THE EFFECT OF FEEDING AND DRUGS ON GASTRIC MUCOSAL BLOOD FLOW ESTIMATED BY THE CLEARANCE OF RADIOACTIVE ANILINE AND ITS RELATIONSHIP TO GASTRIC ACID SECRETION IN DOGS

by

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being a thesis submitted for the degree of Doctor of Philosophy in the University of London

June 1972

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Abstract

A method of measuring gastric mucosal blood flow (MBF) in conscious pouch dogs by the clearance of radioactive aniline is presented. The method has been validated by a detailed comparison with the existing method of amidopyrine clearance. The estimation of aniline in plasma and gastric juice is very much simpler than amidopyrine estimation and is shown to be subject to less error, thus giving greater precision to measurements of clearance. The plasma-bound fraction of aniline is probably not available for clearance across the mucosa but is estimated as part of the plasma concentration. A correction must therefore be made to quantify MBF, though this is not necessary where changes in flow rather than absolute levels are to be studied.

Using the aniline clearance method, MBF has been compared in conscious dogs in response to different secretory stimuli. Histamine was found to cause more MBF, relative to acid secretion, than either pentagastrin or feeding, the effects of which were similar.

Sympathomimetic drugs have been found to decrease gastric acid secretion and MBF. While secretion in response to feeding

or pentagastrin is relatively susceptible to inhibition by isoprenaline and noradrenaline, approximately ten times the dose of catecholamine is required to inhibit histamine-induced secretion. The mechanisms by which isoprenaline and noradrenaline reduce pentagastrin-induced secretion have been shown to be different. The ratio of MBF to secretion is unchanged or decreased by noradrenaline suggesting that noradrenaline probably acts by vasoconstriction. However, during inhibition by isoprenaline the ratio of MBF to secretion is increased. Inhibition by isoprenaline is therefore not secondary to a decrease in MBF.

Acknowledgements

I am most grateful to Dr Pamela Holton for her excellent supervision of and constant interest in this work, and to Professor R. Creese for his interest and encouragement.

I am grateful to Mr J.R. Hancock and the technical staff, especially Jan Spencer and David Lane for much valuable assistance and to Mrs Keatinge for typing this thesis.

I am grateful to St Mary's Hospital Medical School for a scholarship and to the Medical Research Council for supporting the work with a grant to Dr Holton.

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CHAPTER 1.

INTRODUCTION

1.1. GENERAL INTRODUCTION

In 1966, Jacobson, Linford and Grossman described the first satisfactory method of measuring gastric mucosal blood flow using the clearance of amidopyrine across the mucosa. Since then, interest in this subject has increased as disturbances in mucosal blood flow have been implicated in the aetiology of gastric ulceration.

The work presented in this thesis describes the development and validation of a simpler method of measuring gastric mucosal blood flow in conscious dogs using radioactive aniline clearance. Using this method, some investigations into changes in gastric mucosal blood flow in response to various secretory stimuli and inhibitors will be presented, with emphasis on the relationship of mucosal blood flow to gastric acid secretion. I have studied in some detail the effects of catecholamines and some related substances on secretion and mucosal blood flow.

1.2 HISTORICAL SURVEY

1.21 The gastric circulation. Its role in secretion and ulcerogenesis.

The gastric circulation has long been of interest to physiologists. In 1858 Claude Bernard observed colour changes in gastric venous blood during digestion, and in this century many workers have investigated the relationship between gastric secretion and blood flow (Burton-Opitz, 1910; Boenheim, 1930; Cumming, Haigh, Harries and Nutt, 1963).

Any long-lasting energy-requiring process, such as gastric secretion of acid, must depend on an adequate supply of energy substrates and maintenance of a suitable environment which can only be brought about by sufficient capillary blood flow (Holton, 1972). Since gastric secretion is generated in the mucosa, mucosal rather than total gastric blood flow is the relevant function if it is to be correlated with secretory studies.

The measurement of gastric mucosal blood flow has been hampered by the impossibility of isolating the mucosal arterial supply or venous drainage from that of the submucosa and muscle layers. Also, as Jacobson (1965) pointed out, the gross anatomy of the gastric circulation is complex and highly anastomotic. Earlier workers failed in their attempt to reduce gastric blood flow and then study secretion (Bernheim, 1932; Layne and Bergh, 1943). They found that subtotal devascularization of the stomach

had little effect on structure or secretion and that ligation of all blood vessels caused necrosis of the organ.

Gastric secretion and mucosal blood flow are now known to be linked (Swan and Jacobson, 1967; Bynum and Jacobson, 1971). An increase in gastric secretion is accompanied by an increase in mucosal blood flow (Jacobson, Linford and Grossman, 1966), but not caused by this increase in perfusion (Cowley and Code, 1970). Obviously, when mucosal blood flow is limiting gastric secretion, an increase in blood flow may permit an increased secretion (Holton, 1972).

Virchow in 1853 suggested that local vascular insufficiency was implicated in peptic ulcer formation and Jacobson (1965) stated that many workers have since been in agreement with this concept. Its development was, however, hampered by a lack of knowledge of the submacroscopic anatomy and a paucity of techniques for blood flow measurement. Many investigators therefore turned their attention to the study of acid and pepsin secretion in relation to ulceration but could not conclude that excess secretion was correlated with a high incidence of ulcer formation (Palmer and Buchanan, 1953). They suggest that only an anoxic mechanism satisfactorily explains the spontaneous focal devitalization prior to ulceration and cite the part played by stress and the general vasomotor hyperirritability of ulcer patients as indirect evidence pointing

to ischaemia as a primary source of gastric ulceration.

Recently Dragstedt (1971) has forcibly argued the case for acid hypersecretion as being responsible for the majority of gastric ulcers seen clinically. He states that the gastric mucosa is capable of being damaged by pure gastric juice but that normally, secretory control ensures that prolonged exposure to secretion, unbuffered by food or saliva, is rare. Retention of gastric contents, resulting in prolonged exposure of the antrum to food has been shown to cause ulcers in man and animals (Dragstedt, 1971). This retention can be caused by pyloric stenosis or gastric atony which may be a result of vagotomy. Consequently combination of a gastric drainage procedure, such as pyloroplasty, with vagotomy is often recommended for the surgical management of gastric ulcer. Dragstedt suggests that the gastrin phase of secretion, rather than basal secretion, ought to be studied if an attempt to correlate acid hypersecretion with ulcer formation is to be made. Although bile has been shown to damage canine gastric mucosa (Davenport, 1968; Black, Hole and Rhodes, 1971) and is frequently present in the stomachs of ulcer patients, he discounts it as a causative factor.

An extensive literature has, however, accumulated which suggests, but has not established that the microcirculation of the stomach plays a part in gastric ulceration (Moody, 1972). A greatly increased knowledge of the gastric microvasculature has been the result of many studies in man and animals (Barclay and Bently, 1949; Barlow, 1951; Walder, 1952; Sherman and Newman,

1954; Peters and Womack, 1958; Nylander and Olerud, 1961). Of particular interest are the large, functioning, arteriovenous anastomoses found in the submucosa and, in the rat stomach, in the mucosa as well. The arteriovenous shunting mechanism can be very effective with the result that very little blood reaches the mucosal glands (Palmer and Buchanan, 1953).

Disturbances in mucosal blood flow or distribution may play a part in gastric ulcerogenesis but, as yet, quantitative studies of flow at the site of a potential ulcer have not been achieved. In several cases, however, vascular changes have been seen to accompany or precede the development of experimental ulcers (Nylander and Olerud, 1960; Guth and Kozbur, 1968; Nedzel, 1938). The fact that many ulcerogenic drugs are vasoactive indicates that vascular disturbances may be involved in the process. In 1967, Kowalewski found that a vasopressin analogue protected Guinea Pigs from ulceration and mucosal congestion after histamine but that the histamine-induced secretion was not depressed. He concluded that a vascular factor was involved in this type of experimental ulceration. In a study of stress ulceration in rats it has been found that mucosal engorgement is always present when ulceration occurs (Guth and Kozbur, 1968). The findings of Gear and his colleagues that superficial or atrophic gastritis may persist or worsen after ulcer healing also support the hypothesis that ulceration is secondary to a mucosal disorder such as inadequacy of blood flow (Gear, Truelove and Whitehead, 1971).

An interesting theory of ulcerogenesis was put forward in 1948 by Davies and Longmuir. They suggested that the alkali liberated intracellularly by acid secreting bullfrog mucosa, was normally neutralised by a bicarbonate and carbon dioxide buffer system. At high secretory rates, CO_2 in excess of that provided by mucosal metabolism was required to neutralise the alkali and, if this was denied to the tissue, ulceration occurred. Although the results may not apply to mammalian gastric mucosa, one can envisage that excessive arteriovenous shunting of blood might deprive glandular areas of the necessary carbon dioxide to neutralise the intracellular alkali. Since carbonic anhydrase, normally present in oxyntic cells, is necessary for uptake of the carbon dioxide (Davies, 1948), loss or inactivation of the enzyme would provide another mechanism for mucosal ulceration.

Whatever the trigger for gastric ulceration, and there may be several, most theories require the presence of hydrogen ions (Moody, 1972). Injury of the mucosa results in a rapid back diffusion of H^+. When the rate of entry of acid exceeds twice the normal rate, intracellular buffers become inadequate and mucosal destruction occurs (Davenport, 1966).

Gastric blood flow and especially mucosal blood flow is therefore a subject worthy of study not only for its intrinsic interest but also because of its relevance to ulcerogenesis.

Many techniques of estimation have been devised, but meaningful measurements of mucosal blood flow were not possible until 1966 when Jacobson, Linford and Grossman introduced the now well-known amidopyrine clearance method.

Since then a technique using the clearance of radioactive aniline has been developed in this laboratory (Curwain and Holton, 1971). Methods employed in the measurement of gastric blood flow will be briefly reviewed.

1.22 Methods of measuring total gastric blood flow

(a) Measurement of gastric venous drainage.

The most direct method of measuring gastric blood flow is to collect the venous drainage for a known time and measure its volume. Burton-Opitz was the first to do this (Burton-Opitz, 1910). Cutting, Dodds, Noble and Williams (1937) used an anaesthetized cat preparation which was eviscerated from anus to duodenum. When it was desired to monitor gastric blood flow, a clamp was applied to the portal vein and the blood collected from a cannula in the superior mesenteric vein. The effects of anaesthesia and surgical intervention on this preparation must have been considerable and Cumming and co-workers have since devised an improved technique (Cumming, Haigh, Harries and Nutt, 1963). They demonstrated that it was possible to collect all the gastric venous drainage, in anaesthetised dogs, without removal of the intestine and measured

flow in a closed-circuit bubble flowmeter. Considerable vascular surgery was, however, required to achieve this. In our own laboratory gastric venous drainage, in conscious and anaesthetised dogs, has been measured with an electromagnetic flowmeter.

In 1927 Lim, Necheles and Ni measured the blood flow through an isolated stomach, perfused by a trained "donor" dog. More recently Green and Hardcastle (1970) have described an isolated, mechanically perfused stomach preparation. Though a good preparation, its value for blood flow studies is limited since we are interested in measuring flow in a tissue free from trauma and in a normal physiological state.

(b) Indirect methods of measuring gastric blood flow

Hepatic blood flow can be determined by the rate of uptake of heat-denatured ^{131}I Human Serum Albumin (Torrance and Gowenlock, 1962). This method has been used to estimate splanchnic blood flow (Samarji, Watson and Torrance, 1970) and gastric blood flow (Schofield, Ingram and Torrance, 1967). Although gross changes in blood flow are probably detectable, this method does not give a sensitive, continuous measurement of gastric perfusion.

The distribution of radioactive microspheres injected into the circulation has been used to calculate relative distribution of blood between various organs of foetal sheep (Rudolph and Heymann, 1967) and adult rabbits (Duncan, 1967). Two measurements can be obtained from a given animal if different isotopes are used but the

method is limited to measuring blood flow during a steady state.

The indicator fractionation technique was first described by Sapirstein (1956). A bolus of radioactive ^{42}K is injected into an anaesthetized animal which is quickly killed a short time later. The isotope diffuses from the blood as it passes through the capillaries and the amount accumulated in the various organs is related to their blood flow. Unfortunately not all organs completely extract this indicator from the blood and the fraction extracted is variable (Delaney and Grim, 1964). They found that loss of the ^{42}K in the gastric venous blood of anaesthetized dogs was roughly balanced by recirculating indicator but this happy coincidence may not hold under all conditions. The canine stomach is known to have functional arteriovenous shunts (Peters and Womack, 1958) and if a significant amount of blood thus by-passed the capillary circulation, underestimation of flow would occur.

Both Delany and Grim (1964, 1965) and Moody (1968) have used ^{42}K fractionation to study the distribution of blood flow to the layers of the stomach and found that mucosal blood flow was 70-80% of the total stomach flow. The method has several disadvantages. As with microsphere distribution the measurement must be made while flow is steady and only a single estimation is possible in each animal. Since the organs are removed just after death, some abnormal changes may occur during this time. An independent

measurement of cardiac output is required to quantify the blood flow and there must be no significant diffusion barrier to the indicator between the blood and the organ. However ^{42}K accumulation in the stomach wall has been found to be stable for a considerable time after injection (Delaney and Grim, 1964).

Accumulation of markers such as ^{42}K or microspheres reflects the rate at which blood reaches a tissue. In the stomach, however, the situation may be complicated by venous or portal constriction with resultant capillary engorgement. I have previously referred to several cases in which this phenomenon has been observed and it would result in an overestimation of blood flow through the tissue if these methods of measurement were employed.

Thus several methods of measuring gastric blood flow have been described. Blood flow in a single artery to a separated gastric pouch has also been measured by an implanted electromagnetic flow probe (Jacobson, Eisenberg and Swan, 1966; Swan and Jacobson, 1967). This method has the advantage of providing a continuous measurement of gastric blood flow in conscious animals, weeks after surgery. I have carried out some studies using this technique and, once problems of instrument stability and probe construction have been solved it should provide useful information. However, it involves vagal denervation of the pouch and, as previously stated, mucosal, rather than total, blood flow measurements are more valuable, especially when concurrent secretory studies

are undertaken.

1.23 Methods of measuring gastric mucosal blood flow

As I have mentioned in the preceding section, the indicator fractionation technique has been used to determine the proportion of gastric blood flow that perfuses the mucosa (Delaney and Grim, 1964, 1965; Moody, 1968). Several other techniques specifically for measuring mucosal blood flow have been described.

It has long been realised that certain substances appear in the gastric juice in relatively high concentration. These include many dyes (Ingraham and Visscher, 1935), some sulphonamide drugs (Cooke, Davenport and Goodman, 1941) and iodide (Davenport, 1943; Schiff, Stevens, Molle, Steinberg, Kumpe and Stewart, 1947; Honour, Myant and Rowlands, 1952 and Ruegamer, 1953). If a substance, whose plasma concentration were known, was totally cleared from the gastric mucosal blood on one passage through the capillaries, and passed into the secretion, then estimation of the substance in the secretion would provide a measurement of mucosal blood flow. A close analogy can be drawn with glomerular blood flow measured by PAH* clearance into the urine. With this in mind, Brown-Grant and his colleagues measured the clearance of iodide (^{131}I) and simultaneously measured total gastric blood flow with a bubble flow meter (Brown-Grant, Cumming, Haigh and Harries, 1965). They found that iodide clearance was correlated with acid secretion except at high secretory rates

*Para-aminohippuric acid.

where clearance was lower than expected. Acid secretion and iodide clearance were demonstrated to be separate processes and the authors concluded that although iodide clearance did not approximate to total gastric blood flow, it might represent a minimal value for mucosal flow.

In 1966, Jacobson, Linford and Grossman introduced the amidopyrine clearance method for measuring gastric mucosal blood flow. An earlier study had shown that amidopyrine appeared in an acid gastric juice in a concentration twenty to forty times that in the plasma (Shore, Brodie and Hogben, 1957). This substance is an organic base with a pKa of 5.0. Thus at pH 7.4 in the plasma it will exist largely in its unionised, lipid-soluble form. This is free to diffuse through cell membranes and out of the plasma. However, on passing through the mucosa into the acid gastric juice, it ionises and the lipid-insoluble charged molecule is trapped in the juice. The equilibrium concentration ratio of amidopyrine between gastric juice and plasma is 10,000:1 (Shore, Brodie and Hogben, 1957). In vivo this ratio is not approached, probably because the extent of mucosal blood flow limits the amount of amidopyrine presented to the mucosa.

In the work of Jacobson and his colleagues it was found that amidopyrine clearance was closely linked with acid secretion and the authors could not exclude the possibility that clearance was due to active transport across the mucosa (Jacobson et al., 1966).

Indirect evidence however suggested that this was unlikely. Two years later Jacobson compared iodide and amidopyrine clearances in Heidenhain pouch dogs and found clearance of amidopyrine to be about twice that of iodide (Jacobson, 1968). It therefore appears that there is some diffusion barrier to the passage of iodide across the gastric mucosa. To date the use of amidopyrine clearance has not been similarly invalidated by the discovery of an indicator giving a higher clearance.

Shore et al. (1957) also found that aniline is highly concentrated in the gastric juice. Like amidopyrine, it is an organic base with a pKa of 5.0 but, unlike amidopyrine, a stable, ring labelled, radioactive preparation is readily available. Estimation of amidopyrine, by the spectrophotometric method of Brodie and Axelrod (1950) is tedious. I therefore decided to attempt the measurement of gastric mucosal blood flow by ^{14}C aniline clearance in dogs with gastric pouches. The results of this work will be presented in later chapters. A detailed comparison with amidopyrine clearance has been made and the results show good agreement. Both techniques rely on the, as yet unproven, assumption that extraction of the marker from mucosal blood is high and constant, if not complete. It has been shown that this is very likely to be the case for amidopyrine (Jacobson et al., 1966; Harper, Reed and Smy, 1968) and the same arguments apply to aniline.

A few other methods of measuring local blood flow have been suggested. The level of dissolved hydrogen gas in a tissue can be estimated by a platinum and a reference electrode. Aukland, Bower and Berliner (1964) gave dogs hydrogen by inhalation and, after a steady state was obtained, reduced arterial hydrogen to zero and measured its clearance from various tissues. Assuming that the tissue is in diffusion equilibrium with its venous blood and that hydrogen is completely removed from pulmonary blood, the rate of tissue desaturation is related to its blood flow. Unfortunately the platinum electrodes used did not maintain their stability for long and so chronic implantation for use in conscious animals was not feasible.

Clearance of other gases has been used for local blood flow measurements. Nitrous oxide was used to estimate cerebral blood flow (Kety and Schmidt, 1945) and Krypton (^{85}Kr) was used for this by Ingvar and Lassen (1962). The method was adapted to measure gastric mucosal blood flow in anaesthetized dogs (Bell and Battersby, 1967). A disadvantage of this technique is that it does not quantify blood flow but merely detects changes in flow in a small area of mucosa beneath the counting device.

Finally, measurement of heat clearance by the mucosa was described by Bell and Shelley (1968). This involved placing a heating coil and two thermistors on the mucosa and measuring the temperature near to and distant from the heater. Although this

method can be used in conscious fistula dogs, it suffered the same drawbacks as the krypton clearance method. Heat clearance was used to measure flow during blood gas changes and it may not be applicable to concurrent secretory studies when gastric juice would be flowing over and around the heater and thermistors.

Thus although several methods of measuring gastric mucosal blood flow are available, I believe that at present clearance of a marker, such as aniline or amidopyrine provides the most satisfactory results. There is some evidence, however, that under certain conditions, clearance of amidopyrine is not equivalent to mucosal blood flow (Moody, 1968) and this will be discussed later.

CHAPTER 2

GENERAL METHODS

2.1 SURGICAL PREPARATION AND CARE OF POUCH DOGS

Healthy mongrel bitches weighing not less than 10 kg were used. The animals were kept in quarantine for 21 days and vaccinated against hardpad, distemper, infectious hepatitis and leptospirosis (Maxivax; Boehringer). On the day before an operation, the dog was fed a low residue diet of milk, egg and Complan (Glaxo) and any remaining food removed at 5 p.m.

2.11 Surgical Procedures

(a) Premedication and anaesthesia.

Atropine 0.6 mg subcutaneously was given as an antagonist to respiratory and gastric secretion. On rare occasions, when the dog was nervous, chlorpromazine (10-25 mg., s.c.) or Hypnorm (Phillips-Duphar, 0.5 ml/Kg i.m.) was given. Intravenous thiopentone was injected to provide anaesthesia sufficient to permit endotracheal intubation. An intravenous drip was set up and the anaesthesia stabilised with oxygen/halothane in a closed circuit.

The areas to be operated were thoroughly shaved and washed with cetrimide solution, and the skin sterilized with iodine by the surgeon before the incision was made. Full aseptic procedure was used.

(b) Implantation of catheters

In some cases intravenous and intraarterial catheters were

inserted into a branch of the femoral artery and vein. Portex p.p. 120 polythene tubing was used and implanted so that the tip lay in the aorta or vena cava. The catheters were taken subcutaneously to the interscapular area and externalized through a small skin incision.

(c) Vagally denervated (Heidenhain) pouch

Heidenhain (1879) first described the preparation of a vagally denervated fundic pouch and our technique is a modification of his. After careful laparotomy, a splenectomy was performed dividing the splenic blood vessels near the hilum to preserve intact the branch to the stomach. The stomach was delivered and a portion of the greater curvature divided between clamps. The mucosal and serosal layers were closed separately, taking care to invert the mucosa. If necessary, part of the pouch vasculature was divided so that a single large branch of the splenic artery carried the whole pouch blood supply. This was done to permit implantation of an electromagnetic flow probe at a later date. A small incision was made in the pouch and a gastric cannula secured with two purse-string sutures. A titanium cannula, described by Gregory (1950) was used. It was brought through a stab wound in the abdominal wall aligned to point downwards and outwards to prevent the acid secretion from falling onto the animal's legs. To prevent damage to the pouch, the cannula was firmly secured to the interior of the abdominal

wall by silk sutures. Before closing the abdomen, an antibiotic aerosol spray was applied through the incision.

(d) Vagally innervated (Pavlov) Pouch

The first gastric pouch of this type was described by Pavlov (1902) and various modifications have since been advocated by different authors. After laparotomy, the stomach and spleen were delivered and the spleen covered with moist swabs to prevent drying. An incision, 3-5 cm long was made in the stomach wall near to and parallel to the greater curvature. The mucosa was gently pulled through the incision with tissue forceps until several centimetres of the interior and posterior walls had prolapsed through the opening. A rubber-shod, non-crushing clamp was placed across the stomach beneath the prolapsed mucosa. This was found not to be necessary and some operations were performed without clamping the stomach and the consequent risk of damaging vagal nerve fibres.

A solution of adrenaline (10 µg/ml) was infiltrated into a ring of the prolapse to aid separation of mucosa from serosa and to reduce bleeding. The mucosa was then incised along the line of infiltration and carefully undercut at least 0.5 cm on the inner (main stomach) side. This raised mucosa was next closed with an inverting teflon suture. The other mucosal edge was sutured together, carefully everted, to form the inner boundary

of the pouch, the prolapse replaced and the original incision closed. A cannula was secured into the pouch and externalised as before.

2.12 Post-operative Care

The dogs were given antibiotics for five days after the operation to prevent infection while the incision healed. Pethidine (25-50 mg, s.c.) was normally given as the dog regained consciousness and this was occasionally repeated after two hours. Daily checks were made to ensure that the skin sutures were firmly in place. They were removed after 14 days.

Obviously, the diet must be closely regulated after gastric surgery. All dogs were given only glucose and water to drink on the first day after the operation. After this a liquid diet of milk, eggs and Complan was given for 3 or 4 days to Heidenhain pouch and fistula dogs and for a week to dogs with Pavlov pouches.

2.13 Routine Maintenance

The dogs are checked daily for general condition and any change investigated. They are regularly weighed and the amount of food adjusted to enable the dogs to regain and maintain their pre-operative weight. The standard diet is Dog Star (Dog Star Ltd - Fife) supplemented with iron (Fersamel Tablets: Glaxo) and salt. Pavlov pouch dogs however were given proprietary cat food since

this does not contain hard pieces of biscuit that may destroy the delicate mucosal septum. Milk and eggs were given when required and raw beef suet was found to be a highly acceptable caloric supplement. Exercise was encouraged to keep the dogs fit and prevent them from lying in their pens during the time of peak secretion.

If the acid secretion caused damage to the skin, this was treated by bathing with 1% sodium bicarbonate. Water repellent silicone cream was then applied to the area until it had healed.

2.2 MEASUREMENT OF GASTRIC MUCOSAL BLOOD FLOW IN DOGS USING THE CLEARANCE OF RADIOACTIVE ANILINE

2.21 Introduction

The method relies on the complete, or almost complete, extraction of aniline from the blood as it passes through the gastric mucosal circulation. The aniline rapidly diffuses into the acid gastric secretion where it ionises and cannot therefore diffuse back. If the clearance of aniline into the gastric juice is limited only by the extent of mucosal blood flow, which presents the aniline to the mucosa, then measurement of clearance provides a measure of mucosal blood flow.

The method is suitable for use in conscious dogs, prepared with a gastric pouch or fistula and in acute anaesthetised animals. Details of surgical preparation will be found in section 2.1.

2.22 Method

(a) Laboratory Procedure

The dogs were starved overnight before experiments but allowed water ad libitum. After an exercise period they were weighed, brought to the laboratory, and placed in sling stands in which they had been trained to stand quietly. If necessary, a sterile intravenous catheter (O.D.O.63 mm) was inserted, through a needle, into a leg vein for the administration of drugs and another catheter (O.D.O.92 mm) placed in another leg vein for

withdrawal of blood samples. Some of the dogs were prepared with indwelling femoral arterial and venous catheters and in such cases, the catheters were checked for patency before beginning the experiment.

The loading dose of aniline was then given, followed at once by the maintenance infusion. Other drugs to be given during the experiment were mixed with the aniline infusion. All substances for infusion were dissolved in sterile pyrogen-free 0.9% NcCl and the concentration adjusted so that the infusion was administered at 1.0 ml per minute.

Measurements were not begun until one hour after the loading dose of aniline had been given. A 2 ml blood sample was taken every 30 minutes during the experiment and gastric juice was collected at 5 - 20 minute intervals. In order to prevent significant back diffusion of aniline from the pouch, the pH of the contents should be below 3.0. At this value, 1% of the aniline will exist in the unionised form (See Chap. 3.2) and be available for back diffusion. This fraction will increase rapidly if the pH rises.

When a 'spontaneous' acid secretion was not present at the beginning of an experiment, and I wished to study the onset of secretion, then a small dose of pentagastrin (0.5 µg $Kg^{-1}hr^{-1}$) was given to acidify the pouch contents. This procedure was adopted rather than an acid wash because I felt that although

acid at pH 1.0-3.0 would acidify the superficial mucosa, it would be unlikely to substantially lower the pH at the base of the gastric pits. This could probably not be achieved without significant distension of the pouch.

(b) Preparation and doses of ^{14}C aniline

A stock solution containing 35 mg/ml of carrier aniline (Aniline sulphate, B.D.H. Laboratory Reagent Grade) and 2.0 µCi ml^{-1} ^{14}C aniline (Radiochemical Centre - C.F.A. 71) in sterile saline was prepared. Doses were expressed in mg of carrier aniline per kg body weight.

Aniline Loading dose : 10 mg/kg

Aniline Maintenance Infusion : 10-15 mg $kg^{-1}hr^{-1}$

The stock solution contained suitable proportions of radioactive and non-radioactive aniline to give satisfactory levels of ^{14}C for counting in the plasma and gastric juice.

(c) Estimation of ^{14}C aniline in plasma and gastric juice

The heparinised blood was centrifuged at 3000 r.p.m. for 10 minutes and the plasma removed and mixed. 1.0 ml of plasma was added to 0.5 ml M.NaOH and 10 mls diethyl ether and shaken for 5 minutes. 7.0 mls of the ether phase were removed and added to a bottle containing 10 ml of scintillator. This was counted for ^{14}C radioactivity in a Packard Tri Carb Liquid Scintillation Spectrometer.

1.0 ml of gastric juice, filtered to remove mucus if necessary,

was added to 0.5 ml M NaOH and 10 mls of diethyl ether and extracted as above. When the volume of secretion was very small, the juice was diluted with saline and a 1 ml aliquot extracted.

Scintillator:

Dimethyl POPOP - (1,4-bis- [2- (4 Methyl-5-Phenyloxazoyl Benzene)] 52.5 mg

PPO - (2.5-Diphenyloxazole) 4.2 gm

Methanol - I.C.I. Analytical Reagent 300 ml

Toluene - Analar Reagent 700 ml

2.23 Calculation of Mucosal Blood Flow

A graph of ^{14}C aniline concentration in the plasma against time was constructed. The mean plasma aniline concentration during a period of gastric juice collection was estimated from the graph. Aniline concentrations were expressed as net counts per minute of ^{14}C radioactivity.

