OBSERVATIONS ON THE MECHANISM OF STIMULATION OF GASTRIC ACID SECRETION IN MAMMALS AND ON THE USE OF ANILINE FOR THE MEASUREMENT OF MUCOSAL BLOOD FLOW

BY

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Abstract

The development and validation of an in vitro mammalian preparation for the measurement of gastric acid secretion, the guinea-pig isolated stomach preparation is presented.

This preparation has a resting acid production which consists of two components.

(a) an HCl portion of acid secretion, due to the presence of food in the stomach on dissection and sensitive to sodium thiocyanate.

(b) a non-HCl portion of acid production due to either carbon dioxide, lactate or mucin accumulation and insensitive to sodium thiocyanate.

The guinea-pig isolated stomach preparation can be reversibly stimulated to secrete acid by histamine, theophylline, histamine plus theophylline and pentagastrin plus theophylline. The specific $H_2$-receptor antagonists burimamide and metiamide have been found to decrease histamine-stimulated acid secretion and the histamine portion of histamine plus theophylline-induced secretion. Sodium thiocyanate has been shown to abolish all types of stimulated hydrochloric acid secretion in this preparation.

Support has been given to the "second messenger" hypothesis in which cyclic AMP is the final mediator of hydrochloric acid secretion. Histamine is thought to cause acid secretion by stimulating the adenyl cyclase system to produce more cyclic AMP. Histamine mobilisation is envisaged as the common pathway for the action of other stimulants.

In conscious dogs with Heidenhain pouches sodium thiocyanate was shown to decrease both gastric acid secretion and mucosal blood flow, having
a greater effect on acid secretion than on blood flow.
Sodium thiocyanate was also shown to have a significantly greater effect on hydrogen ion secretion than on chloride ion secretion thus giving support to the "separate site" hypothesis for acid secretion and disproving the theory of competition between sodium thiocyanate and chloride ions.
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1:1 General Introduction

The amphibian isolated gastric mucosa preparation has been extensively used for physiological study, and has been particularly useful in studying various aspects of hydrochloric acid secretory mechanisms. This preparation is an excellent model system for studying control and mechanical aspects of HCl secretion, and recent work has demonstrated the value of these preparations in providing fundamental pharmacological information. Isolated organs are generally ideal for studying the action of stimulants and inhibitors, without considering the effect on other organs and blood flow. However, there are also some limitations. By its very nature the isolated system will not permit the kind of total integrative analyses which are possible with intact systems; isolation interrupts important nerves and removal of muscle coats virtually eliminates sub-epithelial neural plexi. The very process of isolation often produces a secretory response itself.\textsuperscript{(Fortc, 1973)} Finally, it is difficult to interpret results due to species differences. Such variations exist within the same genus as well as between major groups of amphibians. In spite of the problems, the cheapness, convenience, simplicity and carefully controlled conditions provided by the isolated amphibian gastric mucosa substantiate its valuable role as an experimental preparation.

Several methods have been employed for preparing and studying isolated gastric mucosae. The heavy, smooth muscle coats are normally removed from amphibian preparations by blunt dissection. The remaining tube
of gastric mucosa may be tied at the oesophageal and pyloric ends producing a closed mucosal sac (Davies, 1948; Crane & Davies, 1951). The tube of gastric mucosa may be turned inside out before tying to produce an everted sac. This secretes acid into the medium surrounding it (Davies, 1948). The isolated mucosal tube is often slit longitudinally and the resultant sheet of gastric tissue is either used directly (Alonso & Harris, 1965) or placed between chambers (Crane et al, 1948a). The "chambered" preparations of gastric mucosa can be thought of as a simplified epithelial system in which secretory, metabolic and electrical events can be correlated simultaneously (Hogben, 1955a; Rehm, 1962; Forte & Davies, 1964).

It has been considered that as far as survival and experimental reproducibility, amphibian mucosa was far superior to isolated mammalian preparations although one or two mammalian preparations have been described.

Davenport and Chavré (1950, 51, 53) used an excised mouse stomach preparation for various secretory studies. Dikstein and Sulman (1965) demonstrated acid secretion in response to histamine in an everted rat stomach preparation. More recently Assem, Schild and Wan (1973) have reported a study of stimulation and inhibition of acid secretion from an isolated rat stomach.

The work presented in this thesis describes the development and validation of an in-vitro mammalian preparation for the measurement of gastric acid secretion: the guinea pig isolated stomach preparation.
The effect of stimulants and inhibitors (including thiocyanate) on acid secretion was tested on this preparation. These experiments have given support to the "second messenger" hypothesis in which cyclic AMP is the final mediator of hydrochloric acid secretion. Histamine is thought to cause acid secretion by stimulating the adenyl cyclase system to produce more cyclic AMP and histamine mobilisation is envisaged as the common pathway for the action of other stimulants.

\[
\text{Pentagastrin} \rightarrow \text{Histamine} \\
\downarrow \\
\text{cyclic AMP} \\
\downarrow \\
\text{HCl}
\]

A simplified scheme showing these events is depicted above. The hypothesis will be discussed in detail in chapter 4.

The effect of thiocyanate on acid secretion and mucosal blood flow was also studied in the conscious dog.

This work was prompted by an investigation carried out by Moody (1968). He studied the relationship between oxygen consumption, blood flow and acid secretion in exteriorised gastric segments of the dog during thiocyanate administration and found that it inhibited histamine-induced acid secretion but did not affect the high respiration rate or decrease total blood flow. However, the mucosal blood flow, measured by the amidopyrine clearance technique of Jacobson, Linford and Grossman (1966) was decreased during thiocyanate administration. He concluded that amidopyrine clearance must be directly linked with
the hydrogen ion secretion, and was dependent on the transport mechanism associated with hydrogen ion transport. Therefore it was not a reliable index of mucosal blood flow during thiocyanate inhibition. The experiments described in Chapter 2.2 were carried out in order to test the hypothesis that amidopyrine clearance was linked with hydrogen ion secretion and to examine the validity of using aniline clearance for the measurement of mucosal blood flow (Curwain and Holton, 1973) during thiocyanate inhibition. The effect of thiocyanate on histamine-induced acid secretion, volume of secretion and the concentration of hydrogen ions and chloride ions was also determined.

This series of experiments was carried out on conscious dogs prepared with Heidenhain (vagally denervated) pouches.

For background information the electrical, metabolic and secretory events associated with the gastric oxyntic cell, which result in the accumulation of hydrochloric acid in the gastric lumen will be reviewed.
1.2 Electrical & Secretory activity of the gastric epithelium

1.2.1 General

Although electrical activity has long been associated with gastric function (Donné, 1834) it is primarily from isolated amphibian mucosa that electro-physiological data has been interpreted in terms of fundamental transport and metabolic events. In amphibia the mucosal layer of the stomach is one cell thick. The mucosal surface faces the gastric lumen and the serosal surface is next to the muscularis mucosae.

The electrical activity of the gastric mucosa is a function of the ion pumps in the tissue, both directly through the electrogenic pumping and indirectly through ion-gradient diffusion potentials (Forte et al, 1963; Harris & Edelman, 1964; Forte 1971; Rehm, 1971). These events are shown schematically in Fig. 1.1.

The predominant ion transport events in amphibian stomach are $H^+$ and $Cl^-$ ions; transmucosal transport of $Na^+$ and $K^+$ ions is relatively low. $H^+$ and $Cl^-$ ions are actively transported into the lumen (Rehm, 1950; Hogben, 1951) which raises the question whether these ions are secreted separately by distinct transport mechanisms, or whether there is a common site to account for translocation of both ions.

1.2.2 'Separate site' and 'Common site' hypotheses

According to the separate site hypothesis there are electrogenic $H^+$ and $Cl^-$ pumps in the mucosal membrane (Rehm, 1965).
Fig. 1.1

**Schematic representation of the electrical and secretory activity in the gastric oxyntic cell**

The membrane at the serosal surface of the cell is assumed to possess properties common to all cells including a sodium pump $\text{Na}^+$ and diffusion potentials due to $\text{Cl}^-$ and $\text{K}^+$ ($E_{\text{Cl}^-}$ & $E_{\text{K}^+}$). In addition the serosal membrane possesses an effective $\text{Cl}^-/\text{HCO}_3^-$ exchange diffusion mechanism.

The mucosal membrane has $\text{H}^+$ and $\text{Cl}^-$ pumps ($\text{H}^+\text{p}$ & $\text{Cl}^-\text{p}$) which may contribute to the total transmucosal potential under certain conditions (After Forte, 1971)
GASTRIC OXYNTIC CELL

Serosal fluid

HCO₃⁻ ← HCO₃⁻ ← CO₂ + OH⁻ → H⁺

Cl⁻

Cl⁻ → K⁺ → Cl⁻

K⁺ → Na⁺ → Na⁺

H⁺

Cl⁻

Cl⁻

Mucosal fluid

+(E⁺)⁻

+(E₉)⁻

-(E₉)⁺
Studies in which Cl⁻ free solution was placed on the serosal side of the gastric mucosa have established this (Forte, Adams and Davies, 1963). Even though the H⁺ and Cl⁻ pumps may be shown to operate in an independent way, a common site mechanism for hydrochloric acid secretion cannot be ruled out. In several studies a tight association between H⁺ and Cl⁻ transport is clearly indicated (Durbin, 1964; Forte, 1969; Solberg & Forte, 1971). Model systems have been proposed whereby a common carrier for the two ionic species is envisaged. (Fig. 1.2A,B & C).

Fig. 1.2A shows a model in which there is no net transfer of charge - i.e. a neutral HCl mechanism.

Fig. 1.2B represents a model in which there is inclusion of a possible electrogenic Cl⁻ pump in addition to the neutral HCl mechanism (Heinz & Durbin, 1953).

Fig. 1.2C shows the separate site model proposed by Rehm (1964).

1.23 Acid-base balance

It follows from the law of conservation of charge that every time a H⁺ ion is secreted into the lumen there must be an OH⁻ ion (or its equivalent) produced in the cells. It has been shown for steady state conditions that for each H⁺ appearing in the lumen, a HCO₃⁻ appears in the serosal solution. (Davies, 1951; Teorell, 1951).
Fig. 1.2

**Schematic models to account for H\(^+\) and Cl\(^-\) transport via an electrogenic mechanism**

(A) Mechanism driven by cellular metabolic energy in which H\(^+\) and Cl\(^-\) become associated with a common carrier (X) within the membrane. Under optimal conditions of H\(^+\) and Cl\(^-\) transport, net transfer of charge does not occur. Removal of Cl\(^-\) from the system would clearly show the existence of an electrogenic H\(^+\) pump (After Forte, 1971).

(B) Adaptation of a system shown in (A) to demonstrate a possible mechanism for the development of an electrogenic Cl\(^-\) pump. The cations in the system (XH\(^+\) and/or H\(^+\)) could leak or exchange back from the mucosal membrane to the serosal side. Net movement of H\(^+\) and Cl\(^-\) would occur only if a shunt leakage e.g. by internal short circuit (or exchange) were present or if external current were passed in the appropriate direction.

(C) Equivalent circuit whereby electrogenic H\(^+\) and Cl\(^-\) transport occur relatively independently at separate sites, and in which current flow represents the only possible continuity between the systems (After Rehm, 1964).
The simplest picture is that OH⁻ ions combine with carbon dioxide and the resultant HCO₃⁻ exchanges with Cl⁻ across the serosal membrane (cf. Fig. 1.1) through high conductance channels. Changes in the resistance across the mucosa are associated with changes in the hydrogen ion secretory rate. Rehm et al (1963) have shown that the increased resistance observed when hydrogen ion secretion falls was a property of the serosal membrane. This throws doubt on the concept of Cl⁻ - HCO₃⁻ exchange occurring via conductance channels and suggests a neutral mechanism.

1.3 Carbon dioxide and carbonic anhydrase: roles in gastric acid secretion

1.3.1 Carbon dioxide

When secreting hydrochloric acid the gastric mucosa requires more carbon dioxide than is produced by tissue metabolism. The 'consumption' of CO₂ occurs by virtue of its incorporation into HCO₃⁻ which is then liberated into the serosal solution or blood, in an amount exactly equivalent to H⁺ ion secretion into the gastric lumen (Davies, 1948; Teorell, 1951). Thus it is not surprising that the rate of secretion by the stomach is sensitive to the availability of CO₂ (Delrue, 1930; Gray et al, 1940).

1.3.2 Carbonic anhydrase

Carbonic anhydrase is an enzyme which catalyses the reversible hydration of carbon dioxide as shown below. It acts on reaction (1.)

\[
\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \quad (1)
\]

\[
\text{H}_3\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^- \quad (2)
\]

It is found in great abundance in mammalian gastric mucosa (Berend, 1938; Davenport & Fisher, 1938; Davenport, 1939) and Maren (1967) reports its
location in high concentrations in the actual oxyntic cell. It was therefore suggested that carbonic anhydrase was involved in the secretion of hydrogen ions. This hypothesis was strengthened when Davenport (1939) found that thiocyanate, which inhibits carbonic anhydrase also inhibits acid secretion. Feldberg, Keilin and Mann (1940) however, found that when acid secretion was largely inhibited by thiocyanate, there was only a 10% reduction in carbonic anhydrase activity.

