OBSERVATIONS ON SOME LOCAL AND GENERAL HORMONES IN THE ALIMENTARY TRACT OF IMPORTANCE TO GASTRIC SECRETION.

A Thesis submitted for the degree of M.D. in the University of Glasgow.

by

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Being a record of work done in the University Department of Surgery, Western Infirmary, Glasgow and the National Institute for Medical Research, London.

"The contemplation of those things which are normal is physiology, and it is the first thing to be learned by medical men. For that which is normal is right and serves as a criterion for both itself and the abnormal."

William Harvey, the first of two essays to Riolan, 1649. (Translation by K. J. Franklin).

'departures from the normal', as Harvey envisaged.

Perhaps it was with this in view that the Medical Research Council decided to initiate in 1951 the first Fellowships in Clinical Research. This was intended to provide a corps of people able to take care of patients in irreproachable manner, yet also prosecuting a serious scientific study of disease. The author was fortunate to be sponsored for one of these fellowships by Professor C. F. W. Illing-:worth, Regius Professor of Surgery in the University of Glasgow, and to be elected by the Clinical Research Board of the Medical Research Council. By permission of Sir Charles Harington, the Director of the National Institute for Medical Research, he joined the staff of the Division of Physiology and Pharmacology under Dr. W. Feldberg, F.R.S. to study gastrointestinal physiology, mostly in relationship to the hormones and "active substances" in the alimentary tract. This work was later continued in the University Department of Surgery at the Western Infirmary, Glasgow, partly while holding a grant from the Department of Health for Scotland and also during the tenure of a University Lectureship in Surgery, at which stage the author renewed his surgical apprenticeship!

One of the outstanding problems afflicting the most responsible citizens of Western civilisation to-day is that of peptic ulceration. It seemed appropriate that a surgical aspirant, turning aside for such/

such a training from surgery for two or three years, should study the background to this disease. Since it is so closely related to the acid secretory process in the stomach, and since so much work has already been done on the neural aspects of gastric secretion, an investigation was begun of the hormones or chemical factors found in gastric tissue which might be of importance in the control of the acid secretory mechanism.

The Thesis itself consists of work done from 1951 onwards and is composed of five main Parts, namely an Intoduction and Review of work done in this field, followed by original work on histamine, 5-hydroxytryptamine, substance P and gastrin. Each chapter in each Part of the Thesis is constructed round an introduction, the methods used, the results and a discussion, while Parts 2 and 3 (consisting of six chapters each) also have a general introduction and a comprehensive discussion. While much of the experimental work has been done exclusively in the laboratory, in several chapters human tissue or spontaneous pathological processes have been examined and the results related to the human case.

The Thesis concludes with a Summary of the experimental work recounted in each Volume, with an Appendix on various special methods used, and a Bibliography. In an acknowledgements section the author extends his thanks to numerous individuals for their help and co-operation during the performance of this work.

EDINBURGH.
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A.N.S.

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PART ONE.

CHAPTER ONE.

Introduction to Thesis.

CHAPTER ONE.

INTRODUCTION.

It was an evolutionary step forward for the organism to be in possession of a stomach, freeing itself from a continuous search for food. In primitive fishes such as Cyclostomes, the digestive tract is tube-like and is lined with ciliated epithelium, but Selachians possess a well developed stomach with three divisions, fundus corpus, and pylorus. At first the primitive stomach fulfilled the passive role of receptacle, but to this was later added pulping activity. As an aid to this mechanical activity came secretion of juice which formed a chyme, readily passed from the pylorus to the duodenum; primitive gastric juice had qualities protective to the organism in a simple form as an alkaline mucoid secretion. The secretory tissues later developed towards an organ of multiple functions producing hydrochloric acid in a juice rich in enzymes, mucus, salts and the factor necessary for the full maturation of the red cells.

In the fully developed secretory mucosa of the stomach the main recognisable cell types are the surface epithelial cells, the cells of the neck of the tubular glands (of mucoid secretion), parietal cells (for secretion of hydrochloric acid) and peptic cells (for secretion of pepsin). As they discharge different secretions the composition of gastric juice will continually vary; this/

this is readily facilitated by the control of cellular activity through separate mechanisms.

Following the work of Pavlov (1910), it is believed that there are two main forms of control of secretory activity in the stomach, one designated the neural mechanism and the other the humoral. The vagus nerve is the secretomotor nerve exciting secretion of acid, pepsin and mucus. The characters of vagal juice, elicited by activity of nuclei in the hypothalamus, are well recognised. Less firmly established is the role of the sympathetic innervation of the stomach which Baxter (1932) believed to be an inhibitory influence on gastric secretion. Babkin (1946), on the other hand, found that these fibres were synergists enhancing the effect of other agents provoking gastric secretion.

The second phase of gastric secretion is generally thought to be controlled by hormones. This has, however, not always been accepted, as it was at one time held that products of digestion, or modification of such products after interaction with the cells of the digestive tract, might be responsible for this phase of secretory activity. If there is indeed a true hormonal mechanism, a comparison between the gastric excitatory hormone and the characteristics of other accepted hormones should be of some interest.

The term "hormone" was first introduced by Starling in 1905 with reference to secretin, a blood borne substance liberated from/

from the duodenal mucosa. In 1914 the same author defined a hormone as "any substance normally produced in the cells of some part of the body and carried by the bloodstream to distant parts, which it affects for the good of the body as a whole". Objections were, however, raised to the word 'hormone' (literally meaning to excite or set in motion), and a further part of the terminology suggested by Sharpey-Schafer was to subdivide the exciting and inhibiting humors into hormones and chalones. Current usage did not sanction the use of these substitute terms, and the term hormone has been retained for both excitatory and inhibitory materials. The features of the accepted hormones may be summarised as follows:-

1) They are special chemical compounds which are produced by restricted areas of the organism, and which diffuse or are transported for variable distances modifying tissues to accomplish co-ordination for the organism.

2) They are usually effective in small quantities.

3) Though they are generally transported for variable distances to modify tissues remote from their place of origin, they may modify the organs producing them. This is particularly true of the adrenal gland, the cortex of which may indirectly be affected by the secretion of the medulla, by action of the medullary secretion through the intermediary of the anterior pituitary. While most hormones act on tissues remote from their place of origin, it would seem that the hormones of the alimentary tract can act on the very tissues producing them; in particular this might be true of a gastric hormone, which in its pure state might act on the organ producing it. In this the gastric hormone, gastrin, would be unique.

It/

It must be acknowledged that the hormones of the alimentary tract are as a group different from the hormones of the ductless glands. While most of the endocrine glands are activated by steroid hormones, as far as is known the exo-endocrine glands of the alimentary tract are activated by proteins, protein fractions or derivatives of amino acids.

They also differ in that they are not readily studied by the techniques usually used for the investigation of endocrine function. In general, such techniques as surgical extirpation, replacement therapy, additive experiments, and the study of natural and artificially induced defects of glandular function, have not been fruitful in the field of hormones of the alimentary tract. Chemical extraction yields some successes, but such preparations are difficult to standardise. Nevertheless chemical extraction has yielded notable results; but such extraction is tedious, may be technically difficult and is expensive.

However, there is another great obstacle present after extraction of such humoral agents, a pitfall confronting workers in this field: the humoral agent so obtained by extraction might well be any secret-:agogue, implying a substance present in food or produced by food digestion. It might excite digestive juices locally, or after absorption into the blood or lymph and might do so by causing, indirectly, the formation of a hormone or its release in the body.

Another/

Another difficulty in the interpretation of results with chemical extracted humoral agents lies in the variability of the responsive cells themselves. The target organs of the alimentary tract - muscles (tunica muscularis and muscularis mucosae) and gland cells may be inhibited by tissue extracts, so that though a possible hormonal agent may be present its secretory effect may be masked when tested for activity.

Grossman (1950) points out that the gastro-intestinal hormones are not indispensable to life. Their place in the body economy is not high, as has been deduced from the fact that no disease has yet been associated with over or under production of a given hormone. They share in the regulation of digestive function with intrinsic or extrinsic nerves, with which they may act in synergism or antagonism. In certain organs the chemical control of secretion by hormones would appear to be almost as great as the neurogenic; such would appear to be the case for the stomach and pancreas, thus providing a brisk secretion of digestive juices for the drastic alteration of foodstuffs.

CHAPTER TWO.

A review of the properties of local and general hormones which have been extracted from gastric tissue.

CHAPTER TWO.

The mechanism of acid gastric secretion has been described in Chapter One. Less is known of the role of chemical mediators of this mechanism. Many pharmacologically active substances extracted from the gastric wall may be important in the secretory or motor functions of the stomach.

These substances may, for descriptive purposes, be divided into two groups.

i) Local hormones - implying substances pharmacodynamically active on formation or release in the tissues, and acting intimately on their glandular structures, smooth muscle or blood vessels.

ii) General hormones - implying substances which circulate and affect the function of distant organs.

The main features of the properties or hormones of the alimentary tract have been listed when discussing the humoral phase of gastric secretion in Chapter One, but a further definition of "local hormones" seems necessary. Feldberg discusses the first historical usage of this term in Gaddum's text book, "Polypeptides which stimulate plain muscle" (1955). He recounts how Sir Henry Dale had given "A Survey Of Present Knowledge Of The Chemical Regulation Of Certain Functions By Natural Constituents Of The Tissues" in his Dohme Lectures of 1933. Gaddum later described various substances which might act as 'gwebestoffe' in his book "Gefasserweitende Stoffe Der Gwebe" (1936); these substances were/

were histamine, acetylcholine, adenosine, kallikrein, vasodilators from blood, intestine and brain, including Euler and Gaddum's Substance P.

Gaddum (1950), discussing the action of local hormones at a Symposium on this topic at the Royal Society, indicated the problem of whether to include acetylcholine which is a known mediator of nerve effects. Burn (1950) in the same Symposium did not consider acetylcholine as a local hormone; yet Burn and co-workers have shewn that in certain tissues as the auricles of the heart and cilia of lower organisms, acetylcholine may be responsible for contractions through local formation in the tissues, quite apart from cholinergic nerve transmission.

In view of the lack of evidence for a local role of acetylcholine in the stomach, it has been omitted from the local hormones investigated or discussed in this thesis.

The local and general hormones distributed in the stomach wall are:

i) local hormones - histamine, 5-hydroxytryptamine, and Substance P.

ii) general hormones - gastrin, (extracts have been made from the stomach containing varying amounts of enterogastrone, secretin, cholecystokinin, villi kinin, and enterocrinin, but all are intestinal rather than gastric hormones).

Histamine/

Histamine is a substance which has been assigned to both groups, either as a local hormone activating the glandular cells of the stomach after restricted release, or as a general hormone similar to gastrin. This anomaly will be discussed below.

LOCAL HORMONES.

HISTAMINE.

Histamine has been isolated chemically from gastric and intestinal mucosa of various species (Barger and Dale, 1911) under conditions which exclude the possibility of bacterial origin or the formation from putrifactive changes during the extraction, (Gerard, 1922; Sacks, Ivy, Burgess, Vandolah, 1932). The amounts of histamine assayed by biological methods were 7-8 ug/G fresh tissue for the small intestine (without mucosa) of the horse (Gaddum and Schild, 1934-35), 35 ug for the dog small intestine, also without mucosa (Gaddum, 1936). The presence of histamine in the wall of the oesophagus, stomach, large intestine may be inferred from Schild's findings of release in the antigen-antibody reaction of anaphylaxis, but no figures are available. On the other hand the histamine of the gastric mucosa has been determined in human beings, cats and dogs (Gavin, McHenry and Watson, 1933: Code, Trach, and Wangensteen, 1944; Emmelin and Kahlson, 1944).

The values for dogs were much higher than those for cats and human beings and in all three species the histamine in fundus is twice/

twice that in pyloric and central region. This has been taken as evidence of higher histamine content of oxyntic cells. The values in ug histamine per G mucosa were as follows:-

Dog fundus - 48-180 (av.80): pyloric region 24-80 (av.42).

Cat fundus - 5-34 (av.16): pyloric region 4-16 (av. 9).

Human beings' fundus - 4-24 (av.10): antral region 3-13 (av. 6).

Gavin et al. also showed that the muscularis propria contained less histamine than the mucosa which contains 80% of the histamine of the stomach wall. Histamine appears also in the gastric juice and is thought to be derived from mucosal histamine (for reference see Babkin, 1950).

Douglas, Feldberg, Paton and Schachter (1951) have examined the histamine content of the different layers of the wall of the gastro-intestinal tract of the dog with the following results:-

> The wall of the oesophagus contained little histamine; the fundus and corpus of the stomach wall were rich in histamine and contained about twice as much as the pyloric region. The intestine was also rich in histamine but the values decreased gradually from duodenum to rectum. In all regions the greater part of the histamine originated from the mucosa but the submucosa contained relatively high histamine values, whereas those for the muscularis externa were lower than those for any other layer.

Feldberg and Harris (1953) have made thin horizontal slices of the gastric mucosa and examined them for histamine. In this way they could build up histamine profiles and correlate them with histological structures.

Since/

Since histamine administered subcutaneously or intravenously has long been known as one of the most active substances stimulating acid gastric secretion, many workers have tried to establish whether its role is that of a true physiological stimulant serving acid secretory function.

The facts suggesting a physiological relationship are as follows:-

(1) Histamine stimulates acid gastric secretion.

Histamine was discovered by Popielski in 1920 to stimulate acid gastric secretion in the stomach of dogs, and this was confirmed in 1931 by Best and McHenry. The dose response curve relating the rate of injection of histamine to the rate of secretion has the customary exponential character which upon probit transformation becomes a straight line (Obrink, 1948).

(2) Site of action of administered histamine.

Histamine stimulates gastric mucosa in vitro, in high concen-:tration (Davies, 1946, Davenport and Chavre', 1950) evidence which suggests that histamine acts directly on the parietal cells without the agency of a further chemical transmitter. It stimulates the action of the secretory cells more briskly if it is brought into contact with their submucosal aspect (Hanson, Grossman and Ivy, 1948) than when applied to their luminal surface. Very large quantities of histamine (e.g. 50 mg. histamine acid phosphate) must be applied to the mucosa to elicit the slightest response (Ivy, Lim & McCarthy, 1925).

Histamine/

Histamine appears to act specifically on the parietal cell leaving the peptic cell little affected since the gastric secretion appears to contain acid but no pepsin after its injection. Acid secretion may wash out some pepsin initially, but this increase does not recur if a second injection of histamine is given (Babkin, 1930; Vineberg and Babkin, 1931; Gilman and Cowgill, 1931; Bjorkman, Norden, Uvnas, 1943).

(3) Histamine is present in high concentration in the gastric mucosa, Principally in the region of acid secretion. Not only is histamine present in the gastric mucosa but the acid secretory area (fundus) has twice the concentration of the antrum (Gavin, McHenry and Wilson, 1933; Emmelin, Kahlson, 1944).

(4) Histaminase is absent from the gastric mucosa and agents which inhibit this enzyme augment acid secretion.

Histamine is destroyed by deamination, through the action of histaminase (Best, 1939; Best and McHenry, 1930; McHenry and Gavin, 1932). Various workers have attempted to demonstrate the presence of this enzyme in the stomach wall, without success (Best and McHenry, 1930; Rose, Karady and Browne, 1940; Dworetsky and Code, 1951; Waton, 1956). Absence of the destroying enzyme would allow every opportunity for histamine to stimulate the parietal cell.

The corollary that drugs which inhibit histaminase might potentiate histamine effects has been confirmed by Schild and co-workers (Mongar and/

and Schild, 1951, Arunlakshana, Mongar and Schild, 1954). Sircus applied one of these inhibitors (β-pyrimidine) to a study of gastric secretion in dogs and cats, and found an enhanced response to histamine, feeding, vagal stimulation and alcohol.

(5) Histamine is present in gastric juice whatever the stimulus.

Histamine has been found in gastric juice by many observers. (Komarov, 1933. Brown, Smith, 1935. McIntosh, 1948. Emmelin and Kahlson, 1944. Code, Hallenbeck and Gregory, 1947. Adam et al., 1954). Emmelin and Kahlson found that the histamine content of the juice was independent of the mode of stimulus employed in exciting the parietal cells. Not only does the gastric juice contain histamine, however, but there is a definite correlation between the amount of histamine in the juice and the intensity of the secretory activity of the parietal cell (Code, 1955).

5-Hydroxytryptamine.

In 1940 Erspamer described the presence of an unknown substance in acetone extracts of gastrointestinal mucosa. He had noticed that the activity of his extracts from the gastrointestinal tract in lower animals varied with the number of argentaffin cells present in the tissue. He therefore named the substance he had discovered as enteramine, a hormone of the argentaffin or Kultchitsky cells which are part of the enterochromaffin system. Its presence was also demonstrated in acetone extracts of the spleen (Vialli and Erspamer, 1942), the posterior salivary glands of the octopus (Vialli and Erspamer/

Erspamer, 1940), the hypobranchial glands of snails (Erspamer, 1947 and 1948), and the skin of amphibia (Erspamer and Vialli, 1951). The following pharmacological reactions have been ascribed to Enteramine. It stimulated the atropinised oestrus uterus of rats and mice, the duodenum of rats, the urinary bladder of dogs (Erspamer, 1940) and the heart of moluscs (Erspamer and Ghiretti, 1951). Enteramine was also found to depress the blood pressure of the atropinised rabbit and cat and inhibit diuresis in hydrated rats (Erspamer and Ottolenghi, 1951). According to Erspamer (1942-1948) when extracts containing Enteramine were boiled for a short time at a pH 7-8 there was increase in activity. He concluded that Enteramine existed in two forms, Enteramine A, active by itself, and Enteramine I, ordinarily inactive but easily activated by treatment with alkali. Erspamer and Boretti (1951) claim to have separated these two by paper chromatography.

Finally Erspamer and Asero announced in 1951 that they had isolated Enteramine as its picrate salt from the salivary glands of octopi and the skin of amphibia. It was identified as 5-hydroxytryptamine and similar to serotonin, isolated by Rapport, Green and Page, 1948, and Rapport, 1949. Dalgliesh, Toh and Work, 1953, independently isolated 5-hydroxytryptamine and another indole derivative not yet identified from the alimentary tract of the dog; Feldberg and Toh (1953) have shown that 5-hydroxytryptamine is present in largest amount in the mucosa of the pyloric region and duodenum, and that there is a gradient of activity distally towards the ileum.

TABLE 1 lists several of the physiological roles, postulated by various authors, for 5-HT.

Role	Hypothesis	Author	Species
Antidiuretic factor	Originates in enterochroma-:ffin cells and may control water excretion in the rat	Erspamer and Ottolengli, (1953)	Rat
Vascular agent	Inhibits vascular neurogenic tone and is possibly import-:ant in hypertension.	Page and McCubbin, (1953)	Dogs and Humans
Platelet factor	Local vasoconstrictor sub-:stance, possibly important in haemostasis.	Reid and Rand (1951)	Rabbits, etc.
Neuro-hormone	Distributed widely in brain, rapidly formed there. Ant-:agonised by lysergic acid, which causes hallucinations.	Amin, Crawford & Gaddum (1953) Woolley and Shaw (1954)	Various
Gastro-intestinal motor hormones	Stimulates intestinal peristalsis; released by motor movements of cut.	Bulbring & Lin (1958)	Guinea-pigs and rabbit
Pain factor	Causes pain on intradermal injection, extracted from cautharides blister fluid.	Armstrong, Dry, Keele, Markham, (1952)	Human

Many physiological roles have been ascribed to 5 HT and the most important claims are listed in Table I. The distribution of 5 HT in the hypothalamic region suggests that it may play an important part in physiological activity at this site. Many important drugs which disturb brain function are also substances which potentiate or block the effects of 5 HT, e.g. resperine, chlorpromazine and lysergic acid.

Bulbring and Lin (1958) have investigated a possible intestinal role of 5 HT, namely its relationship to intestinal motility, as elicited by a peristaltic reflex. They found that 5 HT was formed and stored locally in the mucous membrane and was released as the intra-luminal pressure rose and that it sensitises pressure receptors, lowering the threshold of pressure required to elicit a peristaltic reflex.

5 HT has a rich distribution in the mucous membrane of the alimentary tract. It is present in high concentration in the mucosa of the pyloric region and duodenum, but the concentration lessens in gradient fashion distally. 5 HT might, in view of its distribution in the stomach and duodenum have an important role in gastric secretory function; this forms the main topic discussed in Part III of this thesis.

SUBSTANCE P.

The name Substance P was given by Gaddum and Schild (1934-35) to an unknown substance first found by Von Euler and Gaddum (1931) in extracts from the small intestine and brain, and believed to be a polypeptide. The extracts caused a fall in arterial blood pressure of the atropinised rabbit and a slow contraction of the isolated, atropinised, intestinal preparation of the rabbit.

The substance was destroyed by boiling for some time in strong acid and alkaline solution. It was present in large amounts in the muscle layer of the horse intestine, but there was little activity from the mucosal layers. Extracts from the horse's stomach were relatively active; those from the urinary bladder showed some activity, but those from the viscera and skeletal muscle showed none. In the brain it was found in the basal ganglia. Euler (1934, 1936) found further large amounts of apparently the same substance in human semen and in extracts of the prostatic gland of various animals.

Douglas, Feldberg, Paton and Schachter (1951) found a smooth muscle stimulating substance resembling Substance P in acid saline extracts of the wall of the digestive tract of dogs and studied its distribution in various sites and layers of the gastro-intestinal tract. There was little activity in the oesophagus while there was some activity in the stomach and there was much in the small and large/

large intestine; in the intestine it was to be found in the mucosa and in particular in the muscularis mucosa. The same findings were independently arrived at by Pernow (1951).

Substance P stimulates the smooth muscle of many species studies (Euler, 1936b: Vogt, 1949, 1950) and lowers the threshold for eliciting peristalsis. It, however, has other systemic effects, for instance, injected into rabbits it increased bile flow and caused a fall in arterial blood pressure. It exercises a vasodilator effect on the blood vessels of the frog.

Substance P is precipitated with half saturated ammonium sulphate and destroyed by trypsin. High active powders have been prepared by the method of Euler (1936c, 1942).

Euler (1936 a and b) suggest that the release of Substance P in the intestinal wall may be responsible for its spontaneous movement, but according to Vogt (1949) it, or rather a related substance, is released from the wall of the frog's stomach during vagus stimulation. Recent findings (Vogt, 1950: Fischer and Vogt, 1950) with paper chrom-:atography have shown that Substance P may consist of two related substances, the one with a greater action on the blood pressure of the atropinised rabbit and the other on the atropinised rabbit's intestine. The term Substance P may apply to two polypeptides with differing action.

GENERAL HORMONES.

1. GASTRIN.

Edkins (1905) raised the possibility through his experiments that there was an agent in the gastric mucosa which was produced in the response to mechanical and chemical stimulation, and stimulated secretion of hydrochloric acid by the gastric glands. It was claimed that this humoral agent was liberated specifically from the pyloric region by the products of protein digestion; on extraction it was active on intravenous administration. Popielski, however, showed within a few years that extracts of almost any tissue would stimulate acid gastric secretion (1909, 1913) and later demonstrated that histamine was almost ubiquitously present in many tissues and that it was a potent stimulant of gastric secretion, (Popielski, 1920.) When, in 1933, Gavin, McHenry and Wilson found histamine in rich store in the gastro-intestinal tract, this led to the view that gastrin and histamine were one and the same substance.

i) Evidence that the Humoral Agent is Gastrin.

Edkins (1905:1906) in making acid saline extracts which were active in acute experiments established an experimental fact which has often been disregarded; despite the unwitting contamination of his extracts with histamine, he showed that intravenous administration of the extracts reliably caused acid gastric secretion.* This seems an important finding since it is known that histamine itself is often inactive on intravenous injection* Edkins' gastrin was undoubtedly contaminated/

(* See Part 5).

contaminated with histamine, but Lim (1922) using Edkins' method adequately confirmed his findings.

Komarov in 1938 extracted a protein constituent of the gastric mucosa from the pyloric mucous membrane, free of histamine and highly effective by the intravenous route. In the latter respect he kept closely to the original experiment of Edkins which had been successfully repeated by Lim. His preparation had many simil-:arities with secretin; it could be precipitated by trichloracetic acid by saturation with NaCl, was soluble in ethylalcohol, methylal-:cohol and acetone, while insoluble in pure organic solvents. It was active in anaesthetised and unaesthetised cats and dogs, and acted on the gastric secretory mechanism without lowering blood pressure. Intramuscular injections elicited only slight secretory activity, while that following subcutaneous injection was minimal. Extracts from the pyloric region were the most active, duodenal extracts were less active, while fundic, jejunal and liver extracts were inactive.

Uvnas (1942) repeated much of Komarov's work, using a modification of Komarov's extraction procedure designed to purify the protein constituents and to free them of the nitrogenous bases of small molecular size such as choline and histamine. Uvnas found that following the injection of 1 mgm. of his preparation of gastrin he obtained marked acid secretory activity. He confirmed Komarov's description/

description of the regions of the alimentary tract giving rise to active fractions and found that his active extracts would stimulate acid secretory activity without giving rise to pancreatic or biliary secretion, gastric motor activity, changes in blood sugar or blood pressure. Some of his pyloric extracts were found to contain a secretory depressant material. The active principle was resistant to heating in acid but was destroyed in alkali by pepsin pancreatic juice (activated by duodenal juice), and by ultra violet light. It was dialysable through cellophane and was precipitated by trichloracetic acid, metaphosphoric acid, saturated solutions of NaCl and acetone.

Harper (1946), Friedman and King (1947), Hanson and Grossman (unpublished, cited by Grossman (1950)) have in turn all attempted to confirm this work. Harper, applying Mellanby's method for extraction of secretin, produced pyloric extracts which were not dialysable but were active in eliciting acid secretion, and had no depressor action on the cat; but Hanson and Grossman, applying Ivy's method for the extraction of secretin failed to get active extracts; their successes were few with the method of Komarov.

ii) Evidence that the Humoral Agent is Histamine.

While the work outlined above on the activity of tissue extracts is now accepted as on a fairly firm footing, there is the striking fact that the humoral agent, the purified gastrin of the tissue extracts and histamine/

histamine itself, all cause secretion of the same type of gastric juice. Histamine has accordingly been identified by many authorities with "gastrin" and proposed as the humoral agent; alternatively it has been suggested that it is the local chemical agent present in the gastric wall exciting the parietal cells.

The great sensitivity of the parietal cells to histamine is one of the main factors to be taken into consideration in assessing the role of this substance. Hanson and his co-workers (1948) found that the minimal effective dose was of a very low order, confirmed by Emmelin and Kahlson (1944) who found that it was, in fact, below the level of the currently detectable levels of histamine in plasma. This is evidence for great sensitivity of the parietal cells to histamine.

The position of histamine as a hormone unfortunately cannot yet be clarified because of the relative insensitivity of present methods of extraction of histamine, but various workers have demonstrated its release into the gastric juice. Brown and Smith (1935) extracted a histamine-like substance from gastric juice and MacIntosh studied by the Barsom-Gaddum extraction method the appearance of histamine in gastric juice, after vagal stimulation, sham feeding and histamine injection. MacIntosh (1938) associated the presence of histamine with high acid secretory rates, but Emmelin and Kahlson, though finding histamine in gastric juice, were unable to make this correlation;

Code/

Code, Hallenbeck, and Gregory (1947) agree with the conclusions of the latter. All investigators are agreed that histamine may occur in greater amount in juice than in the plasma, and it remains a possib-:ility, therefore, that the gastric hormone is in fact histamine or that histamine is released for parietal cell activity from fixed stores in the mucosa by the protein fraction now known as 'purified gastrin'.

2. MISCELLANEOUS GASTROINTESTINAL HORMONES.

For completeness, other gastrointestinal hormones are listed here. They do not form material for further discussion since their principal site of localisation is the intestinal tract distal to the stomach, and their target organs are the pancreas, gallbladder and small intestine.

i) Secretin (Bayliss and Starling, 1902) stimulates the secretion of pancreatic juice and is released from the upper intestinal tract by the acid secretion; it is mentioned here solely because it has been extracted from the gastric mucosa.

ii) Pancreozymin stimulates the secretion of enzymes by the pancreas and is extracted from the upper intestinal tract (Harper and Raper, 1943).

iii) Enterogastrone (Kosaka and Lim, 1930) is an inhibitory agent derived from the upper intestinal tract by the action of fats and depresses both acid secretory activity and motility.

iv) Cholecystokinin (Ivy, 1934), Villikinin and Enterocrinin are

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are smooth muscle stimulating substances affecting the gallbladder, intestinal villi and small bowel motility respectively. Intestinal humoral agents, the products of digestions, may cause acid secretion. Le Conte (1900) demonstrated this by the introduction of food into the duodenum of dogs with gastric fistulae, work which Pavlov repeated in 1910. Lim, Ivy and McCarthy (1925) showed clearly an acid secretion on feeding dogs with total stomach pouches; the secretion in the pouch was greater if the food was predigested.

CHAPTER THREE.

Experimental approach to the study of the active substances present in gastric tissue.

CHAPTER THREE.

EXPERIMENTAL APPROACH.

The particular methods employed in the investigation of histamine, 5-hydroxytryptamine, Substance P and gastrin will be described later.

The opportunities for a new experimental approach to the study of gastric hormones will be the main consideration of this chapter.

1) HISTAMINE.

The effects of histamine extracts are rarely studied since the synthesis of this substance (Windaus and Vogt, 1907). This has provided ample quantities of the pure substance for experimental use.

Tissue histamine, however, can be rapidly and extensively altered by synthetic histamine releasing agents of high potency such as Compound 48/80 (Paton, 1951) and Octylamine (Mongar, 1953). This has provided a useful tool for the examination of many problems in which histamine has been assigned a role, often a conjectural one. The histamine liberator is used to reduce the histamine of the tissues, to determine what changes may have been produced by this alteration. Histamine release was however, not always so specifically accomplished.

The study of histamine release began with the perception by Dale of the parellelism between anaphylactic shock and the toxic effects of histamine in guinea pigs, and the release of histamine in this phenomenon was demonstrated by Bartosch, Feldberg and Nagel (1932), when antigen was perfused through sensitised guinea pig lung.

The/

The concept of histamine release as a result of injury to the cell had been advanced by Lewis, and he believed that such a mechanism might explain the similar effects, such as the triple response, of many common injurious agents in the skin (1927). Following his lead, many workers associated the oedema and vascular effects of the allergic or sensitivity reactions of drugs with histamine release. Feldberg and his co-workers pursued the examination of the release of histamine by staphylococcal or clostridium Welchii toxin, mercuric chloride, bee and cobra venom and lysolecithin (Feldberg and Kellaway, 1937: Feldberg and Keogh, 1937: Feldberg and O'Connor, 1937: Feldberg and Kellaway, 1938: Feldberg, Holden and Kellaway, 1938). But the demonstration of histamine release in these experiments was acknowledged to be in some way the result of tissue damage.

The first demonstration of histamine release drugs "without tissue damage" was made by Alam, Anrep, Barsoum, Talaat and Weininger (1939) for d-tubocurarine, repeated by Schild and Gregory (1947), and for strychnine (Schild and Gregory, 1947). Release of histamine without cellular damage became more widely recognised following the conclusion by MacIntosh and Paton (1949) that many organic bases possessed the characters of histamine liberators. Among the bases found to release histamine were diamines, diamidines, diguanidines, di-isothioureas, diquaternaries and some benzamidines. These compounds all produced a sudden fall in arterial blood pressure on injection, but the beginning/

of this fall was in every case delayed till the onset at 20-25 seconds after injection. This accompanied the accumulation of large amounts of histamine in the plasma. Histamine was in all cases detected in large enough amounts to account for the effects on the blood pressure; the characteristic delayed depressor response could be associated with release of histamine in the tissues entering the vascular system a short time after the primary histamine release. These compounds elicited a triple response when injected into human skin, and when infused intravenously in the cat they caused secretion of acid gastric juice, but this was not studied in detail.

Histamine releasing agents have been employed in this study to ascertain whether they elicit an acid gastric secretory response, and to determine the site of the release of the histamine causing acid secretion. They have also been applied to tissues to see whether depletion of tissue histamine modified secretory responses to other hormones, etc.

Reference is made in the text to the histamine-releasing properties, in various chapters, of Compound 48/80, tryptamine, octylamine, d-tubocur-:arine, propamidine and mepyramine, all of which are complex nitrogenous bases. Horse serum, Bolton toxin and gastrin were also found in exper-:iments which are described later to possess histamine-releasing properties.

A full description of the properties of Compound 48/80 which is frequently referred to throughout this thesis is given in the Appendix.

2) 5-hydroxytryptamine (5-HT)

Although enteramine was originally obtained in extract form, it was quickly characterised by Erspamer as 5-hydroxytryptamine. It was soon recognised that this was the same substance as had been extracted from serum as a vasoconstrictor substance shed from platelets. In 1949 Rapport had identified secretonin as 5-hydroxytryptamine, Hamlin and Fischer, in 1951, synthesised this substance, and the synthetic material - as the creatinine sulphate - has been largely used in the experiments reported in this thesis in preference to the crude extract.

Human experiments with 5-HT are complicated by the fact that this substance produces unpleasant and often dangerous effects on systemic injection (Table 2).

It seemed to us that the functioning carcinoid or argentaffinoma provided an alternative means of studying the human effects of 5-HT; just as 5-HT was found by Erspamer to be present in tissue extracts with argentaffin cells, so 5-HT is produced and released into the circulation from tumours of these cells. This causes a remarkable clinical syndrome first recognised by Swedish workers in Malmo (Biorck et al., 1952. Thorsen et al., 1954). This is seen as cutaneous flushing, or a reddish blue cyanosis may be the most striking manifestation. There are often asthmatic like attacks, diarrhoea and occasional giddyness; the development of valvular lesions of the right side of the heart is a feature/

TABLE 2.