Mucosal blood flow per minute was calculated from the following formula:

$$\text{Mucosal blood flow} = \frac{GV}{PT}$$

where G is aniline concentration in gastric juice

V is the volume of juice secreted or, when secretion is small, the total volume after dilution

P is the mean plasma aniline concentration during the collection period

T is the time in minutes over which the juice was collected

Other functions of secretion and mucosal blood flow have been calculated and will be referred to in later chapters. These are:

i) $R_{..}$ The ratio of aniline concentration in the gastric juice and plasma.

ii) $\mu Eq\ ml^{-1}$. This relates mucosal blood flow to gastric acid output. It is calculated from the rate of acid secretion, measured in $\mu Eq\ H^{+} min^{-1}$ and the mucosal blood flow measured in mls min^{-1}

2.24 The use of tritiated (3H) aniline

In some of the later experiments described in this thesis, 3H-aniline replaced the ^{14}C compound. This was found to be less expensive and its use was adopted after an experiment with both isotopes had shown that identical results were obtained with each. The extraction procedure remained unaltered but more radioactivity was required since tritium is counted with a lower efficiency than ^{14}C. The stock solution (see 2.22b) was made up containing 12μCi 3H/ml.

2.3 THE ESTIMATION OF AMIDOPYRINE IN PLASMA AND GASTRIC JUICE

2.31 Introduction

Since radioactive aniline clearance is proposed as an alternative to clearance of amidopyrine for the measurement of gastric mucosal blood flow, the two methods must be closely compared. Many estimations of amidopyrine in plasma and gastric juice were required. The method used, described below, was that of Brodie and Axelrod (1950).

2,32 Method

Samples, each of 1.0 ml, were added to bottles containing 0.5 ml MNaOH and 20 ml of 1,2-dichloroethane. The bottles were shaken for 10 min and centrifuged to separate the aqueous and lipid phases. The upper, aqueous, phase was removed by suction and discarded. The remaining dichloroethane was then washed twice with 5.0 mls of sodium borate (0.05M). The bottle was shaken for 1 min followed by centrifugation and removal of the supernatant each time. 15.0 ml of the dichloroethane was then pipetted into a clean bottle and 5.0 ml of 0.1 M HCL added. The mixture was shaken for five minutes and centrifuged. Approximately 3.0 ml of the supernatant acid was transferred into a quartz cuvette and its optical density measured at 260 nm using an ultravidet spectrophotometer (Unicam SP 1800). A reagent blank and a plasma blank

were generally prepared, free of amidopyrine, to ensure that no substances in the solutions used were interfering with the assay and that the extraction procedure removed any interfering substances from the sample.

2.4 MEASUREMENT OF GASTRIC ACID SECRETION

The secretion from gastric pouches was allowed to drain under gravity into a collection vessel. Secretion was collected over 5-20 min. the volume measured, and an aliquot titrated against 0.1 M NaOH with phenolphthalein as the indicator. Acid secretion was calculated in μEq H^+/min.

CHAPTER 3

VALIDATION OF THE RADIOACTIVE ANILINE CLEARANCE METHOD OF MEASURING GASTRIC MUCOSAL BLOOD FLOW

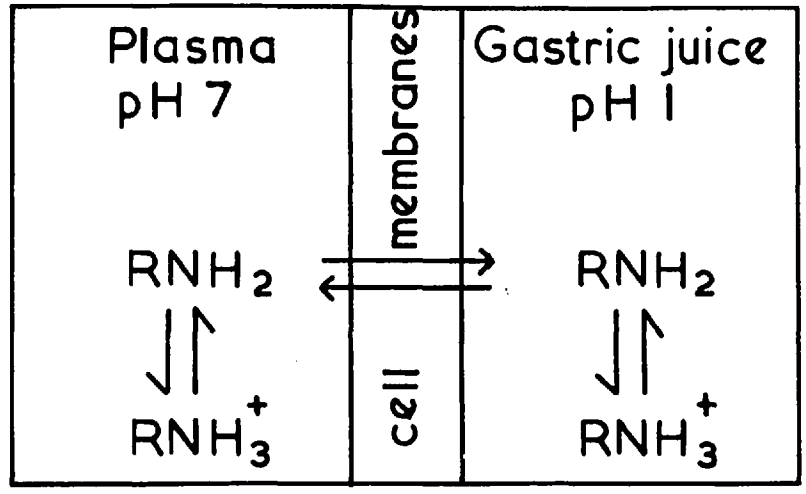
3.1. INTRODUCTION

In 1957 Shore, Brodie and Hogben considered the gastric secretion of several organic compounds. They demonstrated that weakly basic drugs, including aniline and amidopyrine, with a pKa of 5-10 were concentrated in the gastric juice after intravenous administration. They explained their findings by a pH partition hypothesis. At blood pH these bases are lipid-soluble and diffuse freely through cell membranes, including those of the gastric mucosa, but in acid gastric juice they gain a proton and become cations which are not free to diffuse back into the blood. At equilibrium the theoretical concentration ratio between the gastric juice and the plasma of a base with pKa = 5 is 10^4 but in-vivo the ratio attained is 30-40 (see fig. 3.1). It is postulated that, at this ratio, all the base is removed from the gastric mucosal blood in one passage. Hence the clearance of such substances can be used to measure gastric mucosal blood flow.

The use of amidopyrine clearance for the measurement of gastric mucosal blood flow was introduced by Jacobson, Linford and Grossman (1966) who performed an impressive series of experiments validating the technique. In his review, Jacobson (1968) discussed gastric mucosal clearances in detail and came to the conclusion that amidopyrine was the best available marker substance.

3.1. (Adapted from Shore et al. 1957). THE PARTITION OF ANILINE AND AMIDOPYRINE BETWEEN PLASMA AND GASTRIC JUICE.

Theoretical values and those observed of $\frac{G}{P} = R$ are given.



at equilibrium $\frac{G}{P} = \frac{1 + 10^{(pKa-1)}}{1 + 10^{(pKa-7)}}$

	pKa	G/P at equilibrium	G/P found
Amidopyrine	5	10^4	20-40
Aniline	5	10^4	20-40

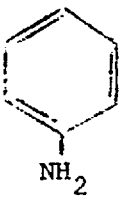
The availability of the amidopyrine clearance method has led to an explosion of interest in gastric mucosal blood flow in the conscious (Swan and Jacobson, 1967; Bynum and Jacobson, 1971; Cowley and Code, 1970) and in the anaesthetized animal (Harper et al., 1965. Moody, 1968; Reed and Sanders, 1971). Most workers agree that amidopyrine clearance is a valid measurement of gastric mucosal blood flow during acid secretion. However if the pH of the gastric content rises above 3.0 a significant proportion of amidopyrine will be present in the unionised form and so some back diffusion into the blood will take place. Moody (1968, 1972) has produced evidence that during inhibition of acid secretion by thiocyanate, amidopyrine clearance does not provide a true measurement of mucosal blood flow. This criticism applies equally to aniline.

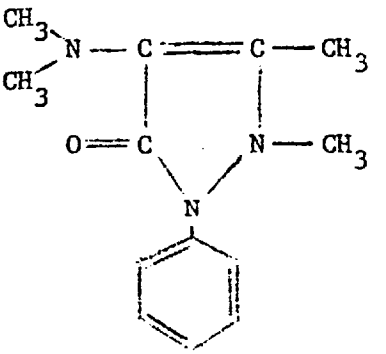
Since the estimation of amidopyrine is tedious (see Chap. 2.3) it was decided to attempt the use of aniline as a marker substance. This has a pKa of 5.0, like amidopyrine and is available with ^{14}C or ^{3}H labelling. It is easy to measure and does not depress bone marrow function. Unfortunately aniline metabolites are capable of forming methaemoglobin and this property prohibits its use in man. However the levels of methaemoglobin observed in the dogs were low and gave rise to no observable toxic effects; Curwain and Holton (1971) found that methaemoglobin reached a mean value of 6% after 4-5 hrs of aniline infusion. Results will

be presented showing that aniline has no effect upon gastric secretion in dogs. Aniline is soluble in organic solvents (Finar, 1961) and, being a weak base, relatively insoluble in alkaline aqueous solutions. It was decided to attempt its extraction from an aqueous solution of high pH into an organic solvent, suitable for liquid scintillation counting.

If ^{14}C-aniline is to be accepted as an alternative to amidopyrine as a gastric clearance marker, a detailed comparison of the results obtained by the two methods must be made. Also the effects of aniline administration in dogs and its behaviour both in vivo and during extraction and estimation must be evaluated. Some aspects of this have previously been published (Curwain & Holton, 1971; Curwain, 1972). In this chapter I describe in full the experiments performed to compare aniline with amidopyrine clearance and to validate ^{14}C-aniline clearance as a method of measuring gastric mucosal blood flow in conscious dogs.

3.2 CHEMISTRY AND METABOLISM OF ANILINE AND AMIDOPYRINE.





Aniline M.W. 93

pKa = 5.0

Amidopyrine M.W. 231

pKa = 5.0

Both aniline and amidopyrine are weak organic bases and, under acid conditions such as prevail in gastric juice, the amine group of aniline and the dimethyl amine fraction in amidopyrine accept a proton, conferring a positive charge on the molecule. Unlike the parent bases, the organic cations thus formed are insoluble in organic solvents and lipid cell membranes and accumulate in acid gastric juice.

Neither aniline nor amidopyrine can be used in man because of toxic effects. Aniline causes methaemoglobinaemia in animals (Spicer, 1950) and man (Polson and Tattersall, 1969) and amidopyrine, though once used as an analgesic and antipyretic, has been found to cause depression of bone marrow function and agranulocytosis (Brodie and Axelrod, 1950). Approximately 50% of an oral dose of amidopyrine (Brodie and Axelrod, 1950) and

aniline (Williams, 1959) can be accounted for in the urine. Brodie and Axelrod (1950) found that in man, the chief metabolites of amidopyrine appearing in the urine were 4-aminoantipyrine (8% of total dose) and its N-acetylated derivative (36% of the total dose). The extraction method devised by Brodie and Axelrod (1950) for amidopyrine includes 4-aminoantipyrine but not the acetylated compound.

Aniline is converted to ortho- and para-aminophenol. In the dog the ortho compound is predominant, 25% of the total dose appearing in the urine in this form (Williams, 1959). The remainder of the urinary excretion is made up of para-aminophenol and unchanged aniline in a free and conjugated form. The fact that dogs are relatively susceptible to the toxicity of aniline is probably due to the predominance of ortho-aminophenol which is thought to be the compound causing methaemoglobinaemia. Williams (1959) states that the lethal dose of aniline in dogs is approximately 200 mg kg^{-1}. The extraction of aniline described in chapter 2.2 does not extract the phenolic derivatives due to the high pH of the aqueous phase from which the aniline is removed.

3.3 METHODS AND RESULTS

3.31 Experiments to find a suitable system for the extraction of ^{14}C-aniline from plasma and gastric juice

(a) Selection of a suitable solvent

^{14}C-aniline hydrogen sulphate from the Radiochemical Centre, Amersham (code C.F.A. 71) containing 204 µCi/mg was used. Initially it was decided to dilute the sample to fifty times its original weight with 'cold' aniline sulphate so that the quantities used need not be too minute. The mixed sample was dissolved in sterile saline (0.9% NaCl) at a concentration of 5 µCi ^{14}C/ml (Solution A). Two solutions of ^{14}C-aniline were used to correspond with the expected concentrations of the substance in plasma and gastric juice.

Solution B = Solution A 1 ml
Sterile Saline to 5000 ml

Concentration = 1×10^{-3} µCi ^{14}C/ml

Solution C = Solution A 0.8 ml
Sterile Saline to 100 ml

Concentration = 4.0×10^{-2} µCi ^{14}C/ml

The Solvents selected for testing were

Diethyl ether - Laboratory Reagent Grade
Petroleum ether 100-120°C - Analar Reagent
Cyclohexane - Laboratory Reagent Grade
Benzene - Laboratory Reagent Grade

Solution B was extracted as follows. 3 ml samples were added to 1 ml MNaoH and 20 ml solvent. After 5 minutes' shaking, samples

of 2, 1 and 0.5 ml of the solvent phase were each added to 18 ml of scintillator (see chap. 2.2) and counted for 5-15 minutes. Solution C was extracted similarly but 0.5 ml instead of 3.0 ml was used, the aqueous phase of the extraction being made up to 4.0 ml with sterile saline. The extracts were compared with counts obtained by adding an equivalent amount of solution B or C directly to the scintillator.

RESULTS

^{14}C-aniline solution	Solvent	Sample Size (ml solvent)	Count expressed as % of Standard
B	Diethyl Ether	2.0	97.6
B	"	1.0	98.8
B	"	0.5	98.2
C	"	2.0	97.6
B	Benzene	2.0	95.7
B	"	1.0	96.7
B	"	0.5	101.0
C	"	2.0	94.3
B	Pet. Ether	2.0	83.6
B	"	1.0	83.6
B	"	0.5	86.2
C	"	2.0	82.7
B	Cyclohexane	2.0	80.4
B	"	1.0	80.6
B	"	0.5	66.1
C	"	2.0	77.4

Table 3.1. The extraction of ^{14}C-aniline from 4 ml alkaline aqueous phase into 20 ml of solvent.

Since these results show that diethyl ether extracted the aniline with consistently high efficiency, it was selected for further use.

(b) The effect upon ^{14}C-aniline recovery of altering the phase volume in the extraction and counting process

0.125 ml of solution C was added to 10 ml ether and 1.0 ml M NaOH. After 5 min.' shaking, 8.0 ml of the ether phase were added to 10 ml scintillator for counting. The standard was 0.1 ml solution C in 10 ml scintillator + 8.0 ml ether.

RESULTS

Extraction: 10 ml ether + 4 ml aqueous pH > 11

Counting: 10 ml scintillator + 8 ml ether

Recovery (single determination) = 102% of standard

The counts obtained in this experiment, together with those from the previous section, showed that 8 parts of ether may be added to 10 parts of scintillator without significantly reducing counting efficiency. Following this experiment, the extraction procedure detailed in chapter 2.2 was adopted. This involves adding 7 ml ether to 10 ml scintillator.

3.32 The extraction of aniline and amidopyrine from plasma and gastric juice

Having decided on a method of extracting aniline it was necessary to quantify its recovery from plasma and gastric juice. Since a comparison with amidopyrine clearance was to be made it was decided to evaluate, in this laboratory, amidopyrine extraction

by the method of Brodie and Axelrod (1950). This method is described in chapter 2.3. ^{14}C-aniline was estimated as described in chapter 2.2.

Since preliminary experiments with aniline had indicated that recovery from saline was complete, recovery of the two bases from plasma and gastric juice was compared with recovery from saline. Plasma and gastric juice were taken from pouch or fistula dogs which had not received aniline or amidopyrine for at least a week. A minimum of five determinations was made in each experiment. The concentration of aniline or amidopyrine in the recovery experiments was approximately equal to that found in the plasma during clearance experiments. In plasma recovery experiments a solution of amidopyrine 400 µg/ml in saline or aniline 70 µg/ml containing 0.0875 µCi ^{14}C/ml was used. Each solution was added to ten times its own volume of plasma or saline from which the base was estimated. For aniline recovery from gastric juice a solution of 875 µg/ml containing 0.05 uCi ^{14}C/ml was used.

RESULTS

	Aniline		Amidopyrine	
	Plasma	Gastric juice	Plasma	Gastric juice
Recovery %	96	98	86	98
s.d %	± 1.17	± 1.0	± 3.16	± 6.2
n	39	6	25	6

Table 3.2 Recovery of aniline and amidopyrine from gastric juice and plasma expressed as a percentage of the recovery from saline.

An analysis of variance of the results of seven groups of aniline extractions from plasma, containing 33 individual estimations, and a similar number of amidopyrine estimations, showed that the variance of the aniline measurements was significantly smaller than that of amidopyrine ($F = 8.9$ $P < 0.01$) - see appendix 1.1.

An experiment was also devised to test whether any substances, extracted from alkaline plasma into ether caused quenching of ^{14}C emissions and a reduction in counting efficiency. 4 ml of fresh canine plasma free from aniline was shaken for 5 minutes with 40 ml ether and 2 ml M NaOH. Five samples of 6 ml of the ether were added to bottles containing 10 ml scintillator. To each bottle was added 1 ml of an ether extract of ^{14}C-aniline. Controls were provided by replacing the ether extract of plasma

with clean ether. The samples were counted for 20 minutes for ^{14}C radioactivity.

RESULTS

	Standard	Ether Extract of plasma
	11234	11296
	11342	11300
	11209	11426
	11150	11478
	11050	11350
	11396	
Mean	11230	11370
Percent of standard	100.0	101.2
	± 0.46% s.e	± 0.31% s.e

Table 3.3 Experiment to establish whether plasma contents, extracted with aniline, quench the ^{14}C radioactivity. Aniline concentration is expressed as net c.p.m. ^{14}C radioactivity.

A t test showed the difference between the groups to be insignificant ($P > 0.05$). From these results it is clear that the observed small reduction of aniline recovery from plasma, compared with controls, is not due to quenching.

3.33 The rate of equilibration of aniline and amidopyrine between erythrocytes and saline and the distribution of the bases between erythrocytes and plasma

(a) Rate of equilibration

If aniline or amidopyrine are completely removed from the blood in one passage through the gastric mucosal circulation, very rapid diffusion from erythrocytes to plasma must take place. The following experiment provides evidence that this is the case. Saline equivalent to the original plasma volume was mixed with the packed cells from blood to which aniline or amidopyrine had been added. The cells were suspended in the saline and an aliquot immediately centrifuged. The supernatant was then examined within approximately two minutes of mixing and aniline or amidopyrine assayed. Further centrifugations were carried out at longer intervals after suspension. Capillary circulation time is much less than two minutes but this was the minimum in which suspension and centrifugation of the red cells could be achieved. Experiments were carried out at 37°C.

RESULTS

Time (min)	Extracellular concentration (optical density at 260 nm)
Amidopyrine - Dog's Blood. Mean of 3 determinations.	
2.25	0.066
4.5	0.062
6.5	0.062
Amidopyrine - Cat's Blood. Mean of 2 determinations.	
2.0	0.064
3.5	0.058
6.25	0.058
Aniline - Dog's Blood. Mean of 2 determinations.	
	net c.p.m. ml^{-1}
1.75	2887
5.25	2896
7.08	2825
9.08	2922

Table 3.4 The equilibration of aniline and amidopyrine from erythrocytes containing one of the bases into saline free of either base.

The results show that the distributions of aniline and amidopyrine reach equilibrium between blood cells and saline within two minutes.

(b) Distribution between erythrocytes and saline.

The distribution of aniline and amidopyrine between red blood cells and plasma was calculated from the initial plasma concentration in blood used in the equilibration studies and the extracellular concentration after equilibration.

Example calculation:

Initial plasma concentration of ^{14}C-aniline = 6222 c.p.m. ml^{-1}

Assuming 96% recovery, actual aniline concentration = 6480 c.p.m. ml^{-1}

Observed extracellular concentration at equilibrium = 2880 "

Volume of cells in equilibrium reaction = 9 ml

Volume of saline " " " = 11 ml

Expected extracellular concentration of aniline at equilibrium assuming equal distribution

$$= \frac{6480 \times 9}{(11 + 9)} = 2910 \text{ c.p.m./ml}$$

$$\frac{\text{Observed aniline concentration}}{\text{Expected " "}} = \frac{2880}{2910} = 99\%$$

RESULTS

Substance	Sample		Expected concentration. $n_E=4$	Observed concentration	n_0	$\frac{O}{E} \times 100$
Aniline	Dog's Blood	R.T.	2940	2630	4	89
Aniline	"	37°	2910	2880	6	99
Amidopyrine	"	37°	0.117	0.065	9	56
"	Cat's Blood	37°	0.088	0.060	6	68
* "	Dog's "	37°	0.111	0.084	9	76

* The amidopyrine was equilibrated from erythrocytes into amidopyrine-free plasma instead of saline.

Table 3.5. Distribution of aniline and amidopyrine between erythrocytes and saline. Aniline concentrations are expressed as net c.p.m. ^{14}C radioactivity and amidopyrine concentrations as optical density at 260 nm.

3.34 Experiments to examine the acute effects of ^{14}C-aniline in dogs in the doses used for clearance experiments

(a) Measurement of urinary excretion of ^{14}C-aniline

Clearly it is important to know the rate of excretion of ^{14}C-aniline after a clearance experiment. On three occasions therefore, when an experiment was over and the dog placed in a metabolism cage, the urine was collected daily for three days and 0.3 ml aliquots were counted by adding them directly to 18 ml of scintillator. Daily urinary excretion of ^{14}C was estimated as a percentage of the total dose given during the experiment. This estimate also included most of the ^{14}C cleared in the pouch secretion.

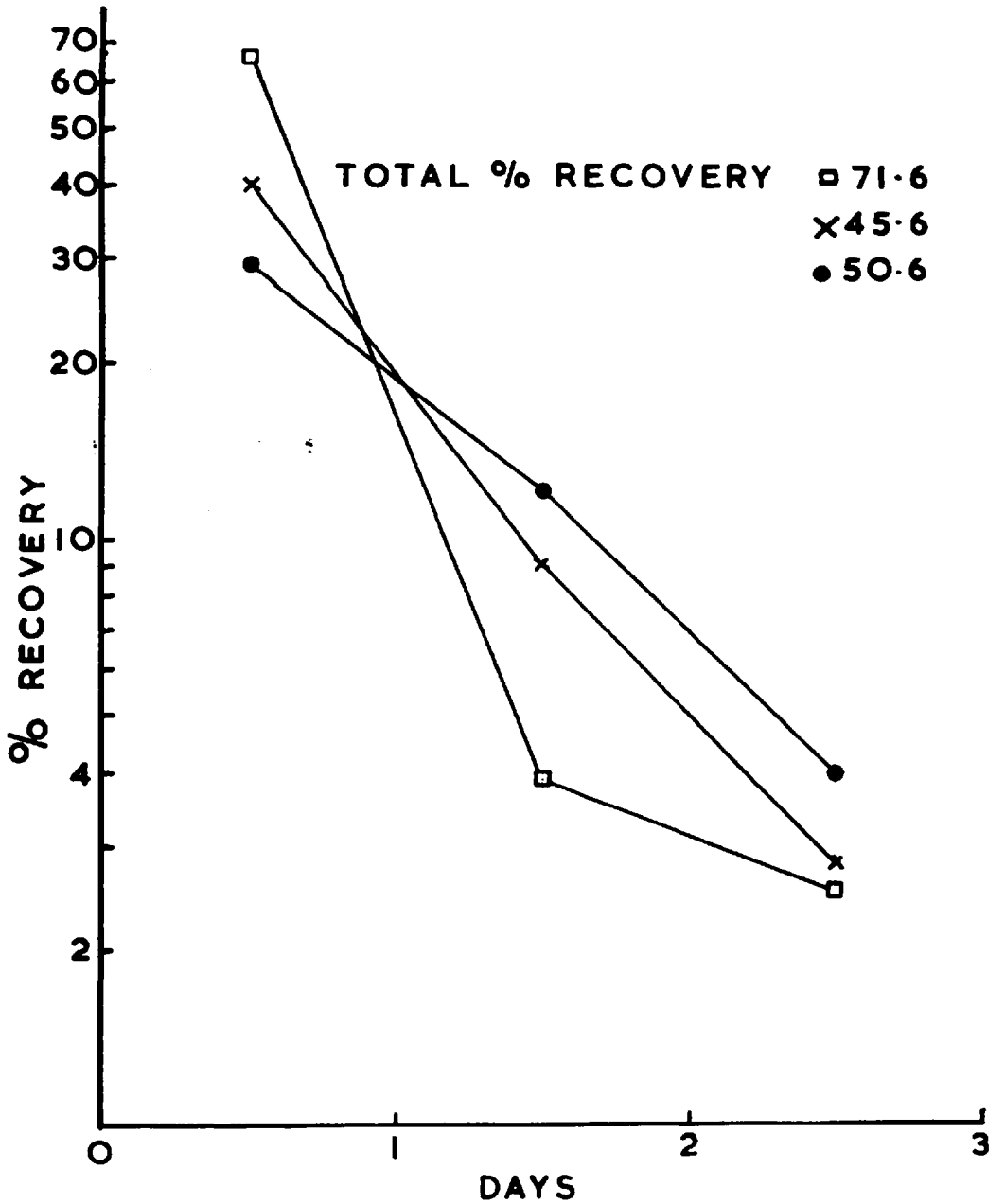
RESULTS

(a) The results are shown in fig. 3.2. Over the first three days 45.6-71.6% of the total ^{14}C administered appeared in the urine and pouch secretion. On the third day, ^{14}C excretion was only 2.5-4% of the total given, corresponding to between 1 and 3 µCi ^{14}C leaving the dog by this route.

3.2. EXCRETION OF ^{14}C-RADIOACTIVITY IN CANINE URINE (+POUCH SECRETION) FOR THREE DAYS FOLLOWING AN ANILINE CLEARANCE EXPERIMENT.

Aniline recovered from the urine is expressed as a percentage of the total dose given during the clearance experiment.

EXCRETION OF ^{14}C IN URINE. (+ POUCH SECRETION.)



(b) The effect of aniline on gastric acid secretion

For aniline clearance to be useful in studies of gastric secretion and mucosal blood flow, it is necessary to demonstrate that it has no effect upon secretion. This was done in two types of experiment. In one series, two dogs with separated pouches were given a standard meal on four or five occasions and the secretory response measured. After one of the test meals a loading dose (10 mg Kg^{-1}) and an infusion (10 mg $Kg^{-1}hr^{-1}$) of aniline were given and the secretion compared with the control experiments. The effect of aniline on histamine-induced gastric acid secretion was also investigated in two dogs with separated pouches. Histamine acid phosphate 2 or 4 µg $Kg^{-1}min^{-1}$ was given intravenously until a plateau of secretion had been maintained for at least an hour. A dose of aniline (10 mg Kg^{-1}) was given and an infusion of 10 mg Kg^{-1} was added to the histamine and the acid secretion measured.

RESULTS

(b) Aniline in the dose used for clearance experiments had no effect upon the acid secretory response of a Heidenhain pouch to a standard meal (see fig. 3.3). Similarly, histamine-induced acid secretion was unaffected by an aniline infusion (see fig. 3.4).

Fig. 3.3. THE EFFECT OF AN ANILINE INFUSION ON THE RESPONSE OF A HEIDENHAIN POUCH TO A STANDARD MEAL.

The broken line denotes an occasion during which a loading dose (10 μg Kg^{-1}) and an infusion (10 mg $Kg^{-1}h^{-1}$) of aniline were given. Secretion was clearly no different from the three control experiments in which no aniline was given.

Acid secretion from a separated pouch on 4 occasions

___ control.

----- during aniline infusion.