1.33 Carbonic anhydrase inhibitors

Studies using other carbonic anhydrase inhibitors (mainly sulphonamides) in mammals have supported the conclusion that carbonic anhydrase activity is absolutely required to catalyse the hydration of CO₂ at high secretory rates. However in amphibian preparations (Hogben, 1955b; Durbin and Heinz, 1957) these inhibitors have been shown to be acting mainly on the Cl⁻ pump rather than directly on H⁺ ion secretion. Hogben (1967b) subsequently concluded that the sulphonamide agents were acting through a specific Cl⁻ receptor and that the effects were not the result of inhibition of carbonic anhydrase per se. Apparent support for this hypothesis comes from studies showing inhibition of Cl⁻ ion transport with sulphonamides in tissues (frog cornea, - Kitahara et al, 1967; turtle bladder, - Gonzalez, 1969) said to lack carbonic anhydrase (Maren, 1947). However, a recent report by Scott (1971) suggests that both these tissues do in fact contain carbonic anhydrase. Therefore it is still possible that the effects on Cl⁻ transport are
initiated through carbonic anhydrase, and some limitation occurs on
the normally rapid Cl\(^-\)/HCO\(_3\)\(^-\) exchange at the serosal surface (Rehm,

The use of carbonic anhydrase inhibitors has shown them to be highly
non-specific inhibiting HCO\(_3\)\(^-\), Cl\(^-\), H\(^+\) and Na\(^+\) transport. The relationship
between this enzyme and ion transport processes is obviously still
unclear but one possible explanation is that the carbon dioxide-
bicarbonate system is closely bound up with transport of several ion
species.

1.4 Metabolism and the energy source for secretory activity

The secretion of hydrochloric acid by the stomach occurs against a
large electrochemical gradient; the electric potential across the
gastric mucosa (-30mV in frogs; -70mV in dogs) and the ion concentration
(more than 10\(^6\): 1 for H\(^+\)) a concentration gradient but is facilitated
by the orientation of the potential difference.

The minimum free energy \(\Delta G\) needed for the secretion of an ion is a
function of the concentration gradient and electrical potential and is
given by the equation:-

\[
\Delta G = nRT \ln \frac{a_1}{a_2} + nzeF
\]

where

- \(n\) = number of gram ions secreted
- \(R\) = gas constant
- \(T\) = temperature in °K

\(a_1\) and \(a_2\) = activities of the ion in the mucosal and serosal solutions
respectively.

\(F\) = Faraday constant (1 faraday = 96,493 coulombs)
\[ z = \text{net charge of the ion} \]

\[ E = \text{electrical potential difference between the two solutions.} \]

It is postulated that the work done in the secretion of acid and production of electrical current by the stomach is derived primarily from metabolism within the gastric mucosa. The stimulated oxyntic cells secrete hydrochloric acid for long periods of time and are among the most metabolically active cells in the body. This is supported by the fact that the oxyntic cells are inhibited by body deficiencies of thiamine (Davenport & Jones, 1949), riboflavin (Lehmann, Rossiter and Walters, 1947) and nicotinamide (Ruffin & Dick, 1939) all of which are involved in energy metabolism.

One of the long standing problems of gastric physiology has concerned the immediate energy source for hydrogen ion secretion. Recently the problem has centred on two major possibilities

(a) metabolically derived ATP is directly coupled to the \( H^+ \) ion translocation mechanism

(b) the \( H^+ \) ions are directly produced from substrate hydrogen atoms by means of a specialised oxidation-reduction in the oxyntic cell. The latter view has been called the Redox hypothesis (Conway & Brady, 1948; Crane & Davies, 1951; Robertson & Wilkins, 1948) and in its simplest form the terminal electron acceptor, molecular oxygen, is directly associated with the translocation event i.e. ATP involvement is not envisaged. Various mechanisms have been postulated which represent these two possibilities viewed either
separately or in conjunction. A good deal of evidence supporting and refuting these mechanisms has been assimilated through the use of metabolites and inhibitors.
CHAPTER 2

METHODS
2.1 Guinea pig methods

2.1.1 Preliminary experiments

Gastric acid secretion in the rat isolated gastric mucosa was investigated in some preliminary experiments prompted by the work of Assem, Schild and Wan (1973). These experiments were abandoned eventually for several reasons. The rat stomach wall was 1mm thick thus reducing the reliability of oxygen diffusion into the secretory cells at a satisfactory rate to support acid secretion. The rat isolated mucosa was obtained by blunt dissection of the muscle coat. This preparation was found to respond variably to pentagastrin 0.3 - 0.5μg.ml⁻¹ and histamine 1-4μg.ml⁻¹ but the dietary pretreatment described below had not been established. Therefore the results were unreliable and not reproducible.

2.1.2 Choice of guinea pig

The guinea pig was subsequently chosen for investigation of gastric secretion as it has a distinct advantage over other isolated mammalian preparations so far investigated (Davenport & Chavré, 1951, 52, 53; Dikstein & Sulman, 1965) in that the stomach wall is only 0.55 mm thick. The rate at which oxygen can be supplied limits the rate of acid secretion in isolated stomachs (Davenport & Chavré, 1950). Therefore the guinea pig stomach is particularly suitable for use as an isolated organ because the diffusion path for oxygen is relatively short. In most previous in vitro studies using isolated gastric preparations the muscle coat has been stripped from the mucosa by blunt dissection for two reasons. The muscle layer restricts ion movement and consumes oxygen thus reducing the P₀₂ at the surface of the secreting cell.
This technique was carried out in a few experiments on guinea pigs but caused no change in observed results obtained using the whole stomach and so was abandoned. Sernka & Hogben (1969) have shown that in the rat and guinea pig the serosal coat does not constitute a substantial diffusion barrier to monovalent ions. The muscle layer can be regarded as only a passive barrier to ion movement.

2.13 Sex of guinea pig

It was considered likely that the gastric secretory responses might vary during the oestrus cycle in female guinea pigs. In order to rule out this potential source of error male guinea pigs were used.

No seasonal variations were observed.

2.14 Pretreatment

The stomachs of guinea pigs on a normal diet were greatly distended with solid food. This had two disadvantages. Distension of the stomach itself causes acid secretion (Lim et al., 1925) and also the stomach had to be handled a great deal in order to wash it out completely.

This would delay setting the preparation up.

A high rate of spontaneous acid secretion has often been observed in these preparations which falls gradually throughout the experiment. Results from such preparations were therefore
unreliable and not readily reproducible. A fasting period of 24 hours alone (Assem, Schild and Wan, 1973) was not sufficient to empty the stomach. Using these guinea pig stomachs which contained different amounts of solid food gave varying results which were not readily reproducible. Often the preparations could not be stimulated at all. Longer periods of fasting (48-60 hours) caused unacceptable weight loss of 50% prefasting body weight and often ulceration of the mucosa. These guinea pigs were in a very bad general condition. A procedure of controlled feeding and fasting was devised. The guinea pigs were given a low residue diet of Complan and sugar cubes for 24 hours, followed by removal of food for a further 24 hours. Water was freely available throughout this period. This procedure resulted in a stomach which contained only a small amount of food in liquid form.

2.15 Method of killing the guinea pig

Several methods are available for killing the guinea pig. Ether anaesthesia has been used (Serrska & Hogben, 1969) but this is very stressful to the animal and is likely to result in hormonal changes which might affect the gastric secretory response. Other methods are nitrogen narcosis (anoxia), cervical dislocation and stunning followed by bleeding; the last method was used for this series of experiments.
2.16 **Dissection**

The stomach was rapidly removed, cut along the lesser curvature, inverted, rapidly washed under the cold tap and placed in ice-cold saline solution in order to decrease metabolic activity. The composition of the solution was 100 mM NaCl, 5 mM KCl, 3.6 mM CaCl$_2$·6H$_2$O, 1.2 mM MgCl$_2$·6H$_2$O, 26 mM NaHCO$_3$, and 16.7 mM glucose (Sermka & Hogben, 1969). The stomach was then cut along the greater curvature into two similar portions and the midglandular portion of each was tied over the end of a short Perspex tube area 1.13 cm$^2$, mucosal side inwards.

2.17 **Setting up the apparatus**

The half stomach preparations were set up at 34°C in separate baths containing 100 ml of buffered saline solution bubbled with 95% O$_2$ and 5% CO$_2$. 4.5 ml of unbuffered saline solution (136 mM NaCl, 5 mM KCl, 3.6 mM CaCl$_2$·6H$_2$O, 1.2 mM MgCl$_2$·6H$_2$O and 16.7 mM glucose) bubbled with 100% O$_2$ and at 34°C was added to the mucosal side.

Care was taken that the levels of solutions either side of the preparation were the same so that no external pressure was acting on it. It was considered that minimal handling of the tissue plus the speed at which the dissection and setting up the preparation was accomplished, was of the utmost importance. The rate of bubbling was many times that required to saturate the solutions at the tissue surface. The stream of bubbles also ensured prompt and vigorous mixing of the solutions.
Results from a recent series of experiments suggest that thorough cleaning of the organ baths is essential for ensuring a quick efficient secretory response by the stomach preparation to the various stimulants and inhibitors administered.

2.18 Reason for using CO$_2$

Carbon dioxide was supplied in the gassing mixture of the serosal solution as the gastric mucosa requires more CO$_2$ to secrete hydrochloric acid than is produced by tissue metabolism (cf. 1.31). Davies (1948) and Davies and Longmuir (1948) found that, in isolated frog gastric mucosa in the absence of external supplies of CO$_2$, spontaneous acid secretion occurred so long as the rate of H$^+$ secretion did not exceed the rate of CO$_2$ produced from metabolism. However, when the mucosa was maximally stimulated by histamine, ulceration occurred which was interpreted as being due to accumulation of alkali within the oxyntic cells (Davies and Longmuir, 1948). Such ulceration did not occur when adequate external supplies of CO$_2$ were available. In contrast Imamura (1967) systematically studied the effect of CO$_2$ on H$^+$ secretion in frog gastric mucosa (R. esculenta) and confirmed earlier observations that H$^+$ secretion was severely reduced at concentrations below 5%.

2.19 Reason for unbuffered mucosal solution

The mucosal solution was unbuffered because the H$^+$ secreted by the mucosa would combine with bicarbonate to produce carbonic acid. This would lower the pH and thus measurement of acid content would not be a true measure of HCl production by the mucosa.
Carbon dioxide diffusing through the stomach from the serosal to mucosal side and CO$_2$ produced by metabolism would also contribute to the acid content of the mucosal solution and must be taken into account. This will be discussed later in section 3.13.

2.110 Choice of temperature
The body temperature of the guinea pig is 38.5°C. Earlier experiments were carried out at 37°C, but reducing the temperature to 34°C, caused no appreciable changes in acid production and has two advantages. A lower temperature decreases the metabolic activity of the cell and therefore the oxygen requirement. Also the solubility of oxygen increases with a fall in temperature. (At 100°C oxygen solubility is 2.3 g/100 ml, at 50°C - 2.46 g/100 ml water and at 0°C - 4.89g/100 ml). Therefore, 34°C was chosen.

2.111 Equilibrium of preparations
The two half stomach preparations were left 30 minutes to equilibrate; the bathing solutions were then renewed and the measurements begun. During the experiment drugs were added to the serosal side of one preparation, while the other acted as a control. The serosal solution was changed at least every hour in order to provide an adequate supply of substrate (glucose). The mucosal solution was removed and replaced every 15 minutes.
2.112 Measurement of acid

It is difficult to compare results from various workers because a standard method of titration has not yet been adopted. Assem, Schild and Wan (1973) expressed their results by calculating the hydrogen ion content of the mucosal sample from the measured pH. This does not take into account the contribution of carbon dioxide accumulation on the mucosal side of the preparation or lactate production or the secretion of mucin by the tissue itself. Not all the acid content measured is due to the secretion of hydrochloric acid. Titration to an arbitrarily chosen pH, the "stat" method, resolves this. Hogben (1972) using rat isolated gastric mucosa, titrated samples to pH 5.6 to eliminate errors due to CO₂ accumulating in the titrated solution either from the serosal bathing solution or from the tissue itself. I have found that sodium thiocyanate always abolishes the stimulated secretion of hydrochloric acid in the guinea pig isolated stomach, leaving a small constant amount of resting acid production. This will be described more fully later, but can be ascribed (at the moment) to accumulation of CO₂, lactate and mucin.

The results presented here are expressed as an increase above this resting acid level thus eliminating the error due to the presence of carbon dioxide. The mucosal samples were, therefore, titrated to pH 7 against 10⁻³M NaOH. The pH was measured with an EIL glass electrode and an EIL pH meter.
2.113 Calculation of results
In order to obtain the amount of hydrogen secreted by the preparation described in $\mu M \text{cm}^{-2} \text{h}^{-1}$ the titre was multiplied by four and divided by the area of the preparation (1.13 cm$^2$).

2.114 Drugs used to test stimulation of acid secretion
The drugs administered to the serosal side of the test preparation to stimulate acid secretion were pentagastrin 0.3-0.5 µg/ml$^{-1}$, histamine acid phosphate 1-4 µg/ml$^{-1}$, theophylline hydrate 0.2 mg/ml$^{-1}$ and histamine and theophylline together.

A few observations were made on the effect of pentagastrin and theophylline administered together.