Human Effects of 5 - HT.

Pain at site of injection, blanching and venospasm, pallor, tachycardia, substernal discomfort.
- - -
Respiratory distress : bronchospasm and hyperpnoea. Flushing and cyanosis.
Abdominal colic, borborygmi, increased frequency of bowel movements and micturition.

After Page et al (1955).

feature. The syndrome is associated with metastasising carcinoids which have hepatic and glandular metastases. Lembeck (1953) established that there was a high concentration of 5-HT in metastatic carcinoid tissue.

Other indirect methods of studying 5-HT which have been employed are those which

a) release 5-HT from its tissue linkage. One of the most powerful agents in this respect is reserpine (Shore et al., 1955, 1957, Brodie and Shore, 1957).

b) augment 5-HT locally by inhibition of the enzyme responsible for 5-HT destruction so that 5-HT preponderates over other local hormones in the tissues. Such an agent is iproniazid, which is a mono-amine-oxidase inhibitor (Zeller and Barsky, 1952).

3) Substance P.

4) Gastrin.

Both of those substances have been studied after extraction from the appropriate tissues. Substance P was studied by the method of Euler (1942). Gastrin was studied mainly by the method of Jorpes, Jalling and Mutt(1952.) A small quantity of an alternative preparation by the method of Uvnas (1945) was prepared for comparison. The features of both methods are outlined in the Appendix.

PART TWO.

INTRODUCTION.

PART TWO.

INTRODUCTION.

Part Two of this thesis deals with experiments conducted to evaluate the role of histamine release in gastro-intestinal activity. Feldberg and Paton in 1951 described the properties of an active histamine liberator, Compound 48/80 (Paton, 1951; Feldberg and Paton, 1951). It seemed possible that by the use of this agent more inform-:ation could be obtained as to the exact means whereby histamine excites gastro-intestinal activity.

Chapter One constitutes an examination of the ability of this particular histamine liberator to produce acid gastric secretion when injected into various tissues. In this way, using acid secretion as an indication of the amount of histamine released, it has been possible to estimate which tissues are most readily affected by histamine liberators. In Chapter Two, the effects of the tissue histamine have been directly studied, in cats. The histamine of the gastro-intestinal was examined in particular, to determine whether local release of histamine in the region of the parietal cells was a prime stimulus for acid gastric secretion.

In Chapter Three the effects of histamine liberators on gastro-:intestinal tone and rhythmic contractions have been recorded. The stimulus which produces these motor effects may be release of histamine in the wall of the intestine, since histamine could be detected in increasing/

increasing amounts in the medium in which these preparations were maintained. These findings, motility change and histamine release, were examined as part of an investigation of the effect of histamine liberators on the gastro-intestinal tract. Part of the work on the intestine was done in collaboration with Dr. W. Feldberg, F.R.S., who examined the effects of histamine liberators on the cholinergic activity of the intestinal preparations.

In the early years of this century Bolton (1908) described some observations which are now regarded as classical ones of their kind. Although the experiments were never supposed to be even remotely analgous to the development of peptic ulcer in man, they demonstrated a serological technique for damaging mucosal cells and in this way predisposing to ulcer. He showed that gastric mucosa from the guinea-pig could on injection into a rabbit, cause the rabbit's own serum to be capable of producing gastric haemorrhages and erosions when re-injected into guinea-pigs. It seemed to us that cellular damage of this sort might be related to a histamine release and the effect of Bolton toxin or gastric toxin in animals pretreated with histamine liberators has been examined in Chapter Four.

In a previous chapter (Chapter Three) reasons were advanced for considering that some antihistamine agents act as histamine liberators; were this the case, the fact that they release histamine might explain why/

why they do not inhibit the effects of histamine on acid gastric secretion. The efficacy of an antihistamine in antagonising the acid secretory process might, furthermore, be dependent on how much anti-:histamine becomes absorbed into the gastrointestinal tissues. Were the antihistamine substances insufficiently absorbed into gastric tissue after injection, this might explain why they fail to antagonise not only exogenous but also endogenously releasedhistamine. An assay technique for the estimation of extracted antihistamine has been devised and the amounts of antihistamine extracted from gastric tissue has been examined in Chapter Five.

In Chapter Six tissue from human subjects has been examined to determine whether histamine is present in the gastric mucosa in higher concentration in subjects afflicted by duodenal ulcer than in patients with a low acid secretion, and how much of it may be released in both groups. The histamine concentration in the secretory portion of the stomach has been examined along the lines adopted by Feldberg and Harris, who constructed histamine profiles which showed that there was a high concentration of histamine in the region of the parietal cells in the dog's stomach.

CHAPTER ONE.

The Effect of Histamine Liberator Compound 48/80, on Acid Gastric Secretion in the Cat.

CHAPTER ONE

THE EFFECT OF HISTAMINE LIBERATOR COMPOUND 48/80, ON ACID GASTRIC SECRETION IN THE CAT.

Histamine liberators elicit acid gastric secretion. This was shown for D-tubocurarine in cats (Feldberg & Holmes, 1941) and for compound 48/80 in dogs (Paton & Schachter, 1951). The acid secretion following administration of such compounds is justifiably assumed to be due to the action of released histamine on the oxyntic cells, but the site of origin of the histamine was not determined. The source of the histamine might have been from such "distant" tissues as skin and skeletal muscle, or from local release in the gastric mucosa from the oxyntic cells themselves.

In the experiments of Feldberg & Holmes (1941) the D-tubocurarine was injected arterially distal to the origins of the coeliac and mesenteric arteries from the abdominal aorta, so that the histamine probably originated mainly from the skin and skeletal muscle of the lower half of the body. In the experiments of Paton & Schachter, compound 48/80 was injected subcutaneously and produced the known manifestations of histamine release from the skin. On the other hand, the gastric mucosa of the body of the stomach is rich in hist-:amine, particularly concentrated in the oxyntic cells (Feldberg & Harris, 1952). Release of this histamine might be expected to be associated/

associated with pronounced acid gastric secretion.

In order to elucidate the origin of the released histamine responsible for secretory activity, a comparison was made of the gastric secretion which occurs when compound 48/80 was injected intravenously, intraportally or intra-arterially into the coeliac vessel. In addition, a few experiments were performed to ascertain the appearance of histamine in the portal blood following the injection of compound 48/80 into the coeliac artery.

METHODS.

Cats, fasted for 18 hours, were anaesthetised with chloralose. A tracheotomy was performed, the oesophagus was ligated in the neck and the vagi were cut on both sides. Artificial respiration was begun at the slightest evidence of respiratory embarrassment. Blood pressure from the carotid artery was recorded throughout, dextran being used to resuscitate cats with severe hypotension in the course of these experiments.

The abdomen was opened and a tube with multiple perforations at its tip inserted into the body of the stomach in retrograde manner from the pylorus.

Intravenous injections were made via a cannula inserted into the right femoral vein. When intra-arterial injections were given into the coeliac artery, the spleen was first removed, preserving the left gastro-epiploic vessel and the vasa brevia. The branches of the coeliac/

artery were then dissected and stripped of surrounding sympathetic nerve filaments and the hepatic artery cannulated, an arterial clip being placed proximal to the site of cannulation. The intra-arterial injections were made from a syringe connected to the arterial cannula which was rigidly clamped to the table. On each injection the arterial clip was opened and blood was allowed to mix with the content of the syringe. The main stem of the coeliac artery was then occluded in such a way that the content of the syringe passed down the hepatic artery into the gastroduodenal artery and left gastric artery to the lesser curvature of the stomach and via the branches of the splenic vessel to the greater curvature. (Fig. 1). Preliminary injection experiments with India ink showed that the stomach was well perfused, except for the marginal areas of the pylorus and cardia. In all experiments as rich a blood supply to the pyloric region as possible was preserved. The anastomosis at the cardia between the portal and systemic circulation was ligated, pancreatic vessels were carefully tied and, in most cases, the animals were eviscerated for convenience below the ampulla of Vater, the common bile duct being tied at its point of entry into the duodenum. For the intraportal injections, a cannula was inserted into the stump of the superior mesenteric vein. (Fig. 2). Surplus tissue, such as the greater omentum, was carefully removed, preserving the gastro-epiploic anastomosis. The layers of the abdominal wound were then lightly apposed around the cannula and the rubber tube from which gastric juice was aspirated. The temperature

of/

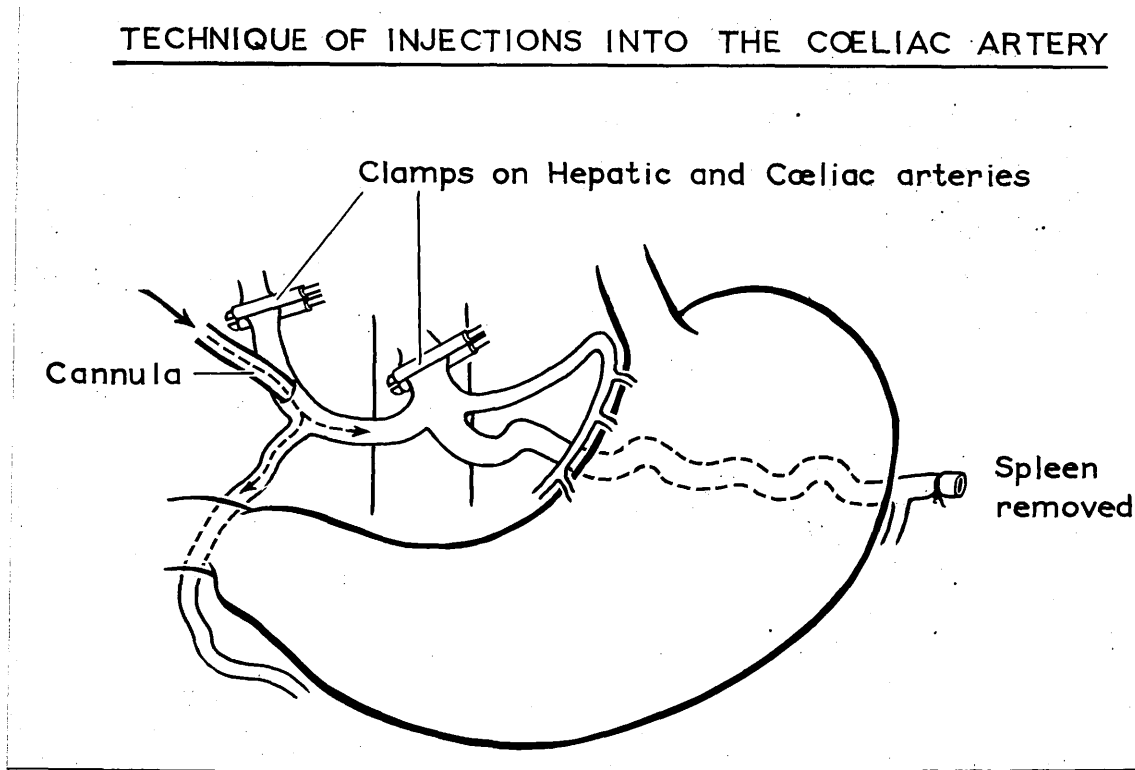

Fig. 1 illustrates the direction of injection from the cannula (occasionally a polythene catheter was used attached to a needle cannula). The injection is made retrograde from the hepatic arterial branch and circulates via the left gastric, splenic and gastroduodenal artery, when the coeliac flow recommences. The diagram shows double clamps momentarily occluding the coeliac and hepatic arteries to ensure that the injection went to the stomach alone. In between injections a tiny clip on the vessel proximal to the cannula tip prevented blood from entering it and occluding it by clot.

TECHNIQUE OF INTRAPORTAL INJECTIONS

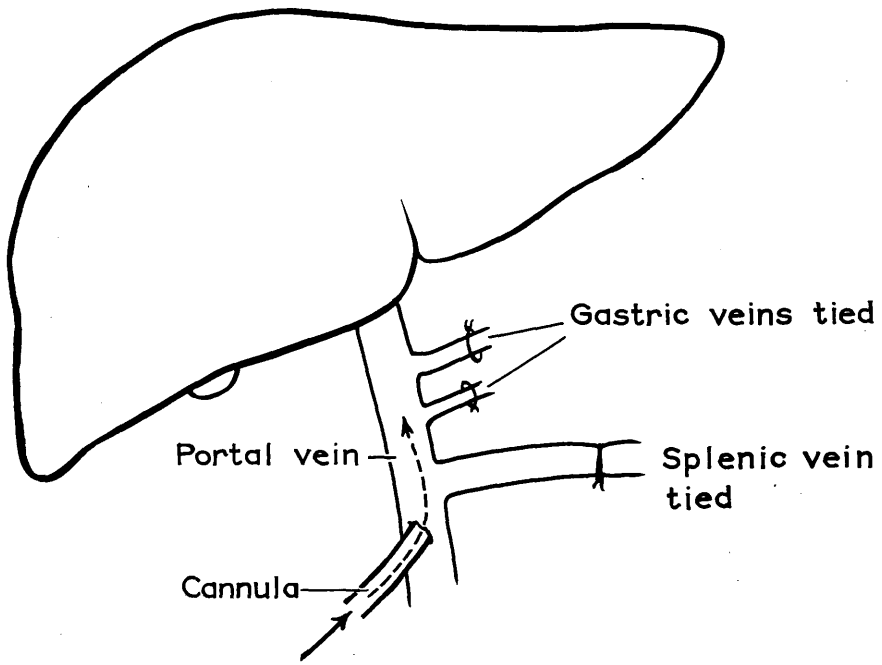

Fig. 2. Intraportal injections were given into the superior mesenteric vein; the splenic vein had been tied firmly in the removal of the spleen. The gastric veins were briefly tied by a slip-knot ligature at the time of injection.

of the animal was taken throughout the experiments by rectal and intraperitoneal thermometers and maintained at 37-38°C by the application of heat, if necessary.

The stomach was filled with 10 ml. saline at 37°C. After 14 minutes the contents were aspirated and replaced with fresh saline. This procedure was repeated every quarter of an hour. The samples obtained were titrated with N/100-NaOH, using Topfer's reagent and phenolphthalein as indicators. Values of secretion were not determined until some time after the admintration of chloralose, indeed until such time as the anaesthetic could have had little depressant activity on gastric secretion. After an appropriate interval of 1-2 hours had elapsed from the time of administration of the anaesthetic, therefore, and with the dissection now completed, the experiment was resumed, and such values as were determined for acid secretion in the first 30 minutes were taken as the basal values in excess of which the secretion elicited was estimated.

In a few experiments compound 48/80 was injected through the superior mesenteric artery into the vascular bed of the small intestine. In these experiments the left kidney was removed and the left renal artery cannulated. The injections were then made into the aorta clamped off above and below the origin of the superior mesenteric artery, while the right renal artery was momentarily occluded. (Fig. 3).

TECHNIQUE OF INJECTIONS INTO THE SUPERIOR MESENTERIC ARTERY

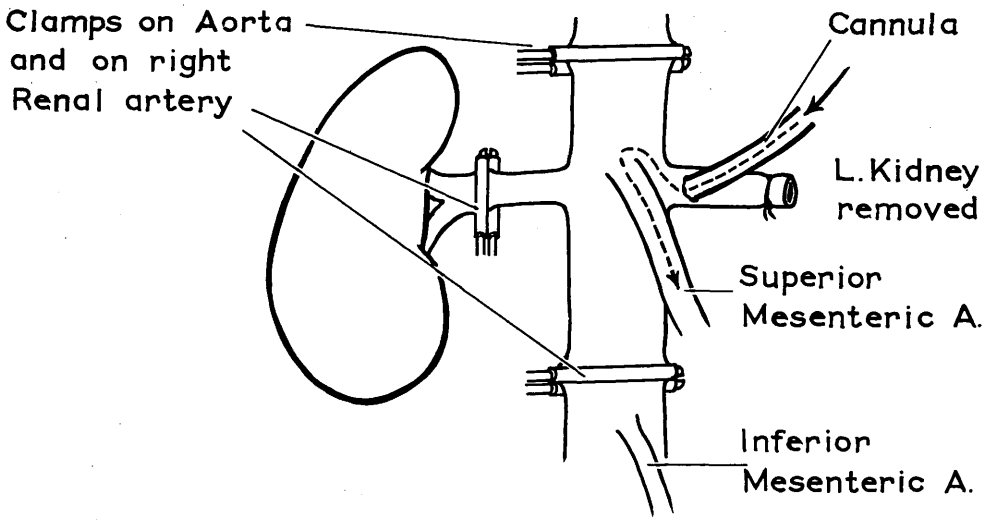

Fig. 3. Injections were made into the superior mesenteric artery via a cannula in the left renal artery (left nephrectomy had been carried out.) The aorta above and below and the right renal artery were momentarily occluded by spring clamps, as shown.

The histamine liberator was thoroughly washed into the superior mesenteric vessel by injection of saline before release of the occluding clamps. The intraportal injections, in these experiments, were given through a cannula inserted into a side branch of the portal vein.

Plasma histamine. In a few experiments portal blood was removed for histamine assay of its plasma. A 5 ml. control sample was removed and heparinized. Compound 48/80 was then injected into the coeliac artery whilst the portal vein was clamped near the liver. Blood was allowed to flow out of the congested portal system via the cannula in the superior vein and collected in 5 ml. samples until the animal died. The plasma obtained after centrifugation was examined on the guinea-pig's ileum suspended in Tyrode's solution containing atropine ($1:5x10^7$). If it caused contraction, the effect was compared with that of known amounts of histamine and shown to be abolished by small doses of mepyramine.

RESULTS.

Compound 48/80, when injected intravenously, caused acid gastric secretion of high acidity, containing practically no mucus and almost devoid of peptic power, measured by Mett's method, and thus resembled closely the juice evoked by the action of histamine on the parietal cells. The secretory effect could be obtained with 5 ug/kg and increased/

with larger doses (Table 1). With doses of 20 ug/kg and more, the secretory response showed measurable but irregular increase until, with doses in excess of 50 ug/kg, the effect lessened and could not always be followed for a sufficient period because such injections caused a profound and lasting fall of blood pressure which proved fatal in several animals within half an hour or less.

When the same dose of compound 48/80 was injected twice intravenously, the secretion following the second injection was greatly reduced (Fig. 4), a diminished response on repeated injections of compound 48/80 and other histamine liberators has been previously described (McIntosh & Paton, 1948; Paton & Schachter, 1951; Paton, 1951). In such experiments no further injection of compound 48/80 was given until acid secretion had returned to basal values.

The acid gastric secretion on intravenous injection of compound 48/80 is unlikely to be due to histamine release from the gastric mucosa, since injections into the coeliac artery were less effective than the intravenous ones. This is strikingly demonstrated in the four experiments recorded in Table 2, in which two intra-arterial injections were followed by an intravenous one. The table shows also the phenomenon observed after intravenous injection: if the same dose is injected twice, in this case by the intra-arterial route, the secretory response to the second injection is greatly reduced. In the experiment illustrated in Fig. 4, intra-arterial and intravenous injections/

TABLE 1.

EFFECT OF INTRAVENOUS INJECTIONS OF COMPOUND 48/80 ON ACID GASTRIC SECRETION.

Weight of cat in kg	ug 48/80 per kg	Total Dose ug 48/80	Acid Secretion		Maximum Secretory Rate. m.equiv. HCl/min.
			m.equiv. HCl	Duration (min)	
3.3	5	16.5	0.08	30	0.004
2.4	7	16.8	0.31	60	0.008
2.8	7	19.6	0.21	30	0.011
2.8	8	22.4	0.61	75	0.019
3.3	10	33	1.06	90	0.020
4.9	10	49	0.01	90	0.020
4.0	15	60	1.20	120	0.021
3.0	20	60	0.65 *	30	0.026
3.2	20	64	1.39	120	0.023
4.0	20	80	1.20	120	0.022
4.0	20	80	0.41 *	30	0.027

* Cat died after 30 min.

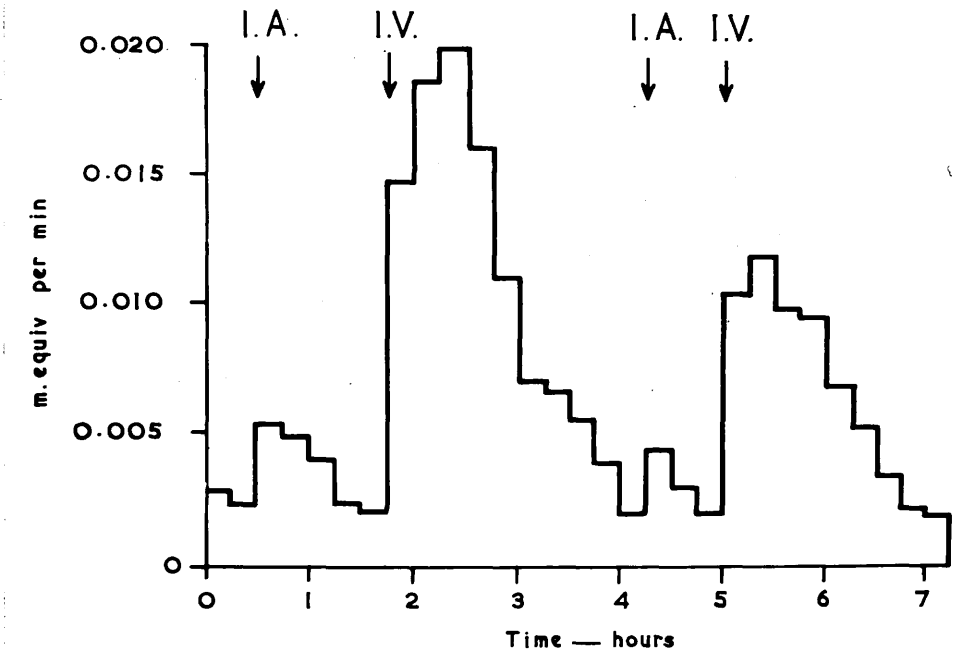

Fig. 4 Acid secretory response in 3 kg cat, following alternative intra-arterial (I.A.) and intravenous (I.V.) injections of compound 48/80; intra-arterial injections into the coeliac artery (10 ug/kg).

injections were given alternately. The results illustrate the greater efficacy of the intravenous route of administration as well as the diminution of secretion on repeated injection whether given intra-arterially or intravenously. When injections of compound 48/80 were repeated three or four times, there was a progressive reduction in secretory effect with each injection, and the dose had sometimes to be increased two or threefold in order to obtain minimal secretion.

While the secretory response rose to very high levels following increased amounts of histamine liberator given by the intravenous route, exceedingly great amounts were required to elicit secretion when compound 48/80 was injected intra-arterially into the coeliac artery. With increased dosage by the intra-arterial route, secretion could be accounted for in two distinct ways. A gradually increasing fraction of the injected histamine liberator passed from the portal into the general circulation, there to act on such distant sites as skin and skeletal muscle; there was, however, an additional contribution to the secretory response from a local action of compound 48/80 on the stomach wall, which could readily be demonstrated by restricting the circulation of the histamine liberator to the vascular bed of the stomach. In several experiments, therefore, higher doses (100-200 ug/kg) were injected into the coeliac artery and, to prevent the passage of the histamine liberator into the systemic circulation, the portal venous system/

system of the animal was obstructed at the porta hepatis at the moment of injection. The animal was then bled of 20 ml. blood from the congested portal vein via a cannula tied into the superior mesenteric vein, while slow intravenous infusion of 20 ml. dextran was given. Normal portal blood flow was then re-established. The results of five such experiments are shown in Table 3. It is unlikely that compound 48/80 escaped in effective amounts into the general circulation, as may be deduced from the following control experiment. The portal vein was punctured after the vene-section and 5 ml blood removed, heparinized, centrifuged and the plasma passed through an isolated, perfused skin preparation (Feldberg & Paton, 1951) from the thigh of the same animal. Histamine release from the preparation was negligible, indicating removal of most of the histamine liberator.

In the above experiments the secretory response was not due to an action of the histamine liberator on distant organs. In the experiments of Table 4, however, in which 20 or 40 ug/kg were injected into the coeliac artery, some of the secretory response may be accounted for in such manner, since no precautions were taken to prevent escape of histamine liberator into the general circulation. But even in these experiments part of the secretory response must be attributed to a local effect of compound 48/80 on the gastric mucosa, for the following reason: if the effects were due in the main to release from distant organs, intraportal injections should be more effective than intra-arterial, an argument valid only on the assumption that the liver, with its low histamine/

TABLE 2.

COMPARISON OF ACID GASTRIC SECRETION PROVOKED BY REPEATED INTRA-ARTERIAL AND INTRA-VENOUS INJECTIONS OF COMPOUND 48/80; INTRA-ARTERIAL INJECTIONS INTO THE COELIAC ARTERY.

Weight of cat in kg	ug 48/80 per kg	Total dose ug 48/80	Route of injection	Acid Secretion	
				m.equiv. HCl	Duration (min)
4.2	8	33.6	I.A.	0.08	30
			I.A.	0.03	30
			I.V.	0.31	60
4.9	10	49	I.A.	0.15	30
			I.A.	0.03	30
			I.V.	0.81	90
3.5	15	52.5	I.A.	0.23	30
			I.A.	0.07	30
			I.V.	0.98	90
4.0	20	80	I.A.	0.58	90
			I.A.	0.19	30
			I.V.	1.08	120

TABLE 3.

EFFECT OF INJECTION OF COMPOUND 48/80 INTO THE COELIAC ARTERY WITH SUBSEQUENT PORTAL VENESECTION.

Weight of cat in kg	ug 48/80 per kg	Total dose ug 48/80	Acid secretion	
			m.equiv. HCl	Duration (min)
3.0	100	300	0.35	60
3.1	100	310	0.32	60
2.5	200	500	0.54	75
3.2	200	640	0.57	60
4.5	200	900	1.07	75

TABLE 4.

SUCCESSIVE INTRAPORTAL AND INTRA-ATRIAL (COELIAC) INJECTIONS OF COMPOUND 48/80 COMPARED.

Weight of cat in kg.	ug 48/80 per kg	Total dose ug 48/80	Total acid secreted (mEq. HCl)				
			Intra-portal	Intra arterial	Intra portal	Intra arterial	Intra portal
3.4	20	68	-	0.53	0.31	0.21	0.20
4.2	20	84	-	0.68	0.11	0.23	0.06
3.4	40	136	0.42	0.76	0.20	0.13	-
4.0	40	160	0.60	1.53	0.44	0.29	-

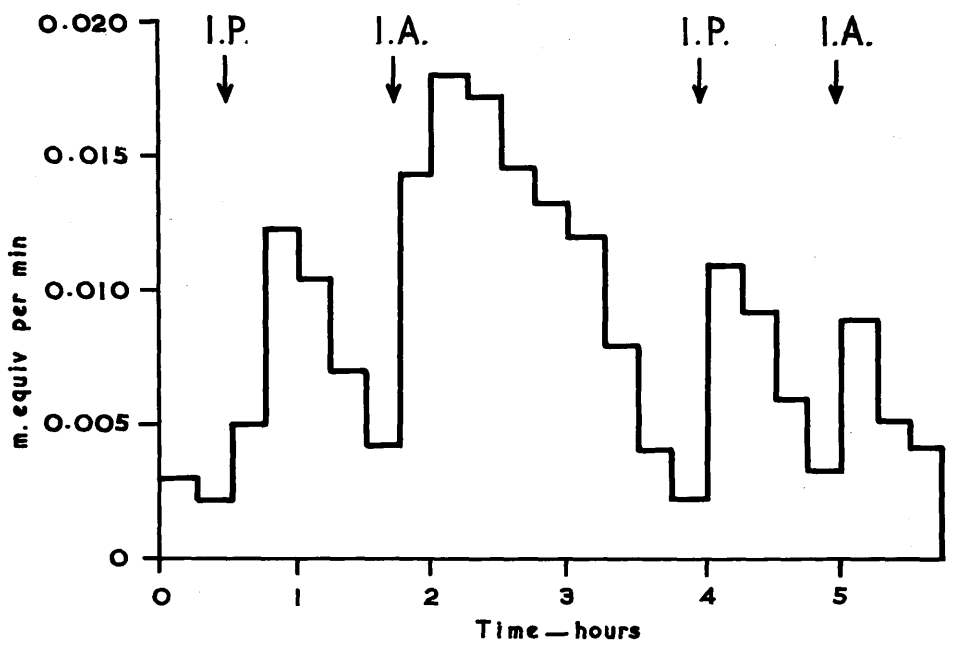

Fig. 5. Acid secretory response in 4 kg cat, following alternate intraportal (I.P.) and intraarterial (I.A.) injections of compound 48/80; intra-arterial injections into the coeliac artery (40 ug/kg).

histamine content, does not participate in the histamine release to any great extent. However, the opposite result was obtained, as seen in Table 4. In the first two experiments the initial injection was intra-arterial, in the second two intraportal, but in all four experiments of Table 4 the intra-arterial injections were more effective than in intraportal ones. The secretory effect after the intraportal injections can be assumed to result mostly from histamine release in skin and skeletal muscle, after circulation of histamine liberator through the liver, and might be compared with a greatly reduced and delayed intra-venous effect; but the greater secretory effect of the intra-arterial injections must be due to an additional action of compound 48/80 on the mucosa. Fig. 5 illustrates the last experiment of Table 4 in greater detail; the intra-arterial injection of compound 48/80 is productive of greater acid gastric secretion than the intraportal injections. The second intra-arterial injection of histamine liberator, however, provokes little acid gastric secretion, and it seems as though a small fraction only of the available hist-:amine in the stomach wall is released under the conditions of this experiment. (Table 5).

The following results obtained by examination of portal blood suggests that compound 48/80 acts by releasing histamine in small amount from the mucosa and not by a direct action on the parietal cells. The plasma histamine in the venous blood from the stomach rose/

TABLE 5.

HISTAMINE ACTIVITY OF PORTAL BLOOD AFTER INJECTION OF COMPOUND 48/80 INTO THE COELIAC ARTERY.

Weight of cat in kg	ug 48/80 per kg	Total dose ug 48/80	Initial plasma histamine (ug/ml.)	Maximum plasma histamine (ug/ml.)	Total Histamine output (ug)
2.6	20	52	-	0.01	0.01
3.4	20	68	-	0.02	0.18
3.2	25	80	<0.01	0.07	0.75
3.6	30	108	<0.01	0.16	1.02
3.3	40	132	<0.01	0.12	1.27

rose irregularly and in small amount after an injection of compound 48/80 into the coeliac artery. In Table 5 is given the maximal histamine content per ml. plasma in the four or five 5 ml. samples of portal blood removed after each injection. The total histamine output from the stomach wall could be assessed by determining the histamine equivalent of each plasma sample. By reading the volumes of each plasma sample in the graduated centrifuge tubes, the total plasma histamine of each sample could be determined. The total histamine output after injection of histamine liberator was obtained by summation of the amounts by which the total plasma histamine of each sample exceeded the values for the control sample. These results suggest that while/

while the main bulk of the histamine in the stomach is probably not released, compound 48/80 certainly gives rise to an output of hist-:amine in the venous effluent from the stomach. This histamine probably exerts its action locally, when released in the neighbourhood of the parietal cells, as well as after circulation from the portal into the systemic circulation. This is suggested by the fact that compound 48/80 on injection into the coeliac artery is more effective, but on injection into the superior mesenteric artery less effective, than intraportal injection. The mucosa of the small intestine is as rich in histamine as the gastric mucosa, and if compound 48/80 were to liberate from both mucosae equally well, or if the acid gastric secretion were due to passage of the histamine liberator from portal to systemic circulation, then both intra-arterial injections should be uniformly either more or less effective than intraportal injections. The results of Table 6 show that compound 48/80 was less effective when given by the superior mesenteric route than by the intraportal route; the enhanced secretion after intracoeliac injections may then be attributed to the local action of released histamine, in addition to the small amounts of histamine released into the portal and passing into the general circulation.

The above deductions are, however, only valid to a certain extent, because the effectiveness of a given dose of compound 48/80 in releasing mucosal histamine would be dependent on the size of the capillary bed in which it acts after injection. The superior mesenteric arterial territory/

territory constitutes a greater bulk in which the histamine liberator is distributed, with consequent diminution of concentration of histamine liberator reaching the intestinal tissues, so that the differences observed could be partly accounted for on these lines as well. Finally, it should be mentioned that it is not possible to compare quantitatively the results given in Table 4 with those of Table 6, because the former results were obtained in partly eviscerate animals.

TABLE 6.

SUCCESSIVE INTRAPORTAL AND INTRA-ARTERIAL (SUPERIOR MESENTERIC) INJECTIONS OF COMPOUND 48/80.

Weight of cat in kg	ug 48/80 per kg	Total dose ug 48/80	Total acid secreted (mEq. HCl)				
			Intra-portal	Intra-arterial	Intra-portal	Intra-arterial	Intra-portal
3.1	20	62	-	0.22	0.52	0.15	0.15
4.0	20	80	-	0.36	0.68	0.16	0.27
3.0	40	120	0.56	0.21	0.21	0.08	-
3.8	40	152	0.86	0.30	0.22	0.15	-

DISCUSSION.

While it had been shown previously that histamine liberators cause acid secretion, it had not been determined whether this secretion was elicited by histamine release locally or from distant sites. The gastric mucosa is rich in histamine, particularly in the region of the parietal cells, and it could be surmised that the release of this histamine is the necessary factor in the acid secretory response to compound 48/80.