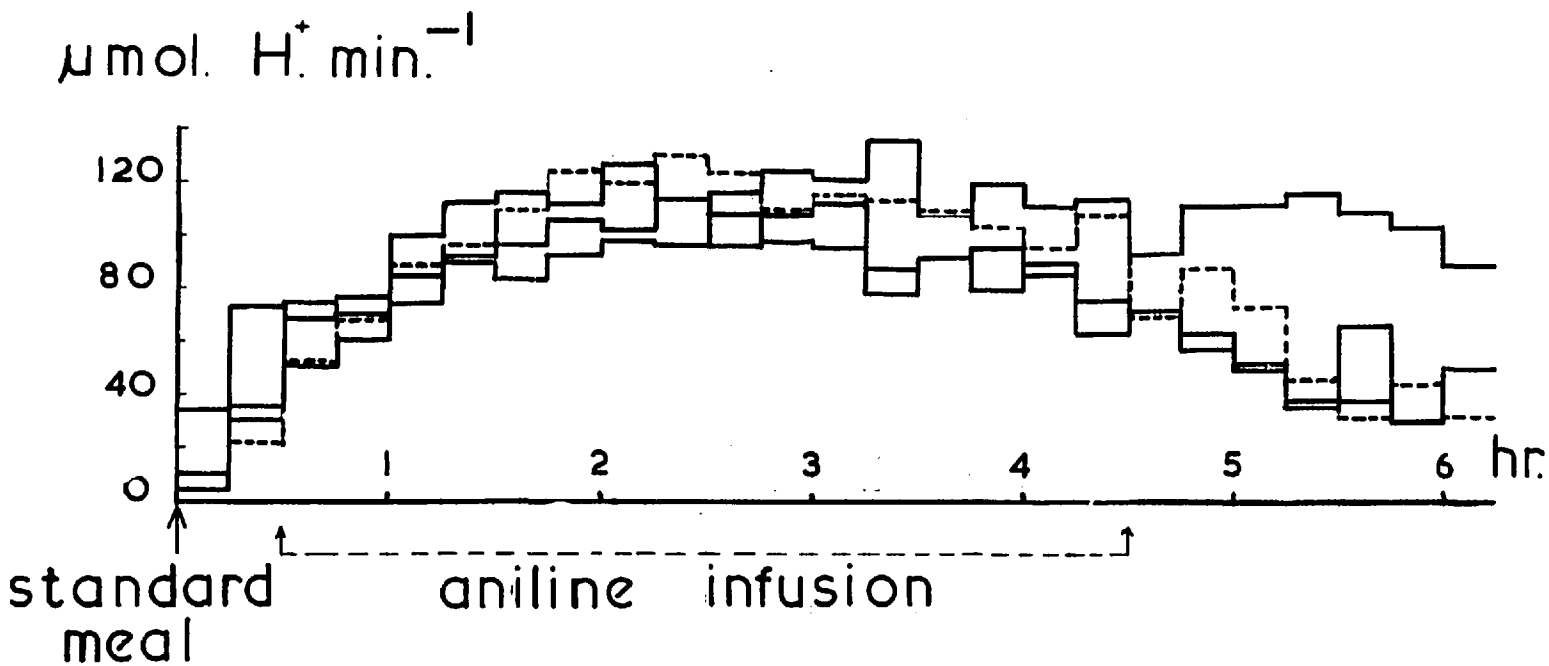
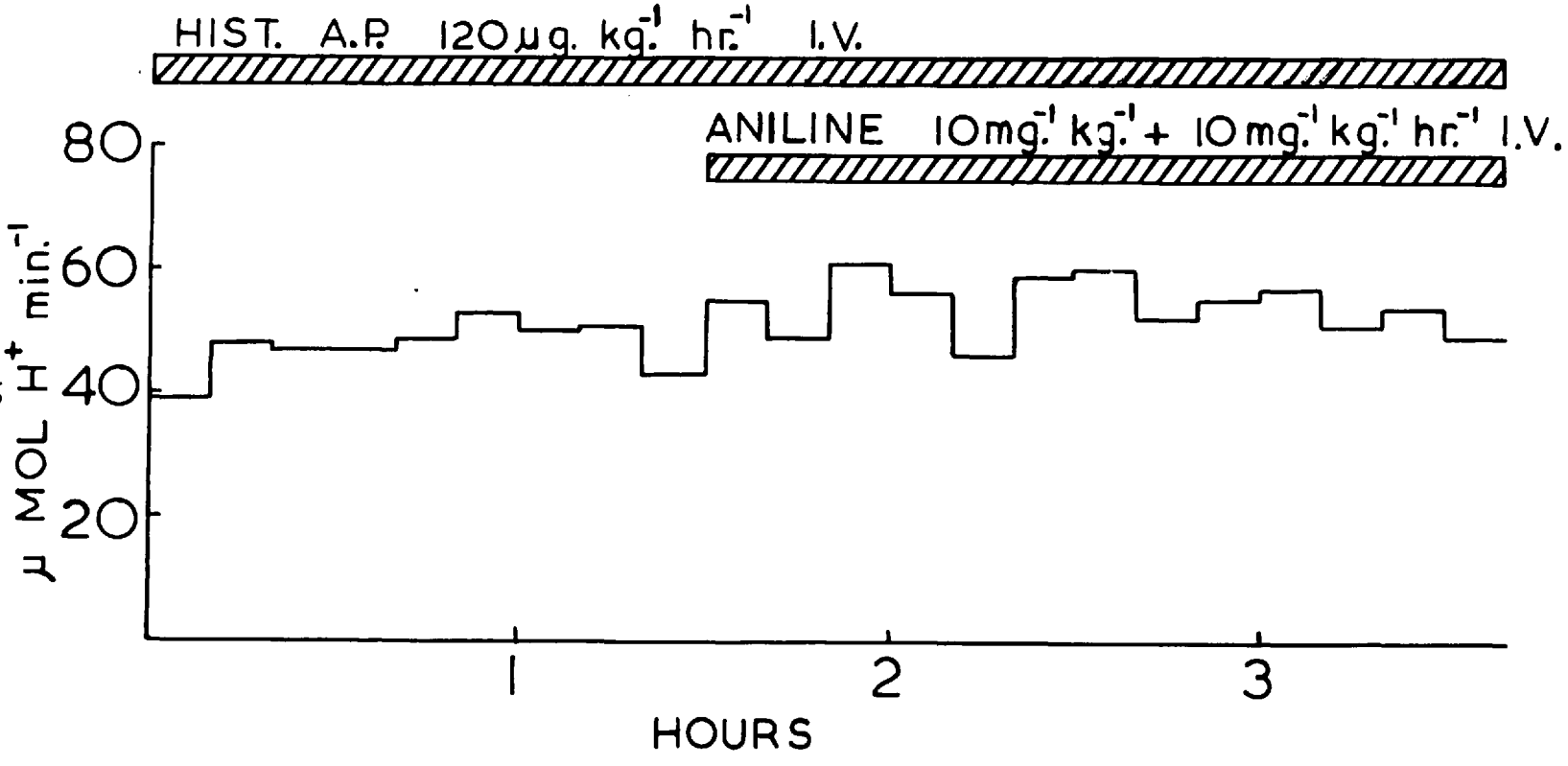


Fig. 3.4. THE EFFECT OF ANILINE ON HISTAMINE-INDUCED ACID SECRETION FROM A HEIDENHAIN POUCH.

Histamine acid phosphate was given throughout and an aniline loading dose and infusion, as described in Fig. 3.3, had no effect on the rate of acid secretion.

THE EFFECT OF ANILINE ON HISTAMINE INDUCED ACID SECRETION FROM A SEPARATED GASTRIC POUCH.



3.35 Pilot clearance experiments with ^{14}C-aniline

Early experiments were directed towards finding the required doses of radioactive and carrier aniline that were necessary to produce the stable plasma levels of radioactivity required for meaningful clearance experiments. When this had been done, measurements of aniline clearance were made during increasing histamine or pentagastrin stimulation of gastric secretion. Jacobson, Linford and Grossman (1966) reported similar experiments with amidopyrine clearance. A histamine (1 µg $Kg^{-1}min^{-1}$) or pentagastrin (2 µg $Kg^{-1}hr^{-1}$) infusion was given, together with aniline and the stimulus was doubled every hour until five doses had been given. Aniline clearance was measured throughout. Seven experiments were carried out, in three Heidenhain pouch dogs.

RESULTS

The results of two experiments are shown in figs. 3.5 and 3.6. These results are very similar to those reported by Jacobson et al. (1966) in that mucosal blood flow was observed to rise as the rate of secretion increased.

Fig. 3.5. PILOT CLEARANCE EXPERIMENTS WITH ^{14}C-ANILINE. I.

Mucosal blood flow (MBF ml min^{-1}) and acid secretion (μEq H^+ min^{-1} = μ mol H^+ min^{-1}) in a Heidenhain pouch measured during increasing histamine stimulation. MBF. measured by aniline clearance, was clearly related to acid secretion.

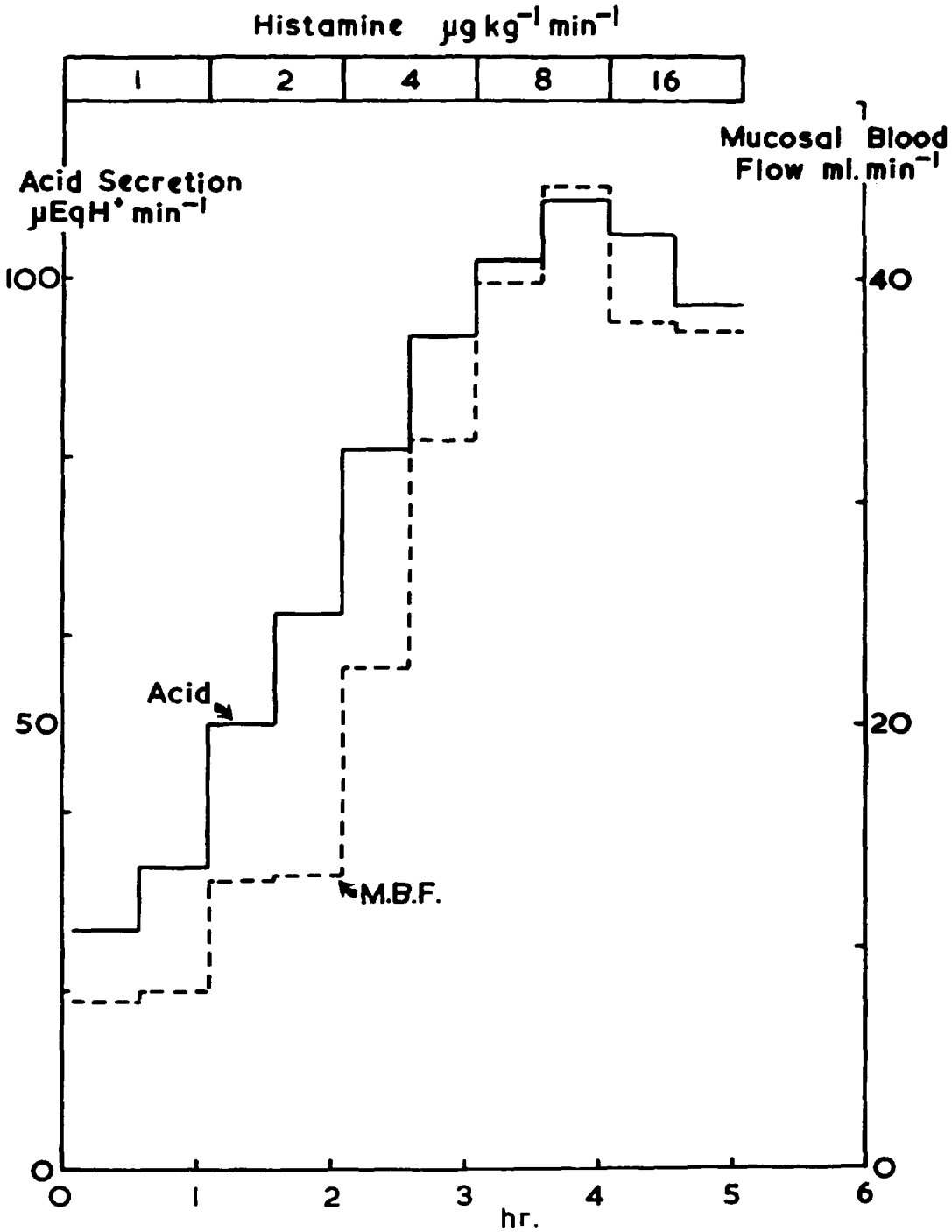
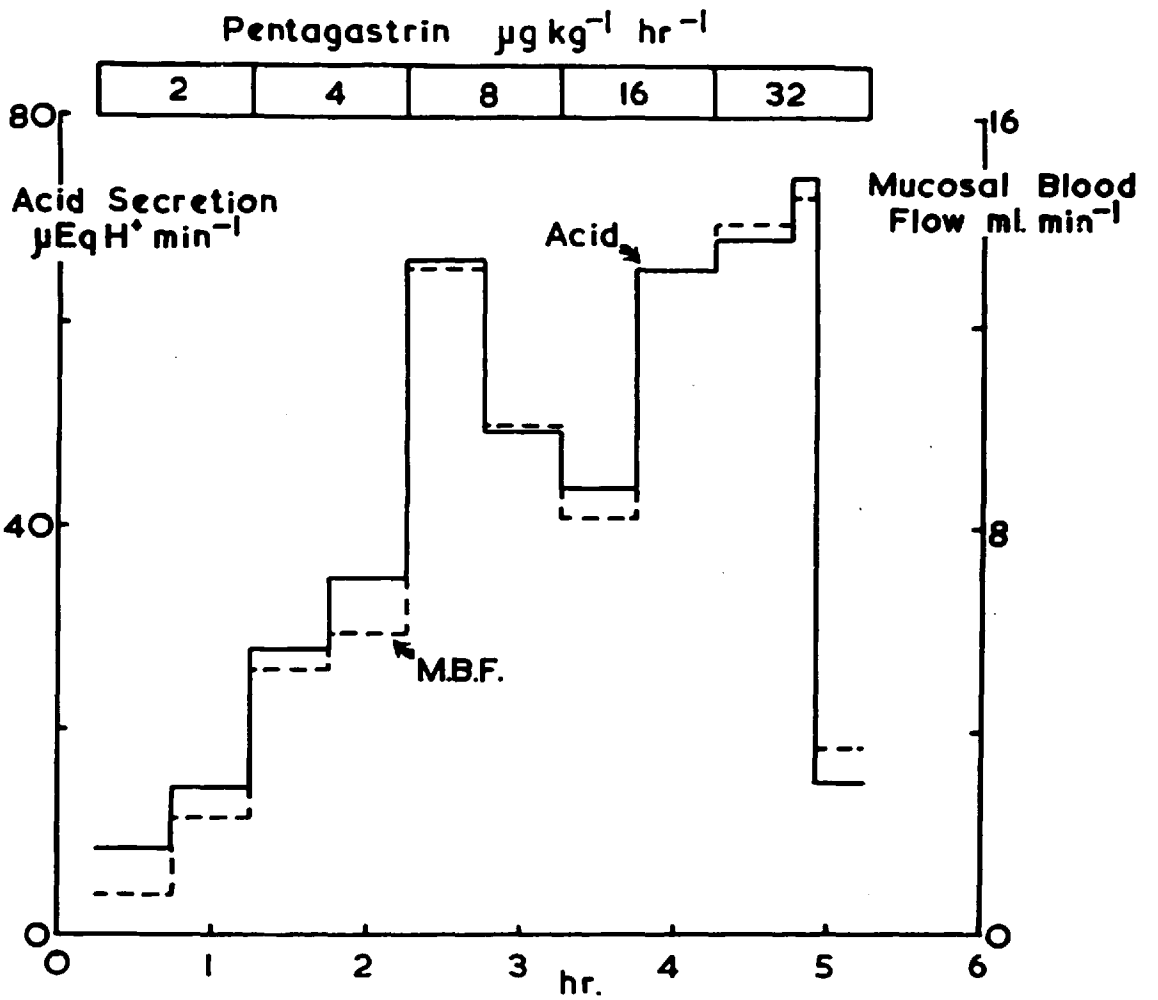

Histamine μg kg⁻¹ min⁻¹
1
2
4
8
16
Acid Secretion μEqH⁺ min⁻¹
Mucosal Blood Flow ml. min⁻¹
100
50
0
40
20
0
Acid
M.B.F.
0
1
2
3
4
5
6
hr.

Fig. 3.6. PILOT CLEARANCE EXPERIMENTS WITH ^{14}C-ANILINE. II.

Mucosal blood flow (MBF) and acid secretion in a Heidenhain pouch measured during increasing pentagastrin stimulation. MBF, measured by aniline clearance, was clearly related to acid secretion.


Pentagastrin μg kg⁻¹ hr⁻¹
2
4
8
16
32
80
40
0
Acid Secretion
μEq H⁺ min⁻¹
Acid
M.B.F.
16
8
0
Mucosal Blood
Flow ml. min⁻¹
0
1
2
3
4
5
6
hr.

3.36 A comparison of aniline and amidopyrine clearance during the response of conscious dogs with Heidenhain gastric pouches to a standard meal

(a) First series of experiments

In this study both bases were infused intravenously together as a loading dose (amidopyrine 20 mg Kg^{-1}, aniline 10 mg Kg^{-1}) and a maintenance infusion (amidopyrine 5 mg $Kg^{-1}hr^{-1}$, aniline 10-12 mg $Kg^{-1}hr^{-1}$). Acid gastric secretion was induced by a standard meal consisting of cooked, minced ox liver 20 gm Kg^{-1} + 10% w/w calcium orthophosphate. A little sodium chloride and water was added to each meal. Effective clearance of aniline or amidopyrine requires an acid gastric juice and if the pouch did not have a small 'spontaneous' secretion at the beginning of the experiments, a very small dose of pentagastrin (0.5 µg $Kg^{-1}hr^{-1}$) was given and maintained throughout. This enabled the clearance to be accurately measured at the time the meal was being eaten. Seven experiments were performed in four dogs.

RESULTS

The results of two of the seven experiments are shown in figs. 3.7 and 3.8. Clearance of both bases were found to be very closely correlated with each other and with the acid secretory response to food. Aniline clearance was, however, somewhat lower than clearance of amidopyrine.

Fig. 3.7. GASTRIC MUCOSAL BLOOD FLOW IN A HEIDENHAIN POUCH AFTER FEEDING, MEASURED SIMULTANEOUSLY BY ANILINE AND AMIDOPYRINE CLEARANCES. I.

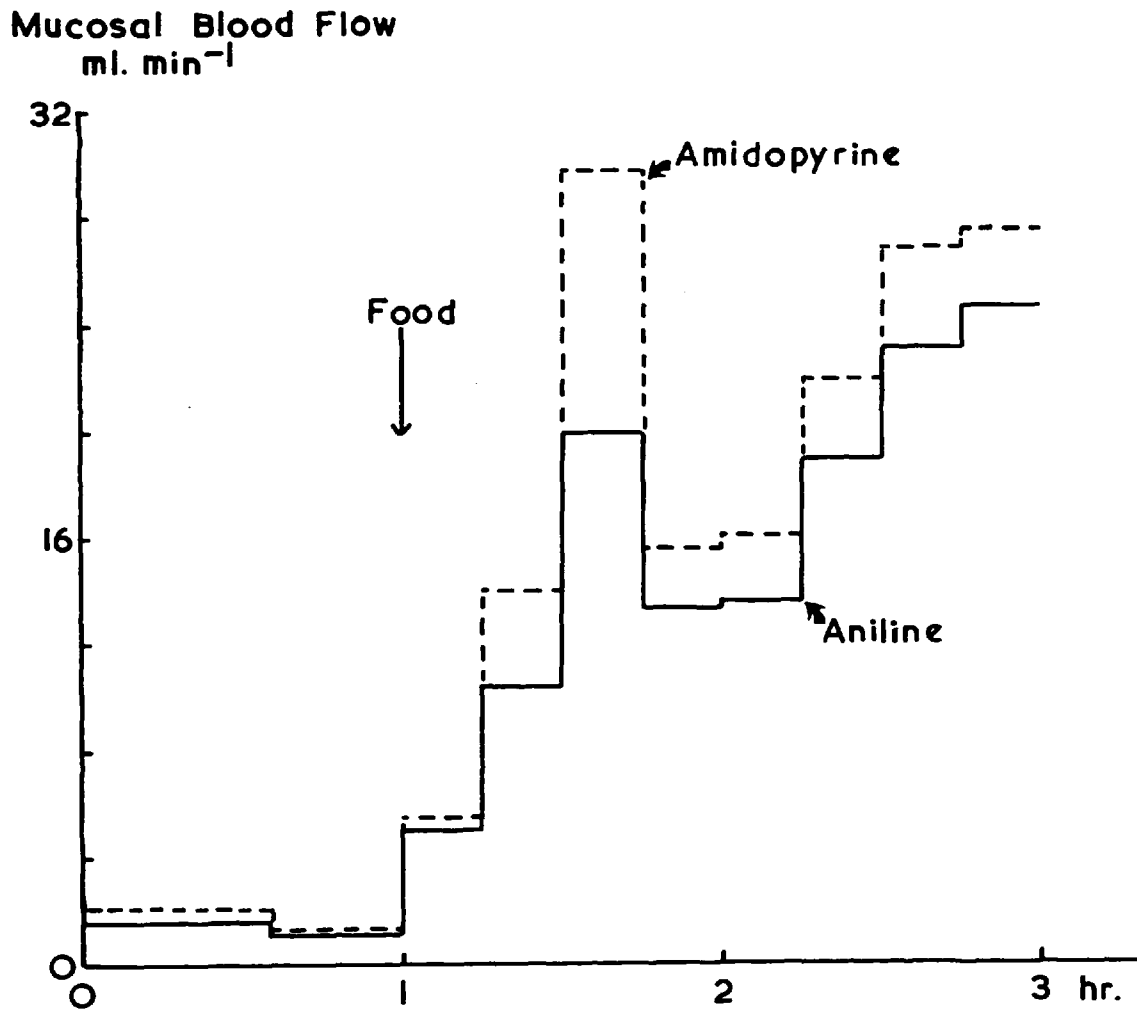
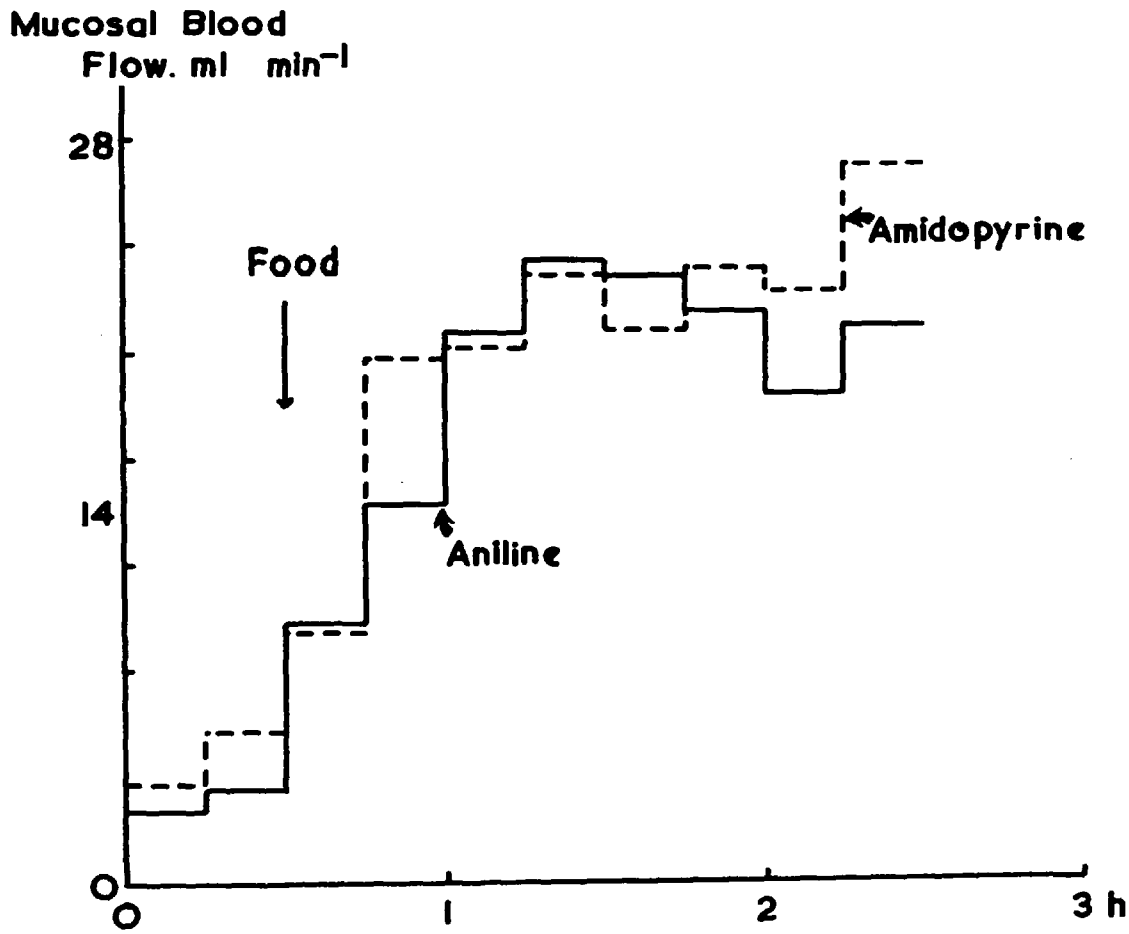

Mucosal Blood Flow
ml. min⁻¹
32
16
0
0
1
2
3 hr.
Amidopyrine
Food
Aniline

Fig. 3.8. GASTRIC MUCOSAL BLOOD FLOW IN A HEIDENHAIN POUCH AFTER FEEDING, MEASURED SIMULTANEOUSLY BY ANILINE AND AMIDOPYRINE CLEARANCES. II.


Mucosal Blood
Flow. ml min⁻¹
28
14
0
0
1
2
3 hr
Food
Amidopyrine
Aniline

The ratios (R) of the concentrations of aniline or amidopyrine in gastric juice and plasma were compared when secretion was steady. It was found from 31 observations that R for aniline was 74% of the amidopyrine value. Standard deviation of $\frac{R(aniline)}{R(amidopyrine)}$ was 19.4% but correlation for observations made within a single experiment were much higher (root mean square of s.d. = 7.0%). Significant differences were noted both between dogs ($P < 0.001$) and between different occasions in the same dog ($P < 0.001$). The full experimental data are given in Table 3.6 and the statistical calculations are shown in appendix 1.2.

Dog	Grocer		Peta		Alex		Chris
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1
	82.1	86.2	103.6	61.8	64.3	70.0	64.7
	65.8	92.5	100.0	66.7	68.3	64.3	63.9
	65.4	94.1	111.5	68.2	71.8	61.7	59.0
	67.1	106.5		68.9	70.7	82.5	
		78.6		68.2		83.3	
						83.3	
						63.2	
mean	70.1	91.6	105.0	66.8	68.8	72.6	62.5
± s.d	8.0	10.3	5.9	2.9	3.3	10.1	3.1

Table 3.6 $\frac{R(aniline)}{R(amidopyrine)}$, expressed as a percentage, in Heidenhain pouch dogs after feeding. Each observation is the result of gastric juice collected over 15 min., analysed for aniline and amidopyrine and compared with the mean plasma concentration of each substance during this time.

(b) Second series of experiments

It became apparent that, under certain conditions, an increased recovery of amidopyrine from plasma, approaching 100% could be obtained. Recoveries of this order were reported by Harper et al. (1968) and it was discovered that, with vigorous shaking during the extraction into dichloroethane, this could be achieved. It was decided to repeat some experiments of the type described in 3.36(a) infusing both aniline and amidopyrine together and measuring both clearances simultaneously. The extractions were checked for efficiency by measuring the recovery of amidopyrine from plasma at the same time as carrying out the experimental estimations. Four experiments were performed in three Heidenhain pouch dogs. A plateau of secretion was obtained with histamine or pentagastrin and pouch secretion collected every 15 minutes. 6-8 serial determinations of the clearances were made.

RESULTS

The clearances of aniline and amidopyrine were compared as before - 3.36(a). The ratios of the concentrations of aniline and amidopyrine in the gastric juice and plasma (R) were calculated for each observation. R(aniline) was then expressed as a percentage of R(amidopyrine). The results are shown in Table 3.7.

Dog	Trudi	Peta	Peta	Minty
	51.3	60.2	69.0	76.7
	54.7	61.8	70.6	78.9
	57.2	55.8	60.3	80.2
	72.6	70.0	56.4	76.7
	63.5	61.0	74.4	71.1
	54.7	57.5	53.3	80.8
	51.6	54.2	68.5	
			65.8	
mean	57.9	60.1	64.8	77.4
$\pm$ s.d.	7.65	5.18	7.75	3.53

Overall mean = 64.5% $\pm$ 1.78% (s.e.) (n = 28)

Table 3.7. $\frac{R(aniline)}{R(amidopyrine)}$ expressed as a percentage, in Heidenhain pouch dogs during steady secretion in response to histamine or pentagastrin. Each observation is the result of gastric juice collected over a 15 min. period, analysed for aniline and amidopyrine and compared with the mean plasma concentration of each substance during this time.

The results of the first and second series of experiments were compared by a t test. The ratio $\frac{R(aniline)}{R(amidopyrine)}$ was found to be significantly lower ($P < 0.001$) in the second series. The second series of experiments were felt to be less reliable for a number of reasons, which will be mentioned in the discussion. Although recovery of amidopyrine from the plasma was measured during the experiments, a very limited number of samples could be tested. This was because the plasma available was limited to that which could reasonably be obtained from the dogs before an experiment. Single

determinations of recovery were made at three amidopyrine concentrations designed to bracket the expected plasma level in the experiment. Measured recovery of amidopyrine from plasma varied between 94.51% and 126.41% and it is clear from this that errors in the extraction procedure must have been made.

3.37 Experiments to investigate the observed difference between the clearances of aniline and amidopyrine

(a) Measurement of gastric venous concentration of ^{14}C-aniline and amidopyrine in anaesthetized dogs

Although it is not possible to isolate gastric mucosal venous drainage, clearance of aniline and amidopyrine from the mucosal circulation would be expected to lead to a detectable difference between the concentrations of the two bases in arterial and gastric venous blood. Four anaesthetized puppies weighing 5-8 Kg were used to investigate this.