2.115 Inhibition of secretion
Once stable maximal secretory plateaux had been established inhibitors of acid secretion were administered for 30 minute periods and then washed out of the bath. It was found that the effect of the inhibitor was reversed more rapidly if the preparation was washed twice after the period of inhibitor administration.

The inhibitors tested were burimamide 50 µg/ml$^{-1}$ and metiamide 10 µg/ml$^{-1}$ (H$_2$ receptor antagonists, Black et al, 1972; Ash and Schild, 1966) and sodium thiocyanate 10 mM, a metabolic inhibitor, whose precise locus of action is discussed later in Chapter 4.

* Throughout this thesis acid secretion is expressed in $\mu M \text{cm}^{-2} \text{h}^{-1}$
Here $\mu M \equiv \mu mol$
The results obtained using these inhibitors are expressed as the percentage decrease of acid secretion due to a named stimulant. (a)
i.e. The level of resting acid production was subtracted from the level of the stimulated plateau (b) i.e. \( b - a \)

The minimum level of secretion obtained with an inhibitor (c) was also subtracted from the level of stimulated secretion (b)

i.e. \( b - c \)  

\[
\text{Therefore percentage inhibition} = \frac{b - c}{b - a} \times 100
\]

The results obtained were readily reproducible.
2.2 Dog methods

2.2.1 Measurement of gastric mucosal blood flow (MBF) in conscious dogs using radioactive aniline and amidopyrine

Food with withheld from the dogs for eighteen hours before the experiment but water was freely available. 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. A sterile intravenous catheter (O.D. 0.63 mm) was inserted through a needle into a leg vein for administration of drugs, and another catheter (O.D. 0.92 mm) placed in another leg vein for removal of blood samples.

The loading dose of aniline or amidopyrine was then given followed at once by the maintenance infusion. Histamine to stimulate acid secretion was mixed with the aniline or amidopyrine infusion.

All substances for infusion were dissolved in pyrogen-free 0.9% NaCl 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 or amidopyrine had been given. A 2.0 ml blood sample was taken every 30 minutes during the experiment and gastric juice was collected at 15 minute intervals. In order to prevent significant back diffusion of aniline or amidopyrine from the pouch, the pH of the contents should be below 3.0. At this value 1% of the aniline and amidopyrine will exist in the unionised form and be available for back diffusion. This fraction will increase with increase in pH.
2.22 Preparation of doses of $^3$H aniline and amidopyrine

A stock solution containing 35 mg ml$^{-1}$ of carrier aniline (aniline sulphate) and 12 $\mu$Ci ml$^{-1}$ $^3$H in sterile saline was prepared. Doses were expressed in mg of carrier aniline per kilogram of body weight.

- Aniline loading dose 10 mg kg$^{-1}$
- Aniline maintenance infusion 12 mg kg$^{-1}$ min$^{-1}$

The stock solution contained suitable proportions of radioactive and non-radioactive aniline to give satisfactory levels of $^3$H for counting in the plasma and gastric juice.

A stock solution of 25 mg ml$^{-1}$ of amidopyrine was prepared in sterile solution. Doses were again expressed as milligrams per kilogram of body weight.

- Amidopyrine loading dose 20 mg kg$^{-1}$
- Amidopyrine maintenance infusion 5 mg kg$^{-1}$ h$^{-1}$
2.23 Experimental protocol

Acid secretion was stimulated by a continuous intravenous infusion of histamine acid phosphate which was mixed with the aniline or amidopyrine infusion. The dose of histamine administered to each dog was chosen to cause submaximal gastric acid secretion which was expressed in $\mu g \text{ kg}^{-1} \text{ h}^{-1}$. Once a stable plateau of secretion had been reached (1-2 hours after sampling had begun) the effect of thiocyanate was studied. Sodium thiocyanate (0.4 - 1.0 mEq.mL$^{-1}$) was injected intravenously over 10 minutes in five one-minute intervals, during which time the infusion was interrupted. Depending on which dose was used 1-5 mls of stock solution (48 $\mu g \text{ ml}^{-1}$) were injected slowly over one minute and then washed in by the infusion mixture.

During the one-minute periods between thiocyanate administration the infusion rate was doubled in order to maintain the correct histamine dose and thus not cause any extraneous fluctuations in the secretory rate. Any inhibitory effect observed would be due only to the administration of the thiocyanate. This procedure was repeated throughout the ten-minute period. The effect of sodium thiocyanate on the acid secretion was followed for 2-3 hours and the various parameters measured and calculated in the manner described below.
2.24a Estimation of $^3$H aniline in plasma and gastric juice
The heparinised blood was centrifuged at 3000 rpm for 10 minutes and the plasma removed and mixed. 1.0 ml of plasma was then added to 0.5 ml M.NaOH and 10 ml diethyl ether and shaken for 5 minutes. 7.0 ml of the ether phase were removed and added to a counting vial containing 10 ml of scintillator. This was counted for $^3$H activity in a Packard Tri Carb Liquid Scintillation Spectrometer.

1.0 ml of gastric juice was added to 0.5 ml M.NaOH and 10 ml of ether extracted as above. When the volume of secretion was very small the juice was diluted with saline and a 1.0 ml aliquot extracted.

Scintillator:
Dimethyl POPOP - (1,4 - bis - [2-(4 methyl-5-Phenyloxozoyl Benzene)]
52.5 mg
PPO - (2.5 - Diphenyloxazole) 4.2 g
Methanol 300 ml
Toluene 700 ml

2.24b Estimation of amidopyrine in plasma and gastric juice
Samples of 0.1 ml were added to bottles containing 0.5 ml M.NaOH and 20 ml of dichloroethane. The bottles were shaken for 10 minutes 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 ml of sodium borate (0.05 M). The bottle was shaken for 1 minute 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 0.1M 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 ultraviolet 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.

2.25 Calculation of mucosal blood flow (MBF)

A graph of $^3$H aniline/amidopyrine concentration in plasma against time was plotted on semilogarithmic paper. The mean plasma aniline or amidopyrine concentration during a period of gastric juice collection was estimated from the graph.

Aniline concentrations were expressed as net counts per minute of $^3$H aniline.

\[
\text{Flow} = \frac{\text{Net Count per Minute}}{\text{Aniline Concentration}}
\]

Mucosal blood flow per minute was calculated from the following formula.
MBF = \frac{GV}{PT}

where G = aniline or amidopyrine concentration in gastric juice (diluted if necessary)

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

P = mean plasma aniline or amidopyrine concentration during the collecting period

T = time in minutes over which the juice was collected.

Other functions of secretion and MBF have also been calculated.

R. The ratio of aniline or amidopyrine concentration in the gastric juice and plasma

This relates MBF to gastric \( H^+ \) ion output. It is calculated from the rate of \( H^+ \) ion secretion, measured in \( \text{mol} \cdot \text{min}^{-1} \), and MBF, measured in \( \text{ml} \cdot \text{min}^{-1} \).

This function was also calculated for \( Cl^- \) ion secretion.

2.26 Measurement of volume and concentration of gastric acid secretion.

The secretion from gastric pouches was allowed to drain under gravity into a collection vessel. Secretion was collected over 15 minute intervals, the volume measured and an aliquot titrated against 0.1 M NaOH with phenolphthalein as the indicator.

Acid secretion was calculated in \( \text{mol} \cdot \text{min}^{-1} \).

Here \( \mu \text{H} \equiv \mu \text{mol} \)
2.27 Measurement of chloride secretion

The amount of chloride secreted into the gastric juice was estimated by measuring the chloride content of a 0.1 mL sample from each 15 minute collection of juice on a chloride meter (EEL 90). This was repeated twice more for each collection and an average value recorded.

2.28 Calculation of $H^+$ secretion

The number of mol/litre of hydrogen secreted into the gastric juice was calculated using the formula

$$ a \text{ mls of acid } = b \text{ mls } N/10 \text{ NaOH} $$

In this case $HCl \text{ } NaOH$ normality - molarity

$$ \therefore \text{ molarity } = \frac{b}{a} \times 10 $$

where $b$ is the titre

$a$ is the volume titrated

$$ \therefore \text{ the secretion contains } \frac{b}{10a} \text{ mol/litre } H^+ $$
CHAPTER 3

RESULTS
3.1. Guinea pig results

3.1.1 Sources of Error

Holes were occasionally made in the preparation during mounting it on the Perspex tube, usually by excessive stretching. On other occasions leaks occurred during the experiment. This was noticed immediately in the first case by a rapid rise in the pH of the mucosal solution to 8 or above. A leakage could be identified by a gradual increase in the mucosal pH to an alkaline value. When either occurred the experiment was abandoned.

On a few occasions once a stimulated secretory plateau had been established the rate of gassing either the mucosal or serosal solution was decreased.

A continuous supply of oxygen to the mucosal solution appeared to be more critical than gassing the serosal solution. Although in both cases reduction of the gassing rate caused the plateau level to fall and this was reversed by returning to the normal rate of gassing, the fall in secretion was larger, more rapid and more prolonged on reducing the supply of oxygen to the mucosal surface.

3.1.2 Resting acid production

The non-stimulated acid production measured from the control preparation (and test preparation before addition of stimulants) was $2-4 \mu \text{mol. cm}^{-2} \text{hr}^{-1}$ and remained stable for the duration (up to 7 hours) of the experiment. As mentioned previously and considered in more detail later, the addition of NaSCN abolishes hydrochloric acid secretion and consistently reduced the non-stimulated acid production level to $1.0 - 1.4 \mu \text{mol. cm}^{-2} \text{hr}^{-1}$. 
This value must therefore represent the true value of resting acid production which is not connected with hydrochloric acid secretion by the gastric mucosa but secretion of a buffer. The HCl secretion portion of the control level might be due to the presence of a small amount of liquid food normally found in the stomach on dissection.

3.13. Factors which might contribute to the non-HCl portion of acid production

(a) As mentioned previously (2.112) the carbon dioxide entering the mucosal solution might be a contributing factor to the resting acid production. The theoretical value of how much acid accumulation of CO₂ would produce could be calculated but could not be applied to this preparation. Here the amount contributed by CO₂ would be much less than the theoretical value as most of the CO₂ is blown out of the mucosal solution by the bubbling of 100% O₂.

(b) Lactic acid produced by cell metabolism might also contribute to resting acid production, although most of the lactate produced in the cell diffuses into the serosal solution (Durbin, 1968).

(c) The buffer secreted by the gastric mucosa has a pKₐ of about 6 and is thought most likely to be mucin (Sernka, PhD thesis, University of Iowa, 1969). This is supported by the observation that unresponsive preparations (normally discarded) often produce samples with a pH of 6.
3.14 **Stimulants**

3.14a **Histamine**

In 15 experiments histamine acid phosphate 1-4 µg ml⁻¹ decreased the pH to 3.5-4.0 in the mucosal solution and increased acid production to 288.9 ± 17% S.E. above resting acid production. (Fig. 3.11, 12 & 14.). The effect was reversible and maintained while histamine was kept in the serosal solution (up to 7 hours).

3.14b **Pentagastrin**

Pentagastrin 0.3 - 0.5 µg ml⁻¹ was used in 7 experiments in which histamine stimulated secretion. Pentagastrin had no effect in 4 experiments (Fig. 3.11), caused a transitory stimulation in 2 experiments and a maintained secretion in one experiment.

3.14c **Theophylline**

In 10 experiments theophylline hydrate 0.2 mg ml⁻¹ decreased the pH to 3.6 - 3.9 in the mucosal solution and increased acid production to 178.2 ± 8.4% S.E. above resting acid production (Figs. 3.13 and 3.14). This effect could be sustained by continued administration of theophylline to the serosal solution and was reversible. Theophylline had no effect in two preparations which responded to histamine.

3.14d **Histamine and theophylline**

The highest secretory rates and lowest pH values were obtained when histamine and theophylline were used together. In 9 experiments theophylline was either added to the preparation during maximal histamine-induced secretion or theophylline and histamine were used together. This caused an increase in acid secretion to 326.3 ± 14.7% S.E.
above resting acid production (Figs. 3.12 & 3.14) which was reversible and maintained while both stimulants remained in the serosal solution (up to 7 hours). The pH of the mucosal solution fell to 3.2 - 3.5.

3.14c. Pentagastrin and theophylline

On a few occasions (6) pentagastrin was added to a preparation secreting acid in response to theophylline stimulation. Twice pentagastrin increased the acid above the theophylline induced plateau but the response was variable and not readily reproducible as it had no effect the other four times it was administered. These preparations were all responsive to histamine.
Acid production by half stomach preparations from a guinea pig
Pentagastrin, 0.5 µg.ml⁻¹, histamine acid phosphate, 2 µg.ml⁻¹
and burimamide, 50 µg.ml⁻¹(B) were added to the serosal side of
one preparation (solid line). Theophylline, 0.2mg.ml⁻¹ was
added to the serosal side of the other preparation (broken line). Note that the resting acid production was the same in both
preparations, that pentagastrin had no effect but that both
histamine and theophylline caused acid secretion; the histamine-
induced secretion was inhibited by burimamide.
Acid Secretion

\[ \text{mol. cm}^{-2} \text{ h}^{-1} \]

\[ \mu \text{M cm}^{-2} \text{ h}^{-1} \]

<table>
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<th>4</th>
<th>6</th>
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<tbody>
<tr>
<td>PENTAGASTRIN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HISTAMINE</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Acid Secretion

\[ \text{mol. cm}^{-2} \text{ h}^{-1} \]

\[ \mu \text{M cm}^{-2} \text{ h}^{-1} \]

<table>
<thead>
<tr>
<th>Time h</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>THEOPHYLLINE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Acid production by half-stomach preparations from a guinea pig
Histamine acid phosphate, 2 μg.ml⁻¹, burimamide, 50 μg.ml⁻¹(B) and metiamide 10 μg.ml⁻¹(M) were added to the serosal side of one preparation (solid line). Burimamide, 50 μg.ml⁻¹(B) and metiamide 10 μg.ml⁻¹(M) were added to the serosal side of the other preparation (broken line.).

