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Gastric mucosal blood flow during pentagastrin- and histamine-stimulated acid secretion in the rat

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Summary

1. Gastric mucosal blood flow was studied in the rat by means of a [^{14}C]-aniline clearance technique.

2. There was a significant correlation between clearance and stimulated acid secretion.

3. No significant difference was found in the relationship between clearance and acid secretion during submaximal stimulation with either pentagastrin or histamine.

4. Estimates of mucosal blood flow per unit acid secretion by [^{14}C]-aniline clearance in the rat were similar to those reported with aminopyrine clearance in the dog and cat.

Introduction

The rat is widely used for the study of gastric function. Elucidation of the mechanism of action of drugs which affect secretion or ulcer formation may require an understanding of their actions on the gastric microcirculation. It was therefore of interest to develop a method of measuring gastric mucosal blood flow (MBF) in this species.

The aminopyrine clearance technique used to determine MBF in the dog is not readily applicable to small animals such as the rat, since it relies on the chemical estimation of aminopyrine in gastric juice and plasma. However, the use of isotopically labelled aminopyrine or a closely similar substance would make such studies feasible. Of the other weak bases which satisfy the necessary criteria for use in gastric clearance measurements, aniline is readily available as the ^{14}C isotope. Moreover, recent studies in the dog have shown that clearance of both aminopyrine and [^{14}C]-aniline gives similar estimates of MBF (Curwain & Holton, 1971, 1973).

In the present investigation we have used a [^{14}C]-aniline clearance technique in the rat to study MBF and its relationship with gastric acid secretion during stimulation with pentagastrin and histamine.

Methods

Female rats weighing 200–250 g were starved for 18 h but allowed water. Anaesthesia was induced with urethane (1·6 g/kg subcutaneously as a 25% solution) and the trachea was cannulated. The gastric lumen was perfused by a technique

similar to that described by Ghosh & Schild (1958). The abdomen was opened by a transverse incision and a polythene cannula was inserted into the stomach via an incision in the duodenum (1–1·5 cm from the pyloric sphincter). The tip of the cannula lay just beyond the pyloric sphincter and was held in place by two ligatures round the duodenum, one at the point of incision and the other close to the pylorus. A rubber catheter (FG8) was passed down the oesophagus so that the tip lay within the gastric lumen and was held in place by a ligature in the neck. To remove any solid contents, the stomach was flushed with 0·9% w/v NaCl solution (saline) and gently massaged, taking care to avoid distension. Saline, or in certain experiments acid saline (0·01 N HCl, pH 2), was perfused through the gastric lumen at a rate of 0·2 ml/min with a Braun slow injection apparatus. Samples of the perfusate were collected at intervals of 20 min and the acid content determined by titration of aliquots with 0·01 N NaOH using phenolphthalein as indicator. The volume of the cannula from the tip in the pylorus to the point of sample collection was 0·4 to 0·5 ml. The time lag between administration of 0·1 ml of 0·1 N HCl via the tip of the oesophageal cannula and detection of an increase in acid was 2 min with a peak acid output at 3 to 4 minutes.

Blood pressure was recorded and blood samples withdrawn via a cannula in a carotid artery. Loss of blood, or dilution of the sample with heparinized saline in the cannula was avoided by drawing blood 5 cm up the cannula and withdrawing the sample via a needle inserted through a rubber connector 1 cm from the blood vessel. Drugs were administered intravenously in a constant volume not exceeding 3·3 ml/h via a cannula in a femoral vein. The dead space of the venous cannula was 0·1 ml and drugs administered via a rubber connector reached the circulation within 1–2 minutes. Body temperature was maintained at 34° C $\pm$0·5° by means of a rectal probe and a warming blanket.

Estimation of [^{14}C]-aniline in blood

One hour after surgery [^{14}C]-aniline was injected i.v. in a loading dose of 2·0 μCi/kg followed by a continuous infusion of 0·033 (μCi/kg)/min to maintain a steady plasma concentration. The loading dose of aniline was either 10 mg/kg or 6 μg/kg and was followed by a continuous infusion of either 170 (μg/kg)/min or 0·1 (μg/kg)/minute.