On/

On the other hand, it is known that the histamine in skin and skeletal muscle is readily released by histamine liberators and this histamine, on entering the general circulation, might also provide an effective stimulus for the parietal cells.

Our results have shown that the strong secretory response of the parietal cells in the cat's stomach, on intravenous injection of compound 48/80, is due not to an effect of the histamine liberator on the gastric mucosa itself but on distant tissues, such as skin and skeletal muscle. The histamine released from these sites acts in turn as a stimulus to the parietal cells. This conclusion is based on comparison of the secretory response on intravenous injections and on injections into the coeliac artery, the intravenous injection being far more effective.

Nevertheless, compound 48/80 is able to act locally on the gastric mucosa, thereby producing additional acid secretion. Otherwise it would be difficult to explain the greater efficacy of the intracoeliac over the intraportal injections. It is, however, unlkiely that compound 48/80, when injected intravenously in usual dosage, reaches the gastric mucosa in a concentration sufficient to produce this local effect.

Since histamine in small amount could occasionally be detected in the portal blood after intracoeliac injection of compound 48/80, the acid gastric secretion resulting from such an injection is probably the result of local release of histamine in the gastric mucosa. In the main, however, the mucosal histamine appears to be resistant to the action

of/

of the histamine liberator, since the secretory activity signifies an amount released constituting only a very small fraction of the available histamine.

The conclusion that the histamine of the gastro-intestinal wall is less susceptible to compound 48/80 than the histamine of skin and skeletal muscle is supported by the findings of Mongar & Schild (1952) on incubation of isolated guinea-pig tissue compound 48/80. A greater fraction of the total histamine was released from skin and skeletal muscle than from stomach and intestinal tissue. Furthermore, Feldberg, Paton & Schachter (personal communication) observed that compound 48/80 released histamine irregularly and in small amount from the stomach and intestine of cats and dogs on intra-arterial injection into these organs in situ, or in isolated perfused preparations.

The reason why the histamine in the gastro-intestinal wall is less susceptible to release than that of skin and skeletal muscle is as yet unknown, nor has it as yet been determined from which layer and from which structures in the wall originates the small amount of histamine which is released by compound 48/80 and detected in the portal blood. The fact that intracoeliac injections cause acid gastric secretion suggests that some of the released histamine is derived from the mucosa.

A possible reason which may be advanced to explain this finding is that much of the histamine of the body is now known to reside in the mast cells. (Riley and West, 1953). Mast cell histamine is readily influenced by histamine liberators; using fluorescent histamine liber-:ators mast cells have been shown to lose their granules when the/

the fluorescent histamine releasing agents enter the cells, with consequent diminution in the histamine content of the tissue. Mast cells were much less frequently seen by Mota (1956) in gastrointestinal tissue than in skin and skeletal muscle; but the gastric mucosa had a higher population of these cells than any other part of the digestive tract. Mota et al. (1956) express the opinion that one part of the histamine of the gastric mucosa is of mast cell origin. If the histamine fraction in gastric tissue, readily released by compound 48/80 to provide acid secretion, is released by other stimuli, its function in gastric tissue might be to initiate the acid secretory process. This histamine fraction might be the one which primes the pump of acid secretion.

The higher resistance to release by compound 48/80 of the main bulk of the mucosal histamine, compared with that of skin and skeletal mucles, may be related to differences in the functions of histamine in these different tissues. Release of histamine in the skin probably constitutes a physiological reaction against injury, as suggested so convincingly for the human subject by Lewis (1927). While histamine could conceivably exert a similar function in the gastro-intestinal tract, it might also be related to local hormonal control of secretion and absorption, as has been suggested by different authors (Sacks, Ivy, Burgess & Vandolah, 1932; McIntosh, 1938; Emmelin & Kahlson, 1944; Douglas, Feldberg, Paton & Schachter, 1951). There is the possibility that/

that the glands of the alimentary tract eliminate the histamine from the circulation by excretion, since it is a noteworthy fact that histamine is a constituent of gastric, pancreatic and intestinal juice. Babkin (1944) believed that histamine was the final common local agent necessary for gastric secretion, regardless of the stimulus used to excite such secretory activity. He further believed that histamine was released in proximity to and passed through the parietal cell under all normal secretory circumstances. Our results are in agreement with such a view, since a small amount of histamine only could be detected in the portal blood by biological assay, in the presence, nevertheless, of strong secretory activity. The possibility exists, therefore, that the histamine is eliminated by the parietal cell, a mechanism which would serve a useful function in helping to protect the organism against the systemic effects of this agent. Emmelin (1951) has recently shown that histamine disappears more quickly from the blood on its passage through the intestine than through the lower limbs, an effect which he attributed to enzymic destruction of the histamine in the mucosa. It might be that some of the histamine was taken up by the mucosa for the purpose of later elimination in addition to destruction by histaminase. Such an activity as this would partly explain the great individual variations in histamine content of normal gastric mucosae.

SUMMARY.

1. Compound 48/80, when injected intravenously in doses of 5 ug/kg or more into cats, causes acid gastric secretion. Repeated injections lessen/

lessen the secretory response.

2. The secretory response to compound 48/80 when injected intravenously is not due to an action of the histamine liberator on the gastric mucosa, because injections into the coeliac artery are less effective than intravenous ones. It is probably accounted for entirely or almost entirely by release of histamine from distant organs such as skin and skeletal muscle.

3. The secretory response to compound 48/80 on injection into the coeliac artery, however, is apparently due to a local effect on the mucosa, since secretion elicited in this way is greater than that obtained with the same dose injected intraportally.

4. Compound 48/80 injected into the coeliac artery probably acts by release of a small fraction of the mucosal histamine, since it may lead to temporarily increased plasma histamine levels in the venous effluent. A small fraction of the histamine in gastric tissue, such as is released by histamine liberators, may be readily released to activate the acid secretory process.

CHAPTER TWO.

Release of Histamine by the Histamine Liberator Compound 48/80.

CHAPTER TWO.

RELEASE OF HISTAMINE BY THE HISTAMINE LIBERATOR COMPOUND 48/80.

Using acid secretion in cats as a measure of the extent of histamine release by compound 48/80, one must conclude that only a part of the histamine of the alimentary tract can be released by this synthetic histamine-releasing agent. In this chapter this opinion is confirmed by measurements of the effect of compound 48/80 on the histamine content of the alimentary tract and other viscera, skin and skeletal muscle of the cat are now described. It was found that the histamine of the skin was most susceptible to the action of compound 48/80; reductions were also observed in skeletal muscle, lungs and in the gastro-intestinal tract, where they occurred principally in the corpus region of the stomach. Control experiments were made in which histamine was administered in such a way as to produce prolonged effects, because intense secretory activity, consequent on the entry of large amounts of histamine into the general circulation, might have been responsible for the diminution in histamine content of the stomach associated with prolonged treatment with compound 48/80.

METHODS.

Cats, 2-4 kg in weight, were used throughout and, unless otherwise stated/

stated, were anaesthetized with chloralose after ethyl chloride and ether induction, followed by tracheotomy. In all experiments involving estimation of acid secretory activity, the right carotid artery was cannulated for the recording of blood pressure; dextran was used to resuscitate animals with profound hypotention.

Intravenous injections were made through a cannula inserted into the right femoral vein, intravenous infusions being administered by attaching this to a burette with a limited air intake at its upper end (devised by means of inserting capillary glass tubing through a rubber bung) so that a constant rate of infusion could be guaranteed. Intra-arterial injections into the coeliac artery were made via a cannula inserted into the hepatic artery and guarded by an adaptor with a two-way stop-cock fitting. Intra-arterial injections into the superior mesenteric and femoral arteries were made by direct puncture of suitable side branches.

Blood samples were obtained as follows: arterial samples were received into a test-tube directly from the severed right femoral artery, heparinized with 0.02 ml. heparin per ml. blood and centri-:fuged at 2000 rev/min for 5 min. Venous blood was obtained by puncture of the external jugular veins and treated likewise.

Several tissues were examined for this histamine content. Duodenal tissue was excised just proximal to the ampulla of Vater, ileum was taken 5 cm from the ileocaecal valve, and colonic tissue at the mid part of the large bowel. The stomach, secured at cardia and pylorus with ligatures, was removed, washed and examined, if

if necessary with binocular lenses. Corpus and pyloric regions were pinned out on a cork board and the muscularis externa dissected free with Mayo scissors. The mucosa, with submucosa adherent on the outer surface, was pinned so that the musocal aspect faced upwards, and removed with a blunt knife from the whitish layer of the submucosa.

Tissue samples were then weighed and ground in a mortar in N or N/3-HCl (2 ml./g) with sand and distilled water (10 ml./g.). The contents of the mortar were washed with saline into a 25 ml. flask and boiled gently for 1 min; B.D.H. indicator was added and the solution neutralized with N or N/10-NaOH and diluted to known concen-:trations which were assayed on the atropinized guinea-pig ileum preparation. (Fig. 1 *). All histamine values refer to the base. When plasma samples were assayed on the guinea-pig ileum, they occasionally produced a slow contraction which made a precise histamine assay difficult. This contraction was not abolished by mepyramine maleate; these samples were assayed on the arterial blood pressure of the eviscerate cat.

Tissue affected by the pathological change was retained in formalin. At a later date wax sections were made and examined under the microscope, after routine staining with haematoxylin and eosin.

Compound 48/80 was injected intraperitoneally dissolved in saline. Histamine was administered as histamine acid phosphate (dosage calcul-:ated as base); in five animals it was administered dissolved in beeswax into/

* Appendix.

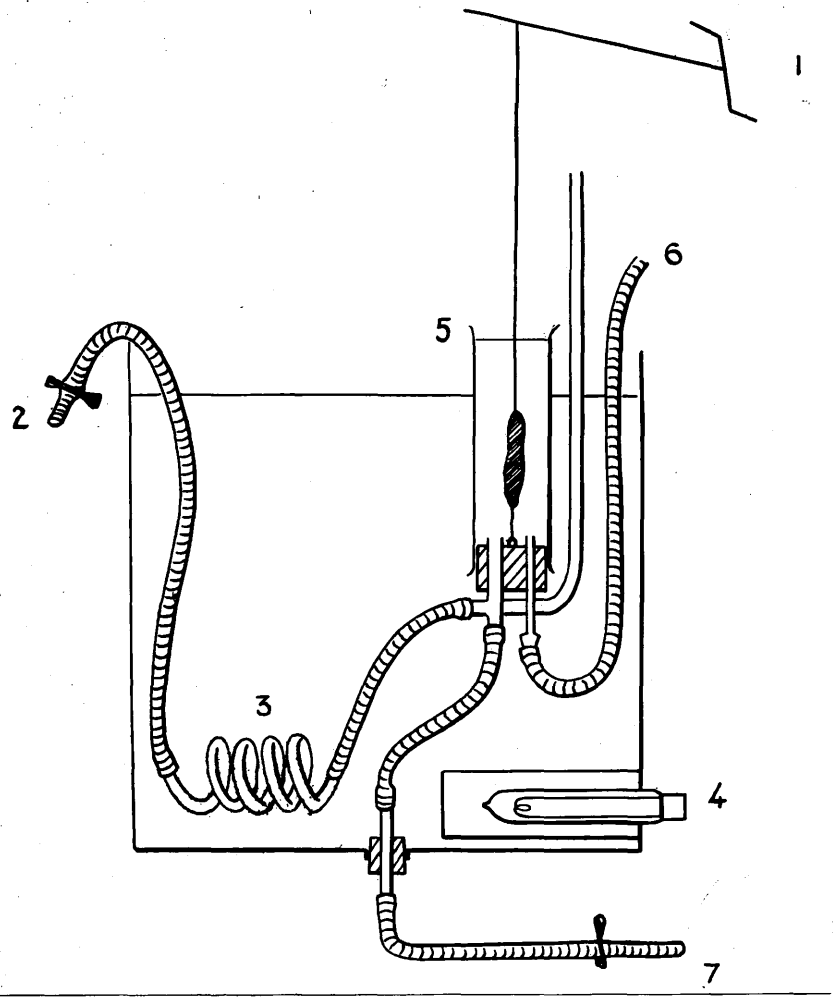

Fig 1 illustrates the outline of the method for biological assay of histamine. The guinea-pig ileum is suspended in Tyrode solution to which the drug is added. The smooth muscle of the intestinal strip contracts and activates a lever carrying a writing point, which records the contraction as a vertical stroke on smoked drum.

1 - frontal writing lever: 2,3,7 - inlet, coil, and outlet for Tyrode solution: 4 - heater: 5 - 18 ml. bath: 6 - O_2 and CO_2 supply. The guinea-pig's ileum is attached by means of an anchoring thread to the bath and pulls on the frontal writing lever.

into the dorsal musculature of the cat, as described by Code & Varco (1942) for dogs. The beeswax was purer than most samples, with too high a melting point, and was accordingly adulterated with yellow soft paraffin which greatly facilitated administration. Daily injections of 20-40 mg were intended to be given, but a fraction of the amount to be injected remained in the syringe, because of the viscous nature of the beeswax. The amounts intended to be injected daily during the first 7-10 days were 20 mg, during the following 5-7 days 30 mg, and during the last 5-10 days 40 mg. If the histamine had been complete injected each time, it would have amounted to a total of between 400 and 700 mg. the actual total amounts injected, however, were between 350 and 450 mg.

RESULTS.

Histamine Content of Cats' Tissues.

Since the histamine content of the tissues varies in individual cats, it was necessary to obtain a sufficient number of values for the normal histamine content in order to have a reliable basis for comparison with the histamine values obtained after treatment with compound 48/80. In Table 1 values are given for various tissues of ten normal cats. The two areas of skin examined in all the animals were the fine skin of the ear and the skin of the abdominal wall, these areas having been chosen because Feldberg & Miles (1953) had found that there are great regional variations in the content of the skin and that the highest/

TABLE 1.

TABLE 1. Histamine equivalent, in $\mu g/g$ tissue, of various organs of ten normal cats

Skin				Skeletal muscle		Stomach						Intestinal wall								
						Corpus			Pylorus											
Ear	Eyelid	Nasal area	Abdomen	Diaphragm	Gastrocnemius	m.	s.m.	m.e.	m.	s.m.	m.e.	Duodenum	Jejunum	Ileum	Colon	Liver	Pancreas	Spleen	Lung	Kidney
92	—	—	22	2·1	1·7	29	17	10	22	17	6	50	—	24	—	3·1	—	—	—	—
80	—	—	27	2·0	2·5	27	14	9	19	12	6	46	—	20	—	5·0	—	—	—	—
81	—	—	23	2·7	2·0	11	8	5	7	6	4	47	—	14	—	1·2	—	—	—	—
80	—	—	18	1·9	1·4	20	12	9	19	12	6	32	—	24	—	1·3	—	—	—	—
71	—	—	20	4·0	2·8	34	26	14	32	27	10	40	—	19	—	3·4	—	—	—	—
96	32	52	27	2·8	2·0	27	23	16	24	22	13	29	24	20	8	1·4	3·1	5·3	35	1·4
106	38	40	24	3·0	2·4	25	20	11	16	12	12	38	19	17	9	2·0	1·2	4·8	43	0·9
112	38	39	20	2·9	2·1	37	25	11	32	21	15	41	38	27	16	6·0	4·5	14·0	35	1·1
105	36	36	22	2·7	1·9	37	20	14	27	22	11	36	30	22	10	2·0	1·8	6·4	15	0·8
136	40	46	19	2·1	1·8	40	21	19	29	23	13	42	32	32	10	3·4	5·8	10·0	48	2·0

m. = mucosa; s.m. = submucosa plus muscularis mucosae; m.e. = muscularis externa.

highest values were obtained from the ear, whereas the abdominal skin represented a region of relatively low histamine content. The skin of the eyelids and nasal area adjacent to the bristles gave values intermediate between those recorded for the skin of the ear and abdomen. Of the viscera, the duodenum and lung contained the highest amounts of histamine. The average values were 40 and 35 ug/g respectively; these values, however, were lower than those for the skin of the ear (96 ug/g). The values for the stomach, ileum and colon lay between 29 and 11 ug/g and were of about the same order as those of the abdominal skin. All other tissues examined yielded low histamine values, the average value for the spleen was about 8 ug/g, whereas the values for skeletal muscle, liver, pancreas and kidney varied between 3.5 and 1.2 ug/g.

HISTAMINE CONTENTS OF CATS' TISSUES AFTER INJECTION OF HISTAMINE LIBERATOR.

i) Effects of intravenous injections of compound 48/80.

A single intravenous injection of 10 or 20 ug/kg compound 48/80 produced little detectable change in the histamine content of the organs, except perhaps in the skin of the ear and nasal area where 20 ug/kg produced a slight reduction. With 50 ug/kg there was a definite reduction in the histamine content of the skin; in the fine skin of the ear and eyelid it amounted to almost 40%, in the nasal area/

area to about 30%, and in the abdominal skin to about 20%, but the histamine of skeletal muscle and of the wall of the digestive tract remained relatively unaffected (Table 2).

The injections caused a transient increase in the histamine of the plasma, maintained longer with 50 ug/kg than with lesser dosage. This increase subsided within 45 min and was thereafter succeeded by subnormal levels in several experiments (Table 2).

ii) Effects of injections of compound 48/80 into the coeliac and into the mesenteric superior artery.

Injections of compound 48/80 into the coeliac artery result in acid secretion which is probably due partly to local release of histamine but mainly to the action of the histamine liberator on the histamine of skin and skeletal muscle. Animals killed after intracoeliac injections of 20-50 ug/kg compound 48/80 showed little detectable change in the histamine content of the stomach wall, skin and skeletal muscle. In animals killed after 100 ug/kg there was a definite change in the distribution of histamine on the stomach mucosa, as well as lowering of the histamine content of skin and skeletal muscle. Normally the histamine content per gramme mucosa of the corpus is greater than of the pyloric region (Table 1). After 100 ug/kg compound 48/80 the reverse was found, the change being accounted for by a reduction in the histamine of the corpus mucosa (Tables 3 and 6). The histamine content of skin of the ear was reduced by about 28%, of the skin of the abdomen by about 27%, and that of skeletal muscle by about 6% (Table 3).

TABLE 2.

TABLE 2. The effect of intravenous injections of compound 48/80 on the histamine content (μg/g) of the tissues and the plasma of cats. Samples obtained from tissues 4–6 hr after injection

Wt. of cat (kg)	48/80 (μg/kg)	Skin				Skeletal muscle (gastrocnemius)	Stomach						Duodenum	Ileum	Colon	Plasma histamine (μg/ml.) before and at intervals after 48/80			
							Corpus			Pylorus									
		Ear	Eyelid	Nasal area	Abdomen		m.	s.m.	m.e.	m.	s.m.	m.e.				Before	10 min	30 min	45 min
3·0	10	96	—	—	24	3·0	28	23	11	25	18	9	34	—	10	0·04	0·04	0·03	0·02
3·3	10	102	—	—	20	2·5	22	15	11	16	11	6	32	—	8	0·01	0·01	0·01	0·01
4·9	10	98	40	39	26	3·1	29	19	10	18	12	7	28	14	8	0·01	0·02	0·02	0·01
3·2	20	86	36	32	20	2·6	30	24	10	18	10	7	44	22	12	0·04	0·07	0·04	0·01
4·0	20	90	40	36	20	2·6	24	18	11	16	8	8	42	20	13	0·01	0·08	0·06	0·02
3·6	50	54	20	30	18	2·2	32	26	15	28	14	10	48	24	9	0·005	0·20	0·16	0·02
3·8	50	60	28	30	17	2·3	37	19	13	22	17	12	40	22	11	0·03	0·26	0·12	0·02

m. = mucosa; s.m. = submucosa plus muscularis mucosae; m.e. = muscularis externa.

TABLE 3.

TABLE 3. The effect of intra-arterial injections of compound 48/80 on the histamine content (μg/g) of the tissues and of the plasma; route of administration (A) into the coeliac artery, (B) into the superior mesenteric artery. Samples obtained from tissues 4–6 hr after injection

	Wt. of cat (kg)	48/80 (μg/kg)	Skin		Skeletal muscle (gastrocnemius)	Stomach						Duodenum	Jejunum	Ileum	Plasma histamine (μg/ml.) before and at intervals after 48/80			
						Corpus			Pylorus									
			Ear	Abdomen		m.	s.m.	m.e.	m.	s.m.	m.e.				Before	10 min	30 min	45 min
(A)																		
	3·1	20	108	19	2·0	36	29	20	23	19	15	48	—	21	0·02	0·02	0·01	0·01
	2·8	50	126	20	2·2	27	17	15	20	16	12	52	—	27	0·04	0·06	0·04	0·02
	3·3	50	102	17	2·1	25	14	10	14	9	7	38	—	26	0·01	0·09	0·01	0·01
	3·2	100	92	16	2·0	19	17	12	20	16	14	43	—	22	0·02	0·10	0·04	0·005
	4·0	100	60	15	2·0	22	12	9	28	16	14	46	—	24	0·05	0·18	0·05	0·005
	3·6	100	54	16	1·9	20	11	9	28	21	10	38	—	19	0·01	0·18	0·05	0·01
(B)																		
	3·0	20	104	19	2·1	32	21	12	21	16	10	41	31	16	0·01	0·04	0·04	0·005
	4·1	50	96	20	1·9	27	18	10	21	13	9	37	26	19	0·02	0·08	0·02	0·005
	4·0	100	86	18	1·9	28	16	12	20	12	7	49	30	23	0·005	0·08	0·01	0·005

m. = mucosa; s.m. = submucosa plus muscularis mucosae; m.e. = muscularis externa.

Injections of 20 and 50 ug/kg into the superior mesenteric artery produced no detectable change in the histamine content of the intestinal tissues (Table 3); 100 ug/kg produced slight reduction of histamine content of skin and skeletal muscle, but no change in the gastro-intestinal tissues.

The plasma histamine remained practically unaltered when 20 ug/kg compound 48/80 were injected into the coeliac artery; sometimes there was a fall. With 50 ug/kg there was a definite rise, following after 10 min by a fall below the initial values. With 100 ug/kg the rise lasted for about 30 min. The injections into the mesenteric superior artery also increased the histamine plasma level, but the rise was smaller than after the intracoeliac injections.

iii) Effects of intraperitoneal injections of compound 48/80.

Initial injection of 1 mg/kg compound 48/80 produced signs of pruritus, salivation, lachrymation and cyanosis of ears and nasal area, soon followed by a state of prostration accompanied by tachy-:pnoea, vomiting, precipitate micturition and defaecation. Recovery was usual in 1-2 hr and as the circulation improved, the nasal area and ears, formerly cold and cyanotic, became red and warm; the facial appearance was then distorted by oedema around the nose and eyelids. In many instances there was bristling of the short hairs of the head, which gave the coat a round appearance. On subsequent injection the following day, there was facial swelling, occasional oedema/

oedema of the nipple and genital area, erythema of the nasal area, lachrymation, salivation and pruritus; prostration was absent or slight. When the same dose was injected on the third or fourth day, the reactions were minimal or entirely absent. The daily injections were then increased by 0.5 mg/kg increments every second or third day until a total of over 30 mg/kg had been given intraperitoneally over two or three weeks.

Post-mortem findings. Five cats were examined post-mortem. Obvious gastric lesions were recognised in three cats by subserous haemorrhages slightly above the incisura on the lesser curvature. These coincided with frank gastric ulceration of the acute penetrating type on the mucosal aspect (Fig. 2). The rougae of the corpus mucosa were swollen and pinker in appearance than normal, so that the contrast with the pallid appearance of the pyloric region was exaggerated. Erosions, both healed and unhealed, were often present, and the mucosa surrounding such lesions was 'peppered' with haemorrhages of pin-point size.

Microscopic section was made through the markedly ulcerated mucosa of one animal(Fig. 3). There was sloughing of the mucosa and penetration by the ulcerative process to the submucosa; in this area high-power magnification showed some infiltration by lymphocytes and plasma cells with little evidence of fibrous tissue reaction.

In two cats the duodenum was the site of erosion, in the region proximal to the ampulla of Vater. Microscopic examination of the erosions showed superficial loss of epithelium with lymphocytic infiltration in the deeper mucosal and submucosal layers.

The/

The chyme of the small intestine was heavily stained with bile as far as or beyond the ileocaecal junction. In this region two animals showed vascular injection of both the muscular and mucosal layers, and the ileocaecal lymph nodes draining the affected segment were noticeably haemorrhagic. Microscopic section of these lymph nodes showed extravasation of blood in the subcapsular area of the gland. In three animals the ileocaecal region and proximal colon were slightly more vascular than normal, with shredding of the engorged mucosa and resulting localized haemorrhage. Microscopic examination showed penetration of the ulcerative process of the submucosal region, where the tissues were heavily invaded by round cells such as lympho-:cytes and plasma cells.

The gall-bladder wall was engorged and oedematous in three animals, but the mucosal lining was everywhere intact. The pancreas in one animal was thickened, vascular and oedematous, and it was thought that evacuation of the gall-bladder, repeated and with maximal contraction, might have forced entry of bile into the pancreatic duct and set up a subacute pancreatitis. Microscopic examination showed oedematous pancreatic tissue with patchy necrosis, infiltrated by lymphocytes. In another animal the liver was the site of subcapsular haemorrhage. Microscopic examination showed extravasation of blood at this site and distension of the central part of the lobule with blood.

In one animal the pericardial sac was the site of moderate effusion, and/

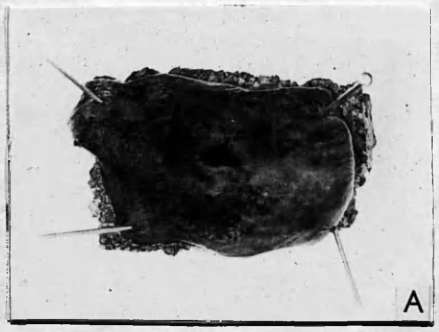

Fig. 2 shows the mucosal aspect of the stomach of a cat treated with compound 48/80, up to 4.5 mg/kg dosage. There is a gastric ulcer, with surrounding diffuse engorgement of the stomach.

Fig. 3 is a photomicrograph of a section through the edge of the gastric ulcer seen in Fig. 2. Ulceration penetrates to the muscularis mucosae; necrotic epithelium is seen near the periphery, beyond which there is transition to relatively normal mucosa.

and the region of the laryngeal folds was swollen by oedematous sacs which almost occluded the aperture of the airway from larynx to trachea. There were no subendocardial haemorrhages. No obvious macroscopic lesions were seen in the kidney and pituitary but the adrenals, macroscopically haemorrhagic showed histologically an increased vascularity, haemorrhage and round cell infiltration.

Histamine content of tissues. The histamine content of various tissues from ten cats with repeated intraperitoneal injections of compound 48/80 over two or three weeks is recorded in Table 4, and in Table 5 the mean values are compared with those of the ten normal cats given in Table 1. General reduction in the histamine content of skin was greatest in the ear; the histamine content had dropped from 96 to 12 ug/g skin: i.e. by 87%. The mean histamine content of the abdominal skin had dropped from 22 to 5 ug/g: i.e. by 77%. In skeletal muscle the histamine was also reduced but the effect was not so striking and amounted to a reduction of 27% for diaphragm and 29% for gastrocnemius. In the stomach wall there was a definite reduction of as much as 37% in the histamine content of the various layers of the corpus region; if there was any change in the pyloric region there was a slight increase of not more than 6% which, considering the great individual variations, may not be significant. The fact that compound 48/80 reduced the histamine content in the wall of the corpus but not of/

TABLE 4.

TABLE 4. Histamine equivalent of tissues in $\mu g/g$, of ten cats, after treatment with intraperitoneal injections of compound 48/80 for 2–3 weeks

Skin		Skeletal muscle		Stomach						Intestinal wall							
				Corpus			Pylorus										
Ear	Abdomen	Diaphragm	Gastro-cnemius	m.	s.m.	m.e.	m.	s.m.	m.e.	Duodenum	Ileum	Colon	Liver	Pancreas	Spleen	Lung	Kidney
15	3	1·8	1·1	14	10	6	15	12	5	33	20	—	1·5	4·2	11	23	1·1
15	2	2·0	1·0	17	12	8	17	13	8	36	14	—	3·2	2·9	6	18	1·9
13	8	3·7	1·3	19	9	7	17	12	6	41	18	—	2·5	1·9	13	28	1·7
8	3	1·3	1·8	23	15	13	24	18	8	33	25	11	2·1	1·7	6	16	1·4
16	9	1·7	2·1	30	20	16	35	30	13	48	13	10	2·0	2·0	8	25	1·3
10	2	1·5	2·1	20	13	11	32	25	16	39	15	12	1·5	—	—	—	—
18	4	1·7	1·3	22	13	10	23	17	9	33	26	13	3·3	—	—	—	—
9	4	1·8	1·6	19	10	8	21	15	8	37	20	11	4·1	—	—	—	—
8	4	1·8	1·4	18	11	14	26	17	12	30	23	—	3·3	—	—	—	—
10	6	2·0	1·6	16	10	13	21	16	10	21	15	—	3·2	—	—	—	—

m. = mucosa; s.m. = submucosa plus muscularis mucosae; m.e. = muscularis externa.

of the pyloric region accounts for a change in the relative distribution of histamine in the stomach wall. Normally the mucosa of the corpus is richer in histamine than that of the pyloric region, but after prolonged intraperitoneal treatment with compound 48/80 the reverse is true. This is shown in more detail in Table 6. The average ratio between the histamine content in corpus mucosa and in mucosa of the pyloric region was 1.3; after compound 48/80 it was 0.87. A similar reversal of this ratio was found after the injection of single large doses of compound 48/80 into the coeliac artery, but not after a single intravenous injection.

The changes in the histamine distribution of the stomach mucosa occurred also in the layer which consists of submucosa plus muscularis mucosae but not in the muscularis externa (see Table 7).

Effects of massive doses of histamine, as a control.

Such changes in the distribution of histamine in the wall of the gastro-intestinal tract as were produced by prolonged treatment with intraperitoneal compound 48/80 could not be reproduced fully by massive amounts of histamine given in beeswax. Histamine itself produced characteristic changes in the histamine content and distribution of the histamine in the stomach wall:-

The daily injection of 20-40 mg histamine in beeswax to a total dosage of 350-450 mg led at first to few detectable signs, but towards the/

TABLE 5.

Changes in histamine content of the tissues in cats after repeated intraperitoneal injections of compound 48/80 and with massive doses of histamine in beeswax. (The normal values are the mean values of the ten cats listed in Table 1, the values after compound 48/80 are the mean values of those cats listed in Table 4, and the values after histamine in beeswax are the mean values of the cats listed in Table 8).

	ug Histamine/g tissue			Histamine content expressed as percentage of normal.	
	Normal	48/80 treated	Histamine treated	(a) 48/80-Treated	(b) Histamine treated
Skin					
Ear	96	12	107	13	111
Abdomen	22	5	23	23	105
Skeletal muscle					
Diaphragm	2.6	1.9	3.3	73	127
Gastrocnemius	2.1	1.5	2.8	71	133
Stomach - corpus region					
Mucosa	29	20	25	69	86
Submucosa	19	12	20	63	105
Muscularis externa	12	11	11	92	92
Stomach - pyloric region					
Mucosa	23	23	30	100	130
Submucosa	17	18	36	106	212
Muscularis externa	10	10	11	100	110
Duodenum	40	35	45	88	113
Ileum	22	19	21	86	95
Colon	11	11	12	100	109
Pancreas	2.9	2.7	2.7	93	93
Spleen	8.1	9.0	8.0	111	99
Lung	35	22	41	63	117
Kidney	1.2	1.5	0.9	125	75

TABLE 6.

Ratio of histamine equivalent of mucosa of the corpus to histamine equivalent of mucosa of pyloric region of the stomach of cats.

- - - - -

Corpus/pylorus

Normal (from Table 1)	After intraperitoneal compound 48/80 (from Table 4)	After intravenous compound 48/80 (from Table 2)	After intra-arterial injection of 100 ug 48/80 into the coeliac artery (from Table 3)	After histamine in beeswax (from Table 8)
1.32	0.93	1.12	0.95	0.76
1.42	0.00	1.38	0.79	0.28
1.57	1.12	1.61	0.71	0.63
0.05	0.96	0.67		0.89
1.06	0.86	1.50		0.88
1.13	0.63	1.14		
1.56	0.96	1.68		
1.16	0.90			
1.37	0.69			
1.38	0.76			

TABLE 7.

Ratio of average histamine equivalents of the corpus to the pyloric values from Tables 1, 4 and 8 for the stomachs of cats.

- - - -

		Corpus/Pylorus	
	Normal	After intraperitoneal compound 48/80	After histamine in beeswax
Mucosa	1.30	0.87	0.83
Submucosa + muscularis mucosae	1.12	0.67	0.56
Muscularis externa	1.20	1.10	1.00

the end of the course of injections the animals became noticeably docile, lost weight and showed marked vasodilatation of the nasal area, and were eventually prostrated , at which stage they were killed by bleeding under anaesthesia.

Histamine content. Table 8 lists the values for the histamine content of tissues from five cats killed after massive histamine administrations. The mean values are compared with those of the normal cats in Table 5. The histamine content of skin, lung, and particularly of skeletal muscle was increased, whereas that of kidney, pancreas and liver was reduced. In the wall of the gastro-intestinal tract, the main change was found in the pyloric region of the stomach where the histamine content of all layers was increased, but the effect was most pronounced in the submucosa where the value was more than doubled; in the mucosa the increase amounted to over 30%. In the corpus region, on the other hand, the histamine content remained either unchanged or decreased slightly. The ratio of the histamine of the mucosa of the corpus to that of the pyloric region is shown in Table 6, and the ratio of the histamine of the submucosa plus muscularis mucosae and muscularis externa of the same regions in Table 7. Like that after compound 48/80, the ratio has fallen in the mucosa and submucosa, but in this case the change is brought about not so much by a decrease in the histamine content of the corpus segment as by an increase in that of the pyloric region. This is particularly striking for the submucosa; the values for the muscularis/

TABLE 8

Histamine equivalent in ug/g tissue, of various organs of five cats, after treatment with massive doses of histamine in beeswax.