Note that again histamine causes acid secretion. Neither burimamide nor metiamide had any effect on resting acid production whereas both antagonists inhibited histamine-induced acid secretion.

The dose of metiamide required to effect the same inhibition as burimamide was one fifth the dose of burimamide.
Acid Secretion

\[ \text{mol} \cdot \text{m}^{-2} \cdot \text{h}^{-1} \]

\[ \mu \text{Mcm} \cdot \text{h} \]

Time h

HISTAMINE
Acid production by half-stomach preparations from a guinea pig

Theophylline hydrate, 0.2 mg.ml$^{-1}$, burimamide, 50 µg.ml$^{-1}$ (B), metiamide, 10 µg.ml$^{-1}$ (M) and sodium thiocyanate, 10mM were added to the serosal side of one preparation (solid line). The same doses of burimamide, metiamide and sodium thiocyanate were also added to serosal side of the other preparation (broken line).

Note that theophylline stimulated acid secretion. Burimamide and metiamide had no effect on theophylline-induced secretion, or on non-stimulated acid secretion. However sodium thiocyanate abolished the theophylline-induced secretion and had a small effect on the non-stimulated secretion reducing it to the level of resting acid production. This indicates the presence of a small portion of spontaneous hydrochloric acid secretion.
Acid Secretion

\[
\text{mol m}^{-2} \text{cm}^{-1} \text{h}^{-1}
\]

Time h

THEOPHYLLINE

Acid Secretion

\[
\text{mol m}^{-2} \text{cm}^{-1} \text{h}^{-1}
\]

Time h
Fig. 3.14

Acid production by half-stomach preparations from a guinea pig

Histamine acid phosphate, 2 μg.ml⁻¹ plus theophylline hydrate, 0.2 mg.ml⁻¹, burimamide, 50 μg.ml⁻¹ (B), metiamide, 10 μg.ml⁻¹ (M) and sodium thiocyanate 10mM were added to the serosal side of one preparation (solid line).

The same doses of histamine acid phosphate, burimamide, metiamide and sodium thiocyanate were added to the serosal side of the other preparation (broken line).

Note that histamine causes acid secretion. The addition of histamine and theophylline together also stimulates acid secretion but to a higher level than histamine alone. This demonstrates the potentiating effect of theophylline. Metiamide again inhibits histamine induced secretion and both burimamide and metiamide inhibit the histamine portion of the histamine plus theophylline-induced secretion. Burimamide had no effect on non-stimulated acid secretion.

Sodium thiocyanate abolished both histamine and histamine plus theophylline induced secretion.
3.15 Inhibitors

3.15a Burimamide

In 7 experiments burimamide, N-methyl N (4 - (4(5) - imidazolyl) butyl) thiourea, 50 µg ml⁻¹ caused an inhibition of histamine induced acid secretion of 81.3± 7.7% S.E. (Figs. 3.11, 3.12 & 3.14). In four experiments in which histamine and theophylline were the combined stimulants, burimamide caused an inhibition of 58.2± 8.7% S.E. (Figs. 3.12 & 3.14). The effect of burimamide was immediate and reversible. On some occasions recovery was gradual taking up to 45 mins to regain pre-inhibitory secretory levels. Burimamide had no effect on resting acid production (8 experiments) nor on theophylline induced acid secretion (3 experiments).

3.15b Metiamide

Metiamide produced similar results to its analogue burimamide but the dose required was one fifth. Metiamide, 10 µg/ml, had no effect on the control level secretion (6 experiments) nor on theophylline induced secretion (4 experiments). In 4 experiments metiamide inhibited histamine stimulated secretion 98.0± 12.1% S.E. (Fig. 3.12). The inhibition of histamine and theophylline induced secretion in 3 experiments was 71.0± 1.8% S.E. (Fig. 3.14). The effect of metiamide was immediate, reversible and recovery occurred over 30 minutes. A summary of the results with burimamide and metiamide is presented in Table 3.11.
Table 3.11
The effect of burimamide (50 µg ml⁻¹) and metiamide (10 µg ml⁻¹) on histamine induced acid secretion, theophylline-induced acid secretion and histamine plus theophylline induced acid secretion. Note that both burimamide and metiamide have a greater effect on histamine-induced acid secretion than on histamine plus theophylline induced secretion, indicating that only the histamine-induced portion of secretion was being inhibited. This is further substantiated by the lack of effect of either antagonist on theophylline-induced secretion alone.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Percentage decrease of stimulated acid secretion</th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>HISTAMINE</td>
</tr>
<tr>
<td>BURIMAMIDE</td>
<td>50μg/ml⁻¹</td>
<td>81.3 ± 7.7 S.E. n=7</td>
</tr>
<tr>
<td>METIAMIDE</td>
<td>10μg/ml⁻¹</td>
<td>98.0 ± 12.1 S.E. n=4</td>
</tr>
</tbody>
</table>
3.15c Sodium thiocyanate

Sodium thiocyanate (NaSCN) 10 mM in 5 experiments had no effect on control levels of secretion but on a further 12 occasions the level was reduced slightly to 1.0 - 1.6 μM cm⁻² h⁻¹. As mentioned previously (3.12) this indicates a small amount of spontaneous hydrochloric acid secretion. This occurred only in stomachs which contained a small amount of liquid food on death. There is a high level of spontaneous HCl secretion from the stomachs of guinea pigs given a normal diet i.e. the stomach is full of food at death.

NaSCN was found to abolish all three types of stimulated secretion investigated (Figs. 3.13 & 3.14) but was not added to the pentagastrin and theophylline induced secretion. The effect was immediate and reversible on washing it from the bath. The actions of NaSCN will be described in more detail later.
3.2 Dog results

3.2.1 The effect of thiocyanate on acid secretion and mucosal blood flow (MBF)

Administration of sodium thiocyanate (0.4 - 1.0mEq/min) decreased the histamine-stimulated acid secretory plateau to 52.0% ± 4.2% S.E. in 21 experiments carried out in 4 dogs. The inhibition was not immediate taking 5-30 minutes to reach the lowest level of secretion, and recovery was variable.

In 13 of 21 experiments the decreased level of secretion was maintained for the duration of the experiment (Fig. 3.21). In the remaining 8 experiments the acid secretion returned to the original plateau level in 30 minutes to 2½ hours (Fig. 3.22).

The effect of the same range of doses of thiocyanate on MBF measured in the 4 dogs by amidopyrine and aniline clearance was considerably less than on acid secretion. In 18 experiments the MBF fell to 66.7% ± 5.3% S.E. of the plateau level in 30 minutes. In only one experiment was this decrease maintained. Figs. 3.21 and 3.22 depict two typical experiments showing that the effect of the same dose of thiocyanate on acid secretion is much greater than on MBF measured in the same animal.

In 10 experiments recovery of MBF to the pre-inhibitory level was obtained in 30 minutes to 2½ hours after thiocyanate administration (Fig. 3.22). In the remaining seven experiments the MBF returned to the level observed before addition of thiocyanate and continued to rise to 130.6% ± 8.8% S.E. of the steady state plateau (Fig. 3.21). The reason for this increase in MBF is not understood. The results...
obtained here for the effect of thiocyanate on acid secretion and MBF do not support Moody's hypothesis (1.1) and will be discussed later (4.72)

3.22 The effect of thiocyanate on R - the ratio of amido pyrine or aniline concentration in the gastric juice and plasma

In 16 of 18 experiments carried out in four dogs, administration of thiocyanate caused R to increase by 67.4% ± 17.1% S.E., showing that the thiocyanate was having a larger effect on acid secretion than on MBF as mentioned above (3.21). The effect of thiocyanate was reversible in only two cases although in a further 5 experiments the initial high value of R fell, but not to the level observed before thiocyanate administration (Fig. 3.23). In 4 experiments the increased level of R remained steady for the duration of the experiment, whereas in the remaining 5 experiments the value of R was still rising when the experiment was terminated (Fig. 3.24).
Fig. 3.21

The effect of sodium thiocyanate on histamine-induced gastric acid secretion and mucosal blood flow from a Heidenhain pouch

Sodium thiocyanate (0.6 μEq.ml⁻¹) was injected intravenously during the time indicated by the bar.

Acid secretion (μM H⁺ min⁻¹) is denoted by the solid line and mucosal blood flow (MBF, ml.min⁻¹) by the broken line. Sodium thiocyanate caused a decrease in secretion which was maintained and a decrease in MBF which recovered to the pre-inhibitory plateau level and then overshot.

The effect of sodium thiocyanate was greater on acid secretion than on MBF.

In this experiment LBF was measured by the aniline clearance technique.
Mucosal Blood Flow

Acid Secretion

\[ \text{μmol min}^{-1} \text{ NaSCN} \]

Time h

0 1 2 3

0 2 4 6 8

0 4 8 12 16 20

ml min \(^{-1}\)
The effects of sodium thiocyanate on histamine-induced gastric acid secretion and mucosal blood flow from a Heidenhain pouch

Sodium thiocyanate (0.6 mEq.ml⁻¹) was injected intravenously during the time indicated by the bar. Acid secretion (μMl.min⁻¹) is denoted by the solid line and mucosal blood flow (MBF, ml.min⁻¹) by the broken line.

Sodium thiocyanate caused a decrease in secretion and MBF. Recovery to the pre-inhibitory plateau level for both parameters took 1.5 hours. The effect of sodium thiocyanate was greater on acid secretion than on MBF.

In this experiment LBF was measured by the aniline clearance technique.
Acid Secretion $\mu$M min$^{-1}$

Mucosal Blood Flow ml min$^{-1}$

NaSCN

Time h
Fig. 3.23

The effect of sodium thiocyanate on $R$, the ratio of aniline \textit{concentration in the gastric juice and in the plasma from a Heidenhain pouch stimulated with histamine.}

Sodium thiocyanate (0.4 mEq.min$^{-1}$) was injected intravenously during the time indicated by the bar. $R$ increased after administration of sodium thiocyanate showing that thiocyanate is having a larger effect on acid secretion than on mucosal blood flow. Recovery was not complete but the initial high value of $R$ fell.

In this experiment $LBF$ was measured by the aniline clearance technique.
The diagram shows the change in property $R$ over time in hours, with the time range from 0 to 3 hours. The property $R$ is measured along the vertical axis, ranging from 0 to 80. The line graph indicates a fluctuating pattern with a peak around the 2-hour mark.
The effect of sodium thiocyanate on $R$ - the ratio of amidopyrine concentration in the gastric juice and in the plasma from a Heidenhain pouch stimulated with histamine.

Sodium thiocyanate ($0.6\text{mEq.min}^{-1}$) was injected intravenously during the time indicated by the bar. $R$ increased after administration of sodium thiocyanate and continued to rise for the duration of the experiment showing that thiocyanate is having a larger effect on acid secretion than on mucosal blood flow.

In this experiment $MBF$ was measured by the amidopyrine clearance technique.
3.23 The effect of thiocyanate on the volume of gastric juice secreted

In 21 experiments carried out in 4 dogs the volume of secretion draining from the pouch over a 15 minute period decreased by 31.6% ± 3.4% S.E. after thiocyanate had been administered.

3.24 The effect of thiocyanate on $H^+$ and $Cl^-$ concentrations

In 9 experiments in four dogs thiocyanate administration caused a decrease in $H^+$ concentration of the gastric juice to 80.6% ± 1.8% S.E. of the pre-inhibitory level. The effect of thiocyanate on $Cl^-$ concentration in the same experiments was slightly smaller causing a decrease to 88.4% ± 1.6% S.E.

A t test was carried out on these two means. The effect of thiocyanate on hydrogen ion secretion was significantly greater than its effect on chloride ion secretion.

(t = 2.29  P < 0.05).