METHOD

Anaesthesia was induced with intravenous pentobarbitone (30 mg Kg^{-1}) and maintained with further doses as required. After tracheal intubation, bilateral splanchnectomy was performed. The abdomen was opened and separated pouches were constructed from the greater curvature. In two of these, which will be referred to as mucosal pouches, the muscle layers of the pouch were carefully

removed using a diathermy after infiltration of noradrenaline. A cannula was secured into the pouch and externalised through a stab wound in the body wall. The spleen was removed and a polythene catheter was inserted into the splenic vein so that its tip lay at the junction with the vein draining the greater curvature of the pouch. Gentle suction permitted gastric venous blood to be sampled. The abdomen was closed and ^{14}C-aniline and amidopyrine were infused together through a catheter in the femoral vein as described in the previous section. Acid secretion was induced by histamine acid phosphate 4 $\mu g\ Kg^{-1} min^{-1}$ added to the infusion. Arterial blood was sampled at intervals from a catheter in the femoral artery

RESULTS

Aniline and amidopyrine concentrations in the gastric venous blood were expressed as a percentage of arterial concentration. The mean venous level taken from 22 observations during acid secretion in four dogs is 37% of arterial concentration for aniline and 27% for amidopyrine. The full data are shown in Table 3.8.

The ratio of the two percentages $\frac{\text{Amidopyrine}}{\text{Aniline}}$ was calculated for each observation (see Table 3.9). This function is directly related to $\frac{R(\text{aniline})}{R(\text{amidopyrine})}$ described in the previous section since it refers to the amount of the substance remaining in the venous blood rather than the amount cleared into the gastric juice.

The data was classified as unbalanced two factor hierarchical (Moore and Edwards, 1965), and an analysis of variance between and within dogs and between the mucosal pouches and those with intact muscle layers was made. The differences between dogs and between the two preparations were found to be not significant. The mean value for the ratio $\frac{\text{Amidopyrine}}{\text{Aniline}}$ was found to be 74.5%. The statistical calculations are shown in Appendix 1.3.

Experiment	Aniline	Mean	Amidopyrine	Mean
Mucosal 1	36 36 36 36 36 36 37 37 39	36.5	28 27 26 26 26 26 26 27 27	26.5
Mucosal 2	36 36 38 42	38.0	29 29 29 29	29.0
Muscle 1	29 29 29 30 30	29.4	20 21 21 21 21	20.8
Muscle 2	45 44 44 43	44.0	44 37 32 24	34.3
Mean	37	37	27	28

Table 3.8 Measurement of gastric venous concentrations of aniline and amidopyrine, expressed as percentages of arterial concentration, in anaesthetized puppies.

Experiment	Amidopyrine / Aniline
Mucosal 1	73%
Mucosal 2	76%
Muscle 1	71%
Muscle 2	78%
Mean	74.5%

Table 3.9 The ratio of amidopyrine and aniline gastric venous concentrations expressed as percentages of arterial levels in anaesthetized puppies.

The results from this series of experiments show that the clearance of amidopyrine across the gastric circulation is slightly greater than the aniline clearance.

(b) An examination of the plasma binding of aniline

It seemed likely that the fraction of aniline that was bound to plasma proteins, was not available for clearance across the mucosa although it was contained in my estimates of plasma aniline concentration. Shore, Brodie and Hogben (1957) stated that 25% of aniline in plasma was bound to proteins. I set out to repeat this estimation and to investigate the variation between dogs. Two methods were used to measure binding, both involving the

use of Visking dialysis membrane which is permeable to small molecules such as aniline but impermeable to proteins.

(i) Measurement of plasma binding by equilibrium dialysis

Blood was taken with sterile precautions from the dogs, heparinised and centrifuged. To 2 ml of plasma was added 0.1 ml of a solution containing 3.5 mg aniline ml^{-1} and 0.2 µCi ^{14}C ml^{-1}. The mixture was then placed inside a sterile Visking membrane and dialysed against 38 ml of sterile saline. Dialysis was carried out at -25 mm Hg. This pressure approximated to the osmotic pressure of the plasma proteins and ensured that net movement of water across the membrane was minimal. It was assumed that the free aniline was in dynamic equilibrium with the bound aniline and that the free aniline could diffuse across the membrane. Thus at equilibrium, the bound fraction is given by the difference in aniline concentration across the membrane, divided by the concentration (bound + free) inside the membrane. The amount of aniline used was adjusted so that the final concentration inside the membrane was within the range of plasma concentration found during clearance experiments. Dialysis was carried out overnight at $4^{o}C$ and then for 8 hr at room temperature. Five 1.0 ml samples were taken for counting from outside the membrane and three 0.5 ml samples from the plasma inside. A control experiment was carried out with saline

instead of plasma inside the membrane.

RESULTS

A control experiment showed no significant difference in aniline concentration inside and outside the membrane ($P > 0.05$). Six dialysis experiments were carried out each using plasma from a different dog. In each case the concentration of aniline inside the dialysis membrane was significantly greater than the concentration outside ($P < 0.001$). The results are summarised in Table 3.10. Standard deviations were calculated from the formula of Armitage (1971) for the variance of a ratio.

Dog	% of total plasma aniline bound $\pm$ s.d
Scruffy	26.3 ± 3.4
Rip	18.5 ± 2.4
Peta	29.1 ± 1.2
Jo	34.5 ± 3.0
Blackie	30.6 ± 2.4
Minty	21.3 ± 3.0
Mean	26.7 ± 2.4 s.e.

Table 3.10 Plasma binding of aniline in dogs measured by equilibrium dialysis.

The mean result of 26.7% of aniline bound in the plasma agrees well with the figure of 25% given by Shore et al. (1957)

(ii) Measurement of plasma binding by ultrafiltration

A modification of the method described by Toribara, Terepka and Dewey (1957) was used. Visking dialysis membrane was boiled for an hour in distilled water. A knot was tied in one end and 7 mls of fresh canine plasma at 37°C and 0.1 ml of a ^{14}C-aniline solution containing 0.5 mg ml^{-1} total aniline were introduced into the bag which was sealed by tying a knot in the other end and placed on a coarse sinter in a centrifuge tube. After equilibration for 30 minutes at 37°C the bag was centrifuged for 90 minutes at 2000 r.p.m. and 37°C in an M.S.E. 6L centrifuge. This manoeuvre produced approximately 1 ml of filtrate from which two 0.5 ml samples were taken for counting. The contents of the bag, well mixed to ensure homogeneity, were also sampled and the percentage of aniline bound to plasma protein was calculated from the following formula

$$\% \text{ Bound} = \frac{B - F}{B} \times 100$$

where B is concentration of aniline inside the membrane and F is concentration of aniline in the filtrate.

The filtrate was tested for albumin by addition of 3% sulphosalicylic acid and, if a precipitate showed albumin to be present, the result was discarded. Control experiments were carried out with saline instead of plasma inside the bag.

RESULTS

In each of three control experiments, the concentration of aniline in the ultrafiltrate was found to be not significantly different from the concentration inside the membrane ($P > 0.05$). Seven experiments were performed on four dogs. Two results were discarded when albumin was detected in the ultrafiltrate. The other five results are shown in Table 3.11. Standard deviations were calculated from the formula of Armitage (1971).

Dog	% of total plasma aniline bound $\pm$ s.d.
Minty	21.1 ± 2.0
Peta	22.2 ± 0.7
Scruffy	19.4 ± 2.7
Scruffy	28.2 ± 1.4
Trudi	33.2 ± 1.2
Mean	24.8 ± 2.6 s.e.

Table 3.11 Plasma binding of aniline measured by ultrafiltration expressed as a percentage of total plasma aniline.

These results agree closely with those obtained by equilibrium dialysis and with those reported by Shore et al. (1957).

(c) Extraction of aniline with 1, 2-Dichloroethane

Since the extraction of amidopyrine from plasma using 1, 2-Dichloroethane evidently does not extract the plasma-bound fraction of the base, it was decided to attempt the use of this solvent for aniline extraction. An experiment was performed as described in Chapter 3.32 in which 1, 2-dichloroethane was substituted for ether.

RESULTS

Recovery of aniline from saline and plasma into ether or dichloroethane was measured. Taking recovery from saline into ether as 100%, recovery into dichloroethane was much reduced, 69.1% from saline and 67.5% from plasma. Each figure is the mean of 5 observations. The fact that aniline could not be effectively recovered from saline indicates that dichloroethane is not a suitable solvent in this system and the similarity of the plasma and saline results shows that any plasma binding is having an insignificant effect upon recovery with this solvent.

3.4 DISCUSSION

3.41 Experiments to find a suitable system for the extraction of ^{14}C-aniline from plasma and gastric juice

Both benzene and diethyl ether were found to extract aniline from alkaline saline and to be compatible with the methanol and toluene-based scintillator. In spite of its inflammability, ether was felt to be less toxic to the experimenter and, when it was found that it could be accurately manipulated, ether was adopted as the solvent. Recently Main and Whittle (1972) have used benzene to extract radioactive aniline from gastric juice and diluted rat plasma. Mixing seven parts of ether with ten parts of scintillator does not significantly reduce the counting efficiency and the large sample of the ether extract that is used increases the accuracy of the aniline estimation.

3.42 The efficiency and accuracy of the extractions of aniline and amidopyrine from plasma and gastric juice

Once a suitable system for aniline extraction had been devised it was necessary to compare it with the method described for estimating amidopyrine by Brodie and Axelrod (1950). A detailed analysis of the accuracy and precision of the two procedures was carried out.

Extraction of both bases from gastric juice was essentially

complete but 14% of plasma amidopyrine was not accounted for. It is likely that this corresponds to the plasma-bound fraction, which Shore et al. (1957) have stated as being 15% of the total plasma amidopyrine. Harper et al. (1968) reported 98% recovery of plasma amidopyrine using this method, but recovery in my hands has not always been as high as this. However, recovery of plasma aniline is consistently high in spite of binding to plasma proteins of 25% of total plasma aniline. Whether this bound fraction is free to be cleared across the gastric mucosa will be discussed in detail in a later section.

Not surprisingly, aniline estimation had the advantage of much greater precision than that of amidopyrine. This is due to the simplicity of the aniline extraction process in contrast to the tedious manipulations required to measure amidopyrine.

Since aniline recovery from plasma was not quite complete, it was felt that some plasma contents, extracted into ether with the aniline, might be quenching the 14-C radioactivity. This was found not to be the case and the small reduction in recovery may therefore be due to a fraction of binding to proteins too resilient to be broken even by exposure to alkaline ether.

3.43 The equilibration of aniline and amidopyrine between erythrocytes and saline and the distribution of the bases between erythrocytes and plasma

Aniline and amidopyrine reach equilibrium between blood cells

and saline within two minutes. Harper et al. (1968) using cat erythrocytes found that amidopyrine equilibrated within one minute. Both these times are far in excess of the circulation time in the gastric mucosa. For clearance to be complete, diffusion from erythrocytes to plasma and out of the capillary would need to be completed within approximately one second. Measurements in such a short time interval require special techniques which were not available and no attempt was made to do so here since the rates at which the two substances are cleared across the mucosa provide strong evidence that complete or almost complete removal of available aniline or amidopyrine takes place in one passage through the mucosal capillaries (Shore et al., 1957).

Aniline was found to be evenly distributed between erythrocytes and saline indicating that binding of the substance in the cells did not occur to a greater or lesser extent than in the plasma. This was not the case with amidopyrine. Extracellular concentration after equilibration was lower than expected indicating that some amidopyrine was bound in the cells and not free to diffuse out or that the initial intracellular concentration was lower than that in the plasma. Harper et al. (1968) reported that amidopyrine was evenly distributed in cats' blood but I was not able to confirm this. This difference may be partly accounted for by the use of

different concentrations of amidopyrine. I have used 40 µg of amidopyrine/ml plasma whereas Harper and his colleagues used between 50 and 100 µg/ml.

One experiment was carried out in which amidopyrine was equilibrated into plasma. The object of this was to test whether removal of free amidopyrine by binding to plasma proteins resulted in an increased extracellular concentration. This in fact was the case but observed extracellular concentration was still only 77% of that expected had distribution between cells and plasma been even.

3.44 Experiments to examine the acute effects of aniline in dogs in the doses used for clearance experiments

Since aniline is known to be toxic to dogs and other species (Williams, 1959; Spicer, 1950) it was important to establish that the doses of aniline used to measure gastric mucosal blood flow were tolerated without serious side effects and to establish specifically that gastric secretion was not affected. The metabolism of aniline is described in section 3.2.

The major toxic effect of aniline is the oxidation of haemoglobin to form methaemoglobin. Bodansky (1951) has reported that the methaemoglobin in normal dogs is, on average, 0.9% of total haemoglobin and Clark, van Loon and Adams (1943) found that not until methaemoglobin reached 40% of the total, did cardiac output

increase. This indicates that in less severe methaemoglobinaemias adequate oxygen supply is maintained by the dissociation characteristics of the residual haemoglobin. Methaemoglobin was found to be up to 2% before an experiment and to rise over four or five hours of aniline infusion to 6-11%. No significant toxic effects would be expected to result from a methaemoglobinaemia of this magnitude and none were observed. Certainly gastric secretion, a process requiring high energy expenditure by the mucosa, was not affected by an aniline infusion as given in clearance experiments. This was so whether secretion was induced by histamine or by feeding.

The effect of methaemoglobin on oxygen dissociation is wholly reversible (Bodansky, 1951), and Williams (1959) has reported that the bulk of a dose of aniline is excreted within 2-3 days. My own measurements of urinary radioactivity due to unchanged aniline and to its metabolites, confirm that two days after an aniline clearance experiment the dog can be safely released from the metabolic cage and returned to the normal exercise and sleeping quarters. In practice dogs are not used for aniline experiments at less than ten day intervals to ensure complete removal of the previous dose.

3.45 Clearance experiments with ^{14}C-aniline and a detailed comparison with amidopyrine clearance in conscious dogs with Heidenhain gastric pouches after feeding

Early aniline clearance experiments produced very similar

results to those reported by Jacobson et al. (1966) using amidopyrine. When increasing histamine or pentagastrin stimulation was applied, secretion and aniline clearance increased. The relationship between mucosal blood flow and acid secretion will be fully discussed in Chapter 4.

Clearances of both aniline and amidopyrine were closely correlated with acid secretion from Heidenhain pouches after feeding and during stimulation by pentagastrin or histamine. Aniline clearance was 74% of amidopyrine clearance in the first series of experiments and 65% in the second series. The results of the second short series of experiments, performed a year after the first series, were somewhat unsatisfactory. Although the values of R obtained for aniline clearance were much as expected, those given by amidopyrine were higher than those previously measured in this laboratory or reported in the literature (Shore et al., 1957; Jacobson et al. 1966). Expected values of R during brisk acid secretion are between 20 and 40 but in three out of the four experiments values well above 40 were recorded. In the fourth experiment (Minty, Table 3.7) normal values of R(amidopyrine) were obtained and the mean figure for the ratio $\frac{R(aniline)}{R(amidopyrine)}$ (77.4%) is in reasonable agreement with the figure of 74.1% obtained in the first series of experiments.

The measured plasma concentrations of amidopyrine in the second series were considerably lower than those obtained in the

first series. This reduces the accuracy of the estimation since the values were near the limit of sensitivity of the assay and, if the actual plasma concentrations were low, might have affected the clearance. The experiments described in 3.33 showed that amidopyrine was not evenly distributed between erythrocytes and plasma but was at a higher concentration in the plasma. It is possible that the red cells may be saturable with respect to amidopyrine. If this is so then at low plasma amidopyrine concentrations the red cells would be expected to contain a greater proportion of the amidopyrine in the blood. If this were free to diffuse across the mucosa an increased clearance would result, and R, which is calculated from the plasma concentration, would be increased.

In addition, the simultaneous estimate of plasma recovery was not satisfactory and variability of the values of R, obtained during steady secretion indicates that errors occurred in the amidopyrine estimations. In two out of the four experiments (Trudi and Peta, Table 3.7) a high plasma blank reading was obtained which would have reduced the apparent plasma amidopyrine concentrations considerably had it been subtracted. The high blank was attributed to an impurity on the glassware or in a reagent and was ignored. Such a finding must throw some doubt on the validity of the other measurements. During the first experimental series, plasma blanks were consistently near zero.

The first series of experiments were carried out during the period of active comparison of aniline and amidopyrine clearances when amidopyrine measurements were made routinely in the laboratory. This was not the case for the second series. The following discussion will refer to the results of the first series of experiments in which clearance of aniline was found to be 74% of amidopyrine clearance.

I felt that plasma binding of aniline might be the cause of the observed difference in clearance of the two bases. In my hands, the extraction of amidopyrine from plasma did not estimate the 15% which is bound to proteins (Shore et al., 1957), but the estimate of plasma aniline did include the bound fraction, amounting to 25% of total plasma aniline (Shore et al., 1957). If it is assumed that the bound fraction is not free to diffuse across the mucosa and the aniline clearances adjusted accordingly, then the clearances of the two substances become identical. Alternatively, it is possible that the amidopyrine clearance value is too high because the bound fraction is free to diffuse but is not estimated in the extraction. However, since bound amidopyrine does not apparently diffuse into dichloroethane from alkaline plasma during 10 minutes' shaking, it seems unlikely that it would pass from neutral plasma to gastric juice in the second or so that it is passing through the capillary.

3.46 Experiments to investigate the observed differences between the clearances of aniline and amidopyrine

In the anaesthetized puppies, the mean arteriovenous difference in aniline concentration was found to be 74% of the a/v amidopyrine difference. This figure correlates remarkably well with the observed difference in clearances in the conscious dogs described in 3.36a. The gastric venous aniline concentration was 37% of the arterial level. The corresponding figure for amidopyrine is 27%. The difference in these figures probably reflects the bound fraction of aniline which is not available for clearance and reaches the venous side intact. If one assumes that 80% of gastric blood flow is passing through the mucosa (Moody, 1968; Delaney & Grim, 1964, 1965), then if 25% of aniline is bound and not available for clearance, the venous level would be expected to be 40% of the arterial aniline concentration. When the same calculation is applied to amidopyrine, assuming the bound fraction is not estimated, the expected venous concentration would be 20%, assuming complete clearance. A complication arises here because with removal of the unbound amidopyrine, the bound fraction would begin to dissociate, thus raising the measured venous concentration. Indeed if equilibrium were reached in the sample before the amidopyrine was extracted, the measured amidopyrine concentration would be expected to reach 28% of the arterial level. The observed figure

was 27%. These calculations are highly speculative and inexact and depend upon a number of assumptions but I believe that the observed differences between aniline and amidopyrine clearances can be accounted for by differences in plasma binding and extraction.

Measurement of plasma binding of aniline by equilibrium dialysis and by ultrafiltration showed that approximately 25% of plasma aniline was protein bound. This is in agreement with the figure given by Shore et al. (1957) but it must be stressed that considerable variations were found on different occasions. Variation was noted both between dogs and between different occasions in the same animal. The dogs were all apparently healthy during the study and so the reason for the day to day differences is not clear. Plasma binding might possibly be affected by the presence or absence of lipids. Although the dogs were always deprived of food overnight before a clearance experiment, this was not always done before taking blood for estimating plasma binding.

The small difference in the results obtained by the two methods may be due to the different temperature at which the measurements were made. Ultrafiltration was carried out at 37°C and dialysis at room temperature (22-24°C). Breckenridge and Rosen (1971) have reported that plasma binding of chlorothiazide decreases as temperature increases and the same is likely to be

true of aniline although the magnitude of the temperature effect has not been investigated. The concentration of aniline used in binding measurements was kept within the range found in clearance experiments since Breckenridge and Rosen (1971) also stated that the proportion of chlorothiazide bound decreased as concentration increased.

The attempt to extract aniline with dichlorethane was not successful. It was hoped that since dichloroethane did not extract plasma-bound amidopyrine, it would behave in the same way for aniline. Unfortunately recovery from saline was much less than with ether, indicating either that extraction was incomplete, even in the absence of protein, or that the dichloroethane was quenching a substantial amount of the scintillation.

3.47 Conclusions

The clearance of ^{14}C-aniline has been put forward as an alternative to amidopyrine clearance for the measurement of gastric mucosal blood flow in conscious dogs. The two methods have been found to give the same results if aniline clearance is corrected for plasma binding.

A significant advantage of aniline over amidopyrine is its ease of estimation. A series of thirty radioactive aniline samples can be extracted in less than an hour and counted automatically, whereas amidopyrine extraction takes several hours and involves

manual operation of a spectrophotometer. The results have shown that aniline estimation is significantly more precise than that of amidopyrine.

Aniline estimation is more sensitive and the method can be adapted for use in small laboratory animals such as rats by increased labelling. This is not possible with amidopyrine as the small quantities involved further reduce the accuracy of the determination.

Although the labelled aniline is expensive, the saving in skilled labour probably justifies this. Pilot experiments with ^{3}H-aniline indicate that this isotope is also suitable for clearance measurements. Although more radioactivity is needed ^{3}H-aniline works out considerably cheaper than the ^{14}C compound.

The methaemoglobinaemia caused by aniline is mild and reversible. Amidopyrine has been found to produce long lasting depression of bone marrow function in some dogs but no such long term toxic effects of aniline have been observed in our animals, some of which have received it repeatedly for several months.

The observation of Moody (1968) that amidopyrine clearance does not reflect mucosal blood flow during inhibition of acid secretion by thiocyanate probably applies to aniline clearance although it has not been verified experimentally. Moody found that oxygen consumption, total gastric blood flow and the distribution of blood to the stomach were unchanged during secretory

inhibition but amidopyrine clearance was reduced. This result emphasizes the fact that these clearance measurements require the presence of acid gastric contents to trap the aniline or amidopyrine. An acid wash alone is probably not effective in lowering the pH at the bases of the gastric pits and glands without causing excessive distension. For this reason a small dose of pentagastrin was used in my experiments when required to provide a minimal background of acid secretion.

CHAPTER 4

THE EFFECTS OF DIFFERENT SECRETORY STIMULI ON GASTRIC MUCOSAL BLOOD FLOW AND ACID SECRETION IN DOGS WITH VAGALLY INNERVATED AND DENERVATED POUCHES

4.1 INTRODUCTION

Since methods of measuring gastric mucosal blood flow have been available, it has been realised that substances which stimulate gastric acid secretion cause an increase in gastric mucosal blood flow (Jacobson et al. 1966; Jacobson, Swan and Grossman, 1967). When the stomach is stimulated to secrete either by food or by secretagogues, mucosal blood flow increases (Bynum and Jacobson, 1971). This indicates that no significant increase in acid secretion can be obtained without an increase in mucosal blood flow. In their original studies, using amidopyrine clearance, Jacobson et al. (1966) found that canine gastric mucosal blood flow was directly related to the rate of acid secretion.

More recently it has become apparent that gastric mucosal blood flow depends not only upon the rate of acid secretion but upon the nature of the secretory stimulus. Jacobson and Chang (1969) found that, at comparable secretory rates, in gastric fistula dogs mucosal blood flow was greater with histamine than pentagastrin or gastrin as the stimulus. Reed and Smy (1971) have reported that, in the anaesthetized, vagotomized cat, the relationship between mucosal blood flow and acid secretion depends upon the stimulus, and that histamine produces more mucosal blood flow per unit of acid secretion than does pentagastrin. They also found that whereas the relationship between

secretion and blood flow is relatively constant during pentagastrin stimulation, the effect of histamine upon this relationship is dependent both upon the dose and the length of time for which the dose has been administered.

I have set out to examine in detail the effects of histamine, pentagastrin and feeding (i.e. endogenous gastrin) on the relationship between mucosal blood flow and acid secretion in conscious dogs with Heidenhain pouches. I have also compared the gastric mucosal blood flow response to food in dogs with Heidenhain and Pavlov pouches. These experiments were carried out to investigate the role, if any, of the vagal innervation of the stomach in the physiological control of gastric mucosal blood flow.

4.2 METHODS

In all experiments mucosal blood flow was measured by aniline clearance as described in Chapter 2.2.

4.21 A comparison of gastric mucosal blood flow after feeding in dogs with vagally innervated and denervated gastric pouches

In these experiments a standard meal of cooked, minced ox liver, 10-20 mg/Kg was used. Calcium orthophosphate (10% w/w) was added to the liver as a buffering agent. A little sodium chloride and water was also added to the mixture. In the absence of a 'spontaneous' secretion the dogs were given a small dose of pentagastrin as described in Chapter 2.22(a) so that the onset of secretion in response to the meal could be studied.

In subsequent experiments, when the response to feeding was being analysed, the following substances were used to increase acid secretion or mucosal blood flow:

Bradykinin (Sandoz), Insulin B.P., Bethanecol chloride (Macarthys).

4.22 A comparison of the effects of Histamine, Pentagastrin and feeding on mucosal blood flow and acid secretion in dogs with vagally denervated gastric pouches

(a) Experiments with increasing doses of histamine and pentagastrin.

Either histamine or pentagastrin was infused and the dose doubled every hour until a maximal secretion was obtained. Histamine

was begun at 0.5 or 1.0 μg $Kg^{-1}min^{-1}$ of the acid phosphate and 8.0 or 16.0 μg $Kg^{-1}min^{-1}$ proved to be maximal. Doses of pentagastrin between 2 and 32 μg $Kg^{-1}hr^{-1}$ were given.

(b) Experiments with equipotent doses of histamine and pentagastrin.

In these experiments a brisk secretion was initiated with either histamine or pentagastrin and mucosal blood flow measured over six 15 minute periods during steady secretion. The other secretagogue was then given in a dose estimated to produce the same level of secretion and mucosal blood flow measured for six further 15 minute periods. If secretion proved to be greatly different from that in the first part of the experiment, the dose was adjusted accordingly and mucosal blood flow measured during the steady state. The ratio of mucosal blood flow to the volume of juice secreted (R) was calculated and compared for the two drugs.

(c) Comparison of gastric mucosal blood flow, relative to gastric secretion during stimulation by feeding and histamine or pentagastrin.

The differences found in mucosal blood flow during histamine and pentagastrin stimulation prompted me to compare flow rates during histamine or pentagastrin stimulation with those elicited by feeding. The information was extracted from experiments previously carried out in three of the Heidenhain pouch dogs. Values

of R were extracted during comparable secretory responses to the three secretagogues in each dog. Between two and six serial estimations were taken and only experiments in which steady values of R were obtained were used.

4.3 RESULTS

4.31 A comparison of gastric mucosal blood flow after feeding in dogs with vagally innervated and denervated gastric pouches

Gastric mucosal blood flow after feeding was measured on three occasions in three dogs with vagally innervated (Pavlov) pouches. The patterns of the blood flow and acid secretion responses were markedly different from those obtained in seven experiments in four dogs with denervated (Heidenhain) pouches. The results of one experiment in each type of pouch are shown in Fig. 4.1. Secretion from the Heidenhain pouches increased gradually after feeding reaching a maximum after approximately one hour. In contrast, the Pavlov pouches approached their peak secretion within 30 minutes and also showed a greatly increased mucosal blood flow relative to secretion (R) during the first half hour. This initial vasodilatation was completely absent in the Heidenhain pouch experiments. Fig. 4.2 shows the relationship between mucosal blood flow and the volume and acid output of the gastric secretion. The increased blood flow in the Pavlov pouch after feeding is clearly seen, whereas in the Heidenhain pouch the ratio of blood flow to secretion (R) falls while secretion is increasing and then remains steady.

It was felt that the vagal innervation of the Pavlov pouch

was probably responsible, at least in part, for the vasodilatation after feeding. Some experiments were designed to investigate this hypothesis. Since insulin hypoglycaemia stimulates gastric secretion via the vagus (Hollander, 1946), secretion induced in this way might be expected to be accompanied by a mucosal blood flow similar to that seen soon after feeding. In two experiments in one of the dogs, insulin 0.4 μ/Kg s.c. was given and mucosal blood flow measured. Mucosal blood flow relative to secretion increased during insulin stimulation. The increased blood flow when related to the acid output was rather smaller than that seen in this dog after feeding but when related to the volume of secretion a larger increase was seen. The results of one experiment are shown in Fig. 4.3.

The cholinergic agonist bethanecol was used to stimulate secretion in three of the Heidenhain pouch dogs. This was done in order to imitate, as far as possible, vagal stimulation to the denervated pouch. A small background stimulation of pentagastrin was given in this and the insulin experiments. The result of one experiment is shown in Fig. 4.4. In all three experiments bethanecol 2.0 μg $Kg^{-1}min^{-1}$ for 30 or 60 minutes stimulated acid secretion and mucosal blood flow. However, in contrast to insulin in the Pavlov pouches, the ratio of blood flow to secretion (R) was in each case lower during bethanecol stimulation than during the preceding control period.

Fig. 4.1. THE EFFECTS OF FEEDING ON GASTRIC ACID SECRETION AND MUCOSAL BLOOD FLOW (MBF) IN DOGS WITH PAVLOV AND HEIDENHAIN POUCHES. In all figures in this Chapter, acid secretion ($\mu eq\ min^{-1} = \mu mol\ min^{-1}$) is denoted by the solid line and MBF ($ml\ min^{-1}$) by the broken line.

A. Pavlov pouch. MBF increased to a maximum within 15 min.of the meal; acid secretion became maximal after 30 min. and then remained steady for 90 min.