- - - -

Skin		Skeletal muscle		Stomach													
				Corpus			Pylorus										
Ear	Abdomen	Diaphragm	Gastro-enemius	m.	s.m.	m.e.	m.	s.m.	m.e.	Duodenum	Ileum	Colon	Liver	Pancreas	Spleen	Lung	Kidney
107	23	2.8	2.4	22	19	7	29	36	7	29	14	7	2.2	2.1	3.4	25	0.7
80	20	2.6	2.0	23	17	12	18	31	10	41	18	11	1.8	3.7	6.8	39	1.1
112	25	4.0	3.1	27	17	11	43	40	12	45	31	14	3.8	5.0	9.6	56	1.0
96	20	3.2	2.8	25	24	14	28	34	13	55	22	12	2.8	1.9	10.4	42	1.2
138	27	3.9	3.7	30	22	12	34	40	12	56	18	18	3.0	2.8	9.8	44	0.7

m.=mucosa; s.m.=submucosa plus muscularis mucosae;

m.e.= muscularis externa.

muscularis externa are unchanged.

Plasma histamine. Before the animals treated with massive doses of histamine in beeswax were killed, 5 ml. samples of blood were collected in heparin from the carotid artery and their plasma assayed for histamine; it was found to be unchanged.

In normal cats an intravenous infusion of histamine causes a transient increase in plasma histamine whereas the intravenous infusion of histamine in the cats treated with histamine in beeswax caused a similar, if somewhat less pronounced elevation of the plasma histamine, as seen from the following results:-

In the normal cat the histamine level of the plasma lay between 0.005 and 0.05 ug/ml. (mean, 0.024). When assayed during a histamine infusion (20 ug/kg/min for 20 min), the values rose to between 0.06 and 0.14 ug/ml. (mean 0.1) and remained at this level after cessation of the infusion for 10-15 min; in many instances thereafter the plasma histamine fell quickly to values below the initial levels. In five cats which had been treated with massive doses of histamine in beeswax histamine was infused. The plasma histamine level rose from between 0.01 and 0.03 ug/ml. (mean 0.02) to between 0.06 and 0.09 ug/ml. (mean 0.08), i.e. to a somewhat lower level than that observed in the normal animals on histamine infusion.

DISCUSSION.

In the previous experiments on the acid secretion of the stomach produced by Compound 48/80, the conclusion was drawn that the secretion was the effect/

effect of circulating histamine released by Compound 48/80 from skin and muscle, and now borne out by the present results, concerning changes in histamine content of various tissues produced by compound 48/80. It was further concluded, from the enhanced secretory response on injections of the histamine liberator into the coeliac artery compared with similar injections into the portal vein, that a small fraction only of the histamine of the gastric mucosa could be released and participate in the gastric secretory response. Again, the present finding that the mucosal histamine of the corpus of the stomach can be reduced by Compound 48/80 is in accord with this conclusion.

The results obtained with Compound 48/80 in cats show that the histamine in the various tissues is susceptible to the effect of this histamine liberator to different degrees. The susceptibility of the histamine in the skin is greater than that of any other tissue examined. As far as the alimentary tract is concerned the histamine in the mucosa of the corpus of the stomach is more readily released by Compound 48/80 than that in any other area of the digestive tract. We do not know why the histamine in the various tissues shows this difference in susceptibility to Compound 48/80, but it is interesting to note that Mongar & Schild (1952), studying the effect of histamine liberators in in vitro experiments on guinea-pig tissue, came, in general, to similar conclusions. Further, Feldberg & Talesnik (1953), using the same method in rats as has been used in the present experiments in cats, also found that the histamine of/

of skin and skeletal muscle was the most susceptible to the Compound 48/80 releasing action. It is certain that the histamine level of a given organ is not the determining factor for susceptibility to Compound 48/80. This is evident when the effect of Compound 48/80 is compared on the histamine of the skin, skeletal muscle and pyloric region of the stomach.

Reasons have been advanced in Chapter one for considering that the labile histamine of skin and skeletal muscle which is affected by histamine liberators is mainly the mast cell histamine. Compound 48/80 has the ability to release the histamine in gastric mucosa but not at other sites in the gastro-intestinal tract. The gastric histamine may therefore be partly of mast cell origin. Mota (1956) has claimed that Compound 48/80 acts mainly through its effects on mast cells and histamine. Mota, Ferri and Yoneda (1955), studying the distribution of mast cells in the gastro-intestinal mucosa, found a higher mast cell population in the mucosa of the body of the stomach than in any other part of the digestive tract. (Fig. 4.)

The effects of intraperitoneal injections of Compound 48/80 in cats can almost all be explained by actions of the released histamine. The refractoriness which develops on repeated daily injections is explained by lack of sufficient histamine readily available for release in/

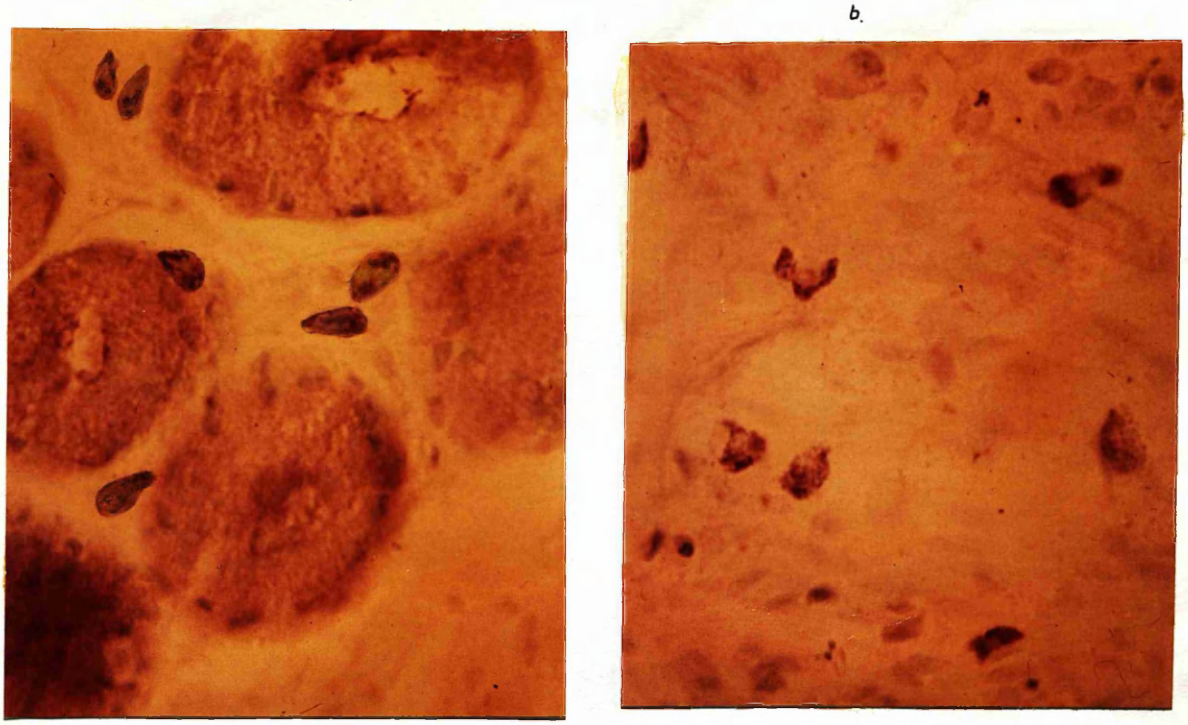

Fig. 4. is a colour photograph of a section of the body of the stomach stained with methylene blue showing numerous mast cells adjacent to the glandular structures in the stomach wall.

in the tissues. One must distinguish between effects of Compound 48/80 produced locally at the site of liberation and those produced by the histamine escaping into the general circulation; the pruritus, the facial swelling, the occasional oedema of the nipple and genital area, and perhaps also the erythema of the nasal area, are probably effects of histamine released in the skin acting at the site of liberation. It is interesting that the skin regions noticeably affected are those yielding, not only in cats but also in other species examined (Feldberg & Miles, 1953), relatively high histamine values on extraction. The fact that characteristic distribution of oedema was often to be seen as much as 1-2 hr after the injection, when the circulation improves, is understandable because, so long as the blood pressure is low after release of histamine, the pressure in the capillaries would be sufficient to cause fluid exudation.

Pathological sequelae in the cats treated over several weeks with Compound 48/80 were attributable to the effects of the histamine released. Gastric ulceration experimentally produced in this way is the result of the outpouring of acid secretion following release of endogenous histamine, and does not appear to differ from the ulceration described by McIlroy (1928) and O'Shaughnessy (1931) following simple injections of histamine, or implantation of the histamine in beeswax (Hay, Code & Wangensteen, 1942). Other changes, such as diffuse engorgement of the rugae of the stomach, increased vascularity of the colon with shredding of its epithelium, and the haemorrhagic necrosis of/

of Peyer's patches and the lymph nodes of the ileocaecal region may also be the result of release of histamine.

Following infusion of histamine itself into 48/80 treated animals, lower plasma histamine values were found in these animals; this was also the case in those animals treated with histamine in beeswax. It appears that large amounts of infused, or released, histamine may be removed rapidly from the vascular system, leading to a sudden fall in plasma histamine level; Schachter (1952) has observed a marked fall in plasma histamine after the administration of neoarsphenamine and bile salts, shown by him to possess, among other characteristics, the ability to release histamine. Comparable sudden lowering of plasma histamine following administration of histamine is described by Dragstedt & Mead (1935), Rose & Browne (1938), Code (1939) and Emmelin (1951).

It was suggested in Chapter one that absorption of histamine might take place into gastro-intestinal tissues. This would be part-:icularly beneficial in the cat, for in this species the intestine is by far the richest source of histaminase (Haeger & Kahlson, 1952). The stomach wall, on the other hand, could do little in the inactivation of histamine; indeed, as a poor source of histaminase, it may be that this very factor explains the marked increase in the histamine content of the pyloric region of cats treated with histamine in beeswax, whence it would be more slowly excreted than from the acid secretory region of the parietal cells in the corpus.

SUMMARY.

1. The histamine content of the skin of the cat shows regional differences similar to those found in other species. It is possible, with single intravenous or intra-injections of Compound 48/80, to reduce the skin histamine particularly in those regions of highest histamine content. Injections of Compound 48/80 into the coeliac artery lead, in addition, to a slight reduction in the histamine of the mucosa of the corpus region of the stomach. In all other tissues examined the histamine content is unchanged. The release of histamine from the skin leads to a transient rise of plasma histamine.

2. Intraperitoneal injections of Compound 48/80 in cats are followed initially by severe symptoms of prostration, vascular effects, respir-:atory distress and alimentary disturbances. On recovery, erythema and facial oedema are noticeable. With repeated intraperitoneal injections of Compound 48/80, the symptoms decrease in intensity and higher doses have to be given to be effective. This refractoriness is mainly accounted for by lack of labile tissue histamine. By these injections it is possible to reduce the histamine in the skin by over 80%. Lung, skeletal muscle and gastric mucosa of the corpus release relatively small amounts. The histamine of the other tissues examined is resistant to release by Compound 48/80. At post-mortem, there are gastric ulcers, intestinal lesions and haemorrhages in the adrenals.

3/

3. In cats treated with repeated intraperitoneal injections of Compound 48/80 and in cats treated with massive doses of histamine in beeswax, an intravenous infusion of histamine produces a much smaller rise in plasma histamine than in untreated cats. This may account in part for the greater resistance of the Compound 48/80-treated cats to histamine. Means for this disposal of released histamine are discussed, among which may be absorption into the gastro-intestinal tissues.

CHAPTER THREE.

The Association of Histamine Release and the Motor Effects of Histamine Liberators on the Guinea-pig's Ileum Preparation.

CHAPTER THREE.

THE ASSOCIATION OF HISTAMINE RELEASE AND THE MOTOR EFFECTS OF HISTAMINE LIBERATORS ON THE GUINEA-PIG'S ILEUM PREPARATION.

Histamine is a normal constituent of the various layers of the gastro-intestinal wall, but, as has been recounted already, little is known about its physiological function in this organ. Since histamine produces strong motor effects on the muscle layers of the digestive tract in many species, the possibility has often been discussed that it may play some role in the motor activity of gastro-intestinal preparations. It seemed feasible that histamine releasing agents would provide a tool by means of which this possible role of histamine for motor activity of the intestinal wall could be re-examined. Were the release of histamine a physiological mechanism for activity in the gastro-intestinal wall of some species, histamine releasing agents might be expected to accentuate the release and to stimulate motor activity.

In the present experiments we have therefore examined whether compound 48/80 exerts motor effects on the isolated guinea-pig's ileum preparation suspended in Tyrode solution and whether histamine can be detected in this solution when the preparation is subjected to treatment with compound 48/80. A few experiments were also performed with propamidine, D/

D-tubocurarine and tryptamine, all of which are known to be histamine liberators.

A perusal of the literature on the pharmacology of histamine-releasing substances shows that at least some of them may cause con-:traction, initiate rhythmic activity and augment the histamine response of the intestinal preparation, but no attempt has so far been made to correlate these effects with the histamine-releasing property of these substances. Mongar & Schild (1953) describe strong contractions with compound 48/80 (1 in 10,000), whereas Dews, Wnuck, Fanelli, Light, Tornaben, Norton, Ellis & de Beer (1953), using smaller concentrations, usually saw only a slight increase in spontaneous activity of the guinea-pig's ileum preparation. Spontaneous rhythmic activity of this prepar-:ation has, further, been seen after large doses of D-tubocurarine (Feldberg, 1951) and Rocha e Silva & Schild (1949) found that D-tubocur-:arine sensitized the preparation to histamine. The ability of tryptamine to contract the guinea-pig's ileum has been known for some time; recently it has been shown that tryptamine produces, in addition, strong rhythmic contractions which persist after the tryptamine receptors in the intestinal wall have been blocked by large doses of tryptamine (Gaddum, 1953; Feldberg & Toh, 1953).

METHODS.

The experiments were performed on the guinea-pig's ileum preparation suspended in a 15 ml. bath of magnesium-free Tyrode solution. The contractions/

contractions were recorded with a frontal writing lever which could be held in position with a photographic release cable when the bath was emptied and refilled. In some experiments the bath fluid of such a preparation (the donor) was siphoned off and tested on another guinea-pig's ileum preparation (the recipient). During the siphoning off the lever was fixed with the photographic release cable to reduce the mechanical effect of emptying and refilling the bath.

In a few experiments the histamine content of the guinea-pig's ileum preparation was determined by grinding the tissue in acidified saline solution, boiling the extract and assaying it for histamine, after neutralization, on the atropinized guinea-pig's ileum preparation. All histamine values refer to the base.

The compound 48/80 which is a condensation of p-methoxyphenylethylmethylamine with formaldehyde was kindly given to us by Dr. Paget and Dr. Trevan from the Wellcome Research Institution. The D-tubocurarine (Burroughs Wellcome) was used as the dichloride, the tryptamine (Roche Products) as the hydrochloride, and the propamadine (May & Baker) as the isethionate. All values refer to the salts.

RESULTS.

When compound 48/80 is added to the bath in which a guinea-pig's ileum is suspended, it produces direct effects during its contact with the preparation and subsequent changes in reactivity and motor activity which persist for some time after the compound 48/80 has been washed out.

The/

The direct effect of compound 48/80 consists of a slow, tonic contraction, often with superimposed, small, rhythmic changes. This effect corresponds to that described by Mongar & Schild (1953) and is sometimes observed on the addition of less than 1 mg of compound 48/80 to the 15 ml. bath. With 2 mg the contraction is obtained in all preparations, although not always on the first application. The method usually employed was to leave 2 mg of compound 48/80 in the bath for 60-90 sec and repeat the administration at 15-60 min intervals. A typical experiment is illustrated in Fig. 1, which shows that the full contractile effect is obtained with the second dose, and that with later administration the contraction becomes again smaller; at this stage the preparation is also less sensitive to histamine.

Mepyramine, in a dose which abolishes strong histamine contractions, reduces but does not abolish as strong or even weaker contractions produced by compound 48/80. The effect of mepyramine on the response to 2 mg of compound 48/80 is shown in Fig. 2.

After washing out compound 48/80, the preparation is less sensitive to histamine for some time. The degree of reduced sensitivity varies from preparation to preparation and occurs independently of whether compound 48/80 has itself caused a contraction and whether the preparation shows a spontaneous rhythmic activity. A pronounced and long-lasting reduction in sensitivity to histamine is illustrated in Fig. 3. In this condition the preparation is also less sensitive to acetylcholine.

A/

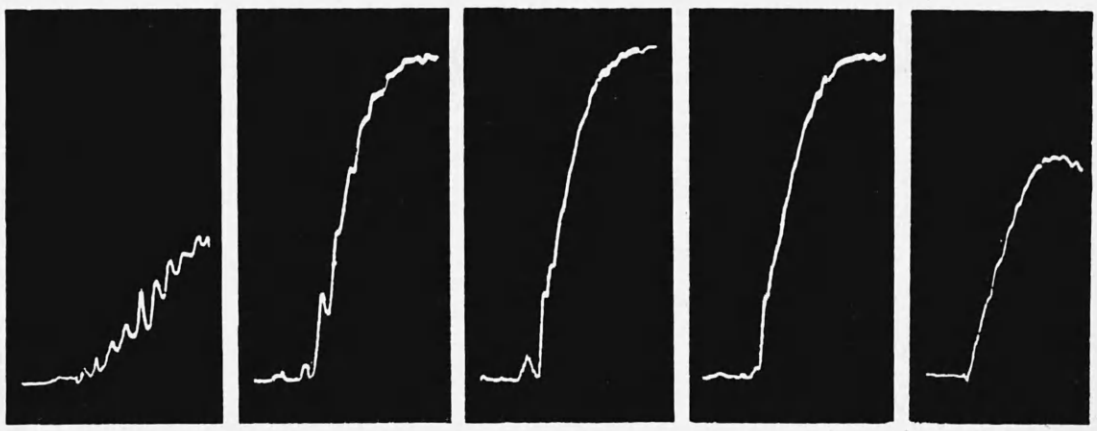

Fig. 1. Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution, 0.04 ug atropine sulphate throughout. Successive applications at 30 min intervals of 2 mg. of compound 48/80 kept in the bath for 90 sec.

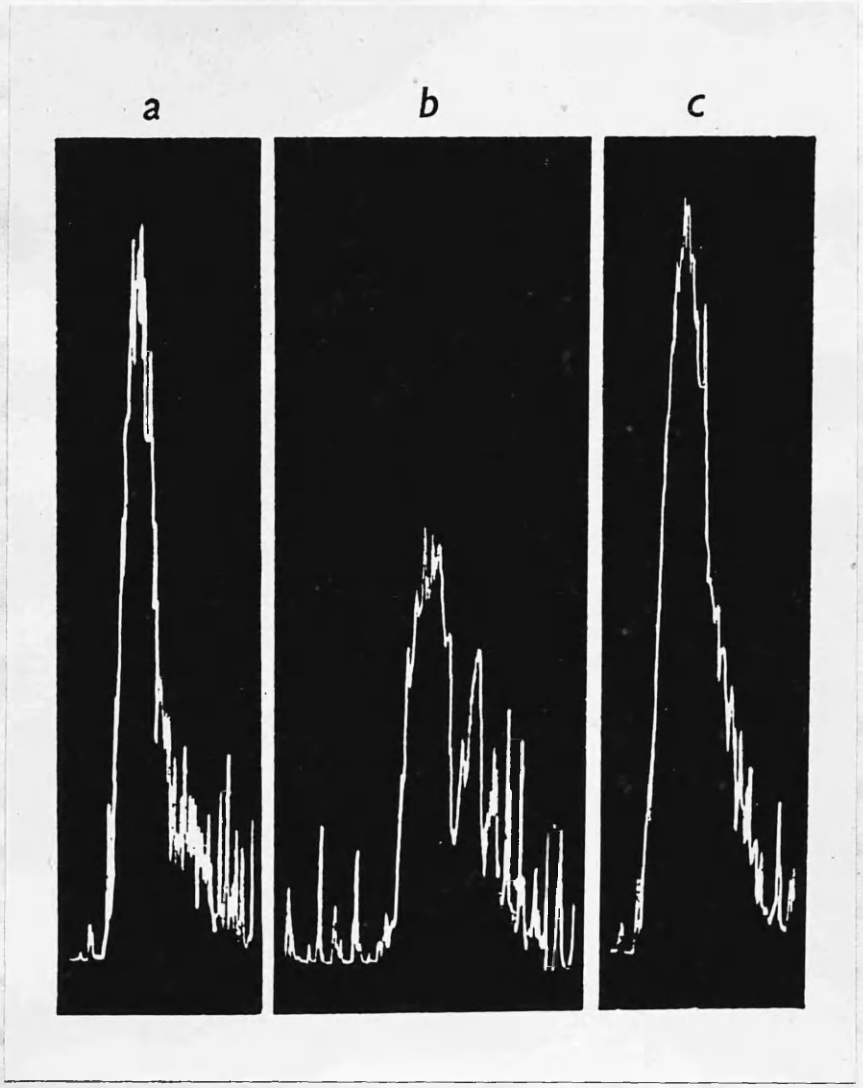

Fig. 2. Guinea-pig's ileum preparation in 15 mg. magnesium-free Tyrode solution. Three contractions due to 2 mg. of compound 48/80 kept in the bath for 90 sec. and administered at 40 min. intervals. The contraction at b in the presence of 0.1 ug mepyramine added to the bath 2 min previously. The contraction at c after washing out the mepyramine.

A similar observation was made by Paton (1951). The reduced histamine sensitivity was often observed after 50 ug of compound 48/80 were added to the 15 ml. bath for 1 min. Sometimes the condition of reduced sensitivity was preceded by a short period of increased sensitivity to histamine.

A characteristic after-effect of compound 48/80 is the development of spontaneous activity and increased done persisting for long periods. Sometimes repeated applications of compound 48/80 are required before this effect becomes apparent, and it is always more pronounced with repeated applications. When this motor activity is not yet apparent, it may be disclosed during a histamine response. Usually histamine causes tonic contractions, but after compound 48/80 rhythmic contractions are superimposed on the histamine contraction. In the experiment of Fig. 3, despite the reduced sensitivity to histamine which was brought about by compound 48/80, the reduced histamine contractions are no longer tonic but rhythmic. The change in the character of the histamine response in an experiment in which compound 48/80 has produced spontaneous activity is illustrated in Fig. 4.

The spontaneous activity and increase in tone develops irrespective of whether the preparation is atropinized or not, but is usually more pronounced when no atropine is given. Fig. 5 illustrates the develop-:ment of rhythmic activity and tone in an atropinized preparation after the first (b), second (c), third (d), fourth (e) and sixth (f) application of/

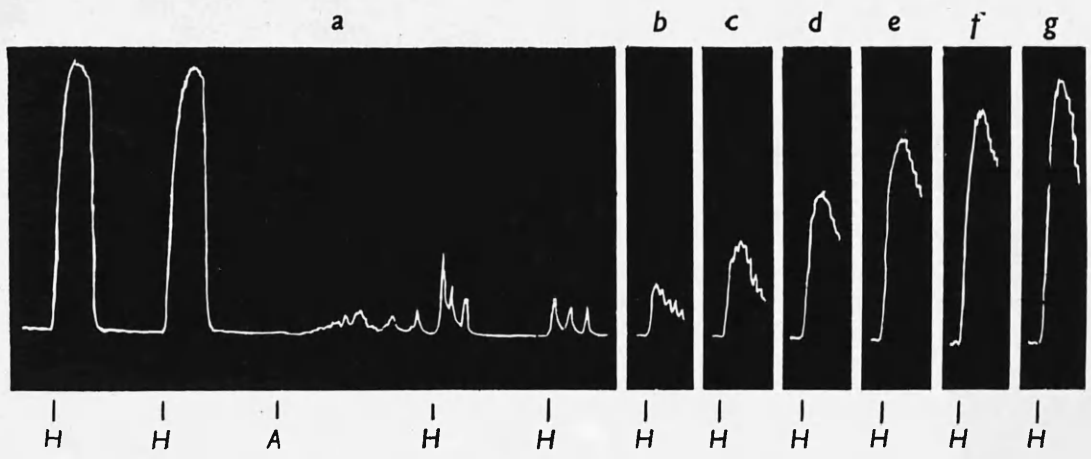

Fig. 3. Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution. 0.04 atropine sulphate throughout. At H successive applications of 0.04 ug histamine. 2 mg. of compound 48/80 added at A and kept in the bath for 90 sec. The histamine was given every 80 sec. but between a, b, c, d, e, f and g two histamine contractions were omitted each time from the tracing.

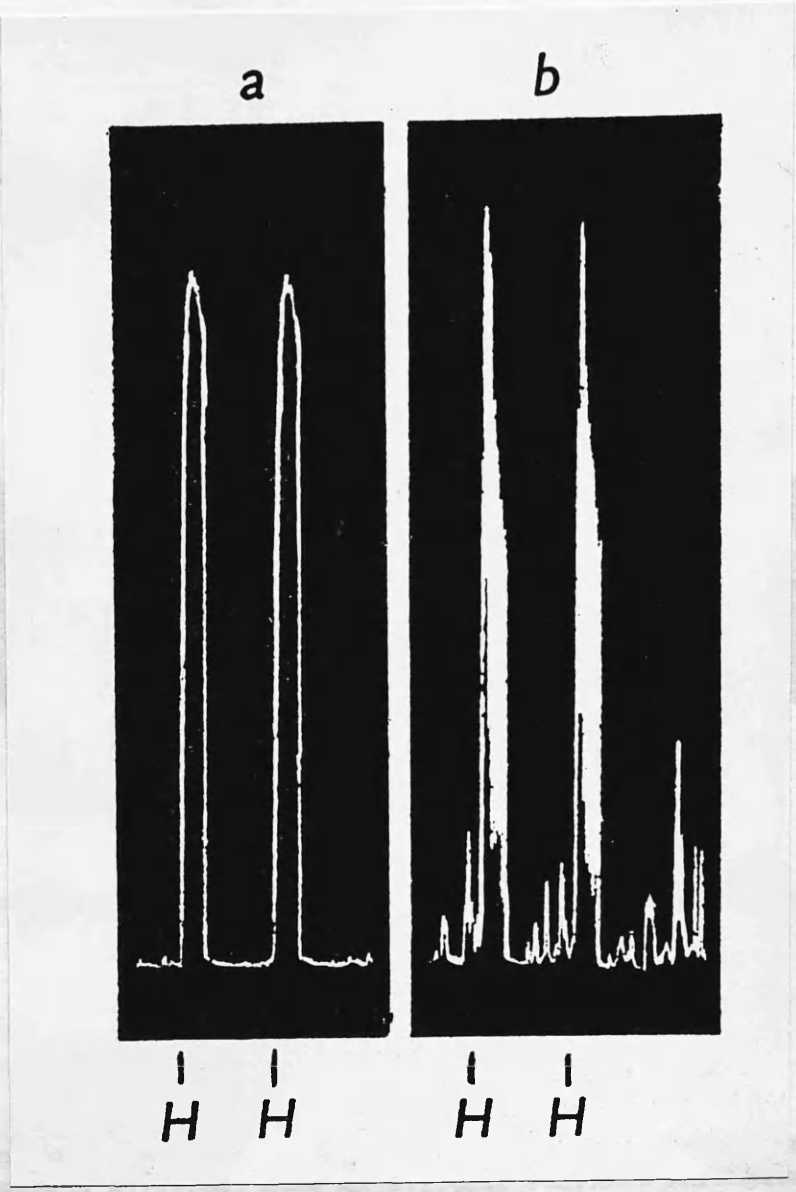

Fig. 4. Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution. 0.04 ug atropine sulphate throughout. At H, 0.06 ug histamine kept in the bath for 20 sec. Between a and b, 2 mg of compound 48/80 were given and kept in the bath for 90 sec.

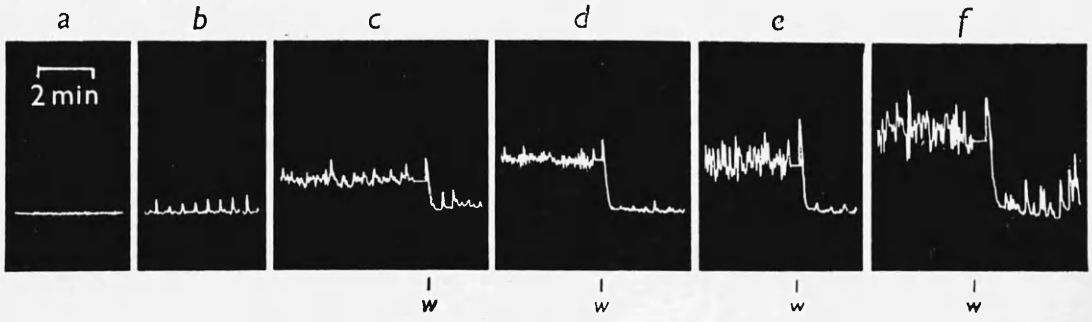

Fig. 5. Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution, 0.04 ug atropine sulphate throughout. a, Before compound 48/80; b-f, development of spontaneous activity and tone after successive doses of 2 mg compound 48/80. The direct contractile effects of compound 48/80 omitted from the tracing. At w the bath fluid was siphoned off whilst the lever was fixed.

of 2 mg of compound 48/80, the direct contractile effects of which are omitted from the tracings. The tracings were obtained about 15 min. after the application of compound 48/80 which was washed out each time after 90 sec contact with the preparation. At (w) the bath was washed out in order to show the relaxation and reduction in activity on replacement with fresh Tyrode solution. Fig. 6 is from a non-atropinized preparation. At (a) there is some slight rhythmic activity before compound 48/80; at (b) the activity and tone are shown about 15 min after the second application of 2 mg of compound 48/80. Again, replacement with fresh Tyrode solution (at w_1) causes pronounced relaxation, but has little effect on rhythmic activity. When the tone has again increased, atropine (At.) and later mepyramine (Me.) are given; together they produce the same relaxation as washing out, which afterwards has no longer any effect (w_2).

It is well known that choline diffuses out from an intestinal preparation suspended in physiological saline solution, and the small contractile effect which we obtained when bath fluid in which a guinea-pig's ileum preparation was suspended (the donor), was tested on another preparation (the recipient) rendered insensitive to histamine, was probably due to choline, because it was abolished by atropine. There was, however, no evidence that this choline output increased after compound 48/80. This was shown by the following procedure. The bath fluid from a donor preparation was collected every half hour and tested

on/

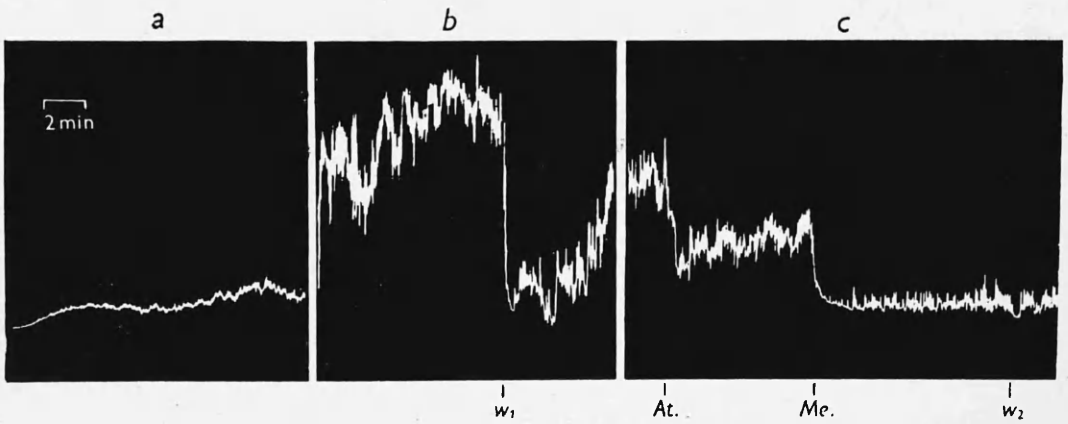

Fig. 6. Guinea pig's ileum preparation in 15 mg. magnesium-free Tyrode solution: (a) before, (b) about 15 min after the second application of 2 mg of compound 48/80 kept in the bath for 90 sec. At w_1 and w_2 bath fluid renewed whilst the lever was fixed. At At. 0.1 ug atropine sulphate; at Me. 0.05 ug mepyramine kept in the bath till w_2.

on a recipient preparation rendered insensitive to histamine by mepyramine. The contractile effect of the bath fluid did not increase when the collection was made after a period of greatly increased rhythmic and tonic activity induced by compound 48/80. However, in order to be certain that there was not some slight increase in choline output, it would have been necessary to acetylate the samples and subsequently determine their acetylcholine content.

A different result was obtained when the bath fluid from a donor preparation was tested on a histamine-sensitive guinea-pig's preparation rendered insensitive to choline and acetylcholine by atropine. The bath fluid collected during 20-30 min periods produced either no contraction or one barely visible, but when corresponding samples were collected during periods of increased activity and tone following the application of compound 48/80, the bath fluid caused strong contractions in several experiments, but not in all. The maximal effect of this kind was obtained when compound 48/80 (2 mg) had been repeatedly applied and had produced strong rhythmic activity and an increase in tone in the donor preparation. An experiment in which the contractile effect of bath fluid increased particularly strongly after compound 48/80 and was assayed against histamine is reproduced in Fig. 7. The figure illustrates the appearance and increase of a histamine-like substance in the bath fluid after two consecutive injections of compound 48/80.