Recovery to the original concentrations observed before thiocyanate administration was never completely effected but in seven experiments the levels were gradually increasing by the end of the experiment. (Fig. 3.25)

It was therefore observed that in each experiment the effect of thiocyanate was larger on the $H^+$ concentration than on the $Cl^-$ concentration. This suggests that thiocyanate is having a different effect on $H^+$ and $Cl^-$ transport and lends support to the separate site hypothesis for acid secretion (1.22). In order to test this suggestion the ratio of MBF to the rate of $H^+$ ion secretion ($R_{H^+}$)
and MBF to the rate of Cl⁻ ion secretion (R_{Cl⁻}) were calculated from results obtained in six experiments using 4 dogs. A typical response is shown in Fig. 3.26 where the ratios are plotted against time. Thiocyanate causes a rise in both R_{H⁺} and R_{Cl⁻} which is to be expected as thiocyanate has a smaller inhibitory effect on MBF than on acid secretion (3.21). It is interesting to note that the two graphs follow almost exactly the same pattern of response before thiocyanate administration, then on addition of thiocyanate R_{H⁺} is increased more than R_{Cl⁻}. This pattern of response was obtained in 3 of 6 experiments and indicates that thiocyanate is having a larger effect on H⁺ transport than Cl⁻ transport. This can be explained in terms of separate site hypothesis for acid secretion which will be discussed later in Chapter 4.73. In the three remaining experiments thiocyanate caused an increase in both R_{H⁺} and R_{Cl⁻} but a difference in effect on the two parameters was not obvious.
Fig. 3.25

The effect of sodium thiocyanate on hydrogen ion and chloride ion concentration in the gastric juice from a Heidenhain pouch stimulated with histamine.

Sodium thiocyanate (0.4 mEq. min⁻¹) was given intravenously during the time indicated by the bar. The filled-in circles represent the concentration of hydrogen ions (mM) and the filled in triangles represent the concentration of chloride ions (mM).

Sodium thiocyanate decreases both the H⁺ concentration and Cl⁻ ion concentration but has a larger effect on the H⁺ concentration.
Concentration

mM

NaSCN

Time h

0 1 2 3

60 80 100 120 140 160
Fig. 3.26

The effect of sodium thiocyanate on the ratios of mucosal blood flow: $H^+ (R_{H^+})$ and mucosal blood flow: $Cl^- (R_{Cl^-})$ calculated from the mucosal blood flow and histamine-stimulated gastric acid secretion from a Heidenhain pouch.

The sodium thiocyanate ($0.6\text{mEq.min}^{-1}$) was given intravenously during the time indicated by the bar. $R_{H^+}$ is represented by the filled in circles and $R_{Cl^-}$ is represented by the filled in triangles.

Sodium thiocyanate causes an increase in both ratios but seems to have a greater effect on $R_{H^+}$ indicating that thiocyanate has a larger effect on hydrogen ion transport than chloride ion transport.

In this experiment $EBF$ was measured by the aniline clearance technique.
CHAPTER 4

DISCUSSION
4.1 Control level of secretion

The control level of acid secretion obtained in these experiments can be considered as being due to two main sources. The portion of acid in the non-stimulated samples which can be inhibited by NaSCN is probably spontaneous HCl acid secretion due to the presence of food in the stomach. The other portion which can be referred to as "resting acid production" is not due to the secretion of hydrochloric acid.

As mentioned previously (3.13) there are three potential sources of non-hydrochloric acid secretion, carbon dioxide, lactate and mucin. The last substance seems the most likely candidate since lactate diffuses preferentially into the serosal solution (Durbin, 1968) and any carbon dioxide entering the mucosal side is likely to be "blown" off by the bubbling of oxygen. The presence of carbon dioxide could be detected by halving samples and titrating one portion as usual. The other portion would be bubbled with oxygen before titration and any difference in titre would indicate the presence of carbon dioxide in the non-bubbled portion. Samples could also be assayed for lactate and mucin content. The nature of the resting acid production cannot really be settled until these studies are carried out.
4.2 The effects of histamine and pentagastrin

Acid secretion can be induced and maintained by histamine. The finding that pentagastrin usually has no stimulating effect is in contrast to the effect on rat isolated stomach (Assem, Schild and Wan, 1973) and may be associated with the difference in histamine metabolism of the species. The rat mucosa contains 81 μg.g⁻¹ (wet weight) of histamine and requires as much as 1 mg.kg⁻¹ exogenous histamine to evoke definite acid secretion, whereas the guinea pig mucosa contains 7 μg.g⁻¹ histamine and is very sensitive to injected histamine needing only 1 x 10⁻² mg.kg⁻¹ histamine base for threshold secretion (Kahlson et al, 1973). Kasbekar et al (1969) made interesting observations with pentagastrin and histamine while studying tachyphylaxis to pentagastrin and other gastric hydrochloric acid secretagogues. The response of bullfrog gastric mucosa to pentagastrin (Ach or mecholyl) faded with time whether the dose was kept constant or increased. Tachyphylaxis was not produced when histamine was used to stimulate acid secretion. Also successively stimulated mucosae, rendered refractory to pentagastrin or Ach responded normally to histamine.

These observations on isolated amphibian gastric mucosa and results with the isolated guinea pig preparation are consistent with the hypothesis that histamine is the final common mediator in the H⁺ ion secretory response (Code, 1965; Blair, 1965; Haverback et al, 1965; Kahlson et al, 1964) and suggest that other secretagogues might act by mobilising mucosal stores of histamine.
4.3 Histamine as the final common mediator of acid secretion

This hypothesis suggests that secretagogues stimulate acid secretion by causing mobilisation of mucosal stores of histamine which are replenished by synthesis of new histamine. The formation of histamine from histidine is catalysed by histidine decarboxylase (Weisbach et al, 1961; Ganrot, 1961) whose activity was identified in rat glandular mucosa by Schayer (1956a, 1957) and Kahlson et al (1964b).

\[
\text{Histidine} \xrightarrow{\text{Decarboxylase}} \text{Histamine}
\]

It is an attractive hypothesis: a single substance acting on the oxyntic cell to mediate the secretory response of a variety of stimuli. A model for local release of endogenous histamine from gastric mucosal "pools" - histamine mobilisation - (Fig. 4.1) has been proposed on the basis of experimental findings using rats (Kahlson, Rosengren and Svensson, 1973).

Kahlson has introduced the term histamine forming capacity for the mucosal cells which have the ability to synthesise histamine (HFC) which is synonymous with histidine decarboxylase activity. Kahlson et al (1973) have shown that there is increased HFC during acid secretion in rats, cats, mice and frogs although a greater proportion of the work in support of this hypothesis has been carried out in rats.
The HC1-secretory device (After Rosengren & Svensson, 1969a)

The HC1-secretory device comprises two distinct components, the parietal cell proper and the HFC cell which forms and contains histamine. Stimulating components of feeding e.g. gastrin, cause histamine mobilisation. It is envisaged that histamine mobilisation:

(a) stimulates acid secretion
(b) decreases mucosal histamine content
(c) releases histidine decarboxylase formation from restraint by decreasing histamine content
(d) contributes to increased urinary histamine (Rosengren and Svensson, 1969a)
FEEDING

FACILITATING INFLUENCES: vagus choline esters

↑HCl

no ↑H.F.C.

↑HCl secretion
↓ histamine content

HISTAMINE MOBILISATION

HISTAMINE STORE
Mucosal histamine-forming capacity [H.F.C.]

↑H.F.C.
↑HCl
GASTRIN

Distention Food
Vagus

PARIETAL CELL

HCl
It has been suggested that the secretory device may not be relevant to gastric secretion in animals with low HFC.

In the guinea pig the low HFC can not be considered functionally insignificant as it is accompanied by high sensitivity to histamine. This model can therefore represent the mode of action of histamine and pentagastrin in the guinea pig. It suggests that histamine acts directly on the oxyntic cell whereas pentagastrin, feeding and exogenous gastrin liberate histamine and increase HFC. i.e. stimulate the histamine forming cell.

Kasbekar (1972) investigated the possibility that acetyl choline and pentagastrin might cause acid secretion by mobilisation of histamine, using C\textsuperscript{14} histamine. He concluded that the transient stimulation of H\textsuperscript{+} secretion observed with pentagastrin and acetyl choline suggested mobilisation of histamine from a bound pool thus enhancing its access to the secretory sites. These observations do not however prove the role of histamine as the final common mediator of acid secretion.

As seen in Fig. 4.1 histamine is not envisaged as mediating all the secretory stimuli. Even in the rat it has been shown that stable choline esters can increase gastric acid secretion without involving any increase in histamine formation, and extra facilitating influences are recruited by feeding not explained by simple histamine mobilisation.

The usual lack of effect of pentagastrin in the results presented (3.14e) might be due to damage of the secretory device during dissection so that it might not function adequately.
The transient stimulatory effects of pentagastrin which was sometimes observed, might be due to depletion of the mucosal histamine stores (cf tachyphylaxis in bullfrog isolated gastric mucosa, Kasbekar et al, 1969) with none formed to support further secretion.

Pentagastrin stimulated acid secretion in only a few of my experiments normally when the preparations had come from stomachs which contained a large amount of liquid food on dissection. This presence of food has already been tentatively connected with the large spontaneous secretion (2.14) observed in some isolated guinea pig stomachs. This suggests that histamine mobilisation is occurring i.e. the mechanism is unimpaired and thus the pentagastrin administered is able to stimulate the histamine forming cell and secretion is supported. The results obtained with pentagastrin and theophylline (3.14e) were rather inconclusive but lend support to the idea that pentagastrin is unable to stimulate acid secretion because there is something lacking in the HFC system.
Supportive evidence for this hypothesis from the use of inhibitors.

Recently further evidence to support the idea of a role for histamine as the final common mediator of acid secretion was supplied by Black et al (1972) who studied the effects of histamine receptor antagonists.

Histamine acts on two types of receptor (Ash & Schild, 1966):

(1) $H_1$ responsible for increased contraction in smooth muscle and vasodilation

(2) $H_2$ responsible for increased gastric acid secretion and heart rate and decreased contraction of rat uterus.

Burimamide, a specific $H_2$ receptor antagonist, was shown to inhibit pentagastrin-induced secretion in anaesthetised rats and cats, and conscious dogs. Black concluded that the antagonism towards pentagastrin, histamine and feeding was quantitatively indistinguishable, so that the actions of gastrin must be involved with those of histamine.

Assem, Schild and Wan (1973) have also shown that burimamide and metiamide inhibit pentagastrin-induced secretion in the isolated rat stomach.
I have made observations demonstrating the inhibitory effect of burimamide and metiamide on pentagastrin-induced acid secretion on only three occasions in the guinea pig isolated stomach. The small number of experiments was mainly due to the variability of the preparation to respond to pentagastrin (3.1) but the fact that inhibition was obtained each time adds support to the hypothesis that the actions of gastrin are involved with those of histamine.

However, relatively little inhibition of vagally-induced or carbachol-induced gastric acid secretion could be shown in rats with these antagonists. Although the work reported above supports the concept of gastrin acting by a two-stage mechanism with histamine as the final step, there is still in existence a second mechanism involving the vagus and stable choline esters, which does not require the involvement of histamine.

It has recently been suggested by Reed, Smy, Venables & Harris (1973) from their studies on anaesthetised cats that burimamide and metiamide act indirectly on acid secretion via effects on the blood flow. However, the effect of burimamide and metiamide on the isolated stomach of the guinea pig reported in 3.15 and on the rat isolated stomach (Assen, Schild & Wan, 1973) provides conclusive evidence that these two inhibitors act directly on the histamine H₂ receptor.
4.5 Role of cyclic AMP, adenyl cyclase and phosphodiesterase in gastric acid secretion

4.51 General

Adenosine 3'-5' monophosphate (cyclic AMP) is a naturally occurring nucleotide that has been implicated as an intracellular regulator of a wide range of physiological activities including ion transport, energy metabolism and hormone secretion. The level of cyclic AMP within the cell is controlled by its synthesis and subsequent breakdown.

The formation of cyclic AMP is catalysed by the enzyme or enzyme system adenyl cyclase,

$$\text{ATP} \xrightarrow{\text{adenyl cyclase}} \text{Mg}^{2+} \rightarrow 3'5' - \text{cyclic AMP} + \text{Pi}$$

(Rall & Sutherland, 1962)

and it is rapidly hydrolysed by a specific phosphodiesterase

$$3'5' - \text{cyclic AMP} \xrightarrow{\text{phosphodiesterase}} 5 \text{AMP}$$

(Rall & Sutherland, 1962)

Adenyl cyclase is a lipo-protein complex with a distinct orientation within the cell membrane, while the specific phosphodiesterase is present in the cell cytoplasm and is not a component of any membrane system.

Adenyl cyclase is thought to consist of two components - a large MW and heat labile part plus an heat stable and dialysible portion. This latter portion is called the activator.
Methyl xanthines (caffeine, theophylline and theobromine) are potent inhibitors, and imidazole a stimulant, of phosphodiesterase activity (Butcher & Sutherland, 1962).

4.52 Second messenger hypothesis

The second messenger hypothesis is a scheme whereby the actions of many hormones are explained by their ability to change intracellular levels of cyclic AMP. Hormones which act in this way produce rapid responses, whose duration is related to the actual concentration of hormone present and are thought to include hormones such as adrenaline, glucagon, insulin and gastrin (Robison, Butcher and Sutherland, 1971).

This hypothesis depends on the hormone inducing the formation of cyclic AMP as shown above. Both adenyl cyclase and phosphodiesterase are found in the "target tissues" of many hormones (that tissue where the hormones has its action).

The first messenger (hormone) reacts in some way with the first target (adenyl cyclase). Production of the second messenger (cyclic AMP) occurs to bring about the physiological response. Fig. 4.2

4.53 Support for the involvement of the second messenger hypothesis in gastric acid secretion

Adenyl cyclase is present in both the mucosa and muscularis of the stomach (Perrier and Laster, 1971).
The first messenger - the hormone, reacts in some way with the first target - adenyl cyclase.

