on solutions without bicarbonate showing ulcers (5 killed each day).	Average number of ulcers in rats not on bicarbonate.
2	4	5·5	5	9·5
4	4	3	5	14
6	2	4	5	20
8	1	2	5	36

fed on the bicarbonate diet had no ulcers, whereas all the rats in the control series showed lesions. Also, in those rats which showed ulceration after bicarbonate treatment the number of ulcers was much smaller than in the control group. The mean pH of the gastric contents in the rats receiving sodium bicarbonate was 6·1 (S.D. 1·49), whereas that of the control group was 2·1 (S.D. 0·35).

Examination of the proventriculus and oesophagus from bicarbonate-treated animals showed some ulcers with a smooth glistening base suggesting possible re-epithelialisation. Microscopically regenerating epithelium was seen growing over the granulation tissue (Fig. 6) and eventually completely bridging the lesion.

DISCUSSION.

The experimental methods described in this work in the investigation of oesophagitis avoided the artificial technique of perfusion employed by Ferguson, Sanchez-Palomera, Sako, Clatworthy, Toon and Wangensteen (1950), and the mutilation of pyloric ligature as in Selye's (1938) work. Early death of the animals was not caused as in these authors' procedures, and it was possible to kill animals at predetermined intervals so that the stages in the development of the lesion could be studied.

The findings suggest that the inflammatory lesion which occurs in rats fed only on glucose and sodium chloride solutions is due to the resultant lowering of the pH of the gastric juice. Proof is supplied by the absence of ulcers when this fall in pH is prevented by the addition of sodium bicarbonate to the solutions. The operation of vagotomy with muscle resection, designed to cause reflux from the proventriculus into the oesophagus, incidentally provides additional proof of the importance of gastric acid in the production of the lesion, for the vagotomy despite glucose and salt solution feeding prevented any fall in the pH and no ulceration or inflammation occurred. The suggestion of Hoelzel and Da Costa (1932, 1937) that the ulceration of the proventriculus produced by partial starvation of rats is directly due to deficiency of some factor in the diet is thus disproved. If solution feeding were maintained indefinitely deficiency diseases would undoubtedly be a complication, but within the time limits of the present experiment they were not seen.

The experiments also illustrate the importance of reflux of acid stomach contents in producing the lesions. It appears that in the rat two mechanisms

normally limit contact between gastric juice and the mucosa of the proventriculus. First, despite the absence of any true sphincter at the gastro-proventricular junction, peristaltic waves aided by the deflector action of the "limiting ridge"—the mucosal lip at the gastro-proventricular junction—prevent excessive reflux of gastric contents (Lodge, 1954). Further protection is afforded by the bolus of food which the proventriculus normally contains. This mass not only acts as a mechanical barrier to the entry of fluid into the proventriculus, but also tends to absorb fluid and prevent its contact with the proventricular squamous epithelium. In a similar manner peristaltic movement and the constriction at the proventriculo-oesophageal junction provide an even more effective barrier to the flow of gastric juice back into the oesophagus.

After about two days without solids, the bolus of food stored in the proventriculus is exhausted and fluid from the stomach can enter the proventriculus and bathe its walls. The mucosa of the proventriculus is thus unusually exposed to a gastric juice of increased acidity and a chemical inflammation with eventual ulceration results.

The operation of vagotomy combined with the resection of muscle from the lower oesophagus destroys the barrier to regurgitation and a similar inflammation occurs in the oesophagus. A close parallel can be drawn between the mechanism of this experimentally induced reflux and that which from the evidence of previous work (Lodge, 1955) appears to operate in oesophagitis in man. In the human series it appeared that oesophagitis occurred when there was incompetence of the cardio-oesophageal junction, due to such local causes as gastric intubation or general causes such as loss of muscle tone and decubitus, which exposed the squamous epithelium to excessive contact with the gastric juice. The results of repeated vomiting which produces a forcible and frequent entry of stomach contents into the oesophagus were similar.

The experimental evidence thus appears a sound confirmation of the hypothesis that non-specific oesophagitis in man is produced by excessive contact between the acid of gastric juice and the squamous epithelium of the oesophageal mucosa.

SUMMARY.

The pH of the stomach contents is lowered when rats are fed on glucose and sodium chloride solutions without solid food. Ulceration then occurs in the oesophagus and proventriculus.

Addition of sodium bicarbonate to the solutions prevents the fall in pH and no ulcers form. Similarly, sodium bicarbonate in the diet produces healing in already established ulcers.

An operation devised to facilitate reflux of gastric contents into the rat oesophagus is described.

This operation, combined with the feeding of acidified solutions, produces lesions maximal in the lower oesophagus.

The appearances of oesophagitis in man are thus reproduced in animals, and the importance of excessive contact between acid gastric juice and the oesophageal mucosa in the aetiology of the disease is demonstrated.

I wish to thank Professor A. C. P. Campbell for his helpful criticism and advice.

REFERENCES.

BUCHNER, F., SIEBERT, P. AND MOLLOY, P. J.—(1928) *Beitr. path. Anat.*, **81**, 391.
FERGUSON, D. J., SANCHEZ-PALOMERA, E., SAKO, Y., CLATWORTHY, H. W., TOON, R. W. AND WANGENSTEEN, O. H.—(1950) *Surgery*, **28**, 1022.
HOELZEL, F. AND DA COSTA, E.—(1932) *Proc. Soc. exp. Biol., N.Y.*, **29**, 382.—(1937) *Amer. J. dig. Dis.*, **4**, 325.
KAMMERAAD, A.—(1942) *J. Morph.*, **70**, 323.
LODGE, K. V.—(1954) M.D. Thesis, University of Manchester.—(1955) *J. Path. Bact.*, in press.
SELYE, H.—(1938) *Canad. med. Ass. J.*, **39**, 447.