At regular intervals (usually one hour) 0·25 ml samples of blood were withdrawn, diluted to 2·5 ml with heparinized saline and centrifuged. The [^{14}C]-aniline content of the supernatant was determined following an aniline-extraction procedure based on the method of Brodie & Axelrod (1948). An aliquot, adjusted to pH 8 with 0·1 N NaOH, was extracted with benzene solvent, and the organic phase re-extracted with 0·1 N HCl. A 4 ml sample of the acidic phase, added to 10 ml 'Instagel' emulsifier (Packard) in a plastic vial, was counted in a Packard Tri-carb liquid scintillation spectrometer. The values obtained were corrected for efficiency of counting and of extraction and were expressed as (d/min)/ml of blood. Recovery of [^{14}C]-aniline from blood by this procedure was 87·8 $\pm$ 5·1% (n=6). The total radioactivity in the blood increased steadily, but the [^{14}C]-aniline content determined after extraction remained constant for 5–8 hours. The concentration of aniline in blood was 16·2 $\pm$ 1·1 μg/ml; 3·2 $\pm$ 0·2 nCi/ml (n=18) and 4·7 $\pm$ 0·4 ng/ml; 1·56 $\pm$ 0·1 nCi/ml (n=24) for the high and low dose of aniline respectively.

Estimation of [^{14}C]-aniline in the gastric perfusate

The output of total radioactivity in the gastric perfusate under resting conditions remained constant during administration of both high and low doses of aniline suggesting that only unmetabolized aniline can diffuse readily into the lumen. Furthermore, with the low dose of aniline, during basal or stimulated secretion, $92 \pm 4\%$ ($n=10$) of radioactivity in the perfusate behaved like aniline in the above extraction technique designed to separate aniline from its more polar metabolites. Thus, for routine studies the [^{14}C]-aniline content of the gastric perfusate was determined directly by liquid scintillation counting without prior solvent extraction, and was expressed as (d/min)/minute.

Estimation of clearance

Clearance was calculated as the ratio of gastric output to blood concentration of [^{14}C]-aniline and expressed as ml/min or percentage of basal (the basal value being the mean of the three values preceding stimulation).

Mucosal weight

Mucosae were removed from the external muscle layers by scraping with a glass slide. The weights of mucosae from perfused stomachs and non-operated rat stomachs were similar (1·44 mg/g body weight $n=22$).

Statistical analysis

Results are shown as the mean $\pm$ standard error of the mean, where (n) is the number of values in the group. The relationship between clearance and acid output was established using the correlation coefficient r and the regression was calculated by the method of least squares. The significance of difference between the slopes and between the grouped data was evaluated by Student's t test. $P<0{\cdot}05$ was taken as significant.

Drugs

Aniline hydrochloride, histamine acid phosphate, (BDH, doses expressed as base) ; pentagastrin (Peptavlon, ICI) ; [^{14}C]-aniline hydrogen sulphate (50 mCi/mmol, Radiochemical Centre).

Results

Clearance of [^{14}C]-aniline was determined during basal and pentagastrin-(0·33 (μg/kg)/min) stimulated acid secretion. The results obtained with both high (170 (μg/kg)/min) and low (0·1 (μg/kg)/min) doses of aniline are shown in Table 1. With the high dose, comparable to that used in the dog (Curwain & Holton, 1971), clearance rose from a basal value of $0{\cdot}11 \pm 0{\cdot}02$ ml/min ($n=4$) or $0{\cdot}33 \pm 0{\cdot}06$ (ml/min)/g of mucosa to a value of $0{\cdot}44 \pm 0{\cdot}04$ ml/min ($n=4$) or $1{\cdot}46 \pm 0{\cdot}12$ (ml/min)/g of mucosa.

With the low dose of aniline, the absolute clearance values were smaller, although when expressed as a percentage of basal, clearance values obtained at comparable

TABLE 1. *Acid output and clearance during successive periods of pentagastrin* (0·33 (μg/kg)/min) *stimulation*

	(a) Aniline 170 (μg/kg)/min			(b) Aniline 0·1 (μg/kg)/min		
Time min.	Acid output μEqu/min	Clearance ml/min	Clearance % of basal	Acid output μEqu/min	Clearance ml/min	Clearance % of basal
0	0·21±0·06	0·11±0·02	99±20	0·24±0·07	0·06±0·01	105±19
20	0·64±0·14	0·23±0·07	211±62	0·79±0·20	0·09±0·02	175±40
40	1·13±0·25	0·32±0·05	296±50	1·26±0·30	0·13±0·03	245±50
60	1·36±0·43	0·36±0·07	336±67	1·42±0·38	0·16±0·03	306±57
80	1·40±0·41	0·41±0·07	384±64	1·64±0·38	0·19±0·03	352±48
100	1·38±0·36	0·45±0·04	417±36	1·58±0·30	0·19±0·02	354±42

Time 0 represents the sample prior to stimulation. Results obtained with (a) a high dose (170 (μg/kg)/min) and (b) a low dose (0.1 (μg/kg)/min) of aniline. Each value is the mean±S.E. of four experiments.

rates of secretion in both series of experiments were similar (Table 1). These low doses resulted in more stable blood levels and minimised possible toxic effects of prolonged infusion of aniline. Most of the subsequent studies were therefore carried out with this low dose, and the results were found to be qualitatively similar to those obtained with the high dose.