Production of the second messenger, cyclic AMP, occurs to bring about the physiological response.

The specific phosphodiesterase inactivates the cyclic AMP and thus, changing the intracellular level of cyclic AMP, regulates the humoral activity.
ENDOCRINE GLAND

HORMONE (1st messenger)

'TARGET CELL' MEMBRANE

(1st target)

ADENYL CYCLASE

Mg^{++} ATP

3',5' CYCLIC AMP (2nd messenger)

2nd TARGET

(kinase enzymes permeability etc.)

PHYSIOLOGICAL RESPONSE

phosphodiesterase

5' AMP inactive
It also has been demonstrated in gastric mucosa homogenates, with highest activity in the plasma membrane fraction; a specific cyclic AMP phosphodiesterase is also present in the supernatant of oxyntic cell homogenates (Forte, Forte & Ray, 1972). It is well known that methyl xanthines enhance hydrochloric acid secretion in the stomach (Roth & Ivy, 1944; Krasnow & Grossman, 1949; Roth & Valdes-Dapena, 1963).

These findings are consistent with the second messenger acting within the oxyntic cell where histamine acts as the first messenger (Fig. 4.3) Robison, Butcher & Sutherland (1968) envisage a model in which adenylyl cyclase and the receptor for the first messenger are part of the same system residing in the cell membrane. Support for the concept that cyclic AMP may have a role in gastric acid secretion comes from the studies of inhibitors of secretion reported in Chapter 3.1 using the isolated guinea pig stomach preparation. When acid secretion was stimulated by histamine plus theophylline the percentage inhibition of secretion obtained with burimamide and metiamide was less than for histamine induced secretion alone. This is interpreted to mean that only the histamine-stimulated portion of the combined secretion had been inhibited. Further evidence comes from the effect of burimamide and metiamide on theophylline-induced secretion. Neither drug was shown to have an inhibitory effect on a theophylline induced secretory plateau, whereas NaSCN consistently abolished it. The lack of effect of
Histamine acting as the first messenger reacts with the specific receptor site, which is thought to be part of the regulatory subunit (R) to stimulate the catalytic subunit (C) of the adenyl cyclase system. This catalyses the rise in concentration of intracellular cyclic AMP and secretion of $H^+$ is effected. Phosphodiesterase which inactivates the formed cyclic AMP is antagonised by methyl xanthines such as theophylline. Thus theophylline potentiates the effect of histamine on acid secretion by maintaining the increased intracellular level of cyclic AMP.
Histamine

Adenyl cyclase

ATP

Mg^{++}

Cyclic AMP

Phosphodiesterase

5' AMP

Imidazole

Methyl xanthines

H^+ secretion, metabolic effects

Cell membrane

Inside cell

Specific receptor site
burimamide and metiamide, but not NaSCN is additional evidence that histamine is not the final mediator of gastric secretion and implicates an additional step involving cyclic AMP.

Further support comes from my experiments (3.14c) in which theophylline was shown to initiate and sustain hydrogen ion secretion in the guinea pig isolated stomach and also potentiate histamine induced acid secretion (3.14d). Harris and Alonso (1963) have shown that methyl xanthines produce their action by increasing the cyclic AMP content in the gastric mucosa.

However, four basic criteria need to be satisfied before implicating cyclic AMP in the action of any hormone (Sutherland et al, 1968).

1. Adenyl cyclase in broken cell preparations should respond to the hormones effective in the intact preparation.

2. Intracellular concentrations of cyclic AMP should change in response to stimulation, and such changes should precede the physiological response.

3. Exogenous cyclic AMP should mimic the actions of the hormone.

4. Submaximal doses of the hormone which stimulate adenyl cyclase should be potentiated by phosphodiesterase antagonists.

1. **Response to adenyl cyclase**

Histamine has been shown to stimulate guinea pig adenyl cyclase in broken cell preparations of gastric mucosa three or four-fold whilst choline esters and gastrin had no effect (Perrier and Laster, 1970).
Nakajima et al (1971b) carried out an investigation into the effects of secretagogues on adenyl cyclase activity in an homogenised preparation of Necturus gastric mucosa. The results suggest that pentagastrin, fluoride, histamine and prostaglandin E₁ stimulate adenyl cyclase. The lack of an additive effect on combination of the last two agents led to the conclusion that a single adenyl cyclase unit is coupled to a distinct receptor site for prostaglandin and histamine.

A specific muscarinic agent AHR 602 (N-benzyl-3-pyrrolidyl acetate methobromide) which stimulates acid secretion in the isolated Necturus gastric mucosa (Nakajima et al, 1970b) was shown to inhibit adenyl cyclase activity.

These results point to the involvement of the adenyl cyclase system in histamine stimulated gastric acid secretion, but not that induced by muscarinic agents, in both amphibia and mammals. The lack of effect of gastrin on adenyl cyclase activity suggests that it has a different mechanism on gastric secretion or that some intermediary is involved. In view of the evidence previously discussed (4.3) the latter appears more likely, and the intermediary is in all probability histamine.

2. Intra-cellular concentrations of cyclic AMP

Using minced gastric tissue of guinea pigs, incubated with 10⁻³M theophylline to prevent breakdown of cyclic AMP Karppanen and Westerman (1973) have shown that histamine increased the content
of AMP in a dose-dependent manner. This increase was competitively inhibited by the H₂ receptor antagonist burimamide: as histamine-induced gastric acid secretion is also inhibited (cf. 4.5) the role of cyclic AMP as the mediator of the action of histamine on gastric acid secretion is supported.

Both histamine and pentagastrin increase the secretion of cyclic AMP into the gastric juice of gastric fistula or Heidenhain pouch dogs (although it cannot be certain whether this derives entirely from the parietal cells or whether it reflects the intracellular picture). From biopsy studies in dog and man histamine increases intracellular levels of cyclic AMP in the intact tissue at dose levels giving rise to physiological response. Both the maximum rate of secretion of cyclic AMP and the maximum rise in intracellular content of cyclic AMP preceded the peak rise of gastric acid secretion (Bieck et al, 1973)

(3) Exogenous cyclic AMP administration

Cyclic AMP (10⁻²M) was found to enhance acid secretion and oxygen consumption in the isolated gastric mucosa of R. pipiens (Harris and Alonso, 1965). Effective stimulation of H⁺ ion secretion using cyclic AMP (10⁻²M) was obtained from R. catesbeiana mucosa (Way and Durbin, 1969) and from rat stomach in vivo (Shaw and Ramwell, 1968).

Nakajima et al (1970b) showed stimulation of H⁺ ion secretion by Necturus mucosa using a lower dose (10⁻⁴M) of the dibutyryl derivative of cyclic AMP. The lower effective concentration was
probably due to the fact that dibutyryl cyclic AMP penetrates the cell membrane more readily, and once inside the cell is less susceptible to inactivation by phosphodiesterase.

In intact dogs and man, exogenous cyclic AMP results in decreased gastric acid secretion: the mechanism for inhibition, however, appears to be related to a decrease in gastric mucosal blood flow (Wilson and Levine, 1969). As such an action does not show up in vitro, it may be that in vivo the action on blood flow obscures any primary action of exogenous cyclic AMP on metabolism or ion transport.

(4) Potentiation by phosphodiesterase antagonists

In the experiments presented in this thesis theophylline was shown to initiate and sustain hydrogen ion secretion in the guinea pig isolated stomach, which lends support to the involvement of cyclic AMP in the secretory process. Support is also provided by the fact that although theophylline alone does not initiate gastric acid secretion in all the species studied, it does invariably potentiate histamine-induced gastric acid secretion (Robertson et al, 1950; Scratcherd and Case, 1969; Mertz, 1969; Bieck et al, 1973). This was also shown in the isolated guinea pig stomach preparation (3.2) where addition of theophylline to a secretory plateau induced by histamine caused a further rise in acid production. Administration of theophylline and histamine together also produced a secretory level above that obtained for either theophylline or histamine alone.
administered in the same doses. These increased levels of secretion could be maintained for the duration of the experiment (up to 7 hours).

So it has been demonstrated convincingly that histamine does cause a rise in intracellular cyclic AMP by stimulating adenyl cyclase and as this rise precedes the physiological event it shows that cyclic AMP can be the causative factor in gastric acid secretion. Also histamine does stimulate adenyl cyclase in broken cell preparations and its effects are potentiated by phosphodiesterase antagonists. The four criteria are thus satisfied.

A scheme incorporating the involvement of cyclic AMP and proposing that gastrin causes transcription of DNA regions for the synthesis of histidine decarboxylase and hence produces histamine to act as the physiological mediator has been proposed (Salganik, Argutinsky & Bersimbaev, 1971) in view of the findings that pentagastrin cannot stimulate adenyl cyclase activity in homogenates but has variable effects in intact tissues (Fig. 4.4).
Fig. 4.4

Scheme for the inter-relationship of gastrin, histamine and adenyl cyclase in gastric acid secretion.

(After Salganik, Argutinsky & Bersimbaev, 1971)
Results reported by Mao et al (1972) suggest that initiation of secretion in dogs does not depend on accumulation of cyclic AMP so although this scheme is fine for amphibia and guinea pigs, species differences do occur.

4.54 How cyclic AMP mediates its action

If, as seems to be well established, cyclic AMP does function as the second intracellular messenger for the mediation of gastric acid secretion in the oxyntic cell, brought about by local release of endogenous histamine, the question remains as to how cyclic AMP effects this stimulation of secretion. The answer would seem to be in the control of intermediary metabolism and its connection with ion transport and the supply of high-energy phosphates. Cyclic AMP has been shown to promote a sustained increase in oxygen consumption in amphibian gastric mucosa, whereas ATP and ADP do not (Harris and Alonso, 1963).

It also stimulates cellular functions other than ion transport e.g. glycogenolysis (Harris & Alonso, 1965).

It was thought that perhaps the increased glycogenolysis was responsible for the increase in acid secretion, but as 5' AMP inhibited glycogenolysis without influencing gastric acid secretion or oxygen consumption it was taken to indicate that the primary effect of cyclic AMP was on the Krebs cycle or oxidative phosphorylation steps of intermediary metabolism (Alonso et al, 1968).
The link between intermediary metabolism and hydrogen ion transport is believed to be at steps involved in dehydrogenation and formation of high-energy compounds such as ATP. However, it would seem that the effects of cyclic AMP are fairly widespread and that control points exist at several stages of intermediary metabolism that might relate to gastric acid secretion; cyclic AMP may also have a direct effect on gastric acid secretion.
4.6 Observations concerned with the potentiating effect of theophylline on histamine-induced secretion.

As reported in 4.53 (4) addition of theophylline to a histamine stimulated plateau caused a further rise in acid production and addition of theophylline plus histamine to the isolated guinea pig stomach caused a level of secretion above that obtained for theophylline or histamine alone. This is not of particular importance until it is noted that the dose of histamine used throughout was that which caused maximal acid secretion.

These results suggest that the oxygen requirement for the continuation of acid secretion is not rate limiting as stated by Davenport & Chavré (1950). In fact oxygen is required at two separate sites involved in the overall mechanism of acid secretion.

(i) for production of ATP from ADP

(ii) by the process involved in translocation and secretion of the hydrogen ion.

It is only at the second stage of events that the oxygen supply is a limiting factor. Oxygen is an essential requirement for the mechanisms involved in hydrogen secretion but not for the availability of cyclic AMP.
4.7 Actions of thiocyanate

4.71 Inhibitory action of thiocyanate on gastric acid secretion in the guinea pig.

As reported in the results section (3.1) sodium thiocyanate was found to abolish hydrochloric acid secretion in the guinea pig isolated stomach preparation whether it was spontaneous or induced by histamine, theophylline or histamine plus theophylline. These effects were readily and rapidly reversed by removing the agent from the serosal bathing solution.

4.72 Inhibitory action of thiocyanate on gastric acid secretion and MBF in conscious dogs.

The series of experiments reported in 3.2 in which thiocyanate was administered intravenously to dogs shows that thiocyanate decreases both acid secretion and MBF measured by either amidopyrine or aniline clearance. However, the effect of thiocyanate on acid secretion was always greater than on MBF as shown graphically for two experiments in Figs. 3.21 & 3.22 and quantitatively for 16 experiments by the calculation of R - the ratio of amidopyrine or aniline concentration in gastric juice and plasma.
Therefore, the hypothesis put forward by Moody (1968) that amidopyrine clearance is directly linked with hydrogen ion secretion is not supported. According to Moody's hypothesis thiocyanate would be expected to have an equal effect on amidopyrine clearance and acid secretion if amidopyrine clearance was dependent on the transport mechanism associated with hydrogen ion transport. Both amidopyrine and aniline clearance therefore remain reliable indices for the measurement of MBF during thiocyanate inhibition.