B. Heidenhain pouch. MBF and acid secretion slowly increased after feeding. Maximal values were reached after 70 min.

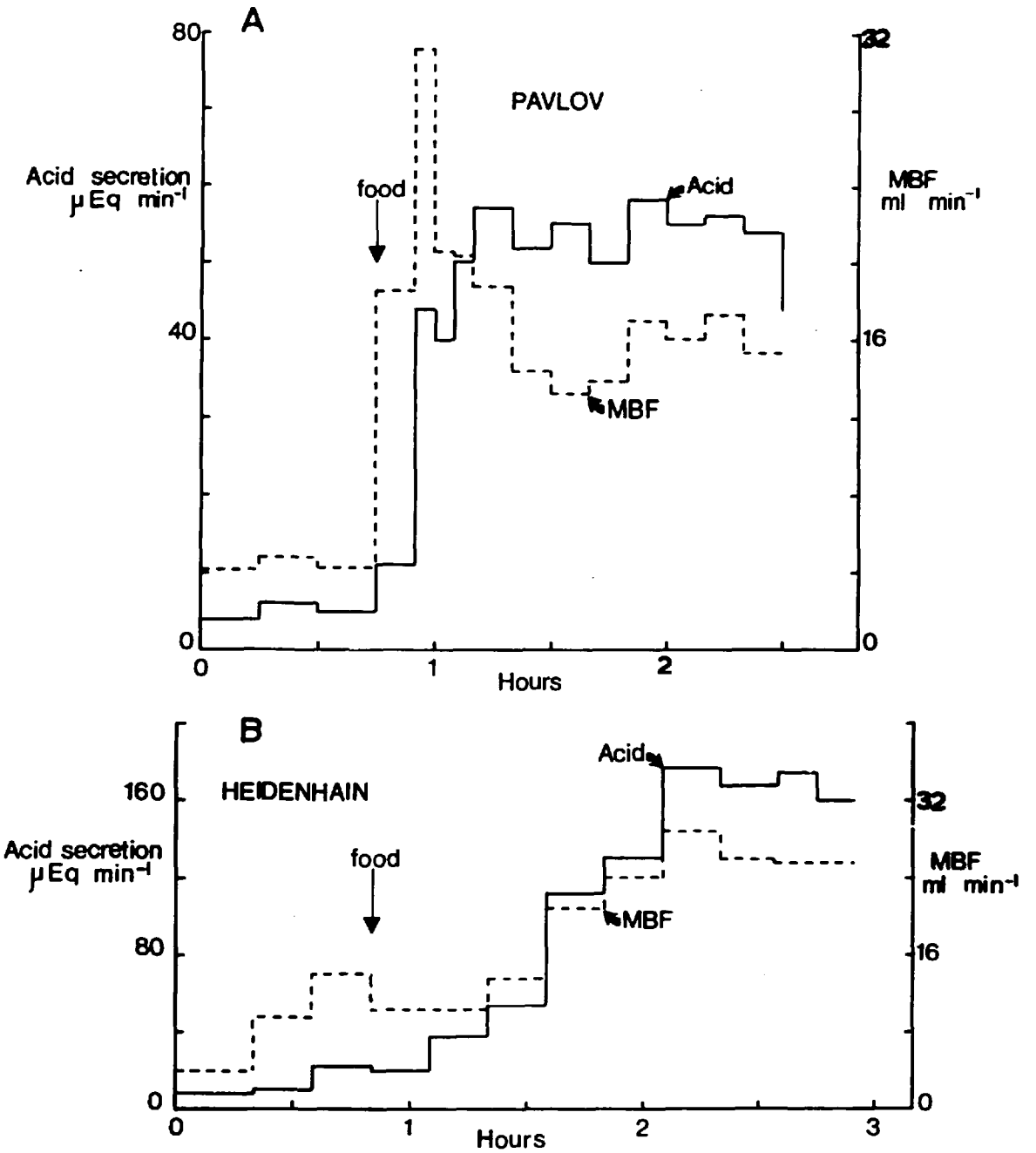

A
PAVLOV
Acid secretion
μEq min⁻¹
MBF
ml min⁻¹
food
Acid
MBF
80
40
0
32
16
0
0
1
2
Hours
B
HEIDENHAIN
Acid secretion
μEq min⁻¹
MBF
ml min⁻¹
food
Acid
MBF
160
80
0
32
16
0
0
1
2
3
Hours

Fig. 4.2. THE EFFECTS OF FEEDING ON THE RATIO OF MUCOSAL BLOOD FLOW TO VOLUME OF SECRETION ($\frac{mbf}{vol}$ = R) AND MBF TO ACID OUTPUT ($\frac{mbf}{acid}$) IN DOGS WITH PAVLOV AND HEIDENHAIN POUCHES. The results are from the experiments depicted in Fig. 4.1.

A. Pavlov pouch. MBF relative to volume and acid output increased after feeding and fell as the steady phase of secretion was reached.

B. Heidenhain pouch. No increase in the ratios after feeding. Both ratios fell to stable values as acid secretion increased.

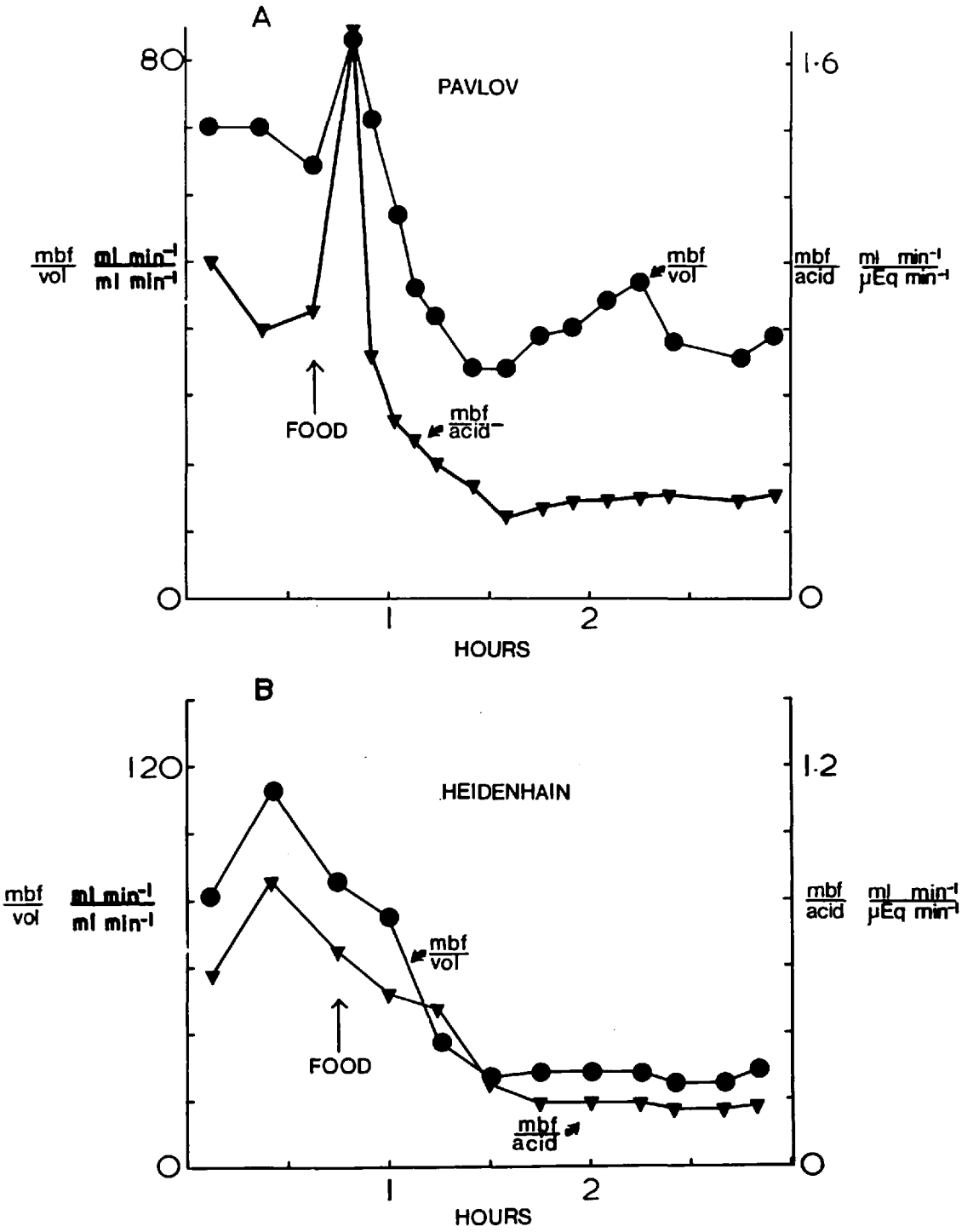

A
80
1·6
PAVLOV
mbf/vol ml min⁻¹ / ml min⁻¹
mbf/acid ml min⁻¹ / µEq min⁻¹
mbf/vol
FOOD
mbf/acid
0
1
2
HOURS
B
120
1·2
HEIDENHAIN
mbf/vol ml min⁻¹ / ml min⁻¹
mbf/acid ml min⁻¹ / µEq min⁻¹
mbf/vol
FOOD
mbf/acid
0
1
2
HOURS

Fig. 4.3. THE EFFECTS OF INSULIN ON ACID SECRETION AND MUCOSAL BLOOD FLOW IN A PAVLOV POUCH. The abbreviations are the same as in Fig. 4.1 and 4.2. A low rate of acid secretion was stimulated by pentagastrin (0.5 μg Kg^{-1} hr^{-1}).

A. Insulin (0.4 i.u. Kg^{-1} i.v.) caused an increase in acid secretion and MBF.

B. Insulin increased the ratios of MBF to secretory volume ($\frac{mbf}{vol}$ = R) and MBF to acid output ($\frac{mbf}{acid}$).

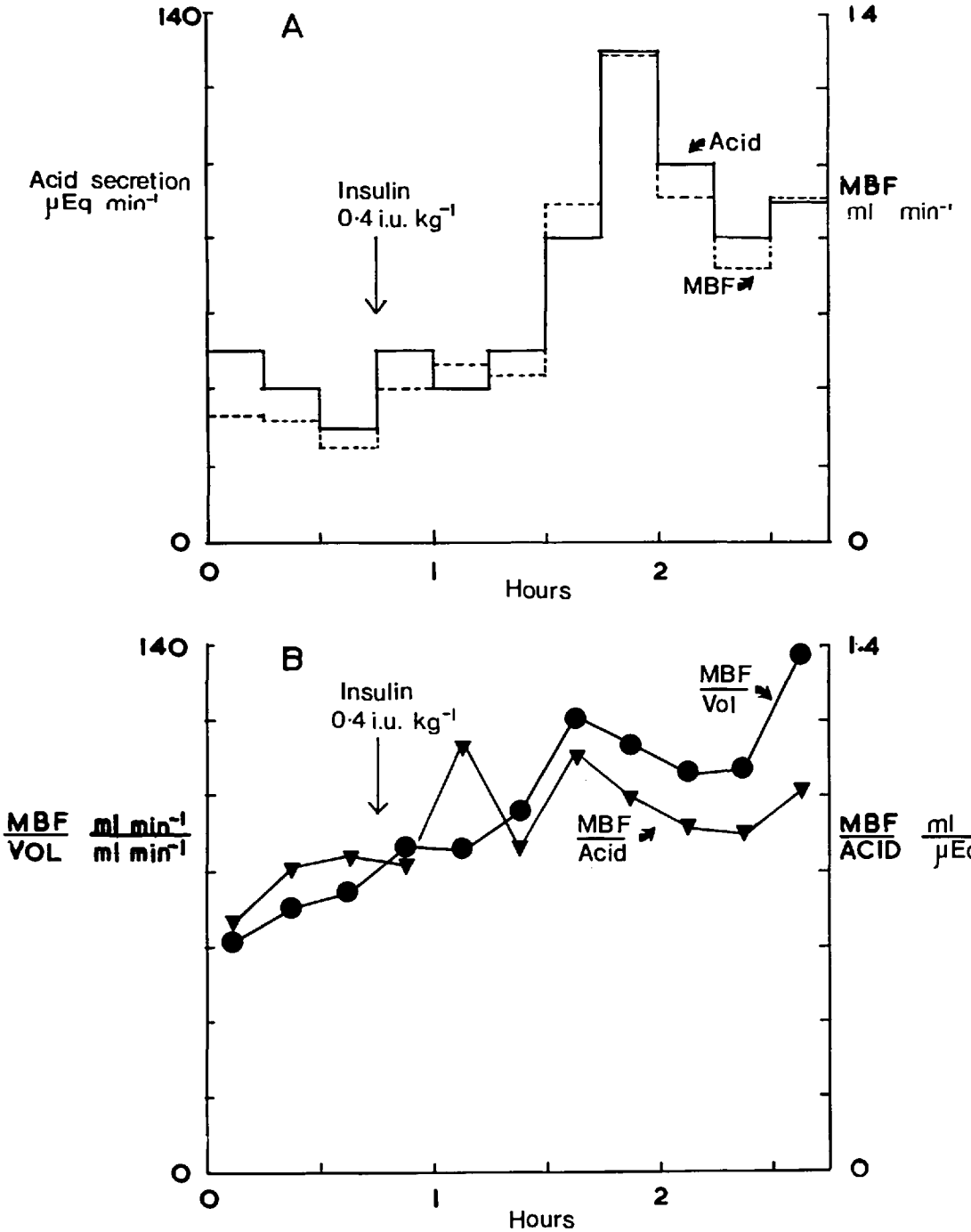
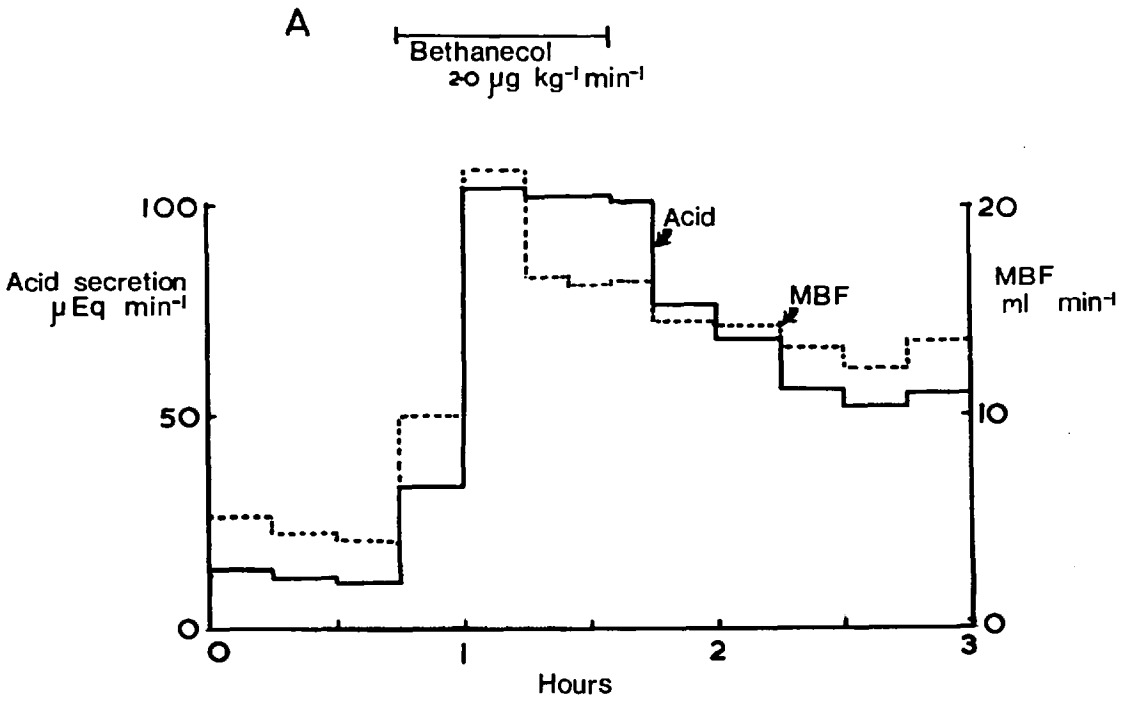

A
140
14
Acid secretion
μEq min⁻¹
Insulin
0·4 i.u. kg⁻¹
Acid
MBF
MBF
ml min⁻¹
0
0
0
1
2
Hours
B
140
1·4
Insulin
0·4 i.u. kg⁻¹
MBF
Vol
MBF
VOL
ml min⁻¹
ml min⁻¹
MBF
Acid
MBF
ACID
ml min⁻¹
μEq min⁻¹
0
0
0
1
2
Hours

Fig. 4.4. THE EFFECTS OF BETHANECOL ON GASTRIC ACID SECRETION AND MUCOSAL BLOOD FLOW IN A HEIDENHAIN POUCH. A low rate of acid secretion was stimulated by pentagastrin (0.5 μg $Kg^{-1}hr^{-1}$) throughout.

A. Bethanecol caused a prolonged increase in acid secretion and MBF.

B. The ratios of MBF to secretory volume ($\frac{mbf}{vol} = R$) and MBF to acid secretion ($\frac{mbf}{acid}$) fell in response to bethanecol and increased as the secretion in response to bethanecol decreased.


A
Bethanecol
20 µg kg⁻¹ min⁻¹
100
50
0
Acid secretion
µ Eq min⁻¹
Acid
MBF
20
10
0
MBF
ml min⁻¹
0
1
2
3
Hours

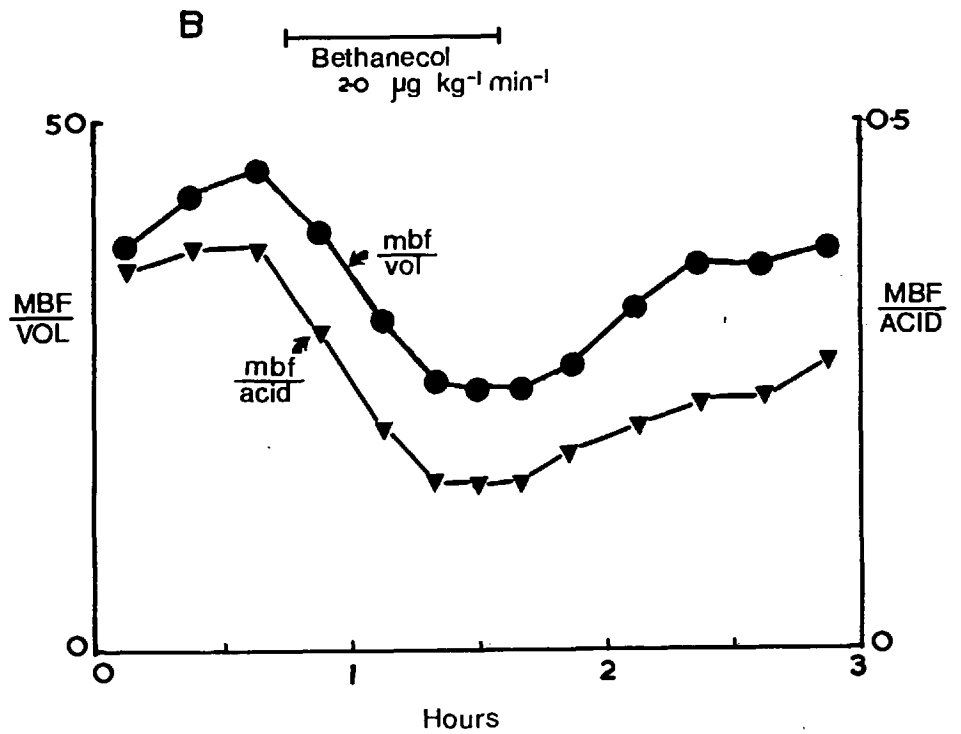
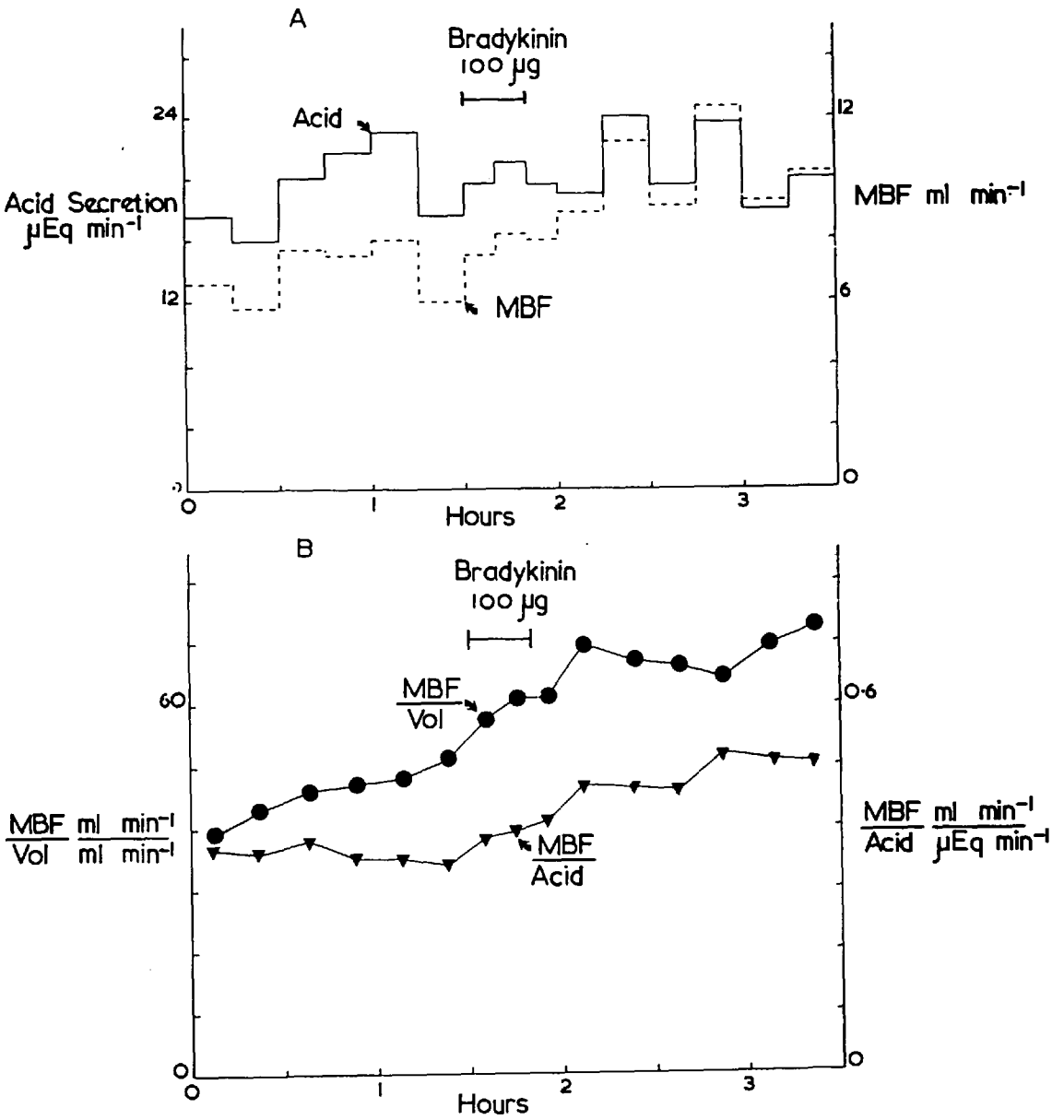

B
Bethanecol
20 µg kg⁻¹ min⁻¹
50
0
MBF/VOL
mbf/vol
mbf/acid
0·5
0
MBF/ACID
0
1
2
3
Hours

Fig. 4.5. THE EFFECTS OF BRADYKININ ON GASTRIC ACID SECRETION AND MUCOSAL BLOOD FLOW IN A HEIDENHAIN POUCH. A low rate of acid secretion was stimulated by pentagastrin (0.5 µg $Kg^{-1}hr^{-1}$) throughout.

A. Bradykinin (100 µg given over 20 min.) did not affect acid secretion but caused an increase in MBF.

B. The ratios of MBF to secretory volume ($\frac{mbf}{vol}$ = R) and MBF to acid secretion ($\frac{mbf}{acid}$) increased after administration of bradykinin 100 µg.


A
Bradykinin
100 µg
Acid
MBF
Acid Secretion
µEq min⁻¹
MBF ml min⁻¹
24
12
12
6
0
0
1
2
3
Hours
B
Bradykinin
100 µg
MBF
Vol
MBF
Acid
MBF ml min⁻¹
Vol ml min⁻¹
MBF ml min⁻¹
Acid µEq min⁻¹
60
0
0·6
0
0
1
2
3
Hours

Finally, Bradykinin was investigated as being potentially involved in the vasodilator response. In one experiment a dose of 20 µg/Kg bradykinin, given over 20 minutes, inhibited acid secretion and mucosal blood flow. On another occasion a dose of 15 µg/Kg in another dog slightly increased secretion and flow. In two experiments, in a third dog, 10 µg/Kg bradykinin, given over 20 minutes, had no effect on acid secretion but increased mucosal blood flow for a considerable time. One of these experiments is shown in Fig. 4.5.

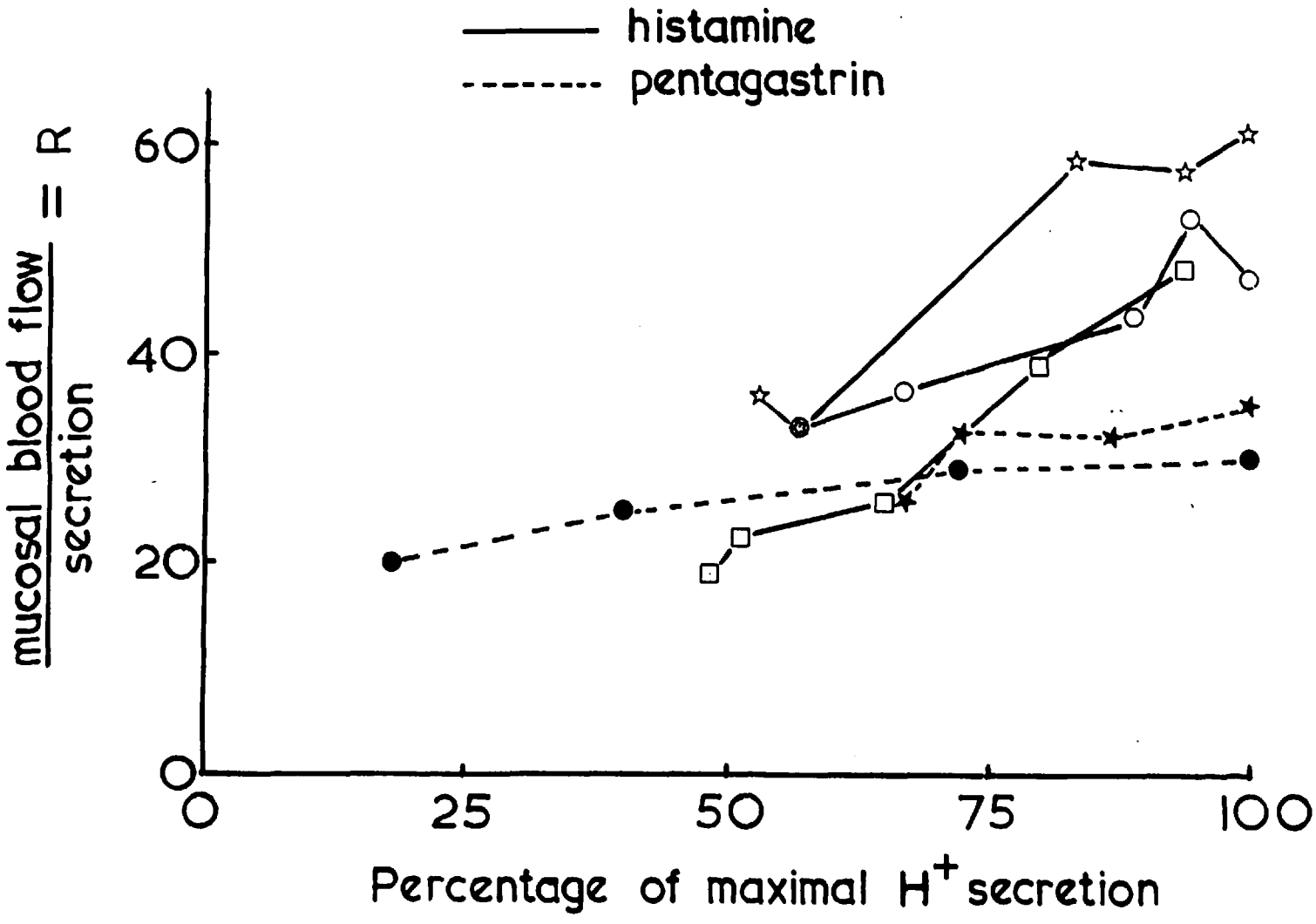
4.32 A comparison of the effects of histamine, pentagastrin and feeding on gastric mucosal blood flow and acid secretion in dogs with vagally denervated (Heidenhain) gastric pouches

(a) Experiments with increasing doses of secretagogue.