The complete pattern of responses is shown in Figure 1. Under resting conditions, clearance and acid secretion were constant. Infusion of pentagastrin caused a progressive increase in acid output accompanied by a rise in clearance, until plateau levels were reached. Both parameters returned to resting levels after the pentagastrin infusion was terminated.

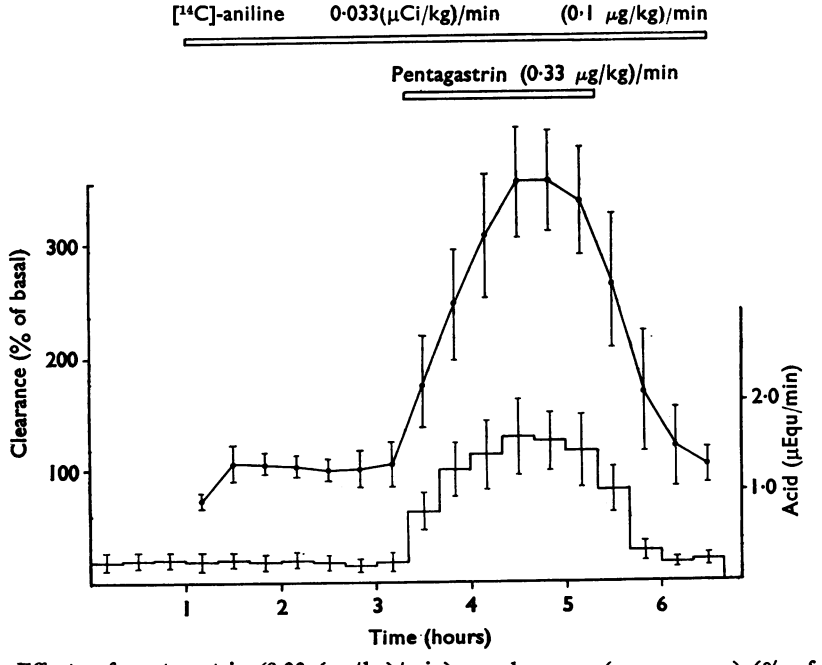

FIG. 1. Effects of pentagastrin (0·33 (μg/kg)/min) on clearance (upper curve) (% of basal) and acid secretion (lower curve) (μEquiv/min). Results expressed as the mean±S.E. of four experiments.

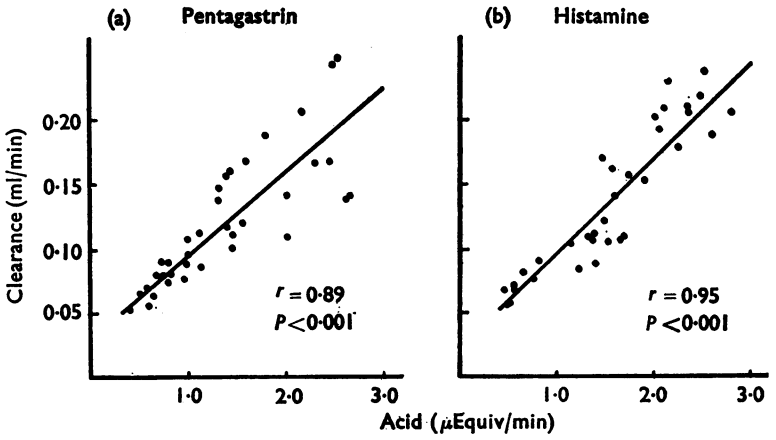

FIG. 2. The relationship between [^{14}C]-aniline clearance and acid secretion in the anaesthetized rat during submaximal stimulation with (a) pentagastrin (0·33 (μg/kg)/min) and (b) histamine (33 (μg/kg)/min) with the lower dose of aniline (0·1 (μg/kg)/min).