4.73 Mechanism by which thiocyanate mediates its action on gastric acid secretion.

The effect of thiocyanate on acid secretion in the stomach has been widely reported (Davenport, 1940b, 1946; Feldberg, Keilin & Mann, 1940; Rehm & Enelow, 1945; Crane, Davies and Longmuir, 1946,1948; Davenport and Jensen, 1948; Obrink, 1948) apart from my observations in guinea pig isolated stomach and conscious dog. The problem facing investigators for the last three decades has been the determination of the precise locus of action of this inhibitor. Originally it was suggested that thiocyanate decreased gastric secretion by its action on carbonic anhydrase. However, this mechanism for inhibition has since been ruled out (Feldberg, Keilin and Mann, 1940; Janowitz et al, 1952; Rehm et al, 1961; Davenport, 1962).
It has been shown that a fall in oxygen consumption was associated with the depression of acid secretion induced by thiocyanate in bullfrog gastric mucosa (Forte and Davies, 1964). This was confirmed by Bannister (1964). There was a considerable time lag between the depression of secretion and respiration which suggests that thiocyanate must operate on hydrogen secretion at some point distal to the terminal oxidation. Support for this proposal comes from work with isolated mitochondria where thiocyanate was ineffective as an inhibitor of mitochondrial respiration and oxidative phosphorylation (Kidder, 1968; Sachs et al, 1970). It is therefore more likely that thiocyanate has some specific effect on ion transport events either by a direct inhibition of ion translocation, or via an "uncoupling" action between the transported species and the energetic intermediates. It was suggested by Hersey and Jobsis (1969) that thiocyanate might act by reversing substrate mobilisation. A decrease in oxygen consumption would be necessary and was observed. However, the cytochrome reduction occurred before the change in secretory rate (LeFevre, Gohmann & Rehm, 1964) which in turn occurred before the fall in oxygen consumption (Bannister, 1964; Forte & Davies, 1964; Moody, 1968) indicating that thiocyanate does not act primarily on substrate metabolism, also the secretory changes may be secondary to the reduction of the cytochromes. There are three current theories for the mechanism of action of thiocyanate in existence at the present time which will now be discussed.
(a) Competition between thiocyanate and chloride:

Durbin and Heinz (1957) found that thiocyanate had a profound effect on acid secretion but very little on the potential difference across the mucosa. They therefore postulated that thiocyanate competes with chloride for a chloride-dependent proton pump, a system quite distinct from the chloride transporting process responsible for the potential difference i.e. supporting a separate-site hypothesis (1.22). Forte (1968) has supplied further evidence in favour of this hypothesis from observations on various components of Cl⁻ flux. My experiments in conscious dogs also support a separate-site hypothesis but rule out the concept of competition between thiocyanate and chloride ions. Thiocyanate decreased the concentration of both H⁺ and Cl⁻ (3.2) in the gastric juice. The finding that thiocyanate appears to have a greater effect on the H⁺ ion concentration than on Cl⁻ ion concentration indicates separate mechanisms for transport of H⁺ and Cl⁻, and also that the thiocyanate cannot be competing with chloride ions. The primary action of thiocyanate is to inhibit the hydrogen ion transport; the inhibition of that portion of chloride ion transport linked to proton transport is therefore a secondary phenomenon. The separate-site hypothesis would thus include a thiocyanate-insensitive chloride transport site for maintenance of the potential difference and a thiocyanate-sensitive hydrogen transport site involving both hydrogen and chloride ion transport.

It has been suggested (Forte et al, 1969) that these sites may even
be localized in separate cells.

(b) thiocyanate sensitive ATPase:
A common site hypothesis can not however be excluded by the observations reported in 4.73(a), where a limited competition between thiocyanate and chloride would exist for a common membrane carrier. The resulting thiocyanate carrier complex would have to inhibit a closely associated enzymic step responsible for separation of hydrogen and hydroxyl ions.

The associated enzyme could be the gastric ATPase which was described originally by Durbin and Kasbekar (1965) in the frog gastric mucosa. As stated previously this is a membrane-bound gastric ATPase not stimulated by addition of both Na⁺ and K⁺, not inhibited by ouabain but sensitive to several anions and inhibited by thiocyanate in doses which inhibit spontaneous acid secretion in vitro. It appears to be a transport ATPase associated with proton translocation. Thiocyanate is thought to inhibit the production of energy from ATP hydrolysis by this enzyme and thus inhibit the secretory process. If thiocyanate is acting in this way no alterations in ATP synthesis can be expected (Forte, Adams and Davies, 1965) whereas anoxia is thought to cause its effect by reducing the availability of ATP to the acid producing machinery. Forte, Forte and Bils (1965) have confirmed that SCN⁻ inhibits microsomal ATPase in rabbit gastric mucosa homogenates. They also showed that SCN⁻ is bound to the microsomes more tightly than Cl⁻, as determined by pH displacement, suggesting a site for
SCN⁻ and Cl⁻ competition observed by Durbin (1964) in intact mucosa (cf. 4.73a).

(c) The other current theory for the mechanism of action of thiocyanate is suggested by Kidder (1970b) on the basis of liquid nitrogen spectrophotometry and supports his hypothetical model for acid secretion. He postulates that thiocyanate combines specifically with the ferric centre of the extra-mitochondrial cytochrome c. Inhibition of oxidation would cause interference with the generation of only 2 moles of ATP/mole of glucose utilised (Lehninger & Wadkins, 1962). This analysis is supported by the fact that in bullfrog gastric mucosa thiocyanate inhibition of spontaneous acid secretion is not associated with any significant alterations in mucosal ATP content (Forte, Adams & Davies, 1965).

However, the results obtained from the studies of thiocyanate on hydrogen and chloride ion secretion in the conscious dog fall heavily in favour of a separate-site hypothesis for the mechanism of acid secretion where thiocyanate inhibits hydrogen ion secretion and the chloride transport linked to it.

The experimental results obtained from the guinea pig isolated stomach preparation give support to the "second messenger" hypothesis in which cyclic AMP is the final mediator of hydrochloric acid secretion and that histamine mobilisation is the common pathway for the action of pentagastrin (and other stimulants).
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4.1 The "HCl-secretory" device

4.2 Schematic representation of the second messenger hypothesis

4.3 Schematic events showing the involvement of the adenyl cyclase system and cyclic AMP metabolism in mediating the acid secretory response

4.4 Scheme for the inter-relationship of gastrin, histamine, and adenyl cyclase in gastric acid secretion.

Table 3.1.1 The effect of burimamide and metiamide on histamine-, theophylline-, and histamine plus theophylline-induced secretion.
Enhancement by propranolol of gastric acid secretion in response to pentagastrin in conscious dogs.

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In conscious dogs, both β-adrenoceptor agonists (Curwain & Holton, 1972; Curwain, Holton & Spencer, 1972) and burimamide (Black, Duncan, Durant, Ganellin & Parsons, 1972; Curwain, Holton & Spencer, 1973) decrease pentagastrin-induced gastric acid secretion. It has also been shown that β-adrenoceptor agonists decrease, and that propranolol increases (Assem & Feigenbaum, 1972), histamine formation in human leucocytes. If histamine formation is involved in the effect of pentagastrin on the gastric mucosa, as has been suggested by Kahlson & Rosengren (1971), the action of β-adrenoceptor agonists on gastric secretion might be secondary to their action on histamine formation. If this were so, propranolol would be expected to increase histamine formation and hence gastric secretion in response to pentagastrin but not in response to histamine. We have therefore investigated the effects of propranolol on gastric acid secretion in conscious Heidenhain pouch dogs.

Secretion was induced by a constant infusion of pentagastrin (1–2 [μg/kg]/h) histamine acid phosphate (1–2 [μg/kg]/min) or bethanechol hydrochloride. (0.5–1.0 [μg/kg]/min). Increasing doses (0.1, 0.3, 0.6 and 1.0 mg/kg) of (+) propranolol hydrochloride were injected intravenously at 30–60 min intervals. In some experiments gastric mucosal blood flow was measured by radioactive aniline clearance (Curwain & Holton, 1971, 1973).

In each of four experiments in four dogs, propranolol (0.4–2.0 mg/kg total dose) caused a prolonged increase of 53% ± 22.7% (S.E. of mean) in pentagastrin-induced gastric secretion (mean maximum increase of 81% over control; range 33–120%). Propranolol also increased gastric mucosal blood flow in parallel with the increased secretion:

During histamine infusion in the same four dogs, propranolol had no effect on the secretory plateau and negligible effect on gastric mucosal blood flow. In another experiment on one of these dogs histamine-induced secretion increased during propranolol administration.

Propranolol had no clear effect on acid secretion induced by bethanechol. In six experiments in five dogs secretion increased twice, decreased twice and was unchanged twice.

These experiments show that propranolol increases gastric acid secretion in response to pentagastrin but not to histamine. This is not inconsistent with the hypothesis that histamine is involved in the secretory response to pentagastrin.

We are grateful to the M.R.C. and the Wellcome Trust for support and to I.C.I. for a gift of propranolol.

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Gastric secretion in the isolated stomach of the guinea-pig

BY JAN SPENCER. Department of Physiology, St Mary’s Hospital Medical School, London W 2 1PG

The rate at which oxygen can be supplied limits the rate of acid secretion in isolated stomachs (Davenport & Chavré, 1950). The guinea-pig stomach is particularly suitable for use as an isolated organ because the stomach wall is thin (0.55 mm) and therefore the diffusion path for oxygen is relatively short.

Male guinea-pigs (300–500 g) were given a low residue diet for 24 hr followed by removal of food for a further 24 hr. The guinea-pigs were killed by a blow on the head, the stomach rapidly removed, cut along the lesser curvature, inverted, washed under the cold tap and placed in ice-cold saline solution (Sernka & Hogben, 1969) (110 mm-NaCl, 5 mm-KCl, 3.6 mm-CaCl₂, 6H₂O, 1.2 mm-MgCl₂, 6H₂O, 26 mm-NaHCO₃ and 16.7 mm glucose). The acid secretory portion of the stomach was then cut along the greater curvature into half-stomach preparations, each of which was tied over the end of a short Perspex tube (diameter 1.3 cm), mucosal side inwards. The half-stomach preparations were set up at 34°C in separate baths containing 100 ml. of saline solution, bubbled with 95 % O₂ and 5 % CO₂; 4.5 ml. of unbuffered solution (136 mm-NaCl, 5 mm-KCl, 3.6 mm-CaCl₂, 6H₂O, 1.2 mm-MgCl₂, 6H₂O and 16.7 mm glucose) bubbled with 100 % O₂ was added to the mucosal side, care being taken that the levels of fluid were the same on either side of the preparation. The mucosal solution was removed and replaced every 15 min; the samples were titrated to pH 7 against 10⁻³ M NaOH using a pH meter.

The resting acid production was 1.0–2.0 μM cm⁻² hr⁻¹. Drugs were added to the serosal side of one preparation while the other portion of stomach acted as a control. The serosal solution was renewed at least every hour.

In fifteen experiments, histamine acid phosphate (1–4 μg/ml.) decreased the pH to 3.5–4.0 in the mucosal solution and increased the acid production to 288.9 ± 17 % S.E. of the control level (as shown in Fig. 1). The effect was reversible and maintained while histamine was kept in the serosal solution (up to 7 hr).

Pentagastrin (0.3–0.5 μg/ml.) was used in seven experiments in which histamine stimulated secretion. Pentagastrin had no effect in four experiments (see Fig. 1), caused a transitory stimulation in two experiments and a maintained secretion in one experiment. Theophylline hydrate (0.2 mg/ml.) increased acid production to 178.2 ± 8.4 % S.E. of control level in
ten experiments (see Fig. 1). Theophylline had no effect in two experiments in which histamine stimulated secretion. In nine experiments, theophylline was added to the preparation during maximal histamine-induced secretion and caused a further increase to $326.3 \pm 14.7\%$ s.e. of resting acid secretion.

![Graph showing acid production by half-stomach preparations from a guinea-pig.](image)

**Fig. 1.** Acid production by half-stomach preparations from a guinea-pig. Pentagastrin, histamine acid phosphate and burimamide were added to the serosal side of one preparation (solid line). Theophylline was added to the serosal side of the other preparation (broken line). Note that resting acid production was the same in both preparations, that Pentagastrin had no effect but that both histamine and theophylline caused acid secretion; the histamine-induced secretion was inhibited by burimamide.

Burimamide (50 µg/ml) in seven experiments (see Fig. 1) and sodium thiocyanate (10 mM) in five experiments inhibited histamine-induced secretion but had no effect on resting acid production.

The results are compatible with the hypothesis that the resting acid production is not due to secretion of hydrochloric acid but that acid secretion can be induced and maintained by histamine and is dependent on the production of cyclic AMP. The finding that Pentagastrin usually has no stimulating effect is in contrast to the effects on the rat isolated stomach (Assem, Schild & Wan, 1973) and may be associated with the difference in the histamine metabolism of the species.

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

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Evidence that the inhibitory effect of burimamide on gastric secretion is not due to decreased gastric mucosal blood flow

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Black, Duncan, Durant, Ganellin & Parsons (1972) introduced burimamide as a drug acting specifically on those histamine receptors (H₂) which are insensitive to mepyramine. The H₂ receptors include those in the stomach and these workers demonstrated that burimamide decreases gastric acid secretion stimulated by histamine or pentagastrin. Gastric secretion is dependent on high mucosal blood flow, and is decreased when mucosal blood flow falls. Black and his colleagues did not exclude the possibility that the antisecretory activity of burimamide was secondary to decreased gastric mucosal blood flow. We have demonstrated that this is not so.