The contractile effect of the bath fluid can be fully accounted for/

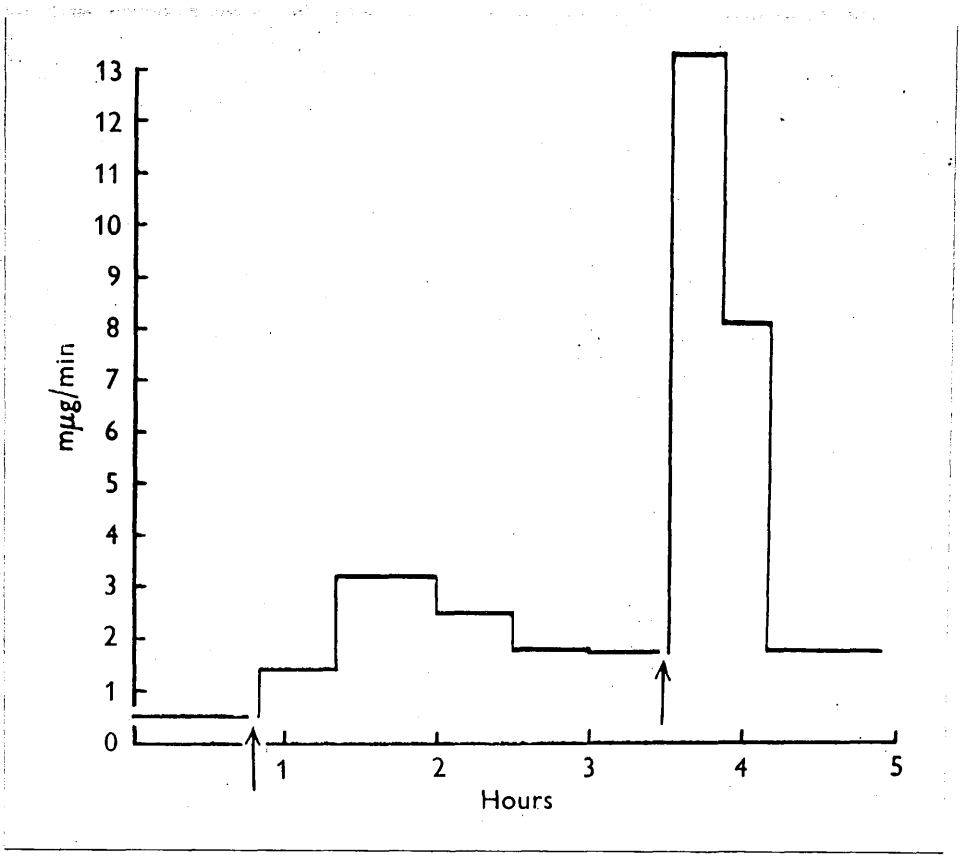

Fig. 7. Histamine output from guinea-pig's ileum preparation after two consecutive administrations of 2 mg. of compound 48/80. Ordinates: output of histamine in mug/min. Abscissae: time in hours. The two arrows indicate application of compound 48/80; the gap corresponds to the time during which the compound 48/80 was kept in the bath and subsequently washed out. The bath fluid was not tested.

for by the action of histamine, because it is affected by mepyramine to the same extent as an equally strong histamine contraction. For instance, in the experiment of Fig. 8, the contraction produced by bath fluid (15 ml.) was stronger than that of 0.04 and weaker than that of 0.05 ug histamine. It will be seen that the contractions of bath fluid and of 0.045 ug histamine were reduced to a minimum by 0.04 ug mepyramine and showed the same recovery when the mepyramine was washed out.

The failure to detect histamine or a histamine-like substance regularly in the bath fluid collected after a period of increased activity was probably due to the fact that the amounts released from a single preparation were so small as to become subthreshold as a result of dilutation in the bath fluid, because in later experiments in which two preparations of the ileum were suspended in the same bath, small quantities of histamine could be consistently detected in the bath fluid collected during periods of increased tone and rhythmic activity elicited by compound 48/80, although the amounts varied from experiment to experiment. This is shown in Table 1.

The injections of compound 48/80 were repeated only after the histamine output had fallen again, although not always to the original level; sometimes the intervals between two injections were as long as 2-3 hr. The maximal histamine output occurred either in the first, second or third 20-30 min sample collected after the compound 48/80, kept in the bath for 90 sec. had been washed out.

The/

TABLE 1. Maximal histamine output in mμg/min from suspended twin preparations of guinea-pig's ileum following successive administrations of compound 48/80 or propamidine

Expt. no.	Before drug administration	1st administration		2nd administration		3rd administration		4th administration	
		mg 48/80 injected	Output of histamine	mg 48/80 injected	Output of histamine	mg 48/80 injected	Output of histamine	mg 48/80 injected	Output of histamine
1	0·1	2	0·2	2	6·5	—	—	—	—
2	0·2	2	0·4	2	0·4	2	0·8	(10)*	3·2
3	0·25	1	0·7	2	2·0	2	1·0	4	5·0
4	0·2	1	—	2	8·6	4	67·0	2	15·0
5	1·2	4	2·4	4	10·0	2	4·0	—	—
6	0·75	4	5·0	4	13·0	(10)*	3·0	—	—
7	1·3	4	2·0	4	7·5	4	17·5	—	—
8	0·1	4	3·0	4	25·0	4	3·0	—	—
9†	0·6	2	2·5	2	7·5	—	—	—	—
10†	0·25	2	17·5	2	4·0	(10)*	4·0	—	—

* The figures in brackets refer to propamidine injections.
† Both ends of the preparations tied.

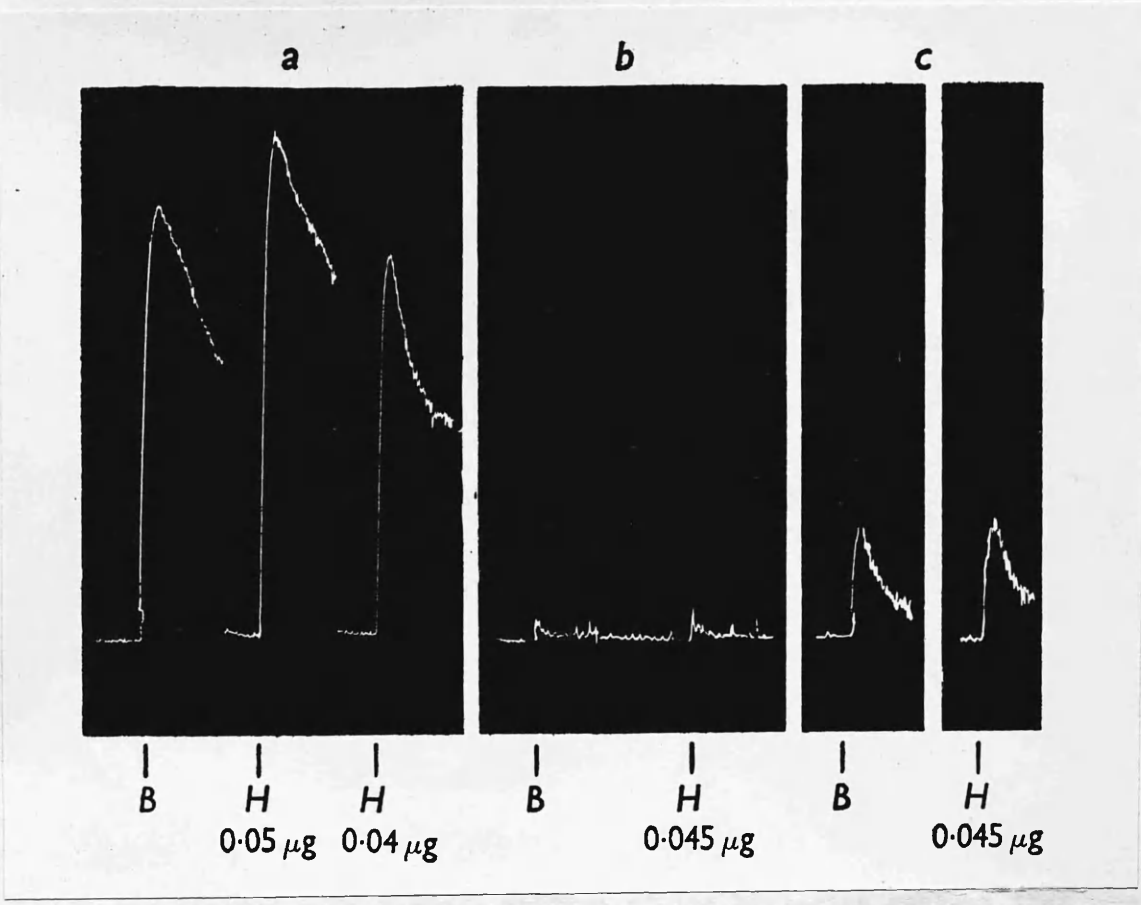

Fig. 8. Contractions of atropinized guinea-pig's ileum to bath fluid B and histamine H before (at a), during (at b) and after (at c) 0.04 ug mepyramine.

The histamine found in the bath fluid must have diffused out from the wall of the intestine, or at least mainly so, and not from the lumen, because there was little difference in the results obtained when the two ends of the donor preparations were tied. This was done in Expts. 9 and 10 of Table 1. In these, compound 48/80 produced the same increase in rhythm and tone as in those preparations in which the lumina were left open.

There was usually good correlation between the degree of tone which developed after compound 48/80 in a 20-30 min. period, and the histamine content of the bath fluid collected at the end of the period. The correlation between increased rhythmicity and histamine content of bath fluid was more difficult to assess, because once rhythmic activity had developed, it was difficult to measure its intensity; moreover, the rhythmic movements often become smaller with a pronounced increase in tone of the preparation.

The amounts of histamine diffusing out from the intestinal preparation represented such a small portion of its histamine content that no evidence was obtained of a reduction of the tissue histamine in the intestinal wall. For instance, the histamine assayed in samples of bath fluid collected after the two injections of compound 48/80, in the Expts. of Fig 7, amounted to 0.84 ug. At the end of this experiment, the histamine content of the intestinal piece, weighing 335 mg, was determined. It contained 12 ug, or about 35 ug/g. A control piece from the same intestine/

intestine contained 25 ug/g. In another similar experiment the histamine content was 39 ug/g for a donor preparation treated with several injections of compound 48/80, and 40 ug/g for a control piece of intestine from the same animal.

Propamidine. The addition of 10-20 mg propamidine to the bath produced a strong contraction which differed from that produced by compound 48/80, in that it was more prolonged and associated with segmental contractions of the circular muscle. These contractions of the circular muscle caused some lengthening of the preparation, and when the propamidine was washed out, the circular muscle relaxed first which produced further shortening of the preparation before the first relaxation of the longitudinal muscle occurred (see Fig. 9). Subsequent rhythmic activity developed in some but not in all preparations. It developed more regularly when the propamidine was given in the course of an experiment after previous injections of compound 48/80, whilst there was still some rhythmic activity left over. This is shown in the experiment of Fig. 9.

Assays of bath fluid after propamidine, during development of tone and rhythmic activity, revealed an increased histamine output; for instance, in one experiment it rose from 0.3 to 1.2, in another from 0.3 to 3 m ug/min. The histamine output initiated by propamidine when added to the bath after previous compound 48/80 administrations is shown in the Expts. 2, 6 and 10 of Table 1.

Tryptamine. Tryptamine produces contraction in the guinea-pig's ileum, followed/

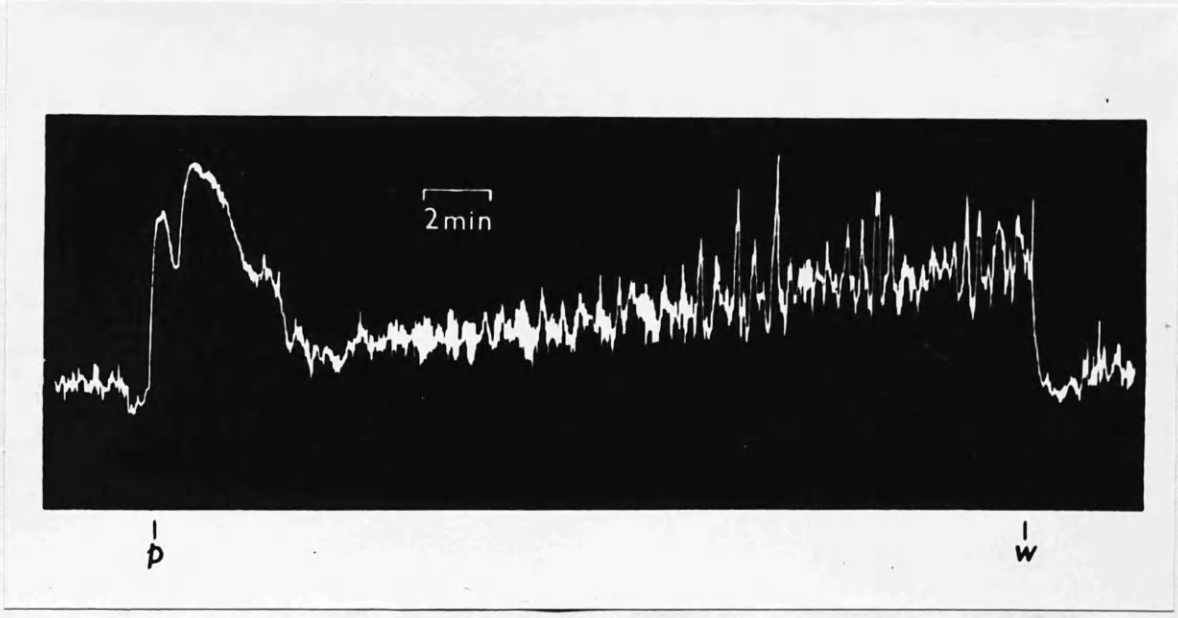

Fig. 9. Guinea-pig's ileum preparation in 15 mg. magnesium-free Tyrode solution. The spontaneous activity at the beginning of the tracing is the result of three previous administrations of 2 mg of compound 48/80 each. At p, 10 mg propamidine isethionate left in the bath for 90 sec. At w, renewal of bath fluid.

followed by increased rhythmicity and tone (Gaddum, 1953; Feldberg & Toh, 1953) and will be shown in Part III of this thesis to be a histamine liberator. In a few experiments it could be shown that the periods of increased rhythmicity were associated with an increased output of histamine into the bath fluid; for instance, in one experiment the histamine output after 20 mg tryptamine rose from 0.6 to 5 m ug/min.

D-Tubocurarine. Rhythmic contractions of the guinea-pig's ileum following administration of D-tubocurarine have been described previously (Feldberg, 1951), and Ambache & Barsoum (1939) have obtained histamine release by curare from isolated pieces of dog's intestine incubated in Tyrode solution. When given in sufficiently high dosage (1 mg/ml.), D-tubocurarine, like the other histamine liberators, has an immediate, strong contractile effect. The period of increased rhythmic activity which develops after washing out the D-tubocurarine is again associated with an increased output of histamine into the bath fluid. In one experiment with twin preparations the output rose from 0.1 to 1.2 m ug/min.

Mepyramine. Mepyramine was shown by Arunlakshana (1953) to be a strong histamine liberator. The fact that it is also a strong antihistamine substance, however, usually masks the stimulating effect of any histamine released in the intestinal wall. In the course of experiments with mepyramine two observations, a direct and an indirect, were made which revealed such a stimulating action

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of mepyramine. (1) It was frequently found that small doses of mepyramine, kept in the bath for only 20 sec, did not at once reduce subsequent histamine contractions. When the histamine was given within a minute or two after the mepyramine had been washed out, the response was potentiated (Fig.10). (2) These small doses of mepyramine, when given to preparations suspended for several hours in the bath and rendered sensitive to histamine by its repeated administration, some-:times produced a small contraction and a short period of increased rhythmic activity, which preceded the period of decreased sensitivity to histamine. Such an effect is illustrated in Fig. II. Further, in a few preparations which had been given compound 48/80 several times and were showing some rhythmic activity, the addition of 0.01 ug mepyramine produced a small contraction with superimposed rhythmicity (Fig. 12a); when the mepyramine was washed out and sufficient time allowed for its antihistamine effect to pass off, a second dose of mepyramine again produced a contraction which, however, was smaller than that produced on its forst application (Fig. 12b).

DISCUSSION.

These experiments show that histamine-liberating substances produce characteristic motor effects on the guinea-pig's ileum preparation which consist of immediate contractions followad, after washing out the histamine liberators, by prolonged periods of increased motor/

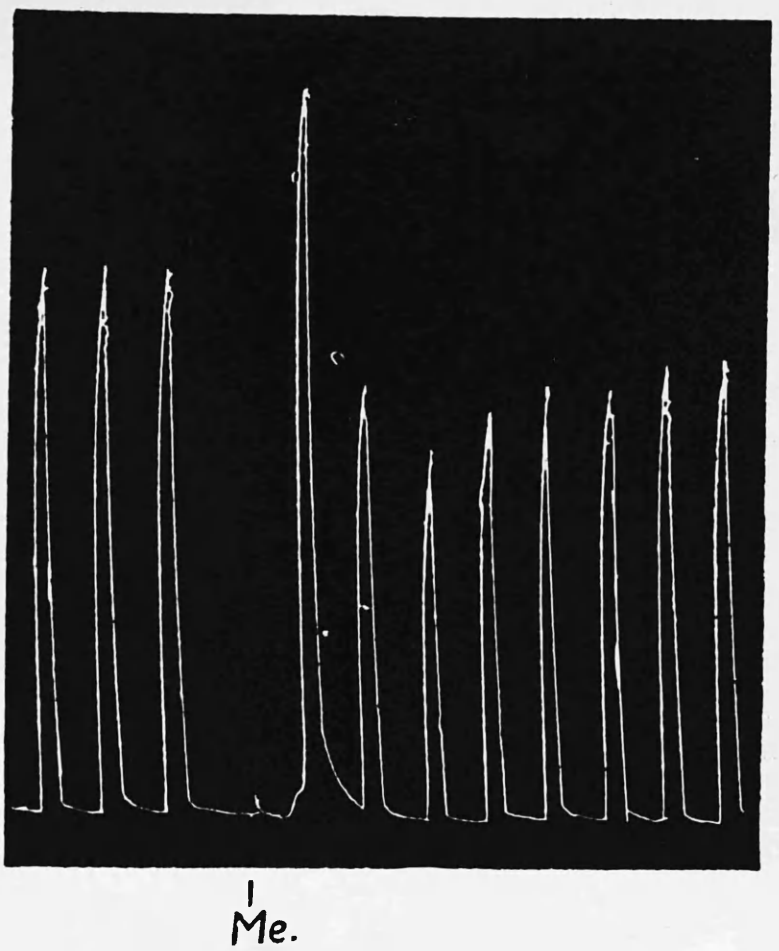

Fig. 10. Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution. Contractions to 0.04 ug histamine kept in the bath for 20 sec. At Me. 0.02 ug mepyramine kept in the bath for 20 sec.

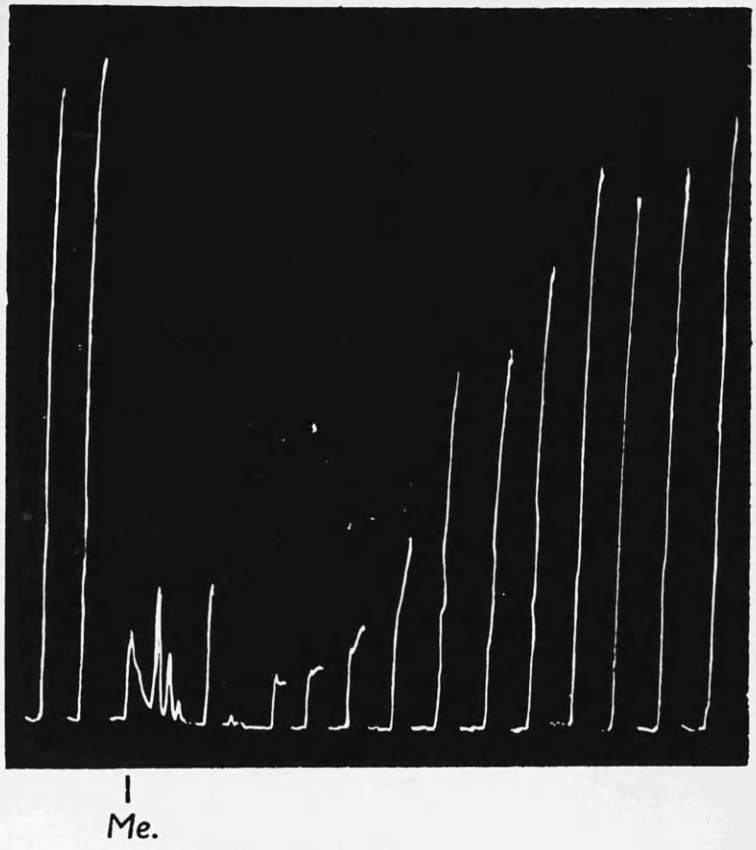

Fig. 11. Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution about 3 hr after having been suspended. Contractions to 0.08 ug of histamine kept in the bath for 20 sec at 90 sec intervals. At Me. 0.05 ug mepyramine kept in the bath for 60 sec.

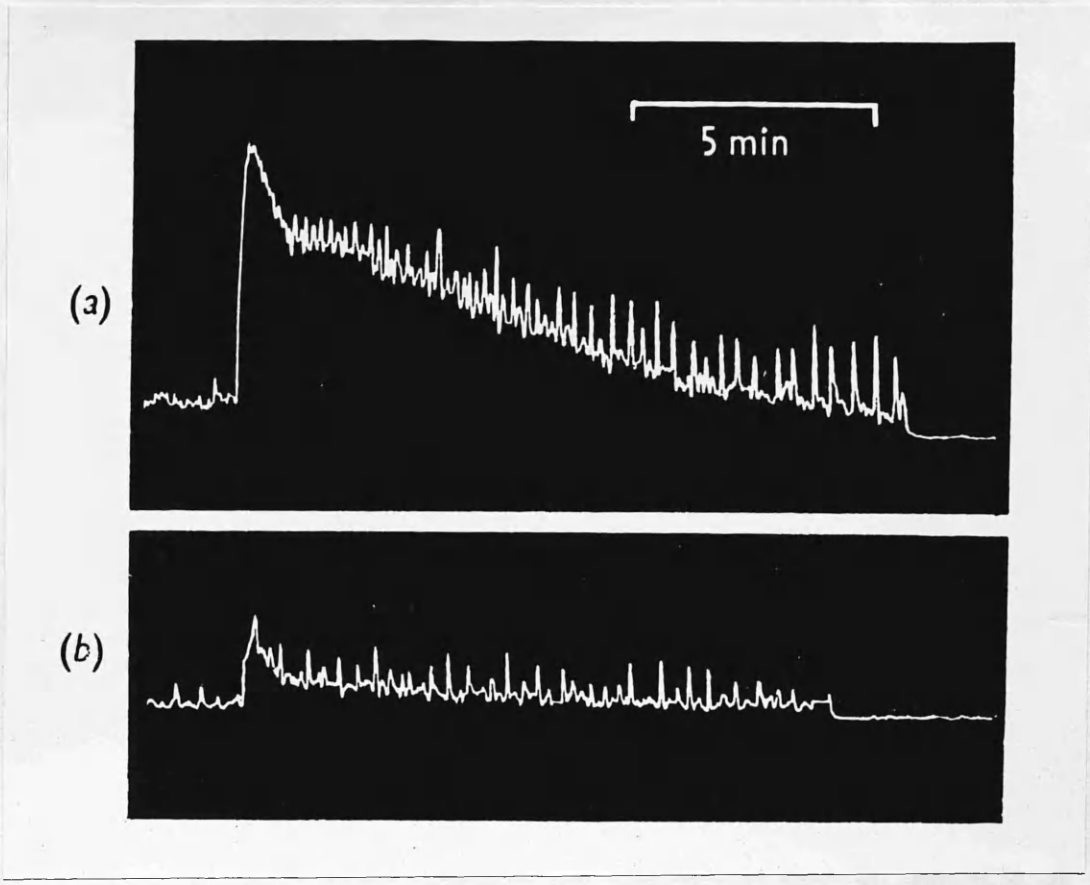

Fig. 12. Guinea-pig's ileum preparation in 15 ml. magnesium-free Tyrode solution 6 hr after having been suspended and given 2 mg of compound 48/80 six times. Two contractions with superimposed rhythmicity produced by 0.01 ug mepyramine left in the bath for 15 min.

motor activity and tone. There is little doubt that the development of tone and the appearance of motor activity is partly or wholly the result of histamine release in the intestinal wall. During the development of spontaneous activity and tone, histamine was shown to diffuse out from the intestinal wall into the surrounding bath fluid, and in amounts sufficient to produce motor effects on the intestinal preparation. The histamine which diffuses into the bath fluid can account fully for the development of tone, because when the bath fluid is replaced by fresh Tyrode solution, or when mepyramine is added to the bath, the tone is removed and the muscle relexes. However, motor activity is only partly affected by these procedures. None the less, it may result from the histamine released, not after it has diffused into the bath fluid but when it acts at the site of its release within the muscle coat. Replacement of the bath fluid would not remove this histamine, and mepyramine added to the bath might be unable to reach this 'intrinsic' histamine acting within the muscle coat at the site of its release. A dinstinction between the action of 'intrinsic and extrinsic histamine' (Dale, 1948), would account for a number of other details observed, for instance, the finding that motor activity always precedes the development of tone and that there is a parallelism between intensity of motor activity and the degree of tone which develops. The possibility cannot be excluded, however, that other factors than release

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of histamine play a part in this development of motor activity.

The immediate contractile effect produced by compound 48/80, propamidine, and large doses of D-tubocurarine may also be an action of such intrinsic histamine, and the finding that mepyramine has a much weaker effect on these contractions than on those produced by histamine added to the bath fluid, would be in accordance with this view. The fact that compound 48/80 produces its main motor effects on the guinea-pig's intestine only when applied in large doses, seems at first sight contradictory to this interpretation. Compound 48/80 is known to be such a potant histamine liberator that one would expect it to act in very small doses. However, the high potency applies to the effect of compound 48/80 on skin and skeletal muscle histamine; the histamine of the viscera is relatively resistant to compound 48/80. It is therefore not surprising that compound 48/80 has to be applied in relatively large doses to produce motor effects on the intestine with concomitant release of histamine. In this connexion it is also interesting to note that the doses of compound 48/80 which regularly produce strong contractions of the guinea-pig's ileum were found to have no effect of this kind on the rat's colon preparation; this muscle preparation is insensitive to histamine.

The concept that the motor effects of histamine liberators are to a great extent due to the action of released histamine receives additional support/

support from the unexpected motor effects which were occasionally seen with mepyramine before its antihistamine action developed, because mepyramine is not only an antihistamine substance but a potent histamine liberator as well.

When considering the motor effects of histamine liberators on the intestine, we have to realize that these substances may have motor effects on the intestine independent of their histamine releasing property; this appears to be so with tryptamine. Its immediate contractile effect, which can be obtained repeatedly with relatively small amounts and even when given at short intervals, is probably not accounted for by release of histamine but by a direct action of tryptamine. Tryptamine also contracts the histamine insensitive rat's colon preparation, and the contractile effect on the guinea-pig's ileum is abolished when the tryptamine receptors are blocked. The spontaneous activity it induces, however, persists in this condition and may well be accounted for by histamine release.

The concept that the increased motor activity seen with so many histamine liberators results from histamine released within the muscular coat has significance with regard to the mechanism underlying atropine resistant, spontaneous activity of intestinal preparations. Release of histamine in the intestinal muscular coat may be one mechanism by which intestinal motor activity can be produced under physiological and pathological conditions in those species in which the muscle coat of the intestine is sensitive to histamine. Ambache & Barsoum (1939) have,

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in fact, obtained some evidence that minute amounts of histamine are released from the guinea-pig's intestine and diffuse into the surr-:ounding fluid when the preparation is made to contract by various means. On the other hand, the fact that atropine resistant, spontan-:eous activity occurs in intestinal preparations which are insensitive, or relatively insensitive, to histamine, for instance the intestine of the rabbit and rat, shows clearly that release of histamine cannot be the only factor involved in the atropine resistant, spontaneous activity of the muscle of the intestinal wall.

SUMMARY.

1. The effect of compound 48/80 on the guinea-pig's ileum prepar-:ation suspended in 15 ml. magnesium-free Tyrode solution was examined. When added to the bath in a dose of 2 mg, it produced a strong, trans-:ient contraction, followed by increased motor activity and development of tone after washing out the compound 48/80. In addition, the sensitivity of the preparation to histamine and acetylcholine decreased.

2. The periods of increased motor activity induced by compound 48/80 were associated with the diffusion of histamine from the intestinal wall into the bath fluid.

3. The motor effects of compound 48/80 can be explained by the release of histamine, when a distinction is made between the action of the histamine diffusing into the bath fluid (extrinsic histamine) and the histamine acting/

acting within the muscular coat at the site of its release (intrinsic histamine).

4. Other histamine liberators, like propamidine, D-tubocurarine and tryptamine, produce motor effects comparable to those produced by compound 48/80, and also associated with the appearance of histamine in the bath fluid; but histamine liberators may have motor effects on the intestine independent of histamine release. This appears to be so with tryptamine.

5. Small doses of mepyramine, which is known to be a potant histamine liberator, sometimes produce, in the guinea-pig's ileum preparation, before the antihistamine effect develops, a transient period of increased sensitivity to histamine and the appearance of small, rhythmic movements. In preparations previously treated with compound 48/80, small doses of mepyramine may occasionally even cause contraction with superimposed rhythmicity. These effects are attributed to histamine release.

6. The possibility is discussed that in some species histamine plays a role in 'spontaneous' motor activity of the intestine.

CHAPTER FOUR.

Histamine-releasing Substances used in the Experimental Production of Gastric Ulcers.

CHAPTER FOUR.

HISTAMINE-RELEASING SUBSTANCES USED IN THE EXPERIMENTAL PRODUCTION OF GASTRIC ULCERS.

Various agents or extracts have been described which have in the past been thought to induce a specific form of gastric ulcer either before or after sensitization of animals to them. It is shown in this chapter that gastrotoxin in guinea-pigs and horse serum in rats, which were formerly thought to induce a specific form of gastric ulcer, do so in virtue of their common property of histamine release. These sera and toxins may be without effect when preceded by moderate doses of histamine liberators planned to release the tissue histamine gradually; loss of the tissue histamine annuls the effect of the sera and toxins and tends to support the argument that they act via histamine release.

METHODS.

Albino guinea-pigs (160-220 g.) were injected intraperitoneally, subcutaneously and intravenously into veins on the extremities, with Compound 48/80 (Burroughs Wellcome), a potent histamine liberator (Paton, 1951). Albino rats were chosen and treated for histamine depletion by the serial injection technique of Feldberg and Talesnik (1953). Tissue extracts were made as described by Smith (1953) and were assayed for histamine on the atropinized guinea-pig's ileum. Blood for plasma-histamine determinations was obtained by exsanguination of/

of the animal from the severed jugular veins into a siliconed vessel, containing 0.02 mg./ml. heparin. The blood was then centrifuged at 2000 r.p.m. and the supernatant tested on the guinea-pig's ileum before and after addition of mepyramine to the bath. Many of these determinations were controlled, extracting the tissues by classical techniques such as Code's (1937) modification of the method of Barsoum and Gaddum (1935).

In perfusion experiments the animals were anaesthetized with 25 per cent urethane 6 ml./kg. and perfusion experiments were carried out with Locke's solution through which was bubbled O_2 and CO_2. The perfusion cannula was inserted into the supradiaphragmatic inferior vena cava for the lung perfusion, and the superior vena cava and the azygos veins were tied; effluent was collected from a cannula in the aorta, from both lungs and the heart in situ. Similarly, by insertion of the perfusion cannula in the upper part of the abdominal aorta, and by tying its lateral branches to the abdominal wall and urinary tract, with further ligation of the aorta at its termination, the alimentary tract and liver could be perfused in situ by inserting the cannula to collect the effluent from the hepatic veins in the supra-diaphragmatic portion of the inferior vena cava; ligation of side vissels of the vena cava and iliac veins was also performed. The tissues of the lower extremities were perfused by inserting the cannula in the lower abdominal aorta/

aorta and collecting the effluent from another cannula tied into the origin of the inferior vena cava (Fig. 1).

Subcutaneous injections of Compound 48/80 were made in guinea-pigs under the shaved skin of the abdomen in volumes up to 0.5 ml. (concentrations of 1 : 500 and 1 : 1000). Intraperitoneal injections were likewise given using this volume, in all but one group of experiments where the volume was increased up to 5 ml. (1 : 10,000 solution). Histamine liberator was also dispersed in a slow release medium consisting of peanut oil with the addition of beeswax as described by Bruce and Parkes (1952). Antihistamine was given as mepyramine maleate. For the purpose of sensitization, horse serum was administered subcutaneously (0.5 ml.) and the antigen repeated 14 days later: 0.2 ml. or 0.5 ml. was now injected and the time of onset and severity of symptoms noted (this dose was repeated, if necessary, since the amount of antigen required to produce anaphylaxis varies somewhat.) For primary injection of rats, without prior sensitization, 1 ml. horse serum was injected; this was compared with the effects of 200 ug. Compound 48/80. Aminoguanidine was injected in 1 ml./kg. dosage as the sulphate. Gastrotoxin was prepared as described by Bolton (1904); 10 ml. were injected intraperitoneally into guinea-pigs and the tissue examined for gastric ulcers (Bolton, 1908)* by standard histological methods.