Relationship between clearance and acid secretion

Pentagastrin stimulation

Clearance plotted as a function of acid secretion is shown in Figure 2a. The values were obtained during plateau rates of acid secretion. The same dose of pentagastrin (0·33 (μg/kg)/min) was used in all experiments. This dose was submaximal (as judged by other experiments in which dose-response relationships were studied) but because of the variation between animals, a wide range of secretory rates could be studied. A significant linear correlation was found between the two parameters, $r=0{\cdot}89$, $P<0{\cdot}001$ ($n=36$).

Histamine stimulation

The results of a similar series of experiments with a submaximal dose of histamine (33 (μg/kg)/min) are shown in Figure 2b. A significant linear correlation was found between both parameters, $r=0{\cdot}95$, $P<0{\cdot}001$ ($n=34$).

The calculated regression lines for histamine or pentagastrin stimulation did not differ significantly ($P>0{\cdot}05$).

Ratio of clearance to acid output $R_{c/a}$

$R_{c/a}$ was determined during submaximal stimulation with pentagastrin and histamine. During the initial period of pentagastrin stimulation, $R_{c/a}$ fell to a value

TABLE 2. *Ratio of clearance to acid output ($R_{c/a}$) during successive periods of pentagastrin* (0·33 (μg/kg)/min) *and histamine* (33 (μg/kg)/min) *stimulation*

	$R_{c/a}$, % of basal			
Time	(a) Aniline 170 (μg/kg)/min		(b) Aniline 0·1 (μg/kg)/min	
(min)	Pentagastrin	Histamine	Pentagastrin	Histamine
0	100·4±14·7	99·6±10·1	98·8±8·2	104·2±10·2
20	54·4± 4·5	65·4± 6·0	50·1±6·7	61·4± 1·5
40	45·6± 6·4	49·7± 3·8	40·1±6·2	39·5± 2·3
60	43·8± 7·4	43·9± 2·2	41·0±6·7	38·6± 2·8
80	41·4± 5·2	42·8± 2·4	39·9±5·8	38·4± 3·1

Time 0 represents the sample prior to stimulation. Results obtained using (a) high dose (170 (μg/kg)/min) and (b) low dose (0·1 (μg/kg)/min) of aniline are expressed as a percentage of the basal value and shown as the mean±S.E of four experiments.

which remained constant throughout the remainder of the rising phase and plateau of the secretory response (Table 2). When expressed as percentage of basal, the values of $R_{c/a}$ obtained from experiments with either the high or the low dose of aniline, were not significantly different ($P>0{\cdot}05$). During submaximal histamine stimulation, $R_{c/a}$ likewise decreased to values which were not significantly different with either dose level of aniline.

Comparison of the pooled values of $R_{c/a}$ obtained with either aniline dose during plateau levels of secretion, did not demonstrate any significant difference between pentagastrin and histamine.

Discussion

The measurement of total gastric blood flow is of limited value in the study of gastric function since it gives little indication of the blood flow through the secretory mucosa. The determination of mucosal blood flow (MBF) necessitates the use of indirect methods, of which the best validated and most widely used is the aminopyrine clearance technique developed by Jacobson, Linford & Grossman (1966).

Theoretically, for the clearance measurement of MBF, aniline would be expected to be as suitable as aminopyrine. Both are weak bases with very similar physicochemical properties, which have been shown to diffuse rapidly from plasma into the gastric lumen, where they are trapped as the non-diffusable, ionized form in the acid environment (Shore, Brodie & Hogben, 1957). It was also proposed that both substances are completely cleared from blood during one passage through the mucosa, and that the rate of accumulation of these bases in the gastric lumen is limited only by the amount of blood flowing through the mucosa. This suggestion led to the development of the gastric clearance technique, the choice of aminopyrine being based mainly on the availability of a simple chemical method of estimating this substance in biological fluids.

Measurement of MBF in small animals cannot readily be carried out with aminopyrine since the quantities needed for accurate chemical determination would require collection of larger volumes of blood and gastric juice than would be compatible with satisfactory experimental design. Aniline, however, is available as the ^{14}C isotope which allows the estimation of small amounts and is therefore potentially useful for clearance studies in the rat. Further, Curwain & Holton (1971, 1973) have shown that MBF estimates in the dog, based on the clearance of aniline and aminopyrine, are very similar.