TABLE 1. The maximum percentage changes in acid secretion, mucosal blood flow (M.B.F.) and the ratio of M.B.F. to acid secretion caused by burimamide

<table>
<thead>
<tr>
<th>Dose of burimamide</th>
<th>2 × 10⁻⁵ mol/kg⁻¹</th>
<th>4 × 10⁻⁵ mol/kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid secretion</td>
<td>M.B.F.</td>
<td>Acid secretion</td>
</tr>
<tr>
<td>Histamine</td>
<td>-40</td>
<td>-22</td>
</tr>
<tr>
<td>Pentagastrin</td>
<td>-40</td>
<td>-16</td>
</tr>
<tr>
<td>Feeding</td>
<td>-58</td>
<td>-43</td>
</tr>
</tbody>
</table>

* Mean of two results.

Four healthy bitches (12-24 kg) with long-established Heidenhain pouches were used. Acid secretion (60-70% maximal) was stimulated by pentagastrin (4 μg kg⁻¹ hr⁻¹ i.v.), histamine acid phosphate (2 μg kg⁻¹ min⁻¹ i.v.) or a standard meal. Gastric mucosal blood flow was estimated by radioactive aniline clearance (Curwain & Holton, 1971; Curwain, 1972). Burimamide (2 or 4 × 10⁻⁵ mole kg⁻¹ i.v.) was injected during the secretory plateau.

As shown in Table 1, both doses of burimamide decreased acid secretion and gastric mucosal blood flow in response to each stimulus. The inhibitory effect usually lasted 45-75 min but on one occasion the effect of the large dose was still apparent for more than 2 hr. In every experiment the ratio of mucosal blood flow to secretion increased after burimamide. These results show that the inhibitory action of burimamide on gastric secretion
is not due to decreased blood flow and support the claim of Black et al. (1972) that it is a specific H₂ receptor antagonist.

We are grateful to Dr Black of Smith, Kline & French Ltd for a supply of burimamide and to the Medical Research Council and the Wellcome Trust for support.

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THE EFFECT OF BETAHISTINE ON GASTRIC ACID SECRETION AND MUCOSAL BLOOD FLOW IN CONSCIOUS DOGS

BY

B. P. CURWAIN, PAMELA HOLTON and JAN SPENCER


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The effect of betahistine on gastric acid secretion and mucosal blood flow in conscious dogs

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In 3 conscious dogs, betahistine (2-(2'-methyl aminoethyl pyridine)) (80 or 160 μg kg⁻¹ min⁻¹) increased acid secretion from Heidenhain pouches to 8.8% and 17.6% respectively of the maximal response to histamine. Betahistine also increased mucosal blood flow (radioactive aniline clearance). The ratio of mucosal blood flow to secretion was greater for betahistine than for histamine but the difference between betahistine and histamine was significant in only one of the dogs.

Betahistine (2-(2'-methyl aminoethyl pyridine)) is chemically related to histamine and like histamine has vasodilator properties (Hunt & Fosbinder, 1942; Konzett, Bost, Bowman, Bowman & McKennis, 1971). It has been used clinically as a vasodilator for the treatment of vascular disorders (Horton, 1962; Horton & von Leden, 1962; Elia, 1966; Esser & Reis, 1968).

Patients receiving betahistine occasionally report dyspepsia as a side effect (Le Pere, 1967). Such an effect could be caused by changes in gastric secretion or motility or possibly by changes in gastric mucosal blood flow since drugs which decrease the ratio of mucosal blood flow to acid secretion are potentially ulcerogenic (Jacobson, 1965). The effect of betahistine (1 mg min⁻¹) on gastric secretion was studied by Allen, Connell, Harries & Roddie (1971) who found that it did not stimulate acid secretion in man. Haigh (personal communication) studied the effects of larger doses (70–150 μg kg⁻¹ min⁻¹ i.v.) of betahistine in anaesthetized dogs and found no change in total gastric blood flow, mucosal blood flow (amidopyrine clearance) or blood pressure. With one exception betahistine did not cause acid secretion. In the exceptional experiment a very small secretion was obtained in response to 150 μg kg⁻¹ min⁻¹. We have extended these observations on secretion using a wide dose range of betahistine (5–160 μg kg⁻¹ min⁻¹ i.v.) in dogs with Heidenhain pouches. We have also studied the effect of betahistine (40–160 μg kg⁻¹ min⁻¹ i.v.) on gastric mucosal blood flow as measured by radioactive aniline clearance (Curwain & Holton, 1971; Curwain, 1972a).

Methods.—Three female dogs (10–14 kg) with long-established Heidenhain pouches were used in 13 experiments. Food was removed 18 h before each experiment but water was available. At the beginning of each experiment an intravenous catheter was introduced and saline (1 ml min⁻¹) was infused throughout the experiment. Drugs were added to the intravenous infusion. After a suitable control period a dose of betahistine was given for one hour after which the dose was doubled. Usually three doses of betahistine were studied in each experiment. In some experiments a small dose of histamine (0.25 μg histamine diphosphate kg⁻¹ min⁻¹) or pentagastrin (0.5 μg kg⁻¹ h⁻¹) was infused throughout. Gastric juice was collected at 15 min intervals and total acid was determined by titration against 0.1 N NaOH using phenolphthalein. In separate experiments, the maximal secretory response of each dog to histamine was determined.

Changes in gastric mucosal blood flow were measured in four experiments in the three dogs using the plasma clearance of radioactive aniline (Curwain & Holton, 1971; Curwain, 1972a). For this measurement, blood samples were taken at 30 min intervals via a second vein catheter and the radioactivity in 1 ml samples of plasma and gastric juice was determined. In these experiments it was necessary to ensure an acid environment at the gastric glands and so pentagastrin was infused throughout in a dose sufficient to cause acid secretion at about 10% of the maximum histamine response. In expressing the results the responses of both secretion and mucosal blood flow to pentagastrin have been taken into account.

Results.—Acid secretion In a total of 18 observations betahistine (5–40 μg kg⁻¹ min⁻¹ i.v.) did not significantly increase
FIG. 1. The effect of betahistine on acid secretion and gastric mucosal blood flow in Heidenhain pouches, in three conscious dogs. (a) Ordinates: Acid secretion expressed as a percentage of the maximum response to histamine for these dogs. Abscissae: Dose of betahistine (log scale) $\mu$g kg$^{-1}$ min$^{-1}$. The line is the calculated least squares regression line $b=26 \ P<0.01$. The points are the mean results of the number of separate experiments (shown in parentheses). (b) Ordinates: Mucosal blood flow (measured as aniline clearance) expressed as percentage of blood flow during the control period. Abscissae: Dose of betahistine (log scale) $\mu$g kg$^{-1}$ min$^{-1}$. The line is the calculated least squares regression line $b=291 \ P<0.01$. The points are the mean results of 16 observations in 4 separate experiments.
the rate of acid secretion. However the larger doses (80 and 160 μg kg\(^{-1}\) min\(^{-1}\) i.v.) increased acid secretion by 8-8% and 17-6% respectively of the maximal response to histamine (Fig. 1a). There was no difference between the effects of betahistine given alone and on a background of secretion induced by a small dose of histamine or pentagastrin: the effect of betahistine was additive and there was no evidence of potentiation or inhibition of other stimuli.

**Mucosal blood flow** Betahistine (40–160 μg kg\(^{-1}\) min\(^{-1}\) i.v.) increased gastric mucosal blood flow at the three dose levels in each of four experiments as illustrated in Figure 1b.

**Relative effects of betahistine on mucosal blood flow and secretion** The ratio of mucosal blood flow to gastric secretion is given by the ratio of the concentrations of aniline in gastric juice and plasma. In these experiments the mean ratios±S.E.M. for betahistine were 41-9±6-2 (n=12) 42-5±1-6 (n=24) and 149±10-2 (n=12) in the three dogs compared with a mean ratio of 37-2±3-4 (n=36) for histamine in the same dogs at comparable levels of secretion.

**The effect of betahistine on pouch drainage** In one of the dogs the pouch drained less well during the largest dose of betahistine so that the volume of secretion collected during the last 15 min of infusion was small. In the subsequent 15 min, after the infusion of betahistine had ceased, a large volume of secretion was collected. The collections from the two periods were combined in the results for acid secretion described above. This observation suggests that this dose of betahistine caused alterations in the muscle tone of the pouch so that the secretion did not drain out into the cannula. This effect was not so marked in the other two dogs. 

Mucosal blood flow measurements were affected in a similar way in two of the dogs. The results shown in Fig. 1b, however, do not include the periods after infusion of betahistine. If they are included the mean percentage blood flow is 502 for the dose of 160 μg kg\(^{-1}\) min\(^{-1}\).

The ratio of mucosal blood flow to secretion depends only on the ratio of aniline concentration and not on the volume of juice collected. Therefore it is not affected by alteration in pouch drainage.

**Discussion.**—These results show that, compared with histamine, betahistine has little activity in stimulating gastric acid secretion. In confirmation of previous work in dogs and man we observed no stimulation of secretion with doses up to 40 μg kg\(^{-1}\) min\(^{-1}\). When larger doses of betahistine were used a small but significant secretion was observed.

The vasodilator effect of betahistine which is known in other vascular beds has been demonstrated in the gastric mucosa. This was an expected result because gastric mucosal blood flow and secretion are intimately linked. In every situation in which secretion is increased mucosal blood flow is also increased (Jacobson, Linford & Grossman, 1966). However, not all secretagogues affect mucosal blood flow and secretion to the same extent. For example, the ratio of mucosal blood flow to secretion is greater for histamine than for pentagastrin (Jacobson & Chang, 1969 ; Reed & Smy, 1971 ; Curwain, 1972b). It is therefore of interest to consider this ratio for betahistine. In none of the dogs was the ratio less for betahistine than for histamine. In one of the three dogs the ratio was more than three times that for histamine and this difference was highly significant (P<0.001). In the other two dogs the ratio was not significantly different from the ratio for histamine. We conclude that there is considerable individual variation in the relative sensitivity of gastric mucosal blood vessels and the secretory mechanism for betahistine and histamine. Since betahistine does not decrease the ratio of gastric mucosal blood flow to secretion, at least in dogs, dyspepsia cannot be attributed to relative mucosal ischaemia. The possibility that dyspepsia might be associated with disturbance of gastric motility needs to be considered. In our experiments there was indirect evidence that betahistine affected gastric muscular tone. The effect of betahistine on isolated stomach muscle has not been reported but on isolated intestine it has about 8% of the activity of histamine (Werle & Palm, 1953).

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(Received May 23, 1972)
The effects of $\beta_2$-adrenoceptor stimulants, salbutamol and terbutaline on gastric acid secretion and mucosal blood flow in conscious dogs with Heidenhain pouches

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Isoprenaline is a potent inhibitor of pentagastrin-induced gastric acid secretion in dogs; this inhibition is not secondary to decreased mucosal blood flow, nor antagonized by propranolol (Curwain & Holton, 1972). In order to investigate whether these properties are shared by other drugs which stimulate $\beta_2$ receptors, we have examined the effect of salbutamol and terbutaline.

Gastric acid secretion was stimulated by pentagastrin [1.0-4.0 (pg/kg)/h] and gastric mucosal blood flow measured by radioactive aniline clearance (Curwain & Holton, 1971; Curwain, 1972). Both salbutamol sulphate [0.1-0.5 (pg/kg)/min] and terbutaline sulphate [0.1-0.5 (pg/kg)/min], infused intravenously for 30 min, decreased gastric acid secretion, and the effect was dose related. In 5 experiments in 3 dogs salbutamol [0.1 (pg/kg)/min] reduced acid secretion to a mean of 54% ± S.E. of mean 12% and the effect was antagonized by propranolol (1 mg/kg; i.v.) given 25 min earlier (3 experiments in 3 dogs).

In 6 experiments in 3 dogs terbutaline [0.2 (pg/kg)/min] reduced acid secretion to 48% ± 4% and in each case the effect was abolished by propranolol. Heart rate, measured by palpation, rose to 163% of pre-dose level during salbutamol infusion and 127% during terbutaline. Propranolol abolished the tachycardia.

The effects of salbutamol and terbutaline on mucosal blood flow were studied in 3 dogs. Salbutamol 0.1 (pg/kg)/min for 30 min in each of 2 experiments in 2 dogs decreased acid secretion and mucosal blood flow, but the ratio of blood flow to secretion (G/P) increased markedly. Terbutaline 0.2 (pg/kg)/min for 30 min gave similar results (2 experiments in 2 dogs).

In a dose of 1 (pg/kg)/min both salbutamol and terbutaline increased gastric mucosal blood flow without affecting secretion when given on a plateau of histamine-induced secretion.

These results are similar to those previously reported for isoprenaline (Curwain, Endersby & Holton, 1971; Curwain & Holton, 1972) except that isoprenaline inhibition of gastric secretion is not sensitive to blockade by propranolol. The inhibition of pentagastrin-induced gastric acid secretion by terbutaline and salbutamol, like the inhibition by isoprenaline, is not secondary to a fall in mucosal blood flow.

The development of $\beta_2$-adrenoceptor stimulants which, in man, have no direct effect on the heart, raises the possibility of using these drugs for their anti-secretory action. A substance which inhibits acid secretion but causes a relative increase in mucosal blood flow might be expected to hasten the healing of some types of gastric lesions.

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