Both histamine and pentagastrin, in the doses given, increased gastric acid secretion and mucosal blood flow. However, it seemed that blood flow was greater, relative to the secretion, during high doses of histamine than when large doses of pentagastrin were given. Five experiments were carried out, in three dogs and the results are shown in Fig. 4.6. Acid secretion was expressed as a percentage of the maximum to each drug and plotted against R, the ratio of blood flow to secretion. It can be seen from Fig. 4.6. that when secretion is above 70% of the maximal to the stimulus;

Fig. 4.6. $\frac{\text{MBF}}{\text{secretory rate}}$ = R DURING INCREASING STIMULATION BY HISTAMINE OR PENTAGASTRIN.

R is plotted against percentage of maximal acid secretion for each stimulus. Each point is the mean of two observations in one dog. Significant linear regressions were found for each stimulus (b pentagastrin = 0.15 $P < 0.01$; b histamine = 0.46 $P < 0.01$) and both were significantly different from the mean regression calculated from the results for both stimuli together ($P < 0.01$). The regression coefficient (b) and elevation for histamine were significantly greater than those for pentagastrin ($P < 0.05$ and < 0.01 respectively).


histamine
pentagastrin
mucosal blood flow / secretion = R
60
40
20
0
0
25
50
75
100
Percentage of maximal H+ secretion

R during histamine stimulation is substantially greater than during stimulation by pentagastrin. Significant linear regressions of R against secretory rate, expressed as a percentage of the maximal to each stimulus, were found. Regression coefficient (b) for histamine was 0.46, $P < 0.01$, and to pentagastrin was 0.15, $P < 0.01$. The slope and elevation for histamine was significantly greater than that for pentagastrin ($P < 0.05$ and <0.01 respectively). Both regressions were also found to be significantly different from the mean regression calculated from the results for both drugs taken together ($P < 0.01$). The comparison of the regression lines was calculated as given in Snedecor and Cochrane (1969) and the calculations appear in Appendix 2.1.

(b) Experiments with equipotent doses of histamine and pentagastrin.

In each of the five experiments, mucosal blood flow relative to gastric secretion (R) was greater during histamine than pentagastrin stimulation. R during histamine was 144.1% $\pm$ 12.8% s.e. of that during secretion stimulated by pentagastrin. The results are shown in Table 4.1. An analysis of variance (see Appendix 2.2) showed that R during histamine stimulation was significantly higher than during pentagastrin ($P < 0.001$).

Dog	Pentagastrin			Histamine			$\frac{R(H)}{R(P.G)} \times 100$
	H^+ µEq/min	R	mean R ± s.d.	H^+ µEq/min	R	mean R ± s.d.	
Peta	53.5	18.2 14.4 18.7 16.3	16.9 ± 1.97	53.4	30.7 30.5 28.1 26.9 25.2	28.3 ± 2.03	167.5
Peta	30.3	21.4 22.1 19.1 20.0	20.7 ± 1.35	38.3	31.0 33.7 39.9	34.9 ± 4.56	168.6
Minty	150	11.5 12.4 13.8 7.7	11.4 ± 2.61	112	18.3 17.8 18.5 17.0	17.9 ± 0.68	157.0
Minty	134	18.8 18.3 20.7 20.6	19.6 ± 1.23	140	20.5 20.6 19.3 22.3	20.7 ± 1.23	105.6
Trudi	46.8	20.5 22.5 22.5 22.5	22.0 ± 1.0	30.5	29.5 27.0 27.4 23.4	26.8 ± 2.53	121.8
							mean=144.1 ± 12.8(s.e.)

Table 4.1. The ratio of Heidenhain pouch mucosal blood flow to acid secretion (R) during stimulation by histamine or pentagastrin.

(c) Comparison of gastric mucosal blood flow, relative to gastric secretion, during stimulation by feeding and histamine or pentagastrin.

Values of R during comparable rates of acid secretion to food, histamine and pentagastrin were compared in three of the Heidenhain pouch dogs. Inspection of the results (Table 4.2 and 4.3) showed that the values of R during histamine stimulation were considerably higher than those after feeding (R Hist = 162.8% $\pm$ 13.7% s.e. of R P.G.). Values during pentagastrin stimulation were very similar to those seen after feeding (R P.G. = 93.2% $\pm$ 1.9% s.e. of R Food). An analysis of variance encompassing all three stimuli was carried out and individual comparisons made within the analysis. The calculations are given in Appendix 2.3.

R after feeding was compared with that during histamine and pentagastrin. It was found that R was significantly higher in response to histamine than in response to food ($P < 0.001$) and that the values for food and pentagastrin were not significantly different from each other ($P > 0.1$).

Dog	Food			Histamine			$\frac{R(H)}{R(F)}$ x 100
	H^+ µEq/min	R	mean R ± s.d.	H^+ µEq/min	R	mean R ± s.d.	
Chris	92	27.3 30.8 29.6 34.6 34.9 33.2	31.7 ± 3.02	97	63.4 54.1 59.2 63.1	60.0 ± 4.35	189.3
Grocer	71	32 33 33 32 28 27	30.8 ± 2.64	81	39 39 48 51	44.3 ± 6.19	143.8
Alex	109	27.0 28.0 28.0	27.7 ± 0.58	80	42.4 43.6	43.0 ± 0.60	155.2 Mean=162.8 ± 13.7(s.e)

Table 4.2. The ratio of Heidenhain pouch mucosal blood flow to acid secretion (R) during stimulation by histamine or feeding.

Dog	Food			Pentagastrin			$\frac{R(P.G)}{R(F)}$ x 100
	H^+ µEq/min	R	mean R ± s.d.	H^+ µeq/min	R	mean R ± s.d.	
Grocer	71	32 33 33 32 28 27	30.8 ± 2.64	26	29.4 25.8 32.6	29.3 ± 3.40	95.1
Alex	109	27.0 28.0 28.0	27.7 ± 0.58	108	23.9 25.0 25.9 26.5	25.3 ± 1.13	91.3 Mean=93.2 ± 1.90(s.e)

Table 4.3. The ratio of Heidenhain pouch mucosal blood flow to acid secretion (R) during stimulation by pentagastrin or feeding.

4.4 DISCUSSION

4.41 A comparison of gastric mucosal blood flow after feeding in dogs with vagally innervated and denervated gastric pouches.

The experiments described have demonstrated that the mucosal blood flow response of a vagally innervated (Pavlov) pouch to a meal is markedly different to that found in a Heidenhain pouch. In the Pavlov pouch mucosal vasodilatation and increased blood flow occurred as soon as the meal was given and reached a maximum before the maximum secretory response was reached. Maximum secretory output was reached much more rapidly in the Pavlov pouches followed by a long-lasting period of stable secretion. In the Heidenhain pouches acid secretion increased more slowly and mucosal blood flow tended to follow a parallel pattern with R falling as a brisk secretion began.

It is reasonable to suppose that part of the response of a Pavlov pouch to feeding is mediated via the vagus and the few experiments so far carried out support this hypothesis. The high blood flow relative to secretion (R) during the vagally-mediated acid secretory response to insulin is similar to that seen during the initial phase of the response to a meal. Hilton and Lewis (1955a, 1955b) showed that a kinin similar to bradykinin was responsible for vasodilatation in the salivary gland of the cat and it was considered that an analogous mechanism might be

functioning in the vagally innervated gastric mucosa. Although the results show that bradykinin is capable of causing an increase in mucosal blood flow I have no direct evidence of its involvement in the physiological response.

The response of the Heidenhain pouch to feeding is due to liberation of gastrin from the antral mucosa. Secretion and mucosal blood flow resemble the gastrin-dominated second half of the Pavlov pouch response. Stimulation of secretion by bethanecol did not result in R values comparable to those found in Pavlov pouches soon after feeding. This does not prevent the conclusion that the initial vasodilatation in Pavlov pouches is due to a vagally mediated component. It is probably a result of the chronic vagal denervation of the Heidenhain pouch and the fact that even under ideal conditions, systemic application of a synthetic mediator may not have the same effects as the excitation of specific nervous pathways that is to be expected during the physiological response.

4.42 Comparison of the effects of histamine, pentagastrin and feeding on gastric mucosal blood flow and acid secretion in dogs with vagally denervated (Heidenhain) gastric pouches.

The results clearly show that histamine can elicit a greater mucosal blood flow relative to secretion than pentagastrin or feeding, the effects of which seem to be indistinguisable from one another. This latter observation is perhaps not surprising and confirms the

previous findings of Jacobson and Chang (1969) in dogs with innervated stomachs and Reed and Smy (1971) in anaesthetized, vagotomised cats. It is further evidence of the "physiological" nature of pentagastrin stimulation.

The results during increasing pentagastrin or histamine stimulation show that when secretion is related to the maximal acid output to each stimulus, R in response to histamine is significantly greater than to pentagastrin and that this difference is most marked at high rates of secretion. This is an extension of the findings of Reed and Smy (1971) that a large dose of Histamine (45 μg $Kg^{-1}min^{-1}$ of the acid phosphate) caused more mucosal blood flow relative to secretion than a small dose of histamine (4.5 μg $Kg^{-1}min^{-1}$) or a range of doses of pentagastrin. The statistical analysis shows that throughout the range studied, R in response to histamine is likely to be greater than during pentagastrin.

Using two doses of pentagastrin, Reed and Smy (1971) found no significant differences in the relationship of mucosal blood flow to secretion. A small, significant increase in R with secretion was found in this study but bearing in mind the differences in species and preparation from which the results are derived, such a discrepancy is not altogether surprising.

It was an incidental observation in this study that the maximal acid secretion of a Heidenhain pouch to histamine was

greater than that obtained with pentagastrin stimulation. This is in agreement with results reported by Andersson and Grossman (1965). They showed that vagal denervation reduces the maximal acid secretory response to pentagastrin but not to histamine and that in innervated pouches both stimulants produce a similar maximal acid output. Reed and Smy (1971) also observed that histamine (45 μg $Kg^{-1}min^{-1}$) in their vagotomised cats gave rise to a greater secretory response than could be obtained with pentagastrin.

The studies during comparable secretory rates in response to feeding, pentagastrin or histamine showed that at the same absolute level of secretion histamine produced the greatest mucosal blood flow. The statistical analysis of the observations demonstrated that R during histamine was significantly different from pentagastrin and feeding which are not significantly different from each other. The results of the experiments with histamine and pentagastrin show that the increased vasodilatation due to histamine is short-lived and that blood flow fell rapidly when stimulation was changed to pentagastrin. The differences in R during brisk secretion to histamine or pentagastrin are similar to those found by Jacobson and Chang (1969) in fistula dogs with innervated stomachs. The observation that histamine elicits a greater mucosal blood flow per unit of acid secreted than pentagastrin or feeding indicates that histamine has a direct

vasodilator action over and above the locally controlled vasodilatation in response to increased secretion. The increase in mucosal blood flow is seen despite the depressor action of large doses of histamine. Clearly blood flow during powerful histamine stimulation is in excess of that required to support the secretion. This is confirmed by experiments with noradrenaline described in Chapter 5.

4.43 SUMMARY

Differences have been observed in the patterns of gastric acid secretion and mucosal blood flow after feeding in Pavlov and Heidenhain gastric pouches in dogs. It is suggested that the rapid, initial mucosal vasodilatation in Pavlov pouches is due to a central nervous system influence, mediated by the vagus.

Differences have also been observed in mucosal blood flow, relative to acid secretion, with different secretory stimuli in Heidenhain pouch dogs. Histamine causes a greater mucosal blood flow, relative to secretion, than either pentagastrin or feeding.

CHAPTER 5

THE EFFECTS OF SOME SYMPATHOMIMETIC DRUGS ON CANINE GASTRIC SECRETION AND MUCOSAL BLOOD FLOW.

5.1 INTRODUCTION

Early studies of the gastric pharmacology of catecholamines were carried out with adrenaline and extracts of adrenal glands. Hess and Gundlach in 1920 found that administration of a probably impure extract of adrenaline caused a reduction in acid secretion in response to food from a Pavlov pouch. Sirotinin (1924) found the opposite effect in Heidenhain pouch dogs but suggested that it may have been due to a delay in gastric emptying.

More reliable results have come from modern work using pure drugs. Forrest and Code (1954) have convincingly demonstrated that adrenaline and noradrenaline cause inhibition of gastric acid secretion induced by histamine. Using dogs with gastric fistulae Harries (1956) confirmed the inhibitory effect of noradrenaline and found that vagally-stimulated gastric secretion was more sensitive to inhibition than histamine-induced secretion. Although Forrest and Code (1954) attributed the inhibitory effects of catecholamines on gastric secretion to mucosal vaso-constriction, the finding of Harries (1957) that isoprenaline decreased secretion in a gastric fistula dog suggested the possibility of a direct effect on the secretory mechanism. Isoprenaline, which is primarily an adrenergic β receptor agonist (Ahlquist, 1948) would not be expected to reduce gastric mucosal blood flow and has been reported as causing mesenteric vasodilatation (Green, Deal, Bardhanabaedya and Denison, 1955).

More support for the idea of a direct action of catecholamines on the mucosa came from Pradhan and Wingate (1962) who found that isoprenaline, adrenaline and noradrenaline, given subcutaneously reduced gastric secretion in pouch dogs stimulated by feeding, histamine or bethanecol. The finding that the effects of isoprenaline were more effectively prevented by β than α blockade was taken by the authors to indicate that the inhibition was not secondary to vasoconstriction.

Although much recent work has confirmed the inhibitory effect of catecholamines on histamine-induced gastric secretion in conscious dogs (Nicoloff, Peter, Stone and Wangensteen, 1964; Holton, Cumming, Putman and Charlton, 1969; Jacobson, Eisenberg and Swan 1966; Jacobson, Swan and Grossman, 1967), the situation regarding the effects in anaesthetized animals is less clear. Noradrenaline in doses of approximately 1 μg $Kg^{-1}min^{-1}$ inhibits histamine-induced gastric secretion in dogs (Cumming, Haigh, Harries and Nutt, 1961, 1963) and this action is prevented by phenoxybenzamine, an α receptor antagonist (Haigh and Steedman, 1968). Both Adrenaline and Isoprenaline have been reported as increasing and decreasing acid secretion and many attempts have been made to correlate secretory effects with changes in total gastric blood flow. Cumming et al. (1961, 1963) found that adrenaline (1-8 μg $Kg^{-1}min^{-1}$) in dogs always increased blood flow but had a variable effect on secretion.

Adrenaline (1-4 μg $Kg^{-1}min^{-1}$) has also been found to increase gastric secretion (Holton and Jacoby, 1966) and to decrease secretion and total gastric blood flow (Peter, Nicoloff, Sosin, Walder and Wangensteen, 1962). Thompson and Vane (1953) reported that the effects of adrenaline on gastric blood flow were dependent upon the dose. This suggests that α receptors are responsible for the effects of larger and β receptors for the effects of smaller doses (Holton, 1972). However, increases in blood flow and concurrent decreases in secretion have been observed (Peters and Womack, 1958; Haigh, Moffit and Steedman, 1967) indicating that under some conditions adrenaline has a direct action on the secretory apparatus.

Isoprenaline has been found to inhibit basal acid secretion in conscious rats (Misher, Pendleton and Staples, 1969). Blockade of adrenergic β receptors with propranolol did not modify the basal secretion and phenylephrine was found to produce an initial increase followed by a reduction in secretion.

In order to avoid the complication of stimulating α and β receptors simultaneously, I have studied the effects of isoprenaline on gastric secretion induced by histamine or pentagastrin in anaesthetized dogs and in conscious dogs with separated pouches, in which secretion was also induced by feeding. The studies have been extended to include measurements of mucosal blood flow by aniline clearance since this seemed more likely than total

gastric blood flow to be related to acid secretion. In some cases a comparison of the effects of noradrenaline and isoprenaline on acid secretion and mucosal blood flow has been made since the two drugs would be expected to act differently on the gastric circulation. Some pharmacological analysis of the isoprenaline responses has been carried out.

5.2 METHODS

5.21 Experiments in conscious pouch dogs

Mucosal blood flow was measured by aniline clearance as described in Chapter 2.2. Drugs were infused intravenously, mixed with the aniline infusion. Where mucosal blood flow was not being measured, a single sterile catheter (O.D. 0.63 mm) was inserted into a superficial leg vein for the administration of drugs.

The following drugs were used:

Isoprenaline sulphate

Noradrenaline acid tartrate (Levophed, Bayer)

Propranolol hydrochloride (Inderal, I.C.I.)

L-Phenylephrine Hydrochloride (Koch-Light Laboratories)

Phentolamine mesylate (Rogitine, Ciba)

Salbutamol sulphate (Allen and Hanbury)

5.22 Measurement of gastric acid secretion in anaesthetized dogs

Two puppies, weighing 9 kg. were used. Anaesthesia was induced by intravenous pentobarbitone (30 mg/kg) and a polythene catheter was introduced into a superficial vein in one fore-leg for the administration of further anaesthetic as required. The stomach was exposed through a mid-line laparotomy and the pylorus

divided between stout ligatures excluding the right gastric artery. A stainless steel cannula (diameter 2 cm) was introduced into the most dependent portion of the greater curvature and secured with two purse string sutures. The cannula was brought out through a stab wound in the abdominal wall and the incision closed. Handling of the stomach was kept to a minimum during the operation.

A femoral vein was then cannulated for the infusion of pentagastrin and isoprenaline and a femoral artery was connected, via a catheter, to a Devices blood pressure transducer. Blood pressure was recorded on an S.E. 2100 Ultraviolet recorder.

A third experiment was carried out using one of the pouch dogs in a terminal experiment. In this case, gastric secretion was collected via a cannula secured into the pylorus.

5.3 RESULTS

5.31 The effects of isoprenaline and noradrenaline on gastric acid secretion induced by histamine, pentagastrin or feeding in conscious dogs with Heidenhain pouches

(a) Histamine-induced secretion.

Histamine acid phosphate was infused intravenously at a rate of 0.5-2.0 μg $Kg^{-1}min^{-1}$ and isoprenaline was given for 10-30 minutes mixed with histamine, after a stable plateau of secretion had been reached. The effect of isoprenaline was found to be dependent on the dose. In 15 experiments in six dogs a small dose of isoprenaline sulphate (0.125-0.2 μg $Kg^{-1}min^{-1}$) increased secretion 8 times by an average of 43% and on 7 occasions had no effect (Fig. 5.1a and b). The increase in secretion, when it did occur, tended to be a transient effect. However, when the dose of isoprenaline was increased to 1.0-2.0 μg $Kg^{-1}min^{-1}$ a decrease, averaging 58%, in acid secretion was observed in each of 7 experiments in five dogs (Fig. 5.1c). In one dog it was not possible to inhibit secretion even with 4.0 μg $Kg^{-1}min^{-1}$ to which a small increase was recorded. Similarly, in 3 experiments in one dog Noradrenaline tartrate given at 2.0, 1.0 and 0.2 μg $Kg^{-1}min^{-1}$ caused a reduction of acid secretion of 80%, 15% and 0% respectively.

In 3 out of 3 experiments in 3 dogs, Propranolol was found to block the inhibition of histamine-stimulated acid secretion by isoprenaline. Propranolol 1-1.5 mg/kg i.v. was given 20-80 min. before isoprenaline 0.5-2.0 μg $Kg^{-1}min^{-1}$ (Fig. 5.1c).

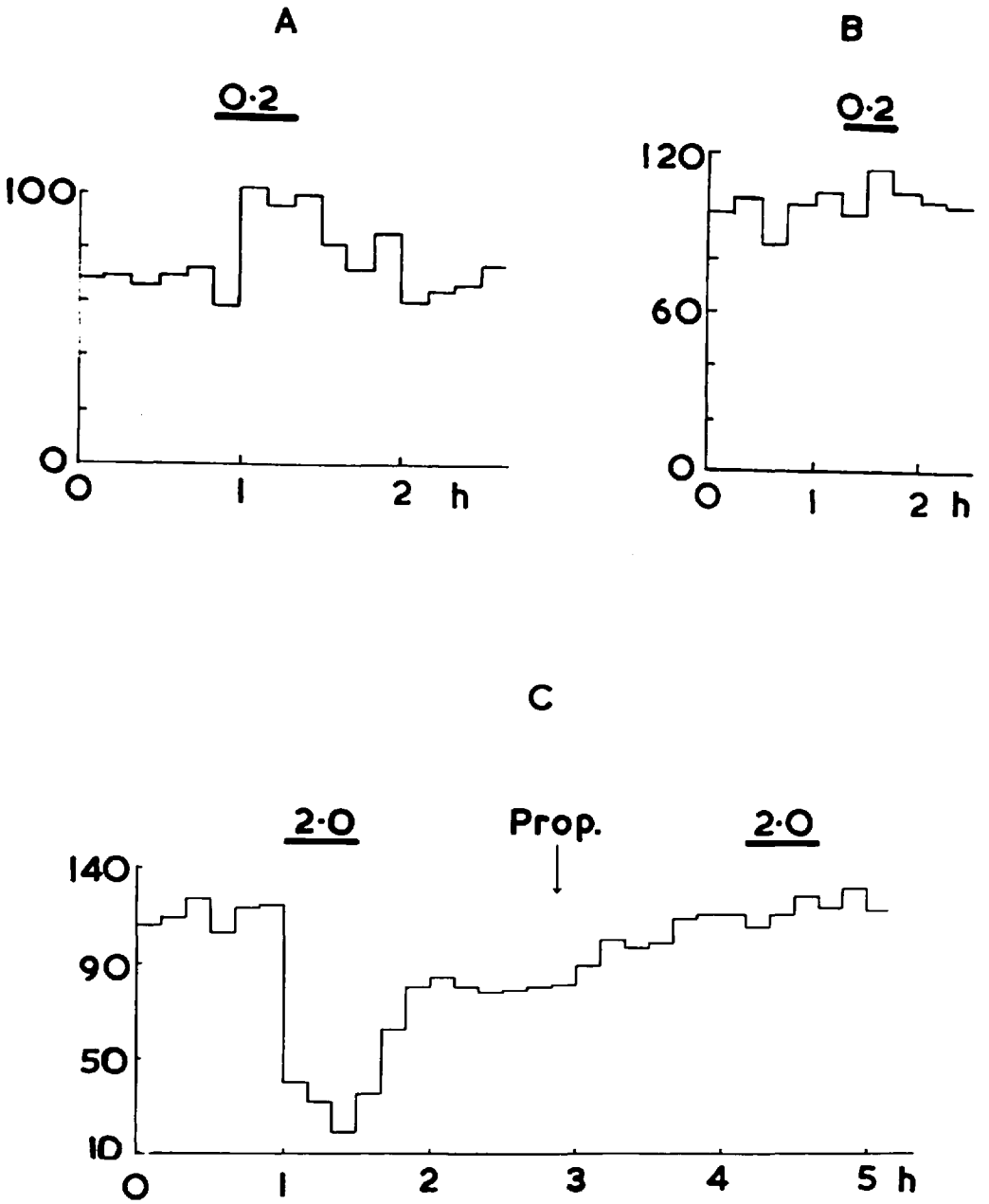
Fig. 5.1. EFFECTS OF ISOPRENALINE ON HISTAMINE-INDUCED GASTRIC ACID SECRETION FROM A HEIDENHAIN POUCH.

In this and all figures in this chapter denoting acid secretion, ordinates are pouch acid secretion in $\mu eq\ H^{+}\ min^{-1}$ and abscissae are time in hours. Isoprenaline was infused during the times indicated by the bars. The doses are given in $\mu g\ Kg^{-1} min^{-1}$.

A. The stimulatory effect of a small dose of isoprenaline.

B. Another experiment in the same dog in which this dose of isoprenaline did not affect secretion.

C. The inhibitory effect of a large dose of isoprenaline on acid secretion and blockade of the inhibition by intravenous propranolol (1 mg/kg).


A
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100
0
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B
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120
60
0
0
1
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C
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2·0
140
90
50
10
0
1
2
3
4
5 h

(b) Pentagastrin-induced secretion

Pentagastrin was infused at 4-5 μg $Kg^{-1}hr^{-1}$. In 10 experiments in four dogs, isoprenaline, in doses ranging from 0.05-2.0 μg $Kg^{-1}min^{-1}$ caused a marked decrease in acid secretion (Fig. 5.2a). At the smallest dose, secretion was reduced by an average of 56% and when 0.2 μg $Kg^{-1}min^{-1}$ of isoprenaline was given for 30 minutes, secretion was reduced by an average of 90% of the pre-dose level. Recovery of secretion was normally complete 30-40 minutes after stopping the isoprenaline infusion.

Noradrenaline was found to have a similar effect on secretion. In each of five experiments in 3 dogs noradrenaline 0.2 μg $Kg^{-1}min^{-1}$ reduced secretion (Fig. 5.2b). The mean reduction was 71%.

The inhibitory effect of isoprenaline (0.05-0.2 μg Kg^{-1} min^{-1}) on pentagastrin-induced acid secretion was found not be be abolished by propranolol (1-2 mg/kg i.v.) in five out of five experiments in four dogs (Fig. 5.2a). Heart rate did not rise during the isoprenaline infusion, suggesting that effective β receptor blockade was present.

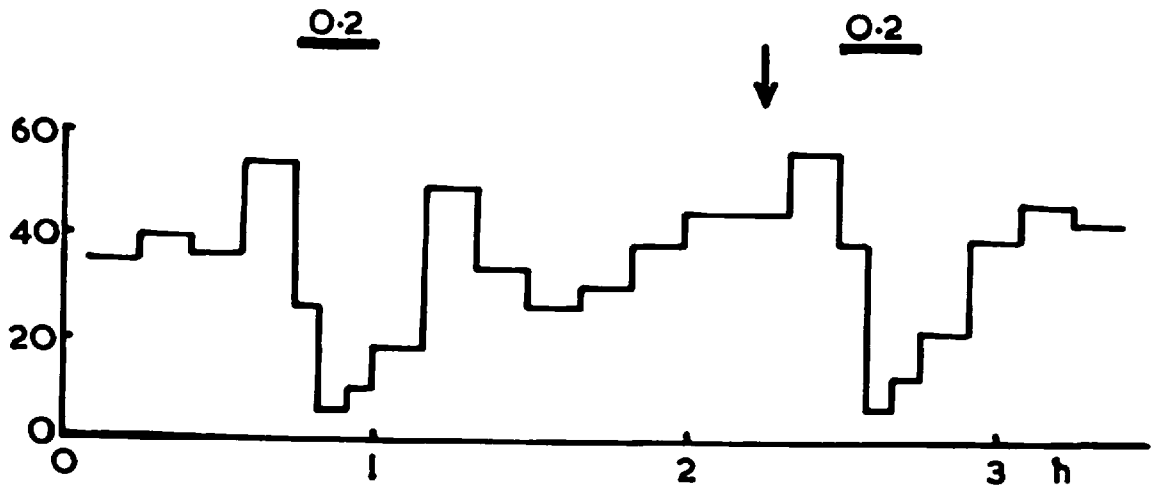
Fig. 5.2. A. THE INHIBITORY EFFECT OF ISOPRENALINE ON PENTAGASTRIN-INDUCED GASTRIC ACID SECRETION FROM A HEIDENHAIN POUCH AND ITS RESISTANCE TO THE β-ADRENERGIC BLOCKING ACTION OF PROPRANOLOL.

Isoprenaline (0.2 μg $Kg^{-1}min^{-1}$) was infused during the times indicated by the bars. Previous administration of propranolol (1 mg/kg i.v.), given at the arrow, did not prevent the inhibition.

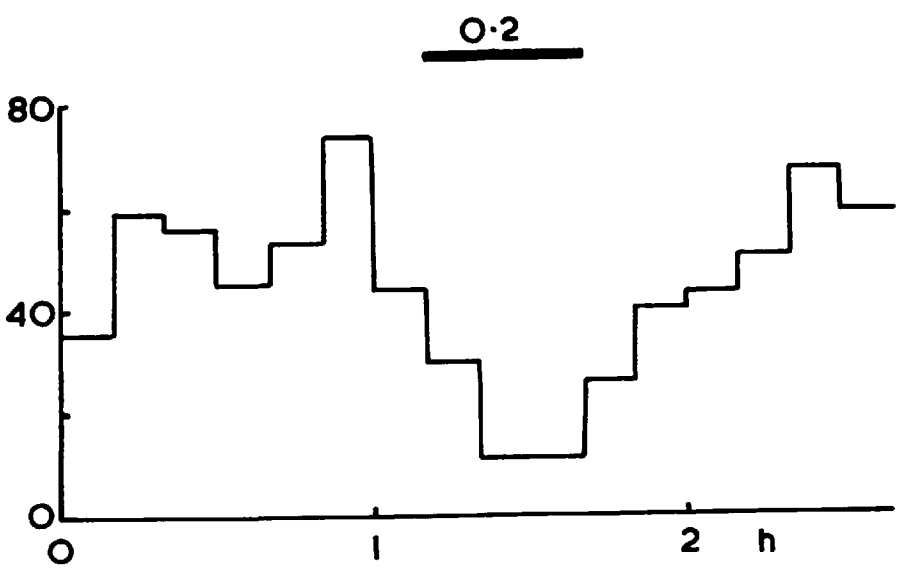
B. REDUCTION BY NORADRENALINE ADMINISTRATION OF PENTAGASTRIN-INDUCED GASTRIC ACID SECRETION FROM A HEIDENHAIN POUCH.