* Appendix.

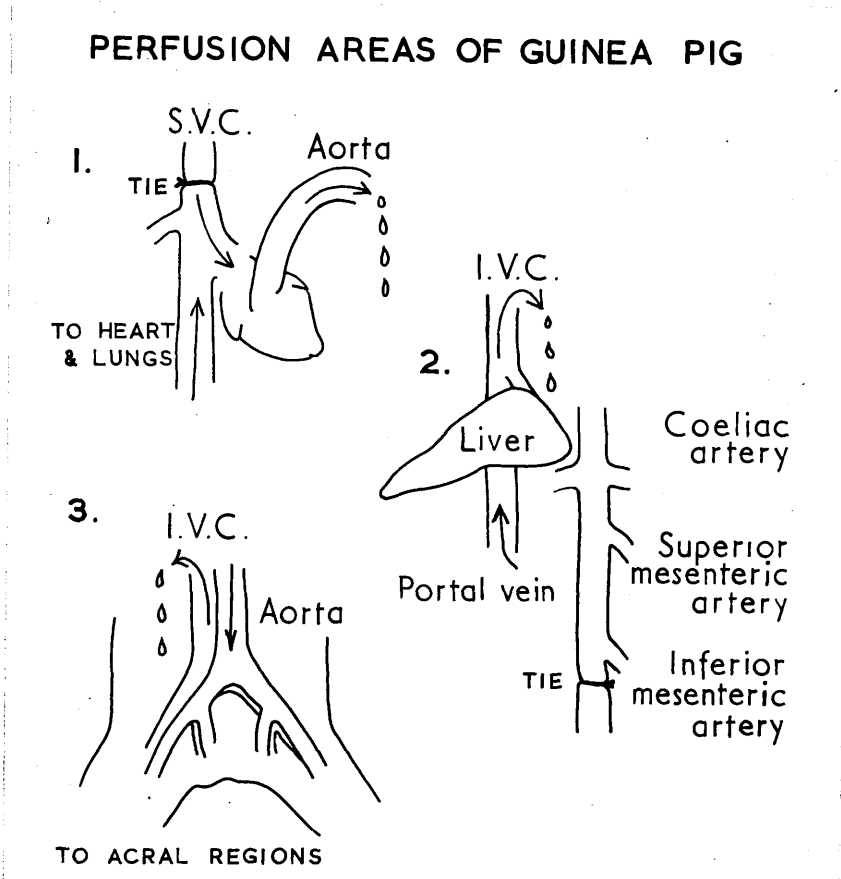

Fig. 1. Perfusion areas of guinea-pig. Areas 1, 2 and 3 were perfused after cannulating the inferior vena cava, coeliac artery, and aorta respectively. The effluent was collected from the aorta and inferior vena cava supra-diaphragmatically and infra-diaphragmatically respectively.

RESULTS.

1. Histamine Release and Gastric Ulcers in the Rat. Feldberg and Talesnik (1953) have described the effects of Compound 48/80 in the rat. They found a marked reduction of tissue histamine, confirmed by the present experiments (Fig. 2), the values for the gastro-intestinal tract showing a much smaller change than the other tissues; they detected the release into the plasma of large amounts of histamine. The general effects are those of prostration, erythema, and oedema (Fig. 3 A, B). These effects are duplicated entirely by horse serum (Fig. 3 C) which acts as a primary histamine-releasing agent in the rat and does not require the agency of prior sensitization (Schachter and Talesnik, 1952). The plasma values for histamine after 200 ug. Compound 48/80 and 1 ml. horse serum are high, highest when the agents are given systematically, and most of all when enzymatic destruc-:tion by histamine is prevented by the antihistaminase, aminoguanidine (Schayer and Smiley, 1954; Table I, A.) The effects on the stomach closely parallel the histamine values; the greater the histamine values the higher the incidence of ulceration and erosion (Fig. 4 B, C compared with F, G), and indeed perforation may be produced (Fig. 4 H). The effects of horse serum are less dramatic (Fig. 4 B, C) than after Compound 48/80 (Fig. 4 F, G) but are greater during treatment by the antihistamine agent, aminoguanidine (Fig. 4 D).

The most severe effects of all follow administration of Compound 48/80 and aminoguanide (Fig. 4 H). Prior treatment with Compound 48/80 resulted/

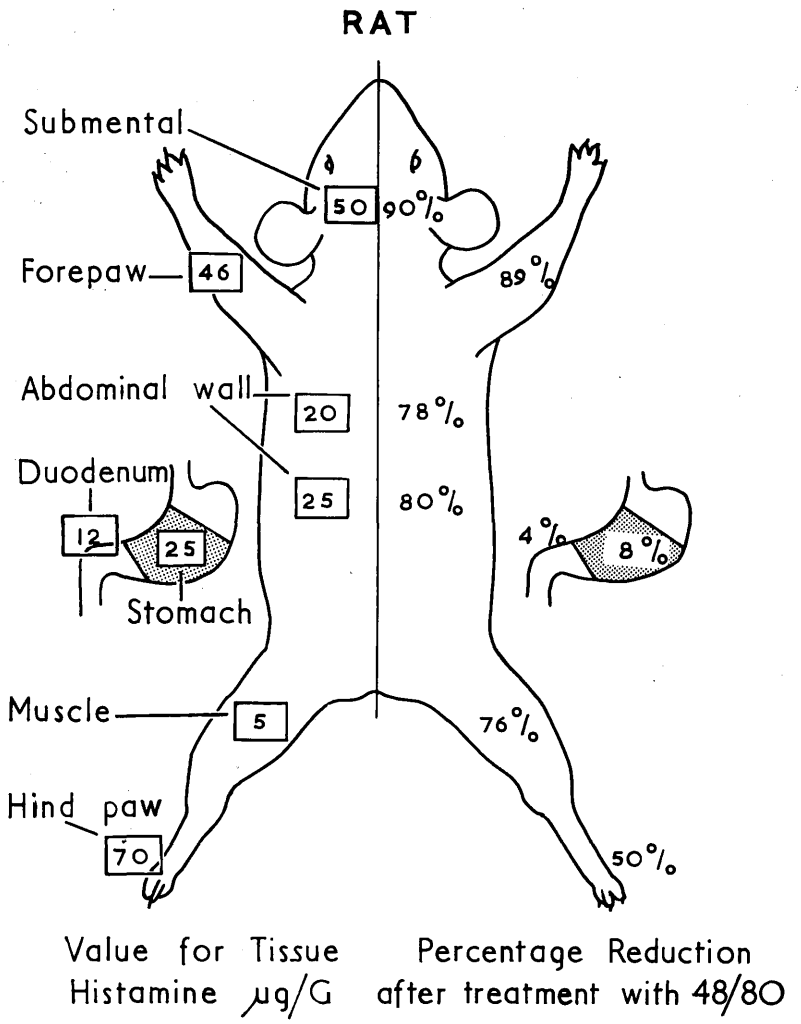

Fig. 2. Histamine values for various tissues of the rat are listed (left); the effect of histamine liberation by Compound 48/80 is to diminish tissue histamine as indicated (right); average of three experiments.

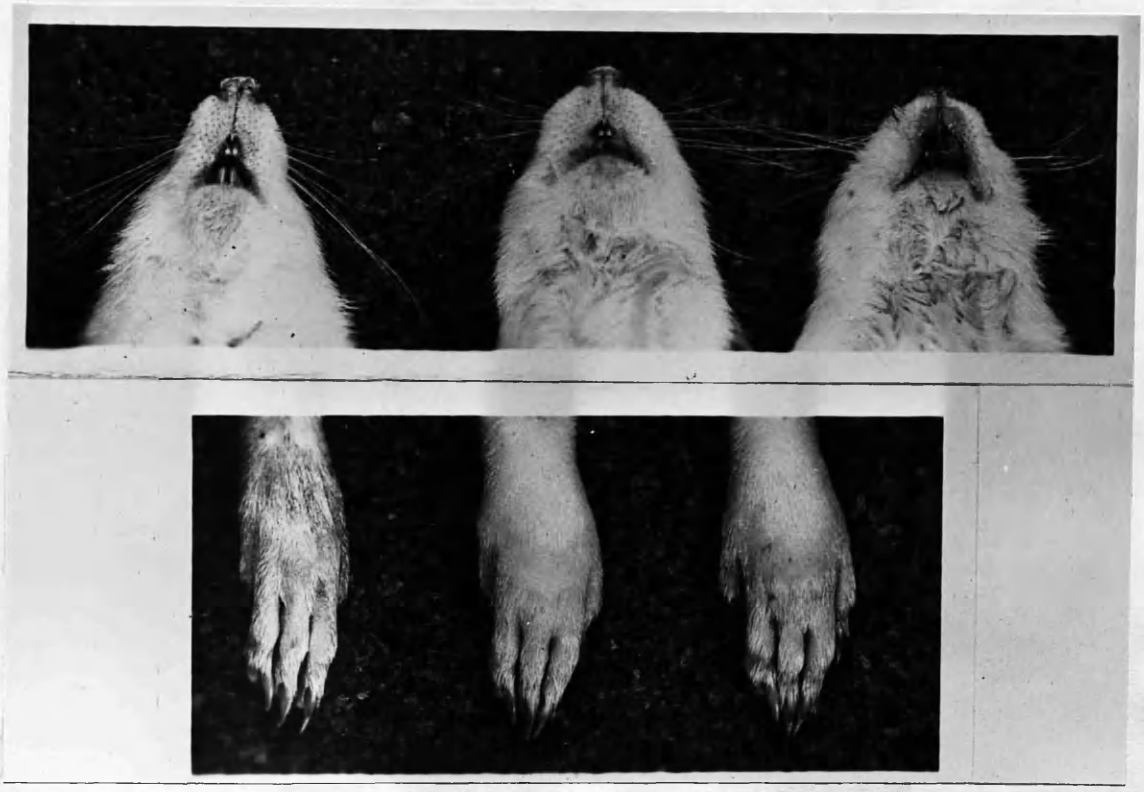

Fig. 3. A, Appearances of snout and paw of normal rat.
B, Rat given injection of Compound 48/80 intra-
:peritoneally with consequent oedema at both sites.
C, Comparable appearances after injection of horse
serum.

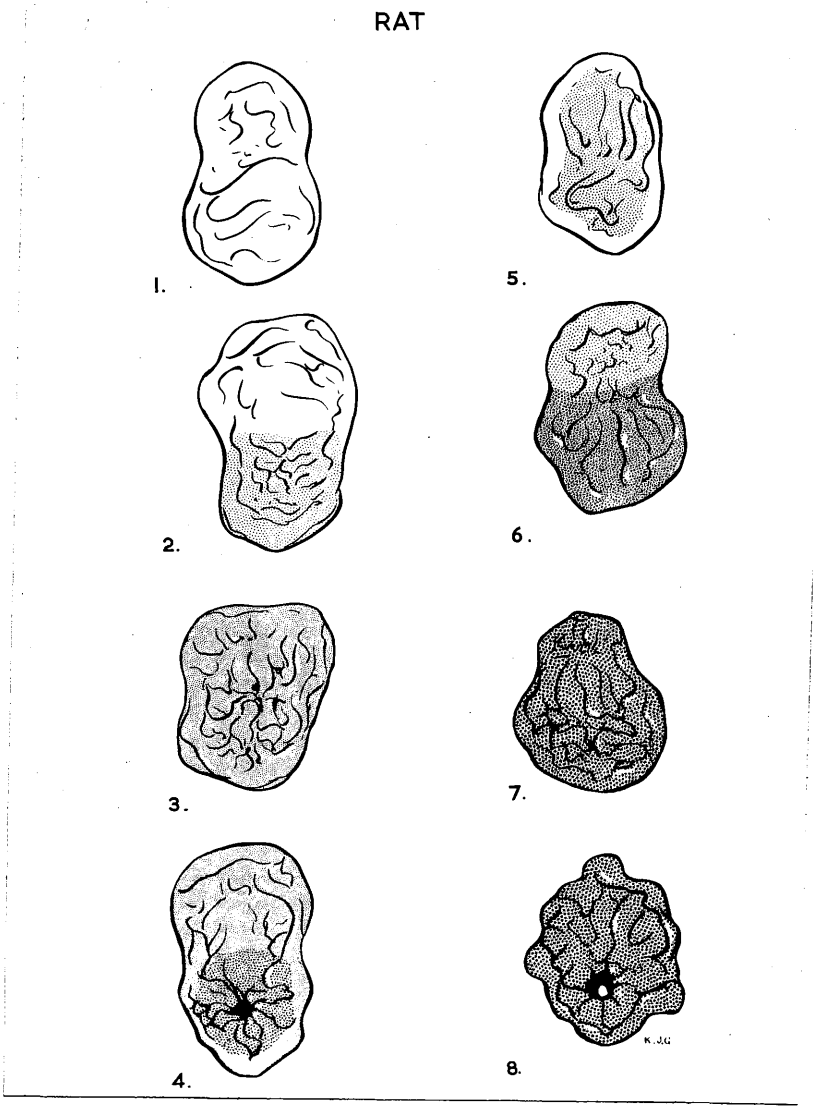

Fig. 4. Diagrammatic representation of rat stomach which normally consists of upper epithelial sac and lower secretory portion. The density of stippling is an attempt to represent mucosal congestion as appearing on colour photographs from 3 animals treated in each group. Same dose as in Table 1. A, Normal stomach. B, After horse serum locally subcutaneously. C, After horse serum intraperitoneally. D, After horse serum + aminoguanidine. E, After horse serum intraperitoneally (previous injections of Compound 48/80 to deplete labile histamine). F, After Compound 48/80 locally subcutaneously. G, After Compound 48/80 intraperitoneally. H, After Compound 48/80 + aminoguanidine.

TABLE 1.

VALUES FOR PLASMA HISTAMINE.

Agent	Route of Administration	Histamine (ug./ml.)	
		A	B
Normal values		0.02	0.005
Compound 48/80	Local, subcutaneous	0.03	0.005
	General, intraperitoneal	0.8	0.24
	General + aminoguanidine	1.2	0.31
Horse serum	Local, subcutaneous	0.02	0.005
	General, intraperitoneal	0.05	0.17
	General + aminoguanidine	0.06	0.28

Values for the plasma histamine: (A) In the rat after 200 ug. Compound 48/80 and 1 ml. horse serum. (B) In the guinea-pig after 4 mg./kg. and 0.5 ml. horse serum repeated after 10-14 days' interval (in each case average of 3 experiments).

resulted in a release of histamine from many tissues, as is shown in Fig. 2. Subsequent attempts to provoke the typical effects of horse serum (Fig. 4 B, C, D) were ineffective (Fig. 4 E).

2. Evidence for Histamine Release in Guinea-pigs. In guinea-pigs there is little information available on the effects of histamine liberators on tissue histamine. Mongar and Schild (1952) in experiments with Compound 48/80 in vitro found as much histamine released by the antigen-antibody reaction in sensitized tissues. On the other hand, Compound 48/80 injected intraperitoneally or intravenously was found by Mota and Vugman (1956) to cause little diminution in the tissues. Nevertheless, they described the appearance of many effects attributable to histamine entering the circulation such as have been observed in the present experiments, for example, vasodilatation in the ear and prostration with respiratory effects. Evidence that there is a release of histamine from the tissues was obtained as follows.

a. Appearance of Histamine in the Plasma. - Guinea-pigs injected intraperitoneally with 10 mg./kg. Compound 48/80 and showing signs of histamine poisoning within 15-30 minutes had appreciable amounts of histamine-like substance present in the plasma (Table II). The activity of the substance was probably that of histamine since the contractile effect of both was abolished by mepyramine; furthermore, the return of the response to the active substance paralleled that of histamine when all mepyramine had been washed out of the bath (Fig. 5).

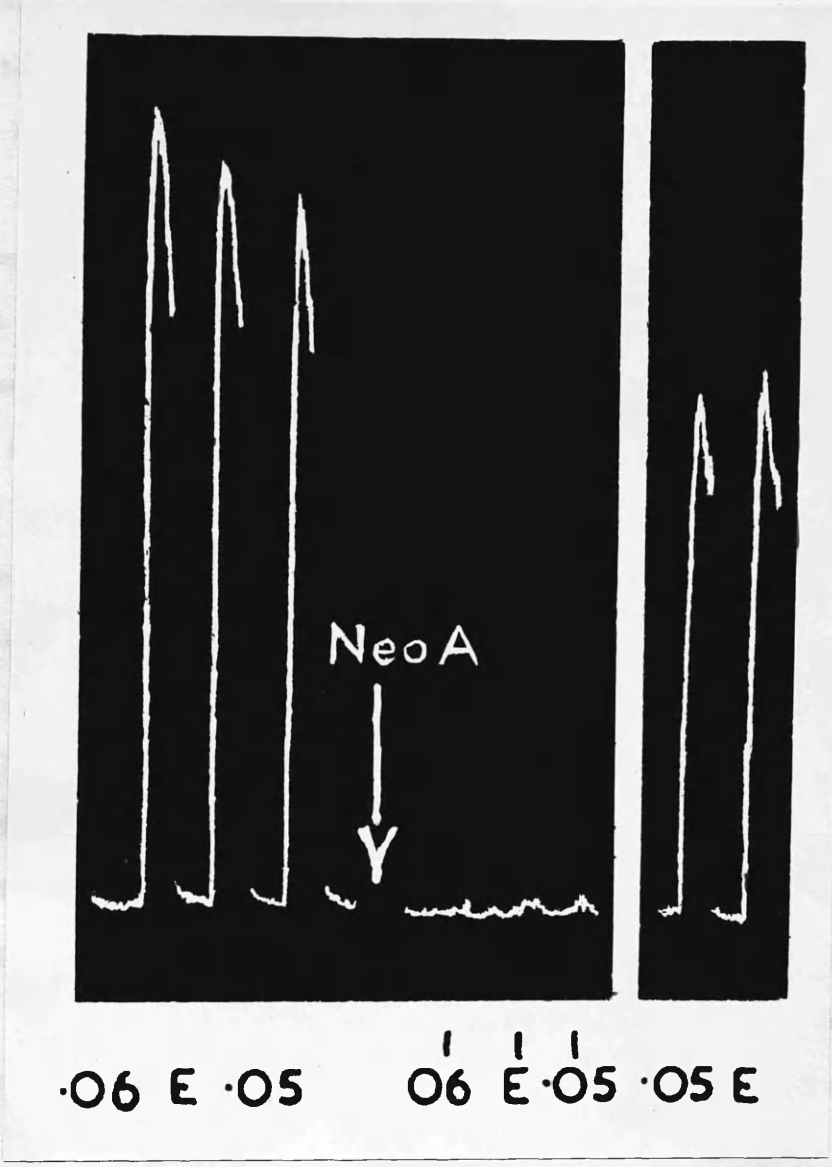

Fig. 5. Effect of 0.06 and 0.05 ug. histamine on guinea-pig's ileum preparation, set up in tyrode solution in 20 ml. bath. At E, 0.2 ml. extract of plasma was given. At arrow 0.4 ug. neo-antergan (mepyramine) added to bath abolishes the contractile effects. The final contractile effects of the extract and of 0.05 ug. histamine were obtained after repeated emptying and refilling of the bath to remove all traces of neo-antergan (mepyramine) (atropine 1: 500,000 solution added in 0.2 ml. solution throughout.)

b. Release of Histamine from Perfused Tissues. - To ascertain the origin of the released histamine various tissues and organs were perfused. Feldberg and Talesnik, (1953) have shown that histamine release in the rat occurs principally in such tissues as skin and skeletal muscles. In the guinea-pig, however, the histamine content of the skin is conspicuously low (Fig. 6), the values for the abdominal skin ranging from 3.2 to 9.9 ug./g. in the present experiments. There are, however, small areas of higher histamine content such as the skin of the ear, the submental region, the genital nipple, basal and orbital regions. On the other hand, in this species other distinct tissues such as lung and intestine are particularly rich in histamine and yielded values on extraction ranging from 42 to 23 ug./g.

Table III lists the values for histamine output after arterial injection of Compound 48/80 into the thoracic and abdominal visceral organs and into the lower extremities. Only when skin and skeletal muscle are perfused is there a substantial release of histamine, the output from the lungs, gastro-intestinal tract being of a much lower order. Vasoconstriction with temporary reduction in perfusion flow accompanied the release of histamine; intestinal peristalsis and distension of the lumen with secretion was observed after injection of Compound 48/80 into the perfused intestine.

c. Reduction in Tissue Histamine following Intravenous and Intraperit-:oneal Administration of Compound 48/80. - Following the injection of substantial/

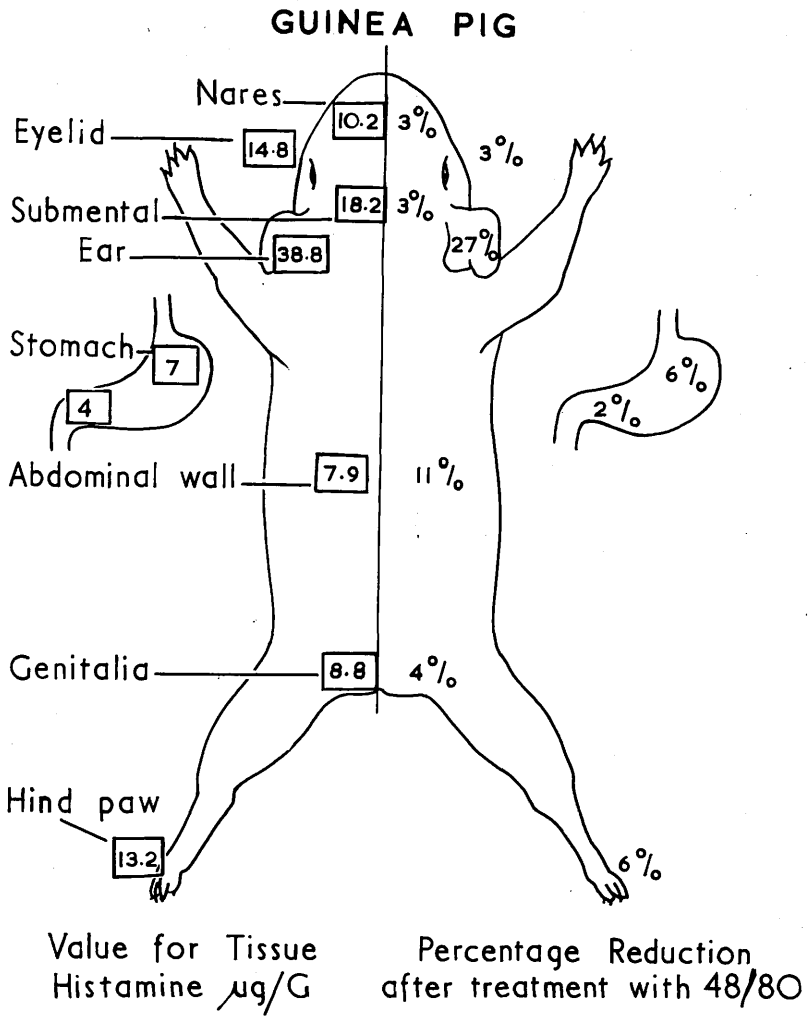

Fig. 6. Histamine values for various tissues of the guinea-pigs are listed (left); the effect of histamine liberation by Compound 48/80 is to diminish tissue histamine to the small extent indicated (right). (Average of three experiments).

substantial amounts of Compound 48/80 into guinea-pigs there was only a slight reduction of tissue histamine. The animals treated intraperitonealls showed a slightly greater reduction in the histamine of their tissues than the animals treated by immediate intravenous injection (Table IV, Fig. 6).

TABLE II.

PLASMA HISTAMINE.

Time after Injections (mins.)	Symptoms	Plasma Histamine (ug./ml.)
15	Prostration	0.24
15	Prostration	0.38
25	Shock	0.5
30	Shock	0.5
Control	-	0.005
Control	-	0.005

Plasma histamine in ug/ml. of 4 guinea-pigs after 10 mg./kg. Compound 48/80 intraperitoneally. As indicated, the animals were killed when seriously affected.

TABLE III

HISTAMINE OUTPUT.

Dose in mg.	Histamine Output (ug./ml.)		
	T	A	E
0.5	1.2	0.8	16.4
1.0	1.8	1.7	21.5
2.0	2.2	1.9	28.3
2.0	2.6	2.4	30.4

Histamine output (ug./ml.) in perfusate from thoracic viscera (T), abdominal viscera (A), and lower extremities (E), after various doses of Compound 48/80.

Several measures were adopted to modity the toxic action of Compound 48/80 which may have been the result of the sensitivity of this species to released histamine or to the toxic effects of the compound itself. This was evident as prostration, respiratory embarrassment, and, on post-mortem examination, gastric ulcers of the acute type. The gastric ulcers and erosions were more pronounced with the combination of Compound 48/80 and aminoguanidine (Fig. 7 B,C). To diminish the intensity and yet permit prolonged action Compound 48/80 was administered by intraperitoneal and subcutaneous injections dissolved in/

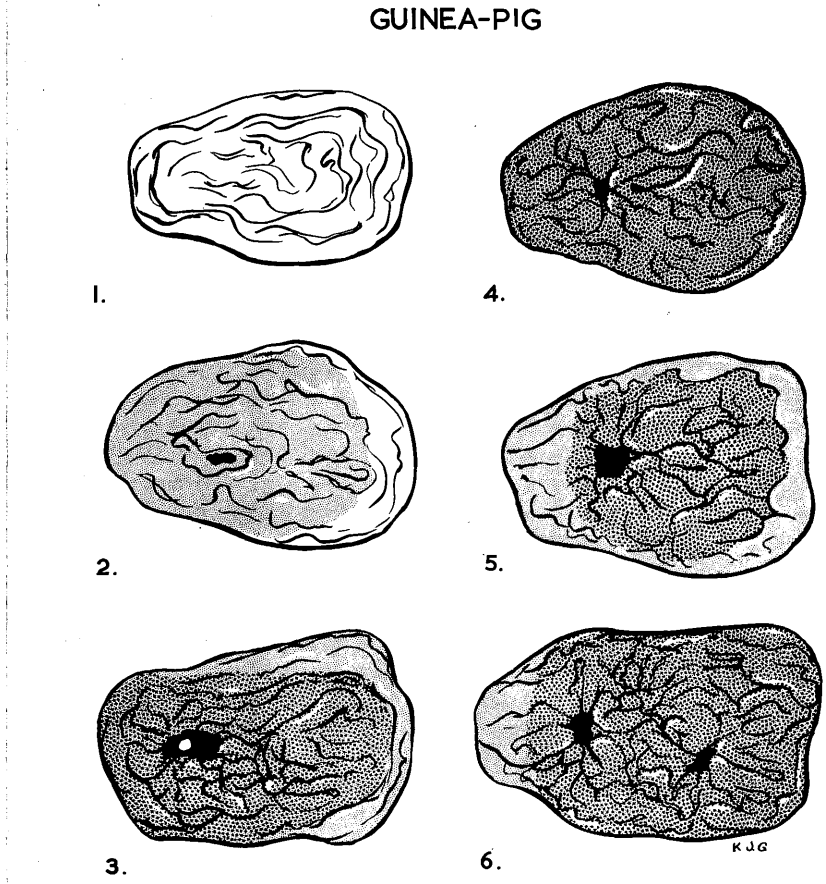

Fig. 7. Diagrammatic representation of guinea-pig's stomach. The density of stippling is an attempt to represent mucosal congestion as appearing from colour photographs from 3 animals, so treated in each group. A, Normal stomach. B, After Compound 48/80 intraperitoneally. C, After Compound 48/80 + aminoguanidine. D, After anaphylactic shock with horse serum intravenously. E, After Bolton toxin (gastrotoxin intraperitoneally). F, After Volton toxin (gastrotoxin) intraperitoneally + aminoguanidine subcutaneously.

TABLE IV

REDUCTION IN HISTAMINE.

Control Values (ug./g.) for Histamine of Skin Regions		Percentage Reduction after Compound 48/80		
		A	B (4-6 days)	C (10 days)
Ear	38.8	27	32	39
Submental	18.2	3	7	14
Abdomen	7.9	11	13	33
Paw	13.2	6	6	13
Eyelid	14.8	3	5	-
Nares	10.2	3	3	10
Genitalia	8.8	4	3	7

Reduction in skin histamine after treatment with Compound 48/80: (A) average values after intravenous injections (1.5-2.5 mg./kg.); (B) of animals examined after 4-6 days and (C) 10 days of intraperi-:toneal injections (total dose administered 20 and 46 mg./kg. respec-:tively; average of three experiments).

- - - - -

in a large bulk of saline or dispersed in a slow-release medium; in another group, histamine intoxication was avoided by antagonizing the released histamine with an antihistamine compound such as mepyramine. With none of these methods was it possible to get as marked a depletion as with rats, and there was little reduction in the rich stores of visceral/

visceral histamine (Table V). Comparison of the groups of animals treated over three weeks with Compound 48/80 shows that the histamine values are lowest for the mepyramine-injected group of animals. This may be accounted for by the enhanced release of histamine in the tissues, since mepyramine has itself the actions of a strong hist-:amine liberator (Arunlakshana, 1953).

Table V

REDUCTION IN HISTAMINE.

		Percentage Reduction after Compound 48/80 (Total Dose 68 mg./kg.)				Antigen-Antibody Reaction	Bolton Toxin (Gastro-toxin)
Control Values		1	2	3	4	5	6
Skin, ear	36.6	21	27	15	46	50	16
Abdomen	7.6	19	16	5	38	48	12
Skeletal muscle	4.1	16	17	1	28	31	6
Lung	38.4	2	9	1	36	33	10
Intestine	26.2	4	8	2	10	10	8
Plasma ug/ml.	0.01	-	-	-	-	0.52	0.38

Reduction in histamine after treatment with Compound 48/80 over three weeks with: (1) Subcutaneous injections; (2) Intraperitoneal, in 5 ml. saline; and (3) Dispersed in oily medium; (4) 5-25 mg./kg. mepyramine; (5) 0.5 ml. antigen in animals sensitized with horse serum; (6) Injection of Bolton toxin (gastrotoxin). (1-6 are the average values from groups of 8 experimental animals.)

3. Comparison of the Effects of Compound 48/80, Gastroxin (or 'Bolton Toxin'), and Sensitization. -

Eight guinea-pigs were treated with Compound 48/80 intraperitoneally in 5 ml. saline as in Table V. Similar groups were sensitized with horse serum and with Bolton toxin. Administration of the antigen produced similar but more severe prostration than that produced by Compound 48/80. There were comparable but less severe effects in those treated with Bolton toxin; for example, in the groups treated with antigen the effects developed within 1-2 minutes after intravenous injection, but were delayed for 2-5 minutes and lasted for 10-15 minutes only following the administration of Bolton toxin. Table V lists the reduction in histamine values in each case. The antigen-antibody reaction produced considerable fall in the histamine content of various tissues; after injection of Bolton toxin there was a small reduction in tissue histamine comparable to that elicited by Compound 48/80 in this species.

The effects of Compound 48/80 on the gastric mucosa were those of erosion and ulceration. Erosions were occasionally produced by single intraperitoneal injections (4 mg./kg.) of Compound 48/80 (Fig. 7 B), but the effects were greatly intensified by pre-treatment with aminoguanidine (Fig. 7 C) and the histamine values were augmented in plasma samples examined (Table I, B). Fig. 7 D, E, F illustrates the appearance of the stomach after anaphylactic shock, after/

after Bolton toxin, and Bolton toxin plus aminoguanidine. In each case there is marked engorgement of the gastric mucosa and the effects have been intensified by the antihistamine agent, which suggests that local release of histamine plays a part in their production. Conversely, the effects were diminished, or were absent, after prior treatment with Compound 48/80, and the appearances were substantially the same as in Fig. 7 A.

DISCUSSION.

On injection of Compound 48/80 and horse serum into rats there was a fair degree of histamine release into the blood-stream, one sequel of which was the occurrence of gastric ulcer-:arion. These effects were intensified by aminoguanidine, which is an agent which prevents the enzymatic destruction of histamine. In contrast, Compound 48/80, which is an agent which releases tissue-histamine stores, could be used to deplete the tissue-histamine stores slowly with minimal toxic effects; subsequent challenging with horse serum was then ineffective. It should be noted that horse serum in rats acts as a primary histamine liberator, that is to say, it can act without prior sensitization of the animal. Gastric ulceration may then be induced by horse serum acting like any other histamine liberator, without the need for sensitization by local administration directly into the stomach wall followed by later injection of antigen systemically at a later date, as described

by/

by Jahiel, Jahiel, and Krakauer (1952) for rabbits.

In guinea-pigs the effect on the histamine of skin and skeletal muscles is, by comparison with rats, not at all striking, but this may be the result of the fact that the local store of histamine in this species is not high at this site. In this species it is well known that the tissues of the lung and gastro-intestinal tract contain the greatest concentrations of histamine. Nevertheless, there was little release of histamine on arterial perfusion of the viscera with Compound 48/80; furthermore, after continuous treatment with it there was still a comparatively small effect on the histamine at these sites. It may be that there is a limiting factor to release of larger quantities of tissue histamine, provided to offset susceptibility of this species to histamine itself. It was estimated for instance by Mayer (quoted by Dubos, 1952) that the guinea-pig was at least 700 times more sensitive to histamine than the rat.