In the present study [^{14}C]-aniline clearance has been used to measure MBF in the rat under various secretory conditions. Unlike most other species, the rat has a resting acid secretion and thus, in our experiments, the pH of the gastric perfusate remained low enough to ensure the efficient trapping of aniline diffusing into the gastric lumen. This was confirmed by the observation that perfusion of acidic saline through the lumen did not alter aniline clearance. The constant gastric perfusion also reduces the chance of back diffusion of aniline from the lumen to the blood. It was therefore possible to study MBF during both basal and stimulated secretion without the necessity of perfusing acid.

To investigate the effect of total aniline concentration on clearance estimates, the dose was reduced from 170 (μg/kg)/min to 0·1 (μg/kg)/min. A comparison of the results from the two series of experiments (Table 1) shows that although the pattern of changes in clearance is similar with the lower dose, the absolute

values are smaller. This implies that not all the extractable [^{14}C]-aniline in the blood is available for diffusion into the gastric lumen. The precise nature of this phenomenon is not yet known, athough it is likely that aniline in such low concentrations is bound to some constituent of blood in a manner not readily reversible during passage through the mucosa. Such a possibility is supported by the experiments which show that clearance values and the ratio of clearance to acid output, when expressed in terms of basal values, were not significantly different with either dose of aniline (Tables 1 and 2). Therefore, clearance values obtained with very low quantities of aniline do not measure absolutely but do reflect MBF.

Changes in acid output were accompanied by parallel changes in clearance, (Fig. 1) as has been shown in other species. The mean clearance value obtained with the high dose of aniline during basal secretion was 0·33 (ml/min)/g of mucosa, rising to 1·46 (ml/min)/g during stimulation with pentagastrin. The basal value may be compared with resting values of MBF obtained in other species using aminopyrine clearance. In the unanaesthetized dog, resting values of 0·4–0·8 (ml/min)/g (Cowley & Code, 1970) and 0·64 (ml/min)/g (Rudick, Werther, Chapman, Dreiling & Janowitz, 1971) were observed in denervated fundic pouches, while in the unaesthetized cat, a value of 0·1 (ml/min)/g was obtained in the gastric fistula preparation (Reed & Smy, 1971).

Comparison of the clearance values obtained during stimulation in different species can best be made in terms of clearance per unit acid output ($R_{c/a}$). During the initial period of stimulation $R_{c/a}$ fell to a steady value (Table 2). A similar fall in the ratio has been observed in the cat (Reed & Smy, 1971) and in the dog (Jacobson, *et al.*, 1966) although in the latter species the ratio of clearance to volume secretion, R, was studied. Under resting conditions, the major component of MBF is likely to be associated with essential non-secretory processes. During stimulation, however, MBF associated with acid secretion predominates, leading to the observed fall in $R_{c/a}$. The values of $R_{c/a}$ obtained in the rat during steady submaximal stimulation with pentagastrin or histamine, although somewhat greater than those in the cat, are very similar to values obtained by several groups of investigators in the dog (Table 3).

The effect of different secretory stimulants on the relationship between MBF and gastric secetion may also be compared in these species. In the gastric fistula dog, Jacobson & Chang (1969) reported significant differences between R values (ratio of MBF to volume secretion) obtained with submaximal doses of pentagastrin and histamine, whereas in a further series of experiments (Jacobson,

TABLE 3. *Clearance per unit acid secretion ($R_{c/a}$) during submaximal pentagastrin and histamine stimulation in rat, dog and cat*

Species	Stimulant	Clearance per unit acid secretion ml/μEqu	Reference
Rat	Pentagastrin	0·28	Present study
Rat	Histamine	0·27	Present study
Dog	Pentagastrin	0·26	Jacobson (1970)
Dog	Histamine	0·25	Jacobson (1970)
Dog	Histamine	0·29	Swan & Jacobson (1967)
Dog	Histamine	0·28	Cowley, Code & Fiasse (1969)
Dog	Histamine	0·28	Rudick *et al.* (1971)
Cat	Pentagastrin	0·16	Harper *et al.* (1968)
Cat	Histamine	0·12	Harper *et al.* (1968)