Secretory rate (μeq H^+ min^{-1}) fell in response to noradrenaline (0.2 μg $Kg^{-1}min^{-1}$) infused during the time indicated by the bar.

A



B



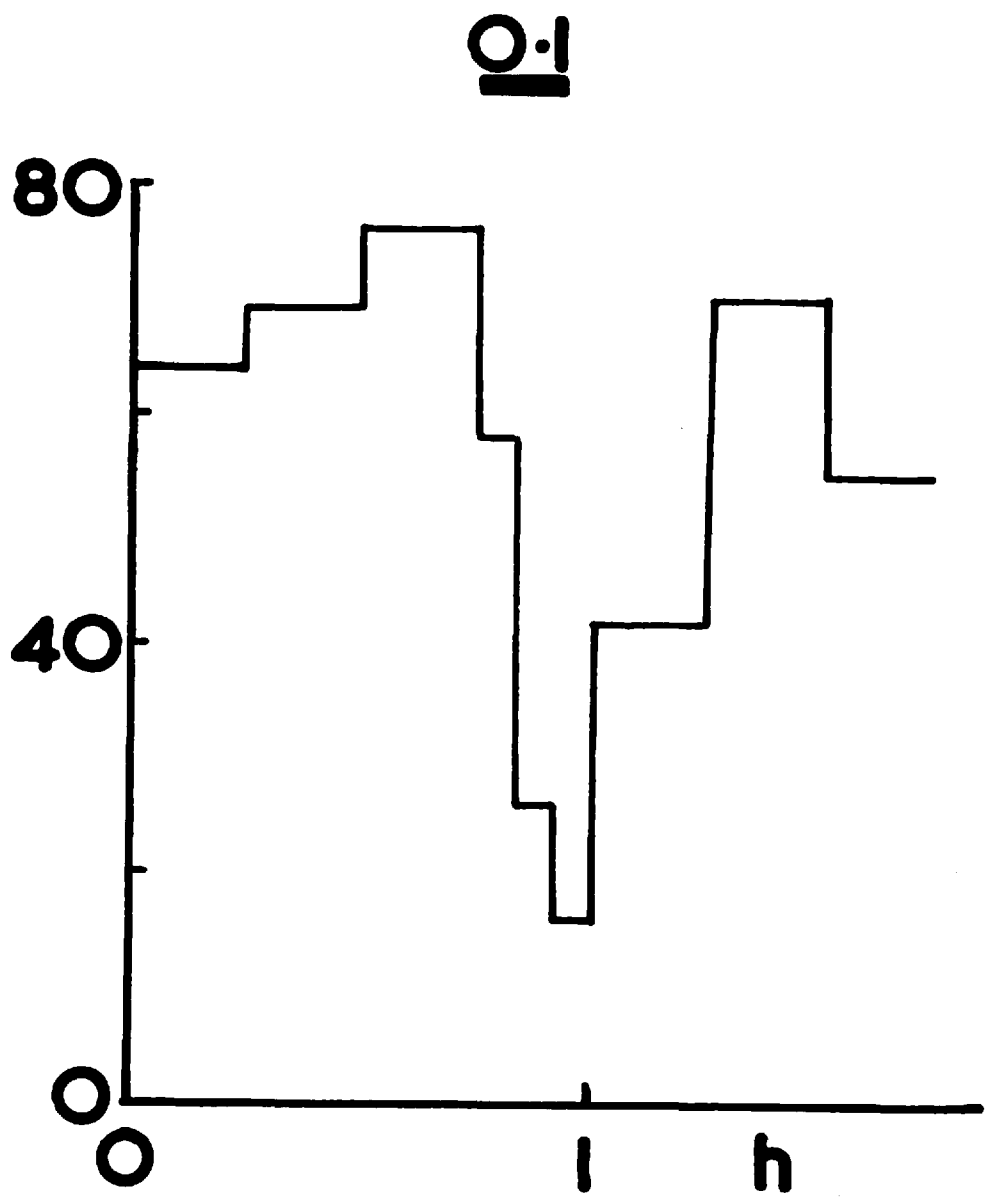
(c) Secretion induced by feeding.

The dogs were fed a standard meal of cooked minced ox liver (10-20 gm/kg) to which 10% w/w calcium orthophosphate and a little sodium chloride and water were added. Isoprenaline was infused when secretion was steady and its effect was similar to that seen when pentagastrin was the secretory stimulant. In 6 out of 6 experiments in four dogs an average reduction of secretion of 83% was seen in response to isoprenaline (0.1-0.25 μg $Kg^{-1}min^{-1}$) given for 10-30 minutes (Fig. 5.3). In two experiments in two dogs noradrenaline 0.1 and 0.5 μg $Kg^{-1}min^{-1}$ given for 10 minutes reduced acid secretion by 33% and 85% respectively.

In summary, both isoprenaline and noradrenaline have been shown to decrease acid secretion from Heidenhain pouches when given intravenously to conscious dogs. When secretion was induced by pentagastrin or feeding, inhibition of secretion always occurred and a smaller dose of either catecholamine was required to produce inhibition than when secretion was stimulated by histamine. Small doses of isoprenaline had varying effects upon histamine-induced secretion, either causing an increase or no change in the acid output. Inhibition of histamine-induced secretion by isoprenaline was blocked by propranolol but this was not the case when secretion was induced with pentagastrin.

Fig. 5.3. THE INHIBITORY EFFECT OF A SMALL DOSE OF ISOPRENALINE ON GASTRIC ACID SECRETION, INDUCED BY FEEDING, FROM A HEIDENHAIN POUCH.

Isoprenaline (0.1 μg $Kg^{-1}min^{-1}$) was infused during the time indicated by the bar resulting in a marked reduction in acid secretory rate (μeq H^{+} min^{-1}).


0·1
80
40
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1
h

Stimulus	ISOPRENALINE			NORADRENALINE	
	0.05-0.25µg Kg^{-1} min^{-1}	0.5-2.0µg Kg^{-1} min^{-1}	After Propranolol	0.05-0.25µg Kg^{-1} min^{-1}	0.5-2.0µg Kg^{-1} min^{-1}
Histamine	Increase or no effect	Decrease	No effect	No effect	Decrease
Pentagastrin	Decrease	Decrease	Decrease	Decrease	Decrease
Feeding	Decrease	Decrease		Decrease	Decrease

Table 5. A summary of the effects of large and small doses of isoprenaline and noradrenaline on gastric acid secretion induced by histamine, pentagastrin or feeding in conscious dogs with separated gastric pouches.

5.32 The effects of salbutamol and phenylephrine on pentagastrin-induced gastric acid secretion

Phenylephrine, an α adrenergic agonist has actions similar to noradrenaline(Goth, 1966; Bowman, Rand and West, 1968). Experiments were performed, in the Heidenhain pouch dogs, to see if its action on gastric acid secretion resembled that of noradrenaline. It was found that in 3 out of 3 experiments in two dogs, phenylephrine (10-20 µg $Kg^{-1}min^{-1}$), infused for 15 or 30 minutes, reduced pentagastrin-induced secretion by 50-75%. A dose of 5 µg $Kg^{-1}min^{-1}$ had no effect on secretion in one of the dogs.

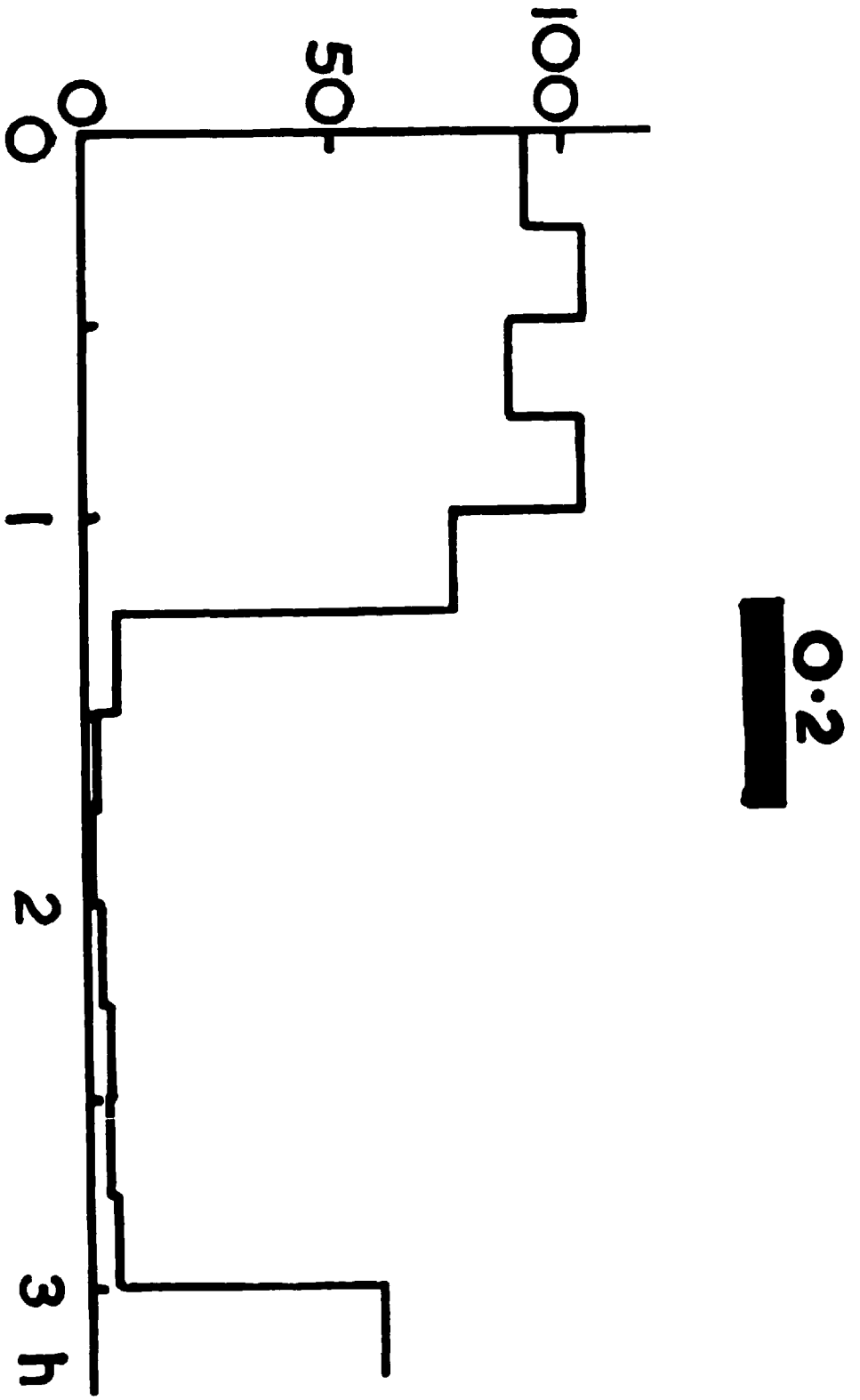
Salbutamol, an adrenergic β receptor agonist (Cullum, Farmer, Jack and Levy, 1969) was also found to inhibit acid secretion. A dose of only 0.1 μg $Kg^{-1}min^{-1}$ given for 15 to 30 minutes reduced secretion by 75% and 72% in 2 experiments in 2 dogs. The action of salbutamol on gastric secretion and its positive chronotropic effect were found to be more prolonged than these actions of isoprenaline. In one experiment heart rate was still increased and gastric secretion reduced three hours after giving salbutamol 10 μg $Kg^{-1}min^{-1}$ for 30 minutes.

5.33 The effects of isoprenaline on acid secretion induced by pentagastrin in anaesthetized dogs acutely prepared for collection of gastric secretion

Experiments previously carried out had shown that isoprenaline always increased histamine-induced gastric acid secretion in anaesthetized dogs (Curwain, Endersby and Holton, 1971). However, when secretion was stimulated by an infusion of pentagastrin, the opposite effect was seen. In each of three experiments a marked decrease in secretion, to an average of 7% of the pre-dose level, followed the administration of isoprenaline sulphate 0.2 μg $Kg^{-1}min^{-1}$ for 30 minutes (Fig. 5.4).

Fig. 5.4. THE INHIBITORY EFFECT OF A SMALL DOSE OF ISOPRENALINE ON PENTAGASTRIN-INDUCED GASTRIC ACID SECRETION IN AN ANAESTHETIZED DOG.

Isoprenaline (0.2 μg $Kg^{-1}min^{-1}$) was infused during the time indicated by the bar resulting in a prolonged fall in acid secretion (μeq H^{+} min^{-1}).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5.34 The effects of isoprenaline, salbutamol and noradrenaline on gastric mucosal blood flow

Since acid secretion induced by pentagastrin had proved to be more susceptible to inhibition by catecholamines than histamine stimulated secretion, pentagastrin (4 µg $Kg^{-1}hr^{-1}$) was chosen as the secretory stimulant.

In 5 experiments in two of the Heidenhain pouch dogs, mucosal blood flow, measured by aniline clearance, decreased when acid secretion fell in response to isoprenaline* but the ratio of blood flow to secretion (R) increased. Inhibition of acid secretion by salbutamol (0.1 µg $Kg^{-1}min^{-1}$) also caused an increase in mucosal blood flow relative to secretion. This effect persisted for over an hour after withdrawing the drug on each of two occasions. When secretion was inhibited by Noradrenaline (0.1-0.5 µg $Kg^{-1}min^{-1}$) the ratio of blood flow to acid secretion remained unchanged (5 experiments in 2 dogs). The results of one experiment, in which both isoprenaline and noradrenaline were given, are shown in Fig. 5.5. The ratios of mucosal blood flow to acid secretion (ml/µEqH^{+}) and volume of secretion (R) during the same experiment are also plotted.

Mucosal blood flow was measured during one of the experiments in which propranolol was found not to prevent inhibition of pentagastrin-induced secretion by isoprenaline. The increase

* (0.05-0.2µg $Kg^{-1}min^{-1}$)

in the mucosal blood flow relative to acid secretion caused by isoprenaline was seen to persist after propranolol administration.

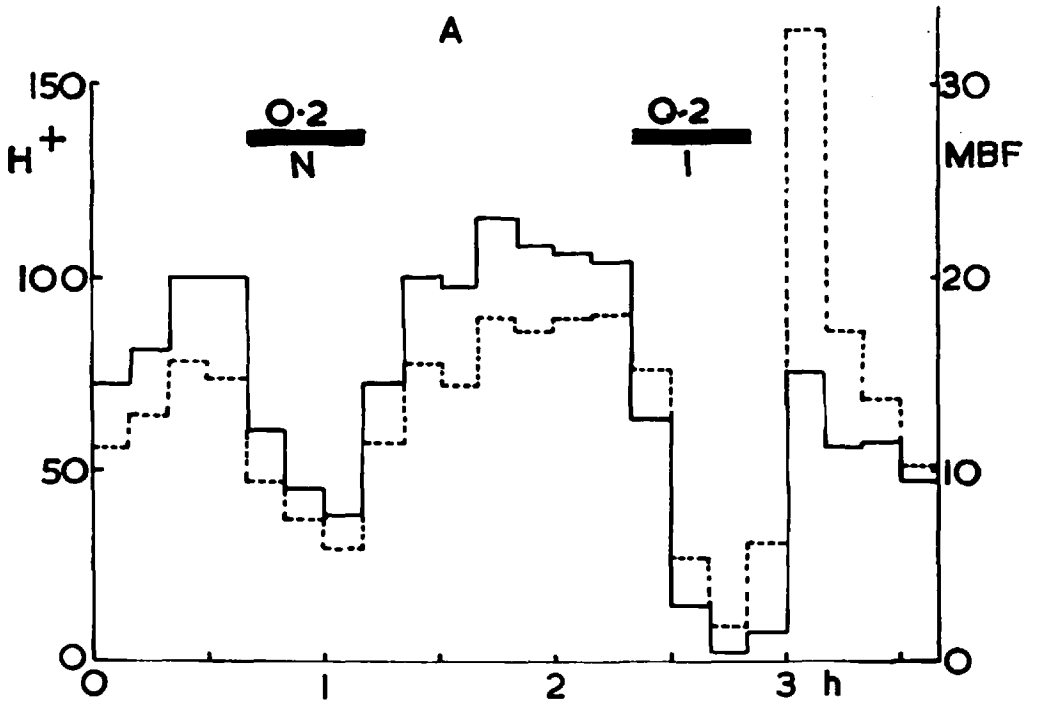
Since histamine causes blood flow in excess of that required for secretion, it might be expected that noradrenaline, acting as a vasoconstrictor, would reduce blood flow relative to secretion induced by histamine. In three experiments in two dogs R fell by 37,59 and 37% in response to 2.0 μg $Kg^{-1}min^{-1}$ noradrenaline infused for 30 minutes.

Fig. 5.5. THE EFFECTS OF NORADRENALINE AND ISOPRENALINE ON PENTAGASTRIN-INDUCED GASTRIC ACID SECRETION AND MUCOSAL BLOOD FLOW FROM A HEIDENHAIN POUCH

Noradrenaline (N) and isoprenaline (I) were infused, each at 0.2 μg $Kg^{-1}min^{-1}$, during the times indicated by the bars.

A. Acid secretion (μeq H^+ min^{-1}) is denoted by the solid line and mucosal blood flow (MBF, ml min^{-1}) by the broken line. Isoprenaline and noradrenaline both caused a decrease in secretion and MBF but the fall in MBF to isoprenaline was less than that in response to noradrenaline when related to the rate of acid secretion.

B. The results of the same experiment as shown in A are depicted. The filled circles denote the ratio of MBF to the volume of secretion in unit time (R) and the open circles denote the ratio of MBF to the rate of acid secretion (ml. MBF/μeq H^+ secreted). Both functions were unaffected during noradrenaline but showed a marked increase in response to isoprenaline infusion.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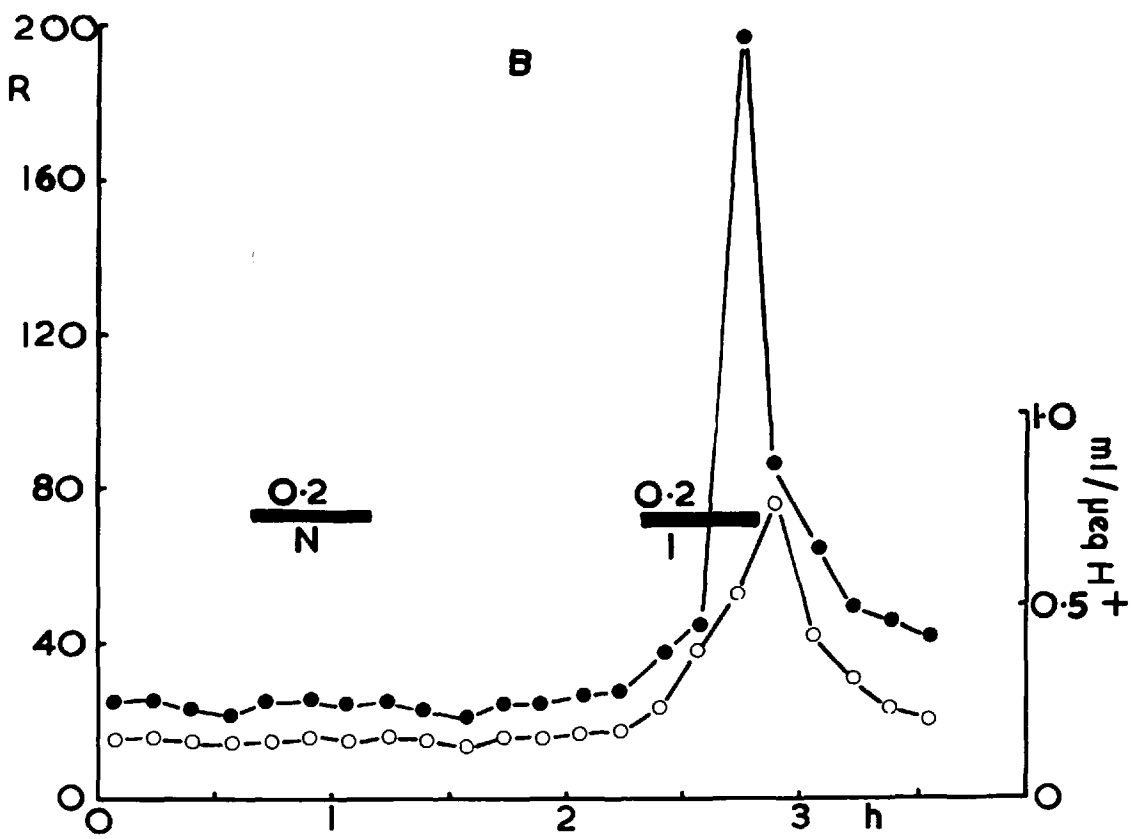
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5.4 DISCUSSION

The results in 5.3 clearly confirm the fact that drugs acting on α and β adrenoceptors are capable of inhibiting gastric acid secretion from separated pouches. Secretion stimulated by pentagastrin or feeding (i.e. by endogenous gastrin) has been shown to be more susceptible to catecholamine-induced inhibition than histamine-induced secretion. These findings, together with those of Harries (1956) concerning vagally stimulated secretion, indicate that histamine-induced secretion is more resistant to inhibition than gastric secretion produced by other means. The results presented in Chapter 4 show that the vascular state of the mucosa secreting in response to histamine is different than when stimulated by pentagastrin or feeding. This point will be referred to later in this section.

5.41 Gastric Haemodynamics

The gastric vasculature is complex and many factors can influence gastric mucosal blood flow. Flow is a product of the pressure drop across the circulation and the resistance of that circulation. In the case of the gastric circulation, the pressure drop is the arterial blood pressure minus the portal venous pressure. The mucosal circulation is in parallel with the muscle and submucosal circulations and with numerous arteriovenous

anastomoses (Fig. 5.7 - from Barlow, Bentley and Walder, 1951). Mucosal blood flow may therefore be increased or decreased by alteration in arterial or portal venous pressure and by changes in the resistance of the parallel circulations as well as vascular changes in the mucosa itself. There is evidence of the existence of pre-capillary sphincters and post-capillary vessels in the gastric mucosa (Jansson, Kamp, Lundgren and Martinson, 1966; Jansson, Lundgren and Martinson, 1970) both of which are sensitive to catecholamines.

5.42 Effects of isoprenaline and noradrenaline on histamine-induced gastric acid secretion

The dose of isoprenaline or noradrenaline required to inhibit histamine-induced acid secretion is approximately ten times that which effectively inhibits secretion to pentagastrin. Another instance of pentagastrin-induced acid secretion being easier to inhibit than histamine-stimulated secretion is given by Pederson and Brown (1972). They found that a gastric inhibitory polypeptide had a greater effect when secretion was induced by pentagastrin but offered no explanation of the finding.

The fact that the inhibitory effect of a large dose of isoprenaline is prevented by propranolol indicates that the inhibition is due to action on adrenergic β-receptors. When

Fig. 5.6. (From Barlow, Bentley and Walder, 1951). A DIAGRAM OF THE VASCULAR ARRANGEMENT ACCORDING TO THE ABOVE AUTHORS IN THE STOMACH WALL.

M = mucosal arteries

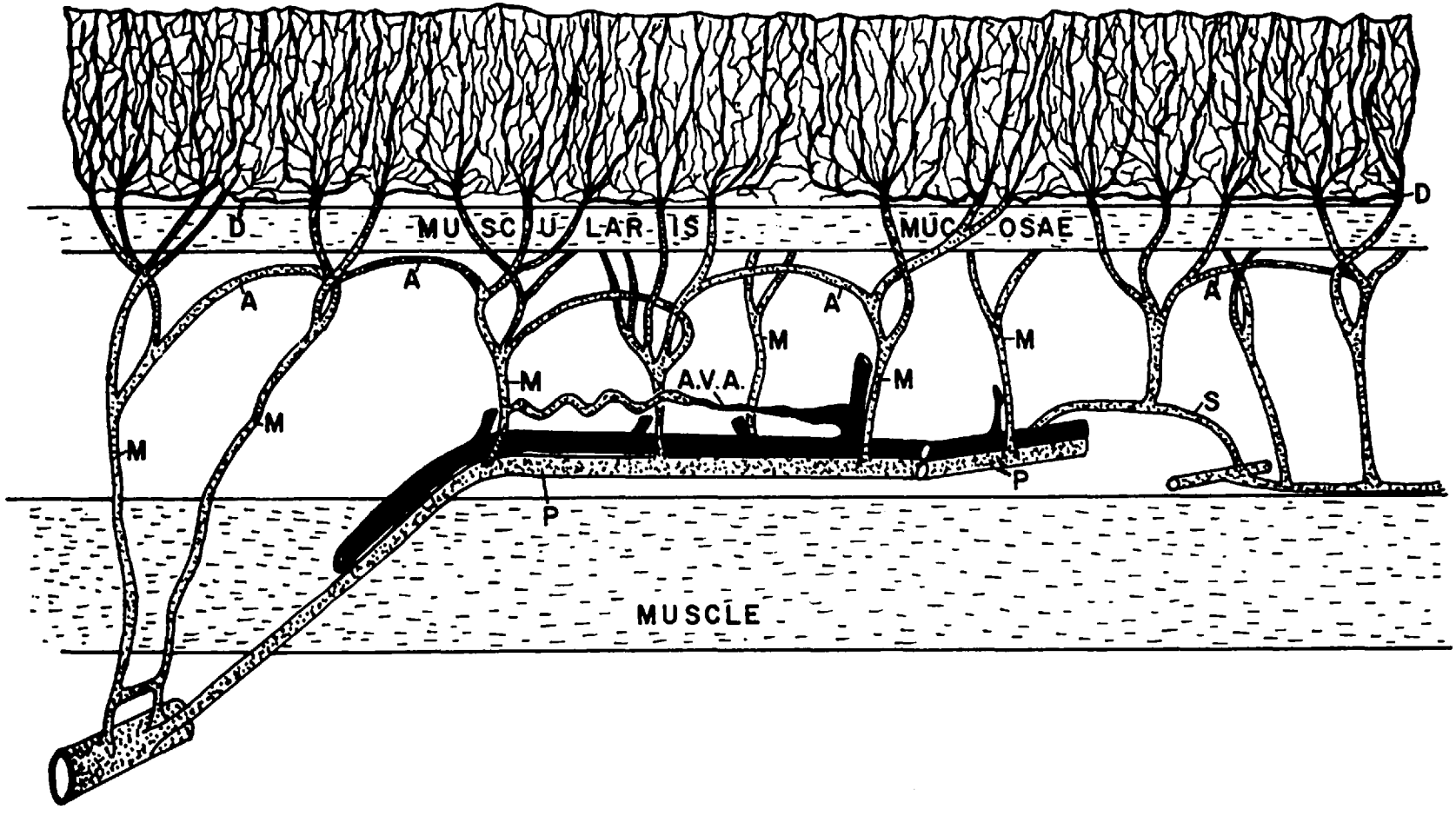
A.V.A. = arteriovenous anastomoses

A = anastomoses between mucosal arteries

S = anastomoses between submucosal arteries

D = network of anastomosing channels on the glandular aspect of the muscularis mucosae from which the mucosal capillaries arise

P = main supply channels of the submucous plexus.


D
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A.V.A.
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P
MUSCLE

isoprenaline is infused in sufficient dose to inhibit histamine-induced acid secretion (2 μg $Kg^{-1}min^{-1}$) heart rate doubles. Other cardiovascular effects which I could not directly observe, principally vasodilatation, may be presumed to occur, resulting in a lowering of central arterial blood pressure. This may bring about a fall in mucosal perfusion reducing its secretory capacity. Although Green et al. (1955) reported increased mesenteric blood flow in response to isoprenaline, their experiments were performed in anaesthetized dogs and single injections of isoprenaline, not exceeding 10 μg were given instead of the prolonged infusions used in my studies.

The dogs are observed to become anxious when receiving a large dose of isoprenaline and the inhibition of histamine-stimulated acid secretion may be invoked by the central nervous system. Although I have found no reference to the effect of emotion on this type of acid secretion in dogs, Bennett and Venables (1920) found that the gastric secretory response of humans to a meal was depressed by emotional disturbances.

The variable effects of small doses of isoprenaline on histamine-induced secretion are hard to explain. It is likely that at these doses (0.05-0.25 μg $Kg^{-1}min^{-1}$) isoprenaline causes mesenteric vasodilatation. However, histamine itself causes mucosal blood flow in excess of that required for secretion (Chapter 4) so this is unlikely to be the mechanism. The fact

that the response varies from dog to dog and day to day indicates that it depends, in part at least, on the initial state of the animal.