Attempts were made to minimize the reaction to the release of histamine by injecting large volumes of Compound 48/80 in dilute concentration, by injecting it in a retard medium, and by injecting mepyramine to antagonize the released histamine. The greater reduction in tissue histamine occurred when Compound 48/80 and mepyramine were administered together. A possible explanation lies in the fact that the antihistamine, mepyramine, is paradoxically a strong histamine-releasing agent (Arunlakshana, 1953) and the two substances may together/

together exert an enhanced effect.

Guinea-pigs treated with Compound 48/80, though showing the slightest evidence only of reduction of histamine in the tissues, showed typical histamine effects, as did others sensitized with horse serum after administration of the antigen; similar effects were produced by gastro-:toxin or Bolton toxin. The effects of gastrotoxin were furthermore aggravated by the use of aminoguanidine which prevents destruction of histamine by histaminase. Such a result strengthens the belief that gastrotoxin exerts its effects via the intermediary agency of histamine. This was again proved likely by preliminary slow release of the labile histamine of the tissues with small doses of Compound 48/80 administered some time before the injections of gastrotoxin. Under these conditions gastrotoxin could be rendered ineffective. It is concluded that the specific gastrotoxin described by Bolton exerts its effects as a histamine liberator.

SUMMARY.

1. Intravenous and intraperitoneal injections of Compound 48/80 in guinea-pigs and rats cause gastric erosions and ulceration which are the result of histamine release.

2. Prolonged treatment with Compound 48/80 effected considerable reduction in the tissue histamine of rats. The reduction of tissue histamine of guinea-pigs was by comparison slight; more thorough release of histamine was prevented by the outstanding sensitivity of guinea-pigs to small amounts of released histamine. Measures to antagonize or

counteract/

counteract the intensity of the histamine-like effects were adopted, but these in turn influenced the process of histamine release. In the case of mepyramine, the histamine liberation from the tissues was even accentuated.

3. Horse serum produces the same effects as a histamine liberator in rats; the gastric effects are intensified with the antihistaminase, aminoguanidine. The effect of horse serum is invalidated by prior histamine release effected by small doses of Compound 48/80 given over a prolonged period.

4. Release of histamine accounts for the action of gastrotoxin or Bolton toxin in guinea-pigs. Comparable effects on the stomach are produced by Compound 48/80 and anaphylactic shock. Aminoguanidine acting as an antihistaminase intensifies these effects. Prior treatment with Compound 48/80 in small doses over a prolonged period invalidates the effects of gastrotoxin given subsequently.

CHAPTER FIVE.

The Distribution of a Synthetic Antihistamine and its Effects on Tissue Histamine, with a Method for its Biological Assay in the Tissue.

CHAPTER FIVE.

THE DISTRIBUTION OF A SYNTHETIC ANTIHISTAMINE AND ITS EFFECTS ON TISSUE HISTAMINE, WITH A METHOD FOR ITS BIOLOGICAL ASSAY IN THE TISSUE.

There is now a unanimous body of opinion that, while the synthetic antihistamines are capable of blocking the systemic affects of histamine, they fail to antagonise the secretagogue action on the parietal cells. This has been strikingly demonstrated by Halpern (1948) who showed that although guinea-pigs could be protected against the systemic effects of as much as 1500 lethal doses of histamine by antihistamine, many died within 48 hours as a result of perforation of gastric ulcers.

Kay and Forrest (1956) examined the action of a synthetic antihist-:amine applied locally to the secretory surface of the gastric mucosa; topical application, which it can be argued, ought to enhance local penetration of the drug, markedly suppressed the acid secretory response to histamine.

Were it possible to measure the quantities of an antihistamine distributed in the tissues after systemic or oral administration of it, it would be of interest to determine how much antihistamine was actually taken up by the stomach. Absence of the antihistamine from gastric tissue after systemic injection might be a reason for the failure of antihistamine to annul the acid secretory effect of histamine. It seemed important to devise a method to study the tissue distribution of/

of antihistamines for this reason.

METHODS.

1) Dose responses of antihistamine substance.

A preparation of the guinea-pig's ileum, to which had been added atropine in $1:10^7$ concentration, was made to contract at 80 second intervals to known amounts of histamine left in the organ bath for 20 seconds. The antihistamine was added to the bath in small amounts with histamine and washed out with it after 20 seconds. The first histamine contraction was unaffected, provided the amount of the histamine antagonist were 0.05 ug mepyramine or less. When, however, further histamine contractions were repeated at 60 second intervals, the second and third contractions were depressed in amount. The antihistamine effect was therefore a delayed one : more than one minute had to elapse before it became evident. The antihistamine effect was estimated by noting the extent of diminution of the second or third contraction, whichever was diminished to the greater extent.

The depressant effects of various amounts of mepyramine maleate on the contractile effect of 0.05 ug histamine are illustrated in Figure 1. In the accompanying diagram, the histamine depressant effect of mepyramine is recorded as follows: the standard 0.05 ug histamine contraction was measured in centimetres and given the value of 100. The reduced contractions after the antihistamine were also measured in/

in centimetres and recorded on a graph as a percentage of the standard height of the original contractions. The effects of 0.025, 0.03 and 0.04 and 0.05 ug mepyramine are recorded in this Figure, both on the original experimental record and after transposition to a diagram are illustrated reading downward from 100%,as unshaded areas on a dark background. It will be apparent from Figure I that the inhibitory effects become greater with increase in concentration of the antihistamine, or vice versa. The minimum reproducible effect of this type was that produced by 0.02 ug mepyramine. Serial administration of 0.04 ug mepyramine demonstrated the constancy of the inhibitory effect. In each case after the antihistamine was washed out of the bath, histamine was added repetitively till the normal contraction height was re-established before beginning a further test.

If antihistamine substances could be obtained in extracts from the tissues, an assay procedure might be devised comparing the effects of the extracts with the effects of small amounts of antihistamine solutions on the histamine stimulated guinea pig's ileum preparation; in such an assay the extracts and standard solutions would have to be alternated till their respective strengths were assessed.

2) Trial extraction of antihistamine drugs from the tissues.

It had been observed that, when histamine was extracted from tissues of guinea-pigs injected with mepyramine maleate, the antihistamine inter-:fered with the assay of histamine by inhibiting its stimulant action on/

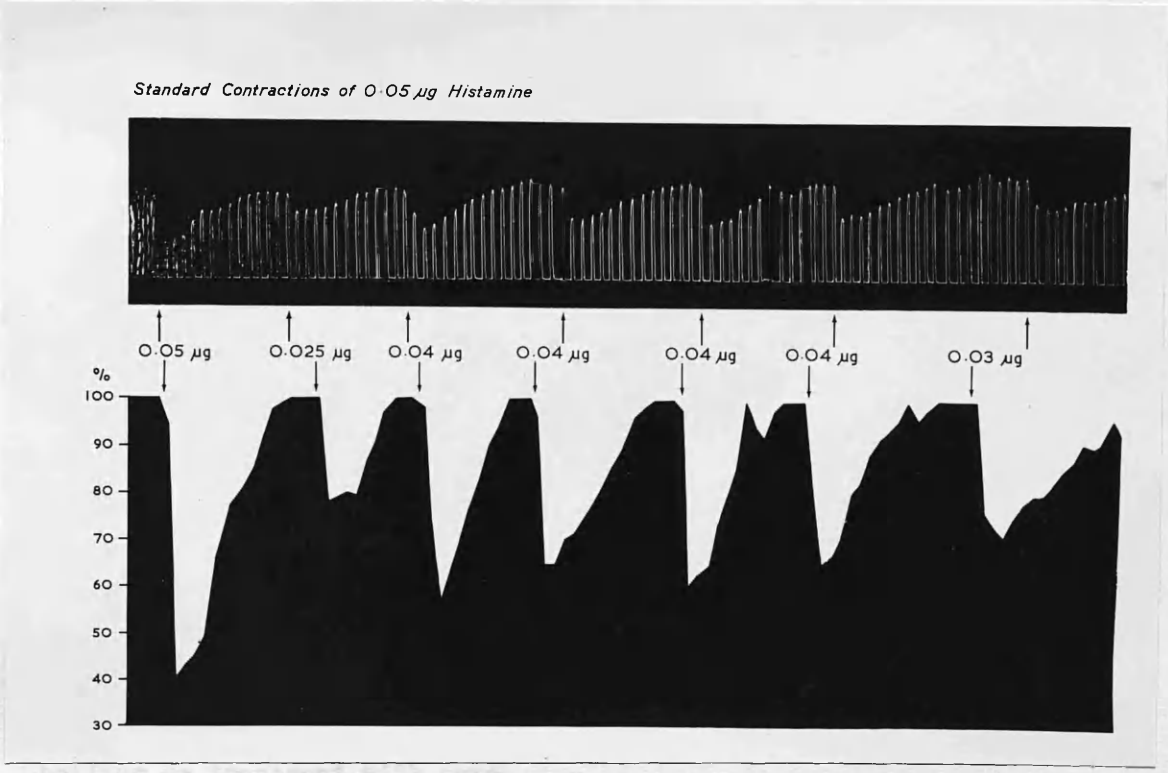

Fig. 1 The top tracing is a record of the depressant effects of 0.025 ug, 0.03 ug, 0.04 ug and 0.05 ug mepyramine maleate on the standard contractile effect of 0.05 ug histamine on the guinea-pig ileum preparation.

In the lower record the depressant effects are recorded as a fall below 100%. The white inverted peaks cutting into the black background give a measure of the % inhibition of the standard effect e.g. 0.05 ug mepyramine reduced the histamine contraction to 40% of what it was formerly.

on the guinea-pig ileum. Since the method of extraction for histamine was the one described by Feldberg and Schachter (1952), the steps of this procedure were applied to the tissue, to see whether the histamine antagonist was extracted and assayed to the full.

a) Effect of boiling in acid on the stability of mepyramine.

20 ml. of 0.1 ug/ml solution of mepyramine maleate was boiled with 2.5 ml. N/HCl for 10 minutes. The solution was cooled, and neutralised to pH 7.4 with BDH indicator. The volume was made up to 25 ml. saline. One ml. of this solution was diluted ten times; 0.5 ml. of this solution ought to contain 0.04 ug mepyramine had it withstood treatment. It was compared with a non-boiled solution treated identically and neutralised, and also with a solution of mepyramine alone, diluted to 1:10 million from stock solution. From the assay recorded in Fig. 2 it may be deduced that the histamine antagonist is in no way reduced by the boiling or treatment with acid.

b) Test extraction of mepyramine added to tissue samples.

Mepyramine was added to patches of abdominal skin taken from a 200 g guinea-pig. The portions of skin were cut into small pieces in a mortar and 18.5 ug/G mepyramine added to each, before adding 1 ml N/HCl. The tissue was then ground with fine sand, boiled and filtered and neutralised. It was finally diluted with saline before testing on the guinea-pig's ileum preparation. It was estimated that 9 ug/G were recovered in a first test on the first sample; repeated assays on the first sample performed, after allowing the/

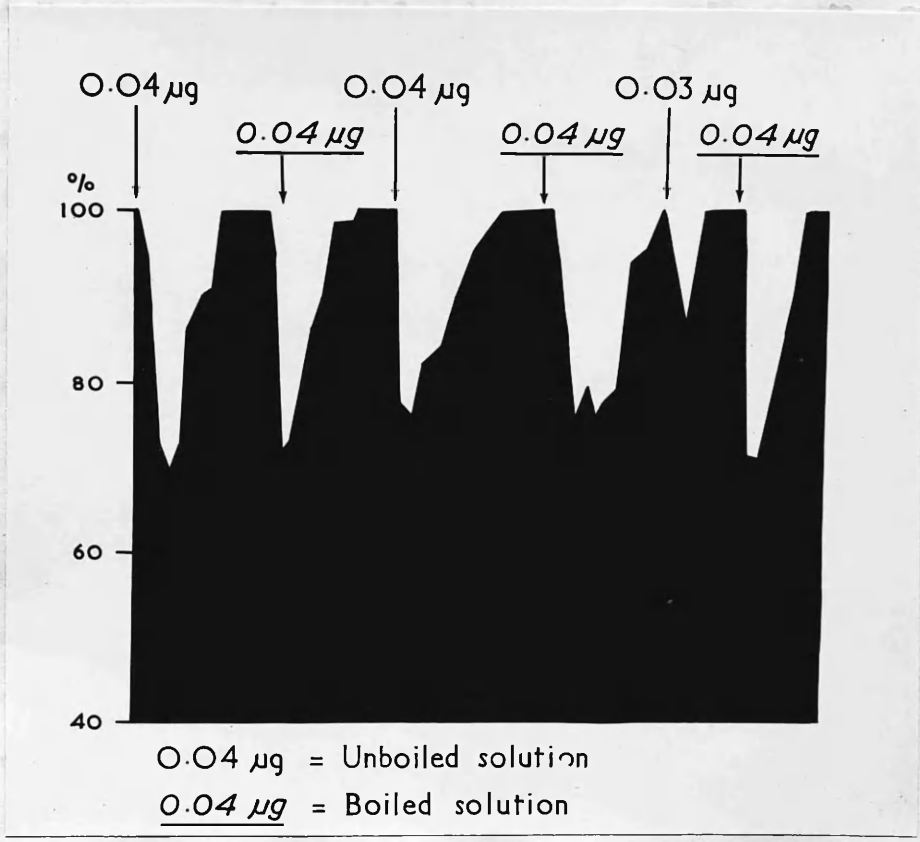

Fig. 2 illustrates inhibitory effects (the inverted white peaks) for 0.04 ug mepyramine maleate, both boiled and unboiled. Boiling does not diminish the inhibitory effect. The effect of 0.03 ug mepyramine is also shown.

the extracts to stand 2 and 4 hours respectively gave values of 12.5 ug/G and 14.3 ug/G. The assay for the four hour sample is presented in Figure 3. The amount of antihistamine detected 12 hours later was not significantly greater (15.1 ug/G). It was concluded that the assay done at four hours gave a sufficient estimate (80%) of antihistamine present since duplicate tests with a second specimen of the same tissue gave values of 10.1 ug/G at the first test, 12.9 ug/G at two hours, 15.8 ug/G at four hours and 15.6 ug/G at 12 hours. A much smaller amount of antihistamine, it will be observed, was detected in the first extraction of both these tissue samples. The concentration, however, increased progressively after allowing the acid extracts to stand for some time. It appeared as though the antihistamine was attached to something in the tissue matrix or colloid and was precipitated; after a period of time the antihistamine seemed to have become free in the supernatant of the extract which would explain why the concentration in this layer increased the longer the acid saline extract was allowed to stand before filtering.

Various factors were investigated to help minimise the loss of antihistamine substance during the extraction procedure. These were as follows:-

1) <u>Possible binding to colloid substance in the extract.</u>

The precipitate was re-extracted. When in a first test there had/

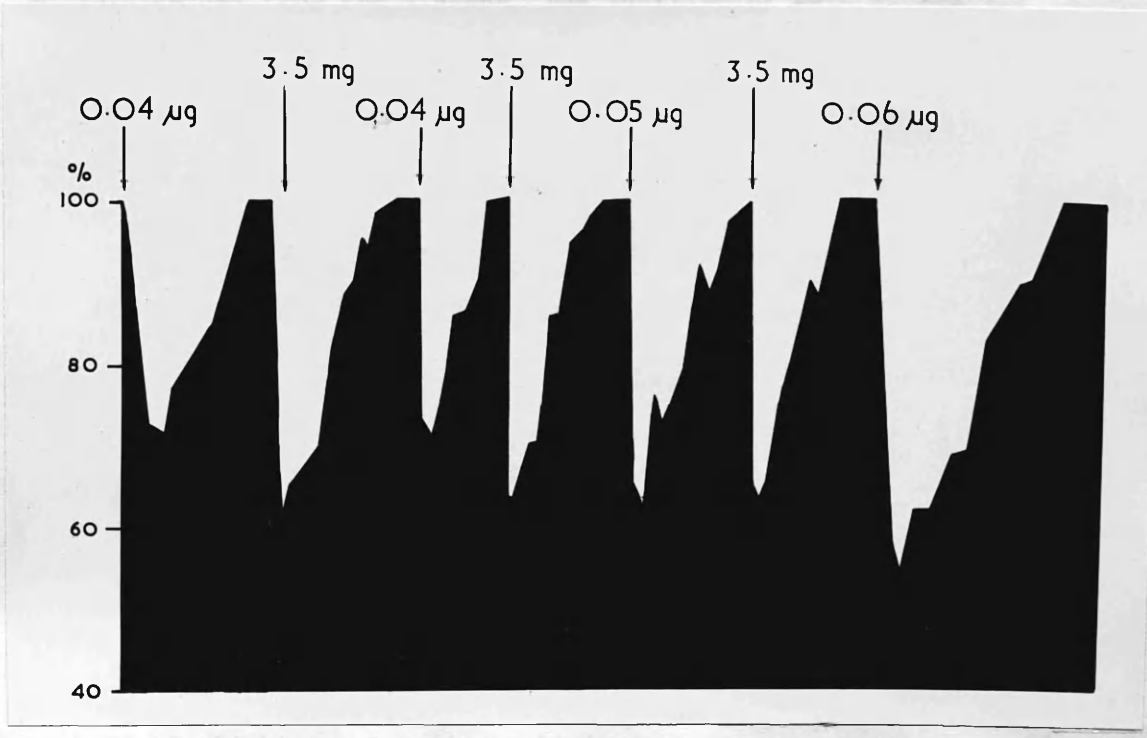

Fig. 3 shows an assay in which the inhibitory effects of various amounts of mepyramine have been compared with that of 3.5 mg. of the extract in solution - 0.04 ug in the first and third applications gave comparable depressant effects. 3.5 mg. was equiv-:alent in its effects to slightly less than 0.05 ug.

had been a loss of 8.5 ug, a second test reduced this to 3.2, thus showing that a double extraction might lead to a recovery of 15.3 ug out of 18.5 ug.

2) Inactivation of antihistamine in the tissues.

If enzymatic destruction played any part in the disappearance of antihistamines from the tissues, the addition of acid before the addition of the antihistamine should have annulled this. N/HCl was added to a piece of skin to which mepyramine was then added to give a concentration of 18.5 ug/G; extraction of the tissue was performed and the extract was allowed to stand for 4 hours before filtration yielded 14.5 ug/G. The converse of this experiment was carried out in which the skin was allowed to stand with mepyramine in contact with it for periods of 30 minutes to 2 hours. At the end of these times acid was applied and the tissue macerated, diluted with saline, boiled, filtered and neutralised; after 30 minutes contact, the mepyramine assay was 14.1 ug/G; after 2 hours contact with mepyramine, the assay figure was 14.3 ug/G.

3) Combination with tissue histamine.

Arunlakshana has shown that mepyramine lowers the histamine values for the guinea-pig's tissues, in 'in vitre' experiments. She has postulated that some antihistamine may also be histamine releasing agents. It might be that some loss of antihistamine substance occurred in the extraction procedure from the tissues were some of the histamine releasing substance and histamine to go into some form of combination at the moment of release. Were loss of histamine antagonist due to an inactivation of this type, a great fraction of it might be

extracted if/

If all the histamine had first been released. A portion of guinea-pig's skin was incubated with 1 mg compound 48/80, which is a potent histamine liberator, (Paton, 1951). The solution, 2 ml. Tyrode was decanted off after 5 minutes and the tissue washed. Mepyramine was added and the extract filtered after four hours, neutralised and tested. The amount recovered was not enhanced; it remained at 14.6 ug/G.

4) Technique of assay; artefacts leading to abnormal results.

In practice it has been found that the most satisfactory assay was obtained by maintaining constant the quantity of extract to be tested and assaying with varying amounts of antihistamine. (See Fig. 3). Greater difficulty in obtaining comparable effects with a standard dose of antihistamine was more usual when the amount of the extract was varied; the erratic effects necessitated the abandoning of the assay. This may have been the result of exciting agents as well as the inhibitory antihistamine being present in the extract. An increase in the first two antihistamine responses (Fig. 4, 0.03 ug mepyramine) was usually associated with failure of the assay; small quantities of extracts were given to attempt to minimise the variability but it will be seen that the responses to the second and third applications of 0.03 ug antihistamine were still irregular.

5) Routine method of assaying antihistamine drugs in tissue extracts and the assay in the presence of antihistamine.

From the above experiment the following technique was evolved:-

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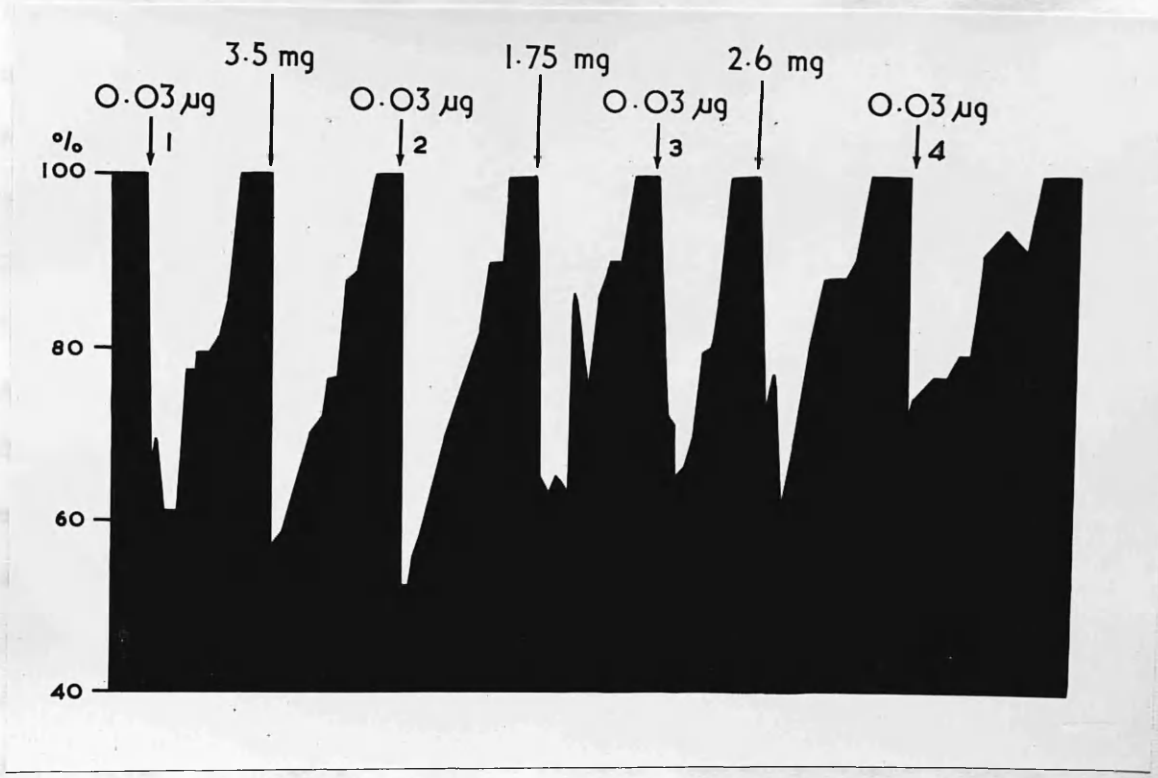

Fig. 4 illustrates a type of attempted assay which usually had to be abandoned. The applications of 0.03 ug mepyramine at 1 and 2 do not give comparable effects. In spite of altering the amount of extract applied a stable effect is not achieved with each successive 0.03 ug.

the assay was not begun on a fresh guinea-pig's ileum preparation but only after it had been suspended in the organ bath for at least two hours. Tissue was taken from the animal which had been injected with the antihistamine, weighed on a torsion balance and covered with N/3 or N/HCl. It was then cut up and ground with sand in a mortar. The acid extract was washed with saline into an Erlenmeyer flask and boiled; the tissue disrupted and a precipitate formed. The contents of the flask were then removed to a measuring cylinder, B.D.H. indicator was added and N/3 NaH added to the neutral point. This solution was allowed to stand for 4 hours and the supernatant was then decanted off. Acid extraction was then twice repeated on the precipitate and the solutions thus obtained were combined and assayed for their antihistamine potency.

It has already been mentioned that antihistamines such as mepyramine did not block the first histamine contraction when they were added to a guinea-pig's ileum preparation contracting to fixed standard doses of histamine. If much histamine were present in the extracts it might be expected to increase the contractile effect of the guinea pig's ileum with the result that the balanced sequence of contractions would almost certainly be upset; before final assay of the antihistamine an approx-:imation to the amount of histamine present in the extract had to be made and this quantity of histamine added to the rest solutions so that like effects would be obtained. (This factor was not taken into account/

account in the assays recorded in Figures 3 and 4 because the tissue extracts in these instances were chosen as having particularly low, almost negligible, histamine values). The histamine present in the extracts was assessed by diluting the extracts to the point where no further antihistamine effects were obtained. It was found in practice that this meant dilution of the antihistamine below a concentration of .02 ug/ml. The antihistamine effect in the solution obtained by extrac-:tion of tissue was thus eliminated and the histamine potency of the solution determined by direct comparison with histamine solution on the guinea-pig's ileum.

RESULTS.

a) Recovery of antihistamine 'in vivo'.

Five mg/kg mepyramine was injected intraperitoneally into three guinea-pigs. One and a half hours later tissue was removed after stunning the animals and bleeding them. Assays for antihistamine were performed as described above. The figures (in ug/G fresh tissue) are given in Table I. It appears that some tissues have consistently more antihistamine in them than others; to illustrate this the value for one area, e.g. abdominal skin, was taken as unity and all other values for each guinea-pig are compared against this. (Table 2). Brain, spleen and intestine contain more antihistamine than other tissues; the skin of the other regions examined (paw, submental and ear) contain amounts approximately the same as the abdominal skin. Liver contains lesser/

TABLE 1.

Recovery of antihistamine (mepyramine maleate) from the tissues of guinea-pigs treated by intraperitoneal injection of 5 mg/kg. Mepyramine is expressed as ug/g wet tissue.

- - - -

Tissue		Animal 1	Animal 2	Animal 3
Skin	Abdomen	2.5	1.5	2.8
	Ear	4.5	8.2	1.0
	Submental	3.0	5.4	1.5
	Paw	2.3	3.4	1.5
Gastro-intestinal	Stomach	1.0	0.8	1.2
	Intestine	5.6	4.8	3.6
	Liver	2.3	1.1	1.5
C.N.S.	Brain	5.2	6.2	7.5
Others	Spleen	3.6	3.0	4.2

TABLE 2.

Recovery index of mepyramine from various tissues relative to the antihistamine in the abdominal skin taken as unity.

- - - -

	Animal 1	Animal 2	Animal 3
Ear	1.8	6.1	0.4
Submental	1.2	3.2	0.54
Paw	0.9	2.2	0.54
Stomach	0.38	0.61	0.41
Intestine	2.2	3.2	1.3
Liver	0.9	0.6	0.5
Brain	2.1	4.1	2.7
Spleen	1.4	2.0	1.5

lesser amounts still, but the lowest values of all were found in the stomach.

Four human subjects about to have a partial removal of the stomach for duodenal ulceration were given 100 mgm. mepyramine maleate intra-:muscularly 30 minutes before operation. Biopsy samples were removed from the margins of the skin wound, from the muscles of the abdominal wall and from the excised stomach about 60 minutes after commencement of the operation. Examination of this material were performed in duplicate. Table 3 shows the relative amounts present in both groups; the amount of antihistamine present in the skin layer is high, muscle layer has much smaller amounts and gastric tissue has a concentration which is still lower.

TABLE 3.

Average recovery of antihistamine in ug/g, in four subjects, listing various tissues. (Skin, skeletal muscle and stomach).

	ug/g mepyramine	Recovery index (abdominal skin as unity)
Abdominal skin	6.3	1.0
Rectus abdominis	3.7	0.58
Body of Stomach	1.1	0.17
Pyloric antrum	0.8	0.13

(b) Release of histamine by mepyramine.

Five mg/ug were injected twice daily into eight guinea-pigs. Two of these were killed 24 hours later. 24 hours later two more animals were killed and further injections of the remainder were given, this

this procedure being kept up till at the beginning of the fifth day the remaining two were killed. Portions of tissue from each pair of animals were therefore removed 24 hours after the last injection. Extracts were made as has been described for the assay of a histamine inhibitory substance, having first determined the amount of histamine present on a fresh guinea-pig's ileum preparation. As a final check the extracts containing the inhibitor, and histamine with the estimated amount of mepyramine added to it were compared with one another.

The results of the injection of mepyramine over four days are tabulated below (Table 4).

Histamine release occurred in substantial amounts after four injections over two days (i.e. in animals examined on day 3); pooled samples taken at this time show that there had been a 41% reduction of the histamine in the abdominal skin, a 64% reduction of histamine in the ear, 71% for the submental region and 44% for the paw. In the viscera the histamine value for lung tissue was reduced by 50% but the liver and gastrointestinal histamine showed no appreciable difference. The highest affinity for antihistamine was shown in the skin of the abdomen and ear and in the lung; the skin of the submental region and paw also showed appreciable amounts of antihistamine. The liver and intestine had amounts which were only one fifth of the amount present/

TABLE 4.

Histamine (H) in ug/g and mepyramine (M) in ug/g detected by assay in the tissues of guinea-pigs injected twice daily with mepyramine. (Each value represents assay for pooled sample from guinea-pigs, the tissue extraction being performed 24 hours after the last injection).

	Controls		2 injections		4 injections		6 injections		8 injections	
	H	M	H	M	H	M	H	M	H	M
Abdomen	4.2	-	4.0	trace	3.8	3.5	2.5	7.5	2.8	3.0
Ear	27.5	-	22.4	"	18.8	3.8	10.5	9.0	14.5	3.5
Submental	18.4	-	16.0	"	9.5	-	5.5	3.0	8.0	1.0
Paw	8.4	-	8.2	"	9.8	4.0	4.7	3.0	4.7	3.2
Stomach	3.2	-	3.2	"	3.8	1.05	3.2	0.8	3.4	1.2
Intestine	28.2	-	26.4	"	32.6	1.9	26.4	1.6	20.5	2.4
Liver	1.0	-	1.05	"	1.0	1.5	1.0	1.6	1.0	2.25
Lung	35.6	-	27.4	"	10.5	2.4	10.9	5.2	18.0	2.8

present in the abdominal skin, and the stomach least antihistamine of all, amounting only to one tenth of the amount present in the abdominal skin.

Pooled samples from the animals injected for four days showed no further reduction of tissue histamine. Indeed the values for histamine had increased slightly by the fourth day; although the values for mepyramine were the same in the peripheral tissues, the recovery of antihistamine had become greater in the visceral tissue samples.

DISCUSSION.

A method has been devised for the assay of antihistamine substances by measuring their depressant effect on known histamine contractions of the guinea-pig's ileum preparation. The method has been extended to the assay of antihistamines in tissue extracts after the investigation of the factors which affect the recovery of an antihistamine added to fresh tissue. Possible factors reducing the amounts of antihistamine present were persistant attachment of the antihistaminic to the colloid precipitate, inactivation at the moment of histamine release or by enzymes in the fresh tissue. Adequate correction for the sources of error were made by early addition of acid to the tissue and re-extraction of the precipitate, after allowing the extracts to stand for four hours at an acid pH before filtering.

Another factor which had to be obviated in assaying these substances was the occasional variability or loss of sensitivity of the histamine-stimulated/

stimulated guinea-pig's ileum preparation to the depressant effect of antihistamine. A preparation highly sensitive to histamine and a standard inhibitory dose of mepyramine which reduced the inhibitory response by not more than 20% were used. The dose of the extract alone was kept constant and the antihistamine dosage varied; mepyramine solution and extract were alternated in the performance of each test.

The recovery of antihistamine 'in vive' from various tissues in the guinea-pig, shows that the tissue with the greatest affinity for mepyramine is that of the central nervous system; this perhaps explains the profound depression of central nervous functions which follows intoxication with antihistamine. The tissue with the least affinity for mepyramine appears to be the stomach wall; the small intestine has slightly greater amounts of antihistamine present. In human tissue samples there was less antihistamine in the stomach wall than in skin or skeletal muscle biopsy specimen removed at operation in the duodenal ulcer patients.

It has already been suggested by Arunlakshana that mepyramine may act as a histamine liberator; our experiments confirm this by finding a marked reduction of the histamine of certain tissues. The relatively poor protection afforded to the effects of 48/80 by mepyramine in certain species may be the result of the fact that mepyramine was not so much acting as an antihistamine substance but exerting histamine releasing effects along with compound 48/80. Numerous workers have

now/

now shown that antihistamines antagonise the effects of histamine in many tissues of the body; one notable exception, however, is that gastric secretion in the stomach in response to histamine is not affected. Halpern has shown that guinea-pigs, injected with hist-:amine and mepyramine develop gastric ulcers as a result of the marked acid secretion and that these ulcers may perforate. Paton and Schachter found that not only was mepyramine unable to prevent the acid secretion following injection of compound 48/80 but that the acid secretion also increased in amount after both injections. Histamine release in the tissues with histaminemia as a result of compound 48/80 and mepyramine both acting as histamine liberators would explain this finding. Little evidence of this histamine release would be expected if mepyramine still manages to exert its antihistamine effects wherever it becomes localised; the finding that mepyramine has a poor affinity for the stomach wall might explain the failure of the antihistamine to exert an inhibitory effect at this site. It is therefore of great interest that Kay and Forrest were able to annul the acid secretory effects of hist-:amine by a technique which ought to have raised the local antihist-:amine concentration greatly in the stomach, by the topical applic-:ation of the antihistamine to the secretory part of the gastric mucosa.

SUMMARY.

1) A method of assaying an antihistamine substance such as mepyramine has been/

been devised; it has been adapted to the assay of histamine and antihistamine in mixtures.