1970) with similar secretory responses, no consistent difference in R or $R_{c/a}$ values was reported. In the Heidenhain pouch dog, Curwain & Holton (1973) obtained higher $R_{c/a}$ values with histamine than with pentagastrin. In the anaesthetized cat, similar $R_{c/a}$ values were found during stimulation with pentagastrin and histamine (Harper, Reed & Smy, 1968). In a more recent study, Reed & Smy (1971) observed that MBF per unit secretion was greater with histamine than with pentagastrin, this effect being more marked with very high doses of histamine. Our experiments in the rat show no significant difference in $R_{c/a}$ values during submaximal stimulation with pentagastrin or histamine, and thus provide no evidence that histamine exerts a net mucosal vasodilator action in addition to that associated with acid secretion. One explanation for these results could be that in submaximal doses any direct vasodilator effect of histamine is largely offset by a concurrent reduction in the functional vasodilator drive. The latter is assumed to be linked closely to the metabolic requirements of the mucosa, and to be responsible for the observed relationship between MBF and acid secretion. The possibility that histamine, in doses greater than those required to stimulate maximal acid secretion, would cause an additional increase in MBF remains to be investigated in the rat. However, interpretation of the results in this and other species may require the complete characterization of the histamine dose-response relationship for both gastric vasodilatation and secretion, which in turn, may necessitate a means of selectively blocking either effect of histamine. Whether functional vasodilatation in the gastric mucosa is mediated by the local release of histamine, by prostaglandins, which can increase MBF but inhibit secretion (Main & Whittle, 1972), or by some other product of cellular activity has not yet been established. The use of drugs which inhibit the synthesis, release or actions of each postulated mediator should help to resolve this important problem.

B.J.R.W. is an MRC scholar.

REFERENCES

BRODIE, B. B. & AXELROD, J. (1948). The estimation of acetanilide and its metabolic products, aniline, *N*-acetyl *p*-aminophenol and *p*-aminophenol (free and total conjugated) in biological fluids and tissues. *J. Pharmac. exp. Ther.*, **94**, 22–28.

CURWAIN, B. P. & HOLTON, P. (1971). Radioactive aniline clearance from canine gastric pouches for the measurement of gastric mucosal blood flow. *Br. J. Pharmac.*, **41**, 384*P*.

CURWAIN, B. P. & HOLTON, P. (1973). The measurement of dog gastric mucosal blood flow by radioactive aniline clearance compared with amidopyrine clearance. *J. Physiol. Lond.*, **229**, 115–131.

COWLEY, D. J. & CODE, C. F. (1970). Effects of secretory inhibitors on mucosal blood flow in non-secreting stomach of conscious dogs. *Am. J. Physiol.*, **218**, 270–274.

COWLEY, D. J., CODE, C. F. & FIASSE, R. (1969). Gastric mucosal blood flow during secretory inhibition by gastrin pentapeptide and gastrone. *Gastroenterology*, **56**, 659–665.

GHOSH, M. N. & SCHILD, H. O. (1958). Continuous recording of acid gastric secretion in the rat. *Br. J. Pharmac.*, **13**, 54–61.

HARPER, A. A., REED, D. J. & SMY, J. R. (1968). Gastric blood flow in anaesthetized cats. *J. Physiol. Lond.*, **194**, 795–807.

JACOBSON, E. D. (1970). Comparison of prostaglandin E_1 and norepinephrine on the gastric mucosal circulation. *Proc. Soc. exp. Biol. Med.*, **133**, 516–519.

JACOBSON, E. D. & CHANG, A. C. K. (1969). Comparison of gastrin and histamine on gastric mucosal blood flow. *Proc. Soc. exp. Biol. Med.*, **130**, 484–486.

JACOBSON, E. D., LINFORD, R. H. & GROSSMAN, M. I. (1966). Gastric secretion in relation to mucosal blood flow studied by a clearance technic. *J. Clin. Invest.*, **45**, 1–13.

MAIN, I. H. M. & WHITTLE, B. J. R. (1972). Effects of prostaglandin E_2 on rat gastric mucosal blood flow, as determined by ^{14}C-aniline clearance. *Br. J. Pharmac.*, **44**, 331–332*P*.

REED, J. D. & SMY, J. R. (1971). Mechanisms relating gastric acid secretion and mucosal blood flow during gastrin and histamine stimulation. *J. Physiol., Lond.*, **219**, 571–585.

RUDICK, J., WERTHER, J. L., CHAPMAN, M. L., DREILING, D. A. & JANOWITZ, H. D. (1971). Mucosal blood flow in canine antral and fundic pouches. *Gastroenterology*, **60**, 263–271.

SHORE, P. A., [redacted], B. B. & HOGBEN, C. A. (1957). The gastric secretion of drugs: a pH partition hypothesis. *J. Pharmac. exp. Ther.*, **119**, 361–369.

SWAN, K. G. & JACOBSON, E. D. (1967). Gastric blood flow and acid secretion in conscious dogs. *Am. J. Physiol.*, **212**, 891–896.

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