5.43 Effects of isoprenaline and noradrenaline on acid secretion and mucosal blood flow induced by pentagastrin or feeding

Doses of 0.2 μg $Kg^{-1}min^{-1}$ of isoprenaline or noradrenaline, given for 10-30 minutes have been shown consistently to cause a reduction in secretion, from separated pouches, induced by pentagastrin or feeding. However, although the effects of the two catecholamines on secretion are similar, their effects on gastric mucosal blood flow are totally dissimilar. This is to be expected from their known pharmacological actions and suggests that noradrenaline and isoprenaline are acting by different mechanisms. I attempted to analyse the mechanism of noradrenaline inhibition of secretion using the α adrenergic blocking drug phentolamine. This proved impossible when it was found that phentolamine itself markedly reduced gastric secretion. However, evidence discussed below that adrenergic α receptors are involved in this response has accumulated from changes in mucosal blood flow seen during inhibition of secretion by noradrenaline. Also phenylephrine, another drug known to act on α receptors (Goth, 1966; Bowman, Rand and West, 1968) has been found to inhibit gastric secretion

induced by pentagastrin.

The inhibitory effects of catecholamines on gastric secretion have been attributed to mucosal vasoconstriction. My results indicate that this is likely to be the case for noradrenaline but not isoprenaline. Three experiments in two dogs with noradrenaline on histamine-induced secretion showed that the ratio of blood flow to secretion fell in response to noradrenaline indicating that vasoconstriction was reducing mucosal blood flow, previously raised by histamine, to below the level required for the maintenance of brisk secretion. With pentagastrin as the stimulus, the ratio of blood flow to secretion is not altered during noradrenaline inhibition. From this, may be inferred two points. Firstly, unlike histamine, pentagastrin does not elicit mucosal blood flow in excess of that required for secretion and that the increase in mucosal blood flow over control levels is a consequence of the metabolic demands of the secreting tissue. Secondly, from the parallel and simultaneous reduction in blood flow and secretion, it seems more likely that the reduced blood flow is the primary factor in the inhibition. If the reduced blood flow were a consequence of secretory inhibition one might expect a slight time lag in the response showing up as a temporary increase in the ratio of flow to secretion. This was not observed. Support for this theory comes from the results of Cowley and Code (1970)

who found that noradrenaline reduced mucosal blood flow in the non-secreting stomachs of anaesthetized dogs. Reed and Sanders (1971), using anaesthetized cats, showed that sphlanchnic nerve stimulation, which would release noradrenaline, reduced mucosal blood flow and acid secretion. This was so whether secretion was stimulated by pentagastrin or histamine. Thus the effect of noradrenaline on acid secretion seems likely to be secondary to mucosal vasoconstriction although this has not been demonstrated conclusively.

During inhibition of secretion by small doses (0.2 μg Kg^{-1} min^{-1}) of isoprenaline, mucosal blood flow is much increased, relative to the secretory output. This indicates that the mechanism of the response is something other than vasoconstriction since blood flow clearly cannot be a limiting factor during inhibition. The inhibition is probably a direct effect of isoprenaline on the secretory apparatus stimulated by pentagastrin and gastrin, and its resistance to pharmacologically effective doses of propranolol demonstrates that it is not mediated by adrenergic β receptors.

The capacity to inhibit gastric secretion however, seems to be a property of adrenergic β receptor agonists since salbutamol, a drug that stimulates mainly β_2 receptors (Cullum et al., 1969), also causes inhibition. The effect of salbutamol on canine gastric secretion is more prolonged than that of isoprenaline but results

in a similar increase in the ratio of mucosal blood flow to secretion.

It should be stressed that although the ratio of blood flow to secretion is increased during inhibition of secretion by isoprenaline, total mucosal blood flow falls. This is to be expected as a result of local auto-regulatory mechanisms. When secretion is inhibited, the demands of the mucosa for oxygen and metabolic substrates will fall. Since oxygen tension seems to be the major factor in local auto-regulation of blood flow (Guyton, 1971) this will result in more capillaries being closed, thus reducing the overall blood flow through the tissue.

5.44 Effect of isoprenaline on pentagastrin-induced acid secretion in anaesthetized dogs

In the anaesthetized dogs acid secretion induced by pentagastrin proved to be susceptible to inhibition by isoprenaline. This is in direct contrast to results we had obtained with histamine as the stimulant (Curwain et al., 1971) when secretion was always increased by isoprenaline. This may be explained by a direct inhibitory effect of isoprenaline on pentagastrin-induced secretion as has been observed in the conscious dogs.

The potentiation by isoprenaline of histamine-induced secretion is likely to be due to vasodilatation in a tissue

which, as a result of surgical intervention, has undergone prolonged sympathetic stimulation and consequent vasoconstriction reducing its secretory capacity and limiting the amount of blood-borne histamine reaching it. It has long been known that laparotomy and surgical trauma cause inhibition of gastro-intestinal motility and this inhibition has been shown to be mediated by the sympathetic nervous system (Markowitz and Campbell, 1927: Neely and Catchpole, 1971). Reed, Sanders and Thorpe (1971) and Reed and Sanders (1971a) have shown that splanchnic nerve stimulation reduces gastric acid secretion and mucosal blood flow in the anaesthetized cat. From these studies and those of Jansson et al. (1966, 1970) concerning catecholamine-sensitive blood vessels in the stomach, it can be deduced that the vascular state of an acutely prepared anaesthetized animal is vastly different from that of a well established pouch in a conscious animal.

Thus the results in anaesthetized dogs lend further support to the theory that isoprenaline has a direct inhibitory effect on canine gastric acid secretion induced by pentagastrin. From the results presented in Chapter 4 concerning the blood flow characteristics during various forms of secretory stimulation it may be suggested that adrenergic β receptor agonists have a powerful inhibitory action on physiological acid secretion during the gastric phase of gastric secretion.

5.45 Summary

Drugs acting on adrenergic α and β receptors have been shown to inhibit gastric acid secretion in conscious and anaesthetized dogs. Histamine-stimulated secretion is more resistant to inhibition by adrenergic drugs. The mechanisms of action of catecholamines with predominantly α and β actions have been shown to be different. Noradrenaline probably acts by vasoconstriction but isoprenaline and salbutamol have a direct inhibitory effect on the secretory apparatus stimulated by pentagastrin or gastrin.

A P P E N D I X 1

Statistical calculations referring to

results presented in Chapter 3.

Appendix 1.1. AN ANALYSIS OF VARIANCE OF ESTIMATIONS OF ANILINE AND AMIDOPYRINE CONCENTRATIONS IN CANINE PLASMA

(a) Aniline. Plasma concentrations expressed as net c.p.m. ^{14}C radioactivity.

Group	1	2	3	4	5	6	7
	6083	5888	5933	6076	5999	6129	6147
	5861	5709	5950	6074	5994	6133	6045
	5981	5654	5600	6015	5979	6050	6155
	6030	5620	5853	6000	5882	6146	6148
	6026		5786		5944	6160	6111
n	5	4	5	4	5	5	5
Total	29981	22871	29122	24165	29798	30618	30606

$n = 33$

$Sx^2 = 1178750527$

$Sx = 197161$

$Sx/n = 5974$

$(Sx)^2 = 38872459921$

$(Sx)^2/n = 1177953330$

$SSx = 797197$

Sx^2/n (groups) = 1178569705

SS (groups) = 616375 (6 d.f.)

Residual SS = 797197 - 616375

= 180822 (26 d.f.)

Variance = $\frac{180822}{26}$ = 6954

s.d. = $\sqrt{6954}$ = 83.39

= 1.4% of the mean

(b) Amidopyrine. Plasma concentrations expressed as optical density at 260 nm x 1000.

Group	1	2	3	4	5	6	7
	162	173	174	152	157	147	159
	162	182	164	168	171	173	156
	158	183	169	159	161	150	159
	154	178	182	165	157	171	154
	150		173	164		162	156
							163
n	5	4	5	5	4	5	6
Total	786	716	862	808	646	803	947

$n = 34$

$Sx^2 = 914912$

$Sx = 5568$

$Sx/n = 163.76$

$(Sx)^2 = 31002624$

$(Sx)^2/n = 911842$

$SSx = 3070$

Sx^2/n (groups) $= 913664$

SS (groups) $= 1822$ (6 d.f.)

Residual SS $= 3070 - 1822$

$= 1248$ (27 d.f.)

Variance $= \frac{1248}{27} = 46$

s.d. $= \sqrt{46} = 6.8$

$= 4.15\%$ of the mean

Comparison of variances of aniline and amidopyrine estimations.

$$F = \frac{4.15^2}{1.4^2} = \frac{17.24}{1.948} = 8.85$$

For $F_{27,26}$

$P < 0.01$

The analysis shows that the variance of the amidopyrine estimations is significantly greater than the variance of the aniline estimations ($P < 0.01$).

Appendix 1.2. AN ANALYSIS OF VARIANCE OF $\frac{\text{R ANILINE}}{\text{R AMIDOPYRINE}}$ AFTER FEEDING IN HEIDENHAIN POUCH DOGS. (Figures are expressed as percentages).

	Grocer		Peta		Alex		Chris
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1
	82.1	86.2	103.6	61.8	64.3	70.0	64.7
	65.8	92.5	100.0	66.7	68.3	64.3	63.9
	65.4	94.1	111.5	68.2	71.8	61.7	59.0
	67.1	106.5		68.9	70.7	82.5	
		78.6		68.2		83.3	
						83.3	
						63.2	
n	4	5	3	5	4	7	3
Sx	280.4	457.9	315.1	333.8	275.1	508.3	187.6
Dog Totals	738.3		648.9		783.4		187.6
Total Observations per dog	9		8		11		3

Grand total = 2358.2

There are 4 dogs thus k = 4

There are 7 days thus m = 7

There are 4 + 5 + ... + 3 observations thus n = 31

cf = correction factor = $\frac{1}{n}$ x (grand total)^2 = 179390.6

Y = Uncorrected dogs SS = $S\frac{(\text{Dog Total})^2}{\text{No.of obs.per dog}}$ = 180722.7

$$L = \text{Uncorrected days SS} = S\frac{(\text{Day total})^2}{\text{No.of obs.per day}} = 184531.1$$

$$A = \text{Uncorrected total SS} = S(\text{Observation})^2 = 185917.4$$

Corrected sums of squares

Between dogs	= Y - cf	= 1332.1
Between dogs within dogs	= L - Y	= 3808.4
Within days	= A - L	= 1386.3
Total SS	= A - cf	= 6526.8

Analysis of variance table

Source	s.s.	d.f.	M.S.	$F_{3,24}$
Between Dogs	1332.1	k-1 = 3	444.0	7.68
Between days, within dogs	3808.4	m-k = 3	1269.5	21.96
Within days (Residual)	1386.3	n-m = 24	57.8	
Totals	6526.8	n-1 = 30		

The analysis of variance shows that very highly significant differences exist between the dogs ($P < 0.001$) and between days in the same dog ($P < 0.001$).

Appendix 1.3. AN ANALYSIS OF VARIANCE OF THE RATIO OF GASTRIC VENOUS ANILINE AND AMIDOPYRINE CONCENTRATIONS, IN SECRETING, ANAESTHETIZED PUPPIES, EXPRESSED AS PERCENTAGES OF THE ARTERIAL CONCENTRATION.

(The figures are the ratio of the percentages $\frac{\text{Amidopyrine}}{\text{Aniline}}$ multiplied by 10^4 for convenience).

	MUCOSAL POUCH		MUSCLE POUCH	
	Dog 1	Dog 2	Dog 1	Dog 2
	7778	8056	6897	9778
	7500	8056	7241	8409
	7222	7632	7241	7273
	7222	6905	7000	5581
	7222		7000	
	7222			
	7027			
	7297			
	6923			
n_d	9	4	5	4
Total	65413	30649	35379	31041
Mean	7268	7662	7076	7760
n_p	13		9	
Total	96062		66420	

Grand Total = 162482

There are 2 types of pouch K = 2

There are 4 dogs altogether M = 4

There are 22 observations n = 22

Correction factor = $\frac{1}{n}$ (Grand Total)^2 = 1200018197

Y = uncorrected pouches SS = $S\frac{(\text{Pouch Total})^2}{n_p}$ = 1200018665

L = uncorrected dogs SS = $S\frac{(\text{Dog Total})^2}{n_d}$ = 1201489901

A = uncorrected total SS = $S(\text{Observation})^2$ = 1212451434

Corrected sums of squares

Between Pouches	= Y - cf	= 468
Between Dogs within Pouches	= L - Y	= 1471236
Residual	= A - L	= 10961533
Total	= A - cf	= 12433237

Analysis of variance table

Source	SS	d.f.	M.S.	F.
Between Pouches	468	1	468	0.0008 N.S.
Between Dogs within Pouches	1471236	2	735618	1.21 N.S.
Residual	10961533	18	608974	
Total	12433237	21		

The analysis reveals that differences between the two types of pouches and between the individual dogs are not significant.

A P P E N D I X 2

Statistical calculations referring to results presented in Chapter 4.

Appendix 2.1. CALCULATION AND COMPARISON OF REGRESSION LINES OF R AND ACID SECRETORY RATE DURING STIMULATION BY HISTAMINE OR PENTAGASTRIN.

Acid secretory rate was expressed as a percentage of the maximal to each stimulus. The original data are shown in Fig. 4.6. The data were analysed by computer by the Intercollegiate Unit in Medical Statistics using a standard library program.

Results:-

(a) Histamine: analysis of variance of regression.

Source	Sums of Squares	d.f.	mean square	$F_{1,13}$
Regression	1447.83	1	1447.83	19.74
Residual	953.68	13	73.36	
Total	2401.52	14		

When $F_{1,13} = 19.74$ $P < 0.01$

The regression is found to be significant.

Regression coefficient $b = \frac{Sxy}{Sx^2} = 0.455$

(b) Pentagastrin: analysis of variance of regression.

Source	Sums of Squares	d.f.	mean square	$F_{1,7}$
Regression	137.82	1	137.82	29.92
Residual	32.24	7	4.61	
Total	170.06	8		

When $F_{1,7} = 29.92 \quad P < 0.01$

Regression coefficient $b = \frac{Sxy}{Sxy^2} = 0.154$

(c) Comparison of the histamine and pentagastrin regressions.

Calculations were carried out as described in Snedecor and Cochrane (1969) P.435 and are shown in Table A.1.

Within	d.f.	Sx^2	Sxy	Sy^2	b	Deviations from regressions d.f.	S.S.	M.S.
Histamine	14	6981.60	3179.34	2401.52	0.455	13	953.68	
Pentagastrin	8	5794.22	893.61	170.06	0.154	7	32.24	
						20	985.92	49.30
Pooled	22	12775.82	4072.95	2571.58	0.319	21	1273.12	60.62
						1	287.20	287.20
Between	1	92.01	281.24	859.63				
Total	23	12867.83	4354.19	3431.21		22	1957.85	
						1	684.73	684.73

Comparison of slopes $F_{1,20} = \frac{287.20}{49.30} = 5.83 \quad P < 0.05$

Comparison of elevations $F_{1,21} = \frac{684.73}{60.62} = 11.3 \quad P < 0.01$

Table A.1. Comparison of histamine and pentagastrin regressions.

Appendix 2.2. COMPARISON OF R DURING SECRETION IN RESPONSE TO PENTAGASTRIN AND HISTAMINE.

The data were analysed by the Intercollegiate Unit in Medical Statistics using program BMDX64 written by the Health Sciences Computing Facility, UCLA, California. The original data appear in Table 4.1.

Analysis of Variance Table

Source	Sum of Squares	d.f	Mean Square	F
Between Dogs	707.1331	4	176.7833	17.4983
Between Stimuli	559.4763	1	559.4763	55.3778
Residual	343.4989	34	10.1029	
Total	1610.1083	39		

The difference between stimuli (Pentagastrin and Histamine) is very highly significant ($P < 0.001$).

Differences between dogs are also very highly significant ($P < 0.001$).

Appendix 2.3. COMPARISON OF R DURING SECRETION IN RESPONSE TO FEEDING OR HISTAMINE AND FEEDING OR PENTAGASTRIN

The data were analysed as described in Appendix 2.2. and individual comparisons made within the analysis of variance.

Analysis of Variance Table

Source	Sum of Squares	d.f.	Mean Square	F
Between Dogs	391.5302	2	195.7651	9.2012
Between Stimuli	2601.1256	2	1300.5628	61.1283
Residual	574.4504	27	21.2759	
Total	3567.1062	31		

The analysis tells us that significant differences exist between the stimuli ($P < 0.001$) and between the dogs ($P < 0.001$).

Comparison of R in response to feeding and histamine:

Mean value for feeding $= \bar{x}_1 = 30.56$ $n_1 = 15$

" " " Histamine $= \bar{x}_2 = 50.28$ $n_2 = 10$

$$t = \frac{\bar{x}_2 - \bar{x}_1}{\text{s.e.of } (\bar{x}_2 - \bar{x}_1)}$$

$$se = \sqrt{21.2759 \left(\frac{1}{15} + \frac{1}{10}\right)} = 1.8831$$

$$t = \frac{19.72}{1.8831} = 10.47$$

With 27 d.f. $P < 0.001$

The difference between R in response to feeding and histamine is very highly significant ($P < 0.001$).

Comparison of R in response to feeding and pentagastrin:

Mean value for feeding = $\bar{x}_1$ = 30.56 n_1 = 15

" " " pentagastrin = $\bar{x}_3$ = 27.01 n_3 = 7

$$t = \frac{\bar{x}_1 - \bar{x}_3}{\text{s.e. of } (\bar{x}_1 - \bar{x}_3)}$$

$$\text{s.e.} = \sqrt{21.2759 \left(\frac{1}{15} + \frac{1}{7}\right)} = 2.11$$

$$t = \frac{3.55}{2.11} = 1.68$$

With 27 d.f. $P > 0.1$.

The difference between R in response to feeding and pentagastrin is not significant ($P > 0.1$).

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Reprinted from BRITISH JOURNAL OF PHARMACOLOGY, *February*, 1971, *Vol.* **41**, *No.* 2, *p.* 384P

Radioactive aniline clearance from canine gastric pouches for the measurement of gastric mucosal blood flow

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Clearance of various organic bases by the gastric mucosa was studied by Shore, Brodie & Hogben (1957) who showed that both aminopyrine (pKa 5·0) and aniline (pKa 5·0) are concentrated in the gastric juice. They postulated that these substances were completely removed from the blood flowing through the gastric mucosa. Jacobson, Linford & Grossman (1966) developed the technique of aminopyrine clearance as a measurement of gastric mucosal blood flow. Although aminopyrine clearance is now a widely used method, the estimation of aminopyrine is tedious. We have therefore investigated the possibility of using radioactive aniline instead of aminopyrine.

Commercially available ^{14}C aniline was estimated in plasma and gastric juice by shaking each 1 ml sample in a sealed bottle for 5 min with 10 ml diethyl ether and 0·5 ml M sodium hydroxide. Seven millilitres of the ether phase was then added to 10 ml scintillator and counted in a Packard Tri-Carb liquid scintillation counter.

Aniline clearance was studied in four dogs prepared with separated (Heidenhain) gastric pouches. A loading dose of aniline (10 mg/kg and 10 μCi) followed by a maintenance infusion [(10 mg/kg)/h and 10 μCi/h] was given intravenously. Blood samples were taken at 30 min intervals from another vein or via an indwelling arterial catheter. Gastric juice was collected from the pouch.

This dose of aniline had no effect on the gastric secretion in response to a meal or to histamine infusions. As would be expected there was detectable methaemoglobinaemia. However, the percentage of methaemoglobin, which varied in different dogs from 5 to 11%, was well below the value of 40% at which toxic effects are seen (Bodansky, 1951).

Gastric secretion was stimulated by infusing various doses of histamine or pentagastrin intravenously. The clearance of aniline closely followed the acid secretory response.

The theoretical basis of the assumption that aminopyrine clearance measures gastric mucosal blood flow applies equally to aniline clearance. Confirmation that aminopyrine clearance and aniline clearance are the same was obtained in experiments in which the clearances were compared in the same dog and shown to give very similar results. The advantage of aniline clearance is that the use of a radioactive substance simplifies its measurement in body fluids.

This work was supported by the Medical Research Council.

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Printed in Great Britain by Fisher, Knight and Co., Ltd., St. Albans.

Reprinted from BRITISH JOURNAL OF PHARMACOLOGY, *February*, 1971, *Vol.* 41, No. 2, *p.* 384P

Effect of isoprenaline on histamine induced gastric acid secretion in dogs

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Isoprenaline is a vasodilator substance and would therefore be expected to enhance the secretory response of the stomach to blood borne stimuli. However, both Harries (1957) using conscious dogs with gastric fistulae and Jacobson, Linford & Grossman (1966) using conscious dogs with separated gastric pouches found that isoprenaline [(0·75–2·75 μg/kg)/min] either decreased histamine induced secretion or had no effect. We have repeated the experiments on dogs with separated gastric pouches and have extended the observations to anaesthetized dogs and by using a wider dose range.

In thirteen experiments on dogs anaesthetized with pentobarbitone, isoprenaline [(0·125–8·0 μg/kg)/min] increased histamine induced gastric acid secretion (HIGAS) on every occasion, although in four of these experiments (in which the gastric secretion was collected through the cannulated pylorus) the increase was apparently preceded by a decrease. The decrease was probably an artefact due to relaxation of the stomach wall which would have produced a temporary change in the drainage of gastric juice.

Two dogs with separated pouches were used. In the first dog isoprenaline [(0·125–4 μg/kg)/min] increased HIGAS in seven experiments. In one experiment with isoprenaline [(4 μg/kg)/min] there was no clear effect. In the second dog increased HIGAS was observed on four occasions with (0·06–0·25 μg isoprenaline/kg)/min and decreased HIGAS on three occasions with (0·5–1 μg isoprenaline/kg)/minute. The inhibitory effect of isoprenaline in this dog was analysed in a further series of experiments. It was not abolished by light pentobarbitone anaesthesia, by pharmacologically effective doses of guanethidine or hexamethonium or by bilateral section of the splanchnic nerves but was abolished both by phentolamine and by propranolol.

We conclude that suitable doses of isoprenaline increase HIGAS in both anaesthetized and conscious dogs. In conscious dogs large doses of isoprenaline decrease the secretory response. The mechanism of this effect is not yet fully understood.

This work was supported by the Medical Research Council.

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Printed in Great Britain by Fisher, Knight and Co., Ltd., St. Albans.

[*From the Proceedings of the Physiological Society*, 17–18 *December* 1971]
Journal of Physiology, **222**, 1–3*P*

Comparison of the plasma clearances of [^{14}C]aniline and amidopyrine for the measurement of gastric mucosal blood flow

By B. P. Curwain. *Department of Physiology, St Mary's Hospital Medical School, London, W. 2*

Amidopyrine clearance is widely used to estimate gastric mucosal blood flow in conscious and anaesthetized animals (Jacobson, Linford & Grossman, 1966; Harper, Reed & Smy, 1968). The estimation of amidopyrine in biological fluids is tedious and the use of commercially available [^{14}C]-aniline which is more easily estimated was introduced by Curwain & Holton (1971). Both methods depend on trapping the organic cation in acid gastric juice. Preliminary experiments showed that the results obtained with the two methods were very similar. The methods have now been more rigorously compared by simultaneous measurements of clearance.

Both amidopyrine and aniline were almost completely recovered from gastric juice [amidopyrine 98 % ± 6·2 % (S.D.) n = 6, aniline 99 % ± 1·00 % (S.D.) n = 6]. Recovery from plasma was 96 % ± 1·17 % (S.D.) n = 39, for aniline and 86 % ± 3·16 % (S.D.) n = 25 for amidopyrine. The variance of the results for aniline was significantly smaller than that for amidopyrine (F = 8·9, P < 0·01).

Harper *et al.* (1968) stated that amidopyrine equilibrated between cat erythrocytes and saline within 1 min. I have found similar results for amidopyrine and aniline in dog's blood.

Clearance experiments were carried out on conscious dogs with Heidenhain pouches. Both bases were infused intravenously together as a loading dose (amidopyrine 20 mg kg^{-1}, aniline 10 mg kg^{-1}) and a maintenance infusion (amidopyrine 5 mg kg^{-1} hr^{-1}, aniline 10–12 mg kg^{-1} hr^{-1}). Acid gastric secretion was induced by feeding. Gastric juice and plasma were analysed for amidopyrine (Brodie & Axelrod, 1950) and aniline (Curwain & Holton, 1971) and acid secretory rate was measured. The plasma clearance of each base is directly related to

$$R = \frac{\text{concentration in gastric juice}}{\text{concentration in plasma}}.$$

For each base R was constant during steady acid secretion. However, the mean R for aniline was 26 % lower than for amidopyrine. The most likely explanation for this discrepancy is the plasma binding of the bases (Shore, Brodie & Hogben, 1957); 15 % of plasma amidopyrine and 25 % of plasma aniline are not available for diffusion through the gastric mucosa. The

[P.T.O.

extraction procedure for amidopyrine does not extract the bound fraction whereas the procedure for aniline does. If the estimated plasma aniline concentrations are corrected by subtraction of 25% the mean clearances of the two bases are identical.

This work was supported by a grant from the Medical Research Council to Dr P. Holton.

I am grateful to St Thomas' Hospital Medical School for providing accommodation for the dogs and facilities for experimental work during the main part of this investigation.

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Effects of isoprenaline on gastric acid secretion and mucosal blood flow during stimulation by pentagastrin or feeding

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Histamine-induced gastric acid secretion in dogs may be either increased or decreased by infusions of isoprenaline. In conscious dogs small doses (about (0·2 μg/kg)/min) increased and large doses (about (2 μg/kg)/min) decreased secretion; whereas in acute experiments in anaesthetized dogs the only effect observed was an increase of histamine-induced secretion (Curwain, Endersby & Holton, 1971). Since acid secretion is more readily depressed when it is induced by means other than histamine (Harries, 1957) we have investigated the effect of isoprenaline on secretion stimulated by infusions of pentagastrin and by feeding (that is by endogenous gastrin).

In eleven experiments on four conscious dogs with Heidenhain pouches, infusion of isoprenaline (0·05–2 μg/kg)/min decreased acid secretion induced by pentagastrin (5 μg/kg)/hour. In three experiments in acute anaesthetized dogs isoprenaline (0·2 μg/kg)/min also decreased pentagastrin-stimulated secretion. Isoprenaline (0·06–0·25 μg/kg)/min also decreased the (gastrin induced) secretion in response to feeding in seven experiments in the four Heidenhain pouch dogs. On no occasion was an increase of acid secretion observed when secretion was induced by these means.

The effects of isoprenaline on mucosal blood flow was studied in five experiments on two of the Heidenhain pouch dogs using the aniline clearance method (Curwain & Holton, 1971). Mucosal blood flow decreased when acid secretion (induced by pentagastrin) fell in response to isoprenaline but the ratio of blood flow to secretion increased. When noradrenaline (0·1–0·5 μg/kg)/min was used to decrease mucosal blood flow and acid secretion induced by pentagastrin or feeding, the ratio of blood flow to secretion remained unchanged (five experiments in two dogs).

We can conclude from these experiments that the inhibitory effect of isoprenaline on pentagastrin (or gastrin) induced secretion is not secondary to a fall in mucosal blood flow and is probably due to a direct antagonism between isoprenaline and the secretory mechanism initiated by gastrin. We cannot exclude the possibility that the inhibitory action of noradrenaline is secondary to mucosal vasoconstriction.

This work was supported by the Medical Research Council.

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CHANGES IN GASTRIC MUCOSAL BLOOD FLOW IN CONSCIOUS DOGS AFTER FEEDING.

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Although it is generally assumed that gastric mucosal blood flow increases in response to feeding, few quantitative data are available. We have investigated the magnitude and time course of changes in gastric mucosal blood flow in conscious dogs after a standard meal and have related these changes to the rate of acid secretion. Gastric mucosal blood flow was measured by the clearance of radioactive aniline (Curwain & Holton, 1971) in conscious dogs equipped with separated and vagally innervated gastric pouches. Acid secretion was also measured. Control experiments showed that the acid secretion was unaffected by the aniline used. Aniline clearance increased parallel with acid secretion from separated pouches. In dogs with vagally innervated pouches aniline clearance increased before acid secretion rose. It is concluded that gastric mucosal blood flow increases within a few minutes of starting to eat, and remains at a high level throughout the cephalic and gastric phases of acid secretion. Initial increase in mucosal blood flow precedes secretory response.

This work was supported by the Medical Research Council.

Ref. Curwain, B.P. & Holton, Pamela. 1971. Proc. of the Br.Pharmac.Soc.,January 1971.

Reprinted from PROCEEDINGS OF THE INTERNATIONAL UNION OF PHYSIOLOGICAL SCIENCES - Volume IX
Printed in the Federal Republic of Germany