2) The amount of antihistamine in guinea-pig tissue has been measured; it was highest in the brain and lowest in the alimentary tract. The antihistamine appears to release histamine mostly from skin and lung, but little change occurs in the gastrointestinal tissues.

3) Tissue from human subjects injected with mepyramine shows higher levels of antihistamine in skin and skeletal muscle than in gastric tissue.

CHAPTER SIX.

The Distribution and Release of Histamine in human Gastric Tissue.

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THE DISTRIBUTION AND RELEASE OF HISTAMINE IN HUMAN GASTRIC TISSUE.

The wall of the alimentary tract of many species has a very high content of histamine (Feldberg, 1956). In the dog, Douglas, Feldberg, Paton and Schachter (1951) found that the histamine was almost all in the mucosa. The concentration was greatest in the body of the stomach, less than half as large in the pyloric antrum and very high in the duodenum; the concentration then fell progressively down the alimentary canal. Feldberg and Harris (1953) attempted to define the precise cellular components in which the histamine was distributed in the dog and showed that the highest values for histamine in the mucosa of the stomach correlated well with the distribution of the parietal cells in the gastric mucosa. They could not exclude, however, the possibility that some of the histamine was distributed in other tissues, such as the mucous cells present in the glands of this region.

The participation of histamine in normal acid gastric secretion in the human stomach has yet to be proved. Nevertheless, histamine is outstanding in its importance as the most powerful substance known to excite gastric secretion (Emmelin & Kahlson, 1944; Obrink, 1948; Teorell, 1933; Teorell, 1937) and if this substance were found in high concentration close to the parietal cells, themselves the sources of the hydrochloric acid of the gastric juice, this association would support the/

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the view that the secretion of acid by the gastric mucosa may normally be brought about through the agency of histamine. Trach, Code and Wangensteen, (1944) found substantial quantities of hist-:amine in excised human gastric mucosa; in their opinion the amounts present were capable of local and humoral stimulation of acid gastric secretion, but they found no consistent difference in histamine concentration between the body of the stomach, the parietal cell-bearing area, and the pyloric antrum.

This chapter records experiments to determine whether the body of the human stomach has more histamine than the pyloric antrum, to ascertain which layer of the stomach wall has the highest histamine concentration, and to assess how much histamine may be released from each layer.

METHODS.

Sixteen specimens of human gastric tissue, obtained fresh from the operating theatre following subtotal gastrectomy, were studied. Eight specimens were from patients suffering from duodenal ulceration (Group A) and eight from patients suffering from gastric carcinoma (Group B).

(a) Prior to operation the basal (non-stimulated) acid gastric secretion and the maximum acid secretion in response to histamine (Kay, 1953) had been studied. For this latter test 100 mgm. of the antihistamine, mepyramine maleate, was injected intramuscularly one hour/

hour before the subcutaneous injection of histamine acid phosphate (0.4 mgms. per 10 kilo body weight). Gastric secretion was collected continuously by aspiration for 30 mins. commencing collection one quarter of an hour after the injection of the histamine. The volumes of secretion was noted and the acidity determined by titration with N/10 using Topfer's reagent.

(b) The excised stomach was laid open along its greater curvature and washed. Full thickness pieces of the stomach wall were removed, weighed, and macerated by grinding with sand in a mortar in N HCl. The pieces were taken from two parts of the stomach wall, namely near the proximal end of the specimen (body of the stomach) and near the distal end (pyloric antrum). In the cancer cases the part selected was confirmed to be free from malignant disease. The extract was then prepared and assayed for histamine as described by Douglas et al. (1951).

(c) In another series of experiments the various layers of the gastric wall were separated. With the tissue spread out on a cork board, the mucosa was first dissected off the outer layers and then the submucosa with muscularis mucosae adherent to it (Feldberg & Harris, (1953), was separated from the tunica muscularis. (Fig. 1). Small portions of tissue from each of these three layers were cut, weighed on a torsion balance, and their histamine content estimated, again using the method of Douglas et al. (1951).

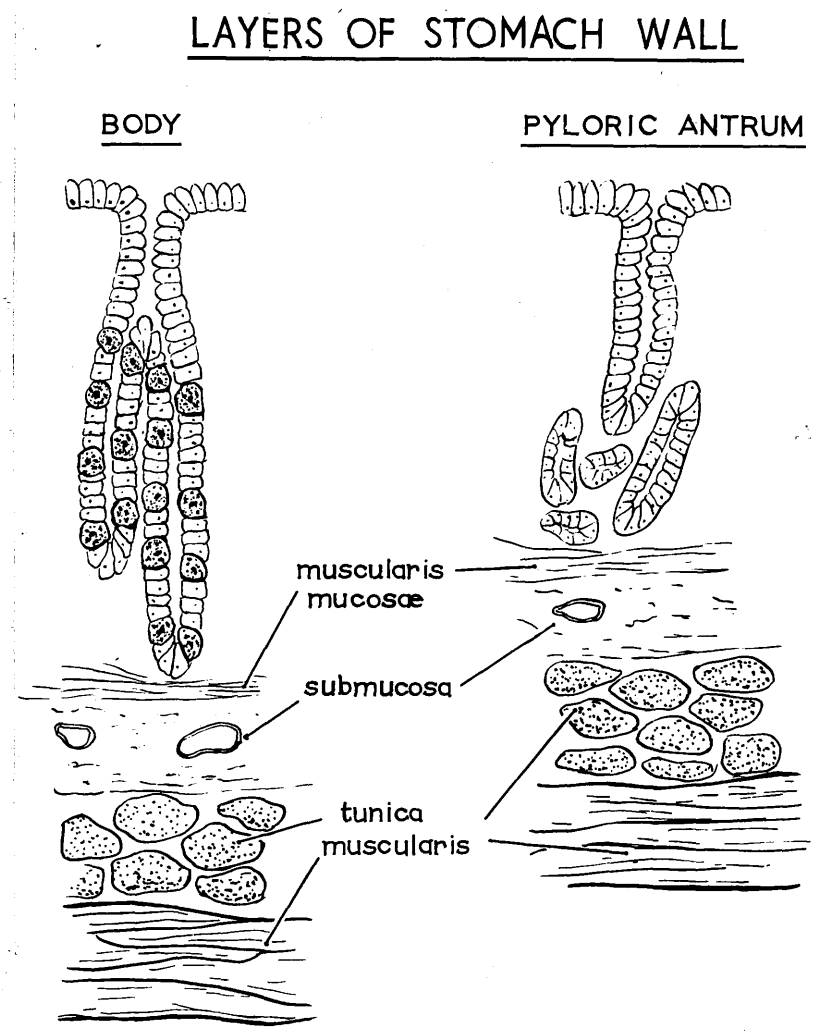


Fig. 1. This diagram outlines the various layers of body of the stomach and of the pyloric antrum. The glandular structures lie in the mucosa, which has at its junctional area with the submucosa the muscularis mucosae. Outwith these structures lies the tunica muscularis.

(d) To study the distribution of the histamine of the stomach wall in relation to its cellular constituents, small flattened pieces of stomach wall were frozen on the stage of a freezing microtome with the mucosal aspect uppermost. (Fig. 2). The tissue was then cut in horizontal planes (i.e. parallel to the mucosal surface). Each section was cut 10μ thick, proceeding through the mucosa in successive layers down through the submucosa as far as was desired. Alternate sections were weighed, extracted and assayed for histamine, or were examined histologically. The sections for histological examination were fixed in 10% formol and, after dehydration, were stained with iron haematoxylin and Van Gieson's stain. By comparing histological features and histamine estimations it was possible to construct curves or "histamine profiles" in which the ordinates represent the histamine concentration and the abscissae the position of the sections in the depth of the stomach wall.

(e) In studying the histamine content of gastric tissue as in (b), (c) and (d), all the histamine present in every cell of the tissue is presumably freed by destruction of the cells. This is a necessary part of the procedure of extracting histamine from the tissue in order to estimate the amount of it present in the tissue. Histamine may, however, be released from the tissues without observable damage, through the agency of chemical releasing substances, one of which, compound 48/80, is a potent histamine liberator (Paton, 1951). The use of such a sub-stance may help to indicate how much histamine is released in the normal functioning of a tissue.

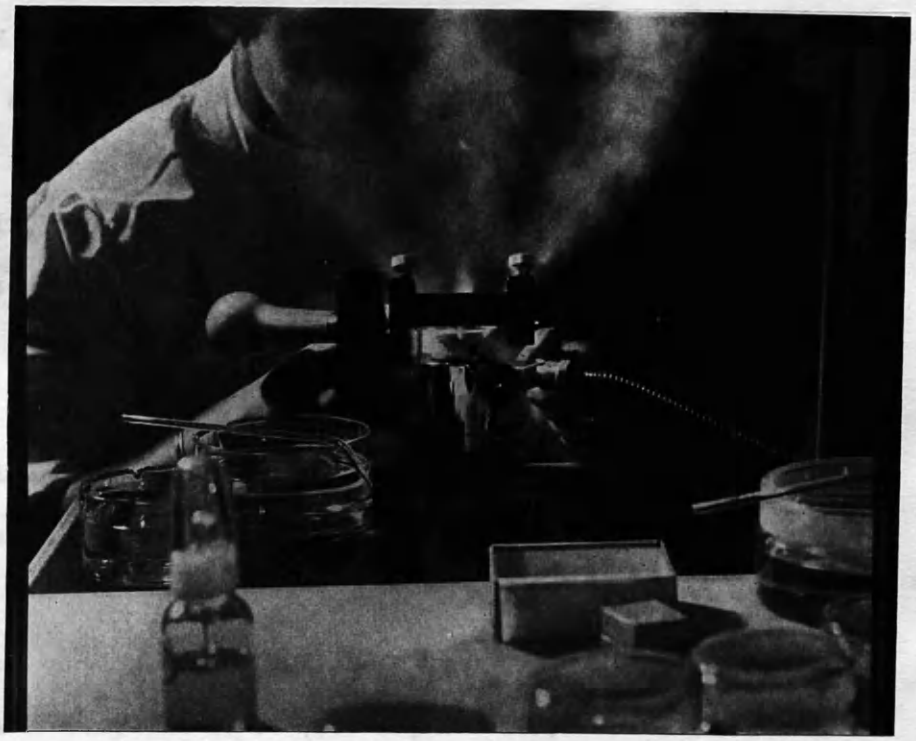


Fig. 2 illustrates the use of the freezing microtome technique to cut fine section of gastric tissue. The tissue, flattened and frozen with its mucosal aspect uppermost, is cut horizontally in thin sections 10 µ thick.

The release of histamine from the separated mucosal, sub-:mucosal and tunica muscularis layers has been quantitatively examined by adopting the methods of Mongar and Schild (1952). Small thin strips of mucosa, submucosa and tunica muscularis were washed for about 20 minutes in 100 ml. Tyrode solution at 37°C and incubated with a histamine liberator (compound 48/80 200 mu/ml.) in 2 mls. Tyrode solution at 37°C for 10 minutes, the solution being well stirred by bubbling oxygen through it. To allow for spontaneous release of histamine, duplicate strips were incubated in Tyrode solution alone; the histamine slowly accumulated in the Tyrode was assessed as a spontaneous release, amounting to 6.3% ± 1.2* of the total histamine content of the strip in Group A, and to 6.2% ± 1.6* in Group B. This has been allowed for when calculating the true release after application of Compound 48/80 (see (e) Results). To exclude the possibility of errors in the assay resulting from the presence of the "slow contracting substance" which is liberated by chemical releasing agents (Paton, 1951), duplicate tests were performed in which mepyramine was added in sufficient quantity to antagonise the contractile effect which had been estimated in the assay and had been presumed to be histamine abolition of the contractile response by the histamine antagon-:ist, mepyramine, confirmed that histamine alone was present.

RESULTS.

(a) In the eight ulcer cases (Group A) the basal secretion of acid was/

* Standard error of the mean.

was high (mean = 230 mg. HCl ± 21). The maximum histamine stimulated secretion was 694 mg. HCl ± 21*. In the eight cancer cases (Group B) the basal secretion of acid was lower (mean = 48 mg. HCl ± 9*) and the maximum histamine stimulated secretion was also lower (224 mg. HCl ± 41 *).

(b) Table I shows the histamine content of full thickness pieces of the stomach wall removed from the body of the stomach and the pyloric antrum in eight cases of duodenal ulcer (Group A) and eight cases of cancer (Group B). It will be seen that in every instance the piece removed from the body of the stomach contained more histamine than the corresponding piece from the pyloric antrum, but there was no more histamine present in the ulcer cases than the cancer cases.

(c) The histamine content of the separate layers of the stomach wall was estimated in four ulcer cases and four cancer cases. In every instance the histamine content of the mucosa was higher than that of the submucosa, which was higher than that of the tunica muscularis (Table 2). This gradient of histamine was just as evident in the layers removed from the pyloric antrum as in those from the body of the stomach. There was no significant difference between the ulcer cases and the cancer cases.

(d) Figures 1 and 2 show typical "histamine profiles" constructed after comparing histological appearances and histamine concentration in slices at different depths of the stomach wall. They are comparable to the appearance of the histamine profiles obtained for the dog by Feldberg and Harris (1953). The/

* Standard error of the mean.

TABLE 1.

HISTAMINE CONTENT OF FULL THICKNESS PIECES OF STOMACH WALL REMOVED FROM THE BODY OF THE STOMACH AND THE PYLORIC ANTRUM.

	GROUP A (ULCER CASES) Histamine ug/g		GROUP B (CANCER CASES) Histamine ug/g	
	BODY OF STOMACH	PYLORIC ANTRUM	BODY OF STOMACH	PYLORIC ANTRUM
1.	42.1	21.4	23.4	18.1
2.	29.7	16.5	36.8	20.6
3.	63.8	31.8	46.2	29.6
4.	26.8	21.1	22.6	16.2
5.	38.5	26.0	33.3	19.7
6.	40.1	27.5	56.5	27.3
7.	20.2	12.8	31.5	17.8
8.	19.8	12.5	19.2	11.1
Mean	35.1	21.2	33.7	20.1
S.E.	± 5.1	± 2.4	± 4.5	± 2.1
DIFFERENCE BETWEEN MEANS (A - B)	BODY OF STOMACH 1.4		PYLORIC ANTRUM 1.1	
S.E. OF DIFFERENCE OF MEAN	±6.8		±3.2	
SIGNIFICANCE	0.9> P> 0.8		0.8> P> 0.7	

TABLE 2.

HISTAMINE CONTENT OF SEPARATE LAYERS OF THE STOMACH WALL (MUCOSA, SUBMUCOSA, TUNICA MUSCULARIS) REMOVED FROM BODY OF STOMACH AND PYLORIC ANTRUM IN 4 ULCER CASES and 4 CANCER CASES.

	GROUP A (ULCER CASES) HISTAMINE ug/g						GROUP B (CANCER CASES) HISTAMINE ug/g					
	BODY OF STOMACH			PYLORIC ANTRUM			BODY OF STOMACH			PYLORIC ANTRUM		
	M.	S.M.	T.M.	M.	S.M.	T.M.	M.	S.M.	T.M.	M.	S.M.	T.M.
1	28.4	19.2	8.2	24.6	14.4	2.9	29.3	16.1	10.4	28.4	14.0	7.9
2	56.1	34.1	10.2	44.2	28.2	9.8	38.4	28.4	7.2	22.8	18.2	6.8
3	36.8	17.3	5.8	20.5	12.1	5.3	99.8	32.4	10.1	40.6	27.5	10.0
4	33.7	25.4	6.9	25.6	18.0	6.5	27.6	16.8	6.5	18.4	14.5	6.3
Mean	38.8	24.0	7.8	28.7	18.2	6.1	48.8	23.4	8.6	27.6	18.6	7.8
S.E.	±6.1	±3.8	±.95	±5.3	±3.6	±1.4	±17.2	±4.1	±.99	±4.8	±3.1	±.82

	BODY OF STOMACH			PYLORIC ANTRUM		
	M.	S.M.	T.M.	M.	S.M.	T.M.
Difference between means (A = B)	10	0.6	0.8	1.1	0.4	1.7
S.E. of difference of means	18.2	5.6	1.4	7.1	4.7	1.7
Significance	P=0.6	P>0.9	0.6>P>0.5	0.9>P>0.8	P>0.9	P = 0.4

The body of the stomach (Fig. 3) had a profile with two peaks, one approximately in the region of the parietal cells and the other in the region of the muscularis mucosae of the submucosa. In the pyloric antrum (Fig. 4) the profile had only one peak and this was in the region of the depths of the pyloric glands.

The total amount of histamine located in the peak areas was calculated from the histamine concentration in sections cut in the region of each peak, using data from histamine profiles of the first four specimens in Groups A and B. The total histamine in ug of the sections was obtained by dividing its histamine concentrations ug/g by its weight. The total histamine of each peak zone was the summation of the values for all the sections cut in the region of the peak, counting the alternate sections used for histological purposes as having the same histamine value as the preceding one used for assay. The peak regions, two for the body and one for the pyloric antrum, have been labelled 1, 2 and 3 respectively in Table 3, which lists the total histamine present in each peak. There was no significant difference in the histamine content of the peaks from the stomachs with a high acid secretion obtained from duodenal ulcer cases (Group A) over the peaks from stomachs with a low acid secretion from cancer cases (Group B).

(e) The histamine released by a histamine liberator from thin strips of mucosa, submucosa and tunica muscularis was estimated by examining the histamine accumulating in the surrounding medium when gastric/

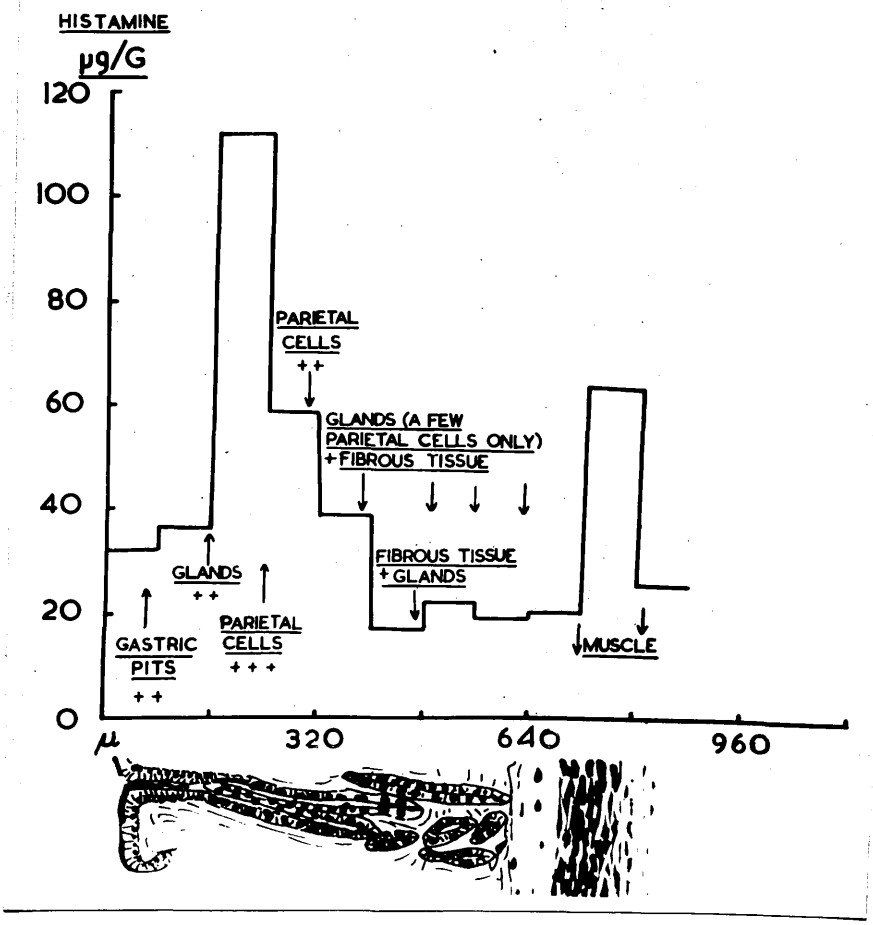


Fig. 3. Histamine profile for the corpus or body of the stomach (3A, Table 1). The vertical axis gives the concentration in ug/G histamine: the horizontal axis the depth in u at which the histamine concentrations or various histological structures were found. (The tissue has been sectioned through the mucosal layer as far as the muscularis mucosae and submucosa).

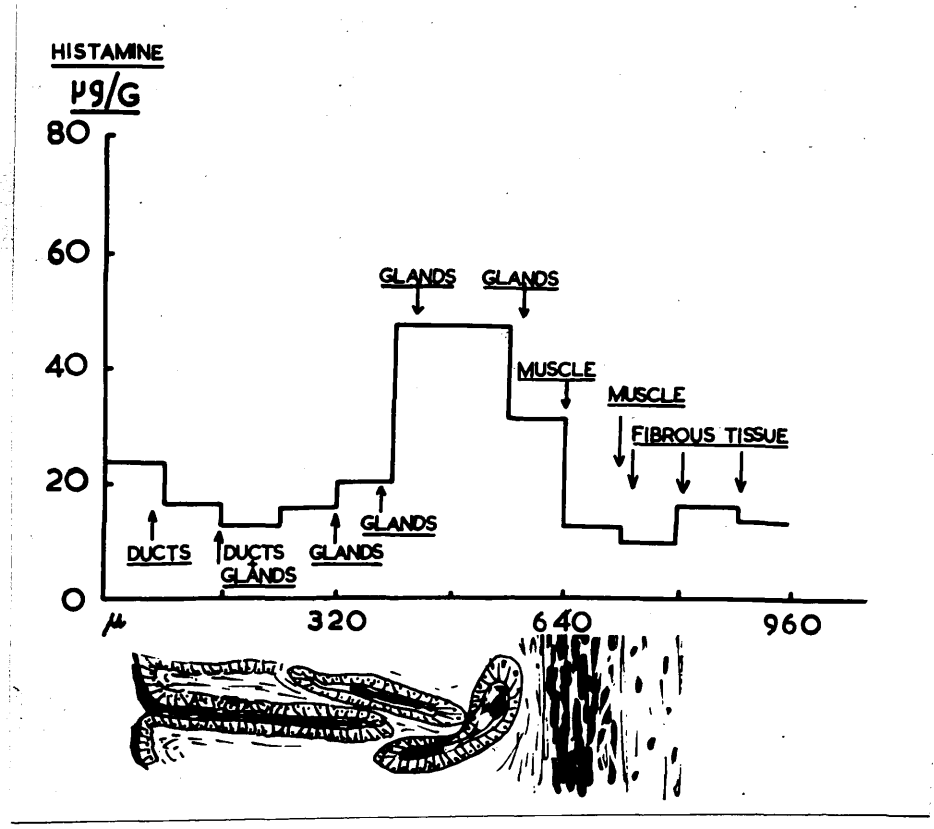


Fig. 4. Histamine profile for the pyloric antrum of the stomach (3A Table 1). The tissue has again been sectioned as far as the submucosa.

TABLE 3.

THE TOTAL HISTAMINE IN THE THREE PEAKS OF THE HISTAMINE PROFILES FOR STOMACH NOS. - 1 4 OF TABLE 1 HAS BEEN ESTIMATED IN GROUPS A AND B.

	GROUP A (ULCER CASES) ug HISTAMINE PER PEAK				GROUP B (CANCER CASES) ug HISTAMINE PER PEAK			
	1	2	3	Total	1	2	3	Total
1.	34	17	18	69	32	21	24	77
2.	41	19	26	86	41	26	29	96
3.	51	33	41	125	37	23	25	85
4.	30	26	31	87	26	19	24	69
MEAN				92				82
S.E.				± 11.9				± 11.6
DIFFERENCE BETWEEN MEANS (A - B)				10				
S.E. OF DIFFERENCE OF MEANS				± 13.1				
SIGNIFICANCE				P = .05				

gastric tissue, histamine liberator and Tyrode solution were incubated together, after discounting the spontaneous release from the tissue (see Methods).

Table IV records the percentage of the total histamine released by Compound 48/80 in the various layers of the stomach wall. Much more histamine was released from the mucosa and submucosa than from the tunica muscularis. More was released in each case from the sub-:mucosa than from the mucosa. The values for the body of the stomach were higher than those for the pyloric antrum. The histamine liberated from the mucosa of the body and pyloric antrum in Group A was not significantly different from the release at the same sites in Group B. The liberation of histamine in the submucosal layer, however, was significantly higher in Group A than in Group B; thiswas the case for both the body of the stomach and the pyloric antrum.

DISCUSSION.

Histamine has been studied in the mucosa of the stomach of many species (Douglas et al., 1951; Emmelin & Kahlson, 1944; Feldberg, 1956; Feldberg & Harris, 1953; Gavin et al., 1933; Trach, Code & Wangensteen, 1944). For the dog, it is agreed that there is usually more histamine in the region of the body of the stomach than in the pyloric zone, and that the mucosal layer contains the highest concentration. Trach, Code and Wangensteen (1944), examining tissue from the human stomach found histamine values ranging from 3.5 to 24.1 ug/g for mucosa of the body/

TABLE 4.

THE HISTAMINE RELEASE FROM TISSUE TAKEN FROM STOMACHS WITH A HIGH ACID SECRETION (GROUP A) HAS BEEN COMPARED WITH TISSUE FROM GROUP B, WHICH HAD A LOW ACID SECRETION. THE PERCENTAGE RELEASE FROM EACH LAYER (M = Mucosa, S.M. = submucosa, T.M. = tunica muscularis) for THE TWO SITES, NAMELY BODY OF STOMACH AND PYLORIC ANTRUM, HAS BEEN COMPARED IN THE TWO GROUPS A AND B; THERE IS A SIGNIFICANTLY GREATER RELEASE OF HISTAMINE FOR THE SUBMUCOSAL LAYER.

	Group A (Ulcer cases)						Group B (Cancer cases)					
	Body of Stomach			Pyloric Antrum			Body of Stomach			Pyloric Antrum		
	M.	S.M.	T.M.	M.	S.M.	T.M.	M.	S.M.	T.M.	M.	S.M.	T.M.
1.	9.2	22.6	2.1	3.1	19.1	1.6	10.5	16.5	1.8	3.5	0.8	1.3
2.	6.6	28.9	2.8	6.2	17.4	1.8	12.6	16.9	3.1	5.8	11.8	2.8
3.	8.5	29.5	3.5	4.8	22.3	2.7	6.3	16.8	1.2	2.2	12.9	1.3
4.	9.1	26.0	1.8	4.9	20.2	1.3	8.5	14.0	2.1	4.6	13.1	1.4
Mean	8.4	26.8	2.6	4.8	19.8	1.9	9.5	16.1	2.1	4.0	11.9	1.7
S.E.	±0.6	±1.6	±0.38	±0.65	±1.05	±0.3	±1.35	±0.7	±0.4	±0.75	±0.75	±0.37

	Corpus			Pylorus		
	M.	S.M.	T.M.	M.	S.M.	T.M.
Difference between means (A-B)	1.1	10.7	0.5	0.8	7.9	0.2
S.E. of difference of means	1.5	1.8	0.55	0.99	1.3	0.47
Significance	$P = \overline{0.5}$	P<0.01	P = 0.4	0.5>P>0.4	P<0.01	0.7>P>0.6

body of the stomach and from 3.0 to 12.5 ug/g for the mucosa of the pyloric antrum. They compared tissue from the body of the stomach and antral region but did not find that the histamine in the mucosa of the body of the stomach regularly exceeded that of the pyloric region. In the present experiments an increased amount of histamine was found in the region of the body of the stomach in the great majority of experiments. This is the region in which the parietal cells are active in the formation of hydrochloric acid. Since histamine is the most active substance eliciting acid gastric secretion, additional histamine at this site suggests that it may serve a purpose in evoking secretion from the gastric glands if locally released.

Construction of histamine profiles for the region of the body of the stomach showed that there was a high concentration of hist-:amine in roughly the same region as the parietal cells. This suggests, but does not prove, that the histamine is in these cells to help promote their normal function, which is the formation of hydrochloric acid. A second region of high histamine concentration was found opposite the smooth muscle of the muscularis mucosae layer. In the pyloric antrum, histamine was found to be present in greater amounts in the region of the depths of the pyloric glands, where these glands penetrate to the level of the muscularis mucosae.

Tissue from duodenal ulcer patients was examined to determine whether/

whether a higher local concentration of histamine might explain the persistently high rate of acid secretion associated with this condition. No significant difference in histamine concentration could be found between the tissue from stomachs with a high acid secretion and the tissue obtained from stomachs with a low acid secretion.

The release of histamine took place to a greater extent in the mucosa and submucosa of the body of the stomach than in the corresponding layers of the pyloric antrum. Much of the histamine in the body resides in the mast cells (Riley, 1954), and the histamine of these cells is the most readily released by histamine releasing agents (Fawcett, 1954; Riley & West, 1955). Riley and West (1955) have shown that there are large numbers of mast cells in the mucosa, muscularis mucosae and submucosa of the gastric tissue of the pig. The fact that a histamine releasing agent caused a copious release of histamine from the mucosa and submucosa of the body of the human stomach suggests that there may be mast cells at this site.

It is not clear why the submucosa should release so much more of its histamine than the mucosa, which has a richer histamine content. Feldberg and Harris (1953) found that when the mucosa and submucosa were separated by dissection that the muscularis remained attached to the submucosa; the conclusion may be drawn that release of submucosal histamine may be taking place in the smooth muscle cells of the muscularis mucosae. This seems unlikely since Walder (1953) found that the/

the muscularis mucosae of the human stomach was comparatively insensitive to histamine.

It may be that the greater quantity of histamine released in the submucosa is related to the plentiful supply of blood vessels in the vascular plexus of this region and also to the mast cells in the vessel walls. The release of histamine occurred in greater amount in the submucosal layer of the duodenal ulcer stomachs, which are always held to be exceedingly vascular in comparison with normal stomachs. It seems quite unlikely that the release of histamine is related to the additional dissection at operation on ulcer cases, since the spontaneous release from damaged cells was not higher in the ulcer cases than in the cancer cases. The enhanced release of histamine is more attributable to some change in the submucosa itself

SUMMARY.

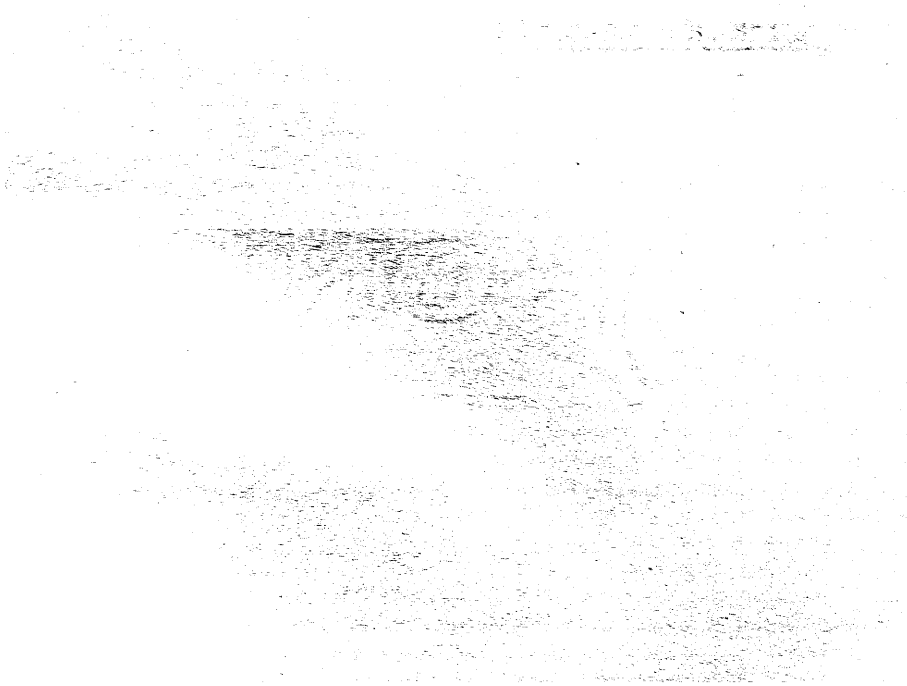
(1) Histamine was present to a greater extent in tissue form from the body of the stomach than from the pyloric antrum.

(2) Histamine was present in higher concentration in the mucosa than in the submucosa or tunica muscularis: on construction of histamine profiles it seemed likely that much of the histamine of the body of the stomach was in the same region as the parietal cells.

(3) The amount of histamine present in the stomachs with high acid/

acid secretion from duodenal ulcer cases was not greater than in stomachs with a low acid secretion obtained from cancer cases.

(4) The release of histamine, following application of a histamine liberator to the tissues, was greater in the mucosal and submucosal layers of the body of the stomach than in the correspond-:ing layers in the pyloric antrum; the release of histamine was highest in the submucosae, and was also greater in duodenal ulcer cases than in cancer ones.



CONCLUDING DISCUSSION.

CONCLUDING DISCUSSION.

There is scarcely any organ in the body which does not contain some histamine. Yet fifty years after its isolation there is much disputation as to whether this substance has or has not a physiol-:ogical role. The experiments described in the various chapters of Part 2 of this thesis give an account of how the gastrointestinal role of this substance has been investigated with the aid of histamine liberators.

In the years 1951 to 1955 many papers dealing with histamine liberators and their effects were written describing the application of such substances to problems such as the histamine release in anaphylaxis, the methods for depleting tissues of histamine were evolved, and a range of chemical histamine liberators was evaluated. It was appropriate that these concepts and experiments should come from the National Institute for Medical Research where Sir Henry Dale had elaborated so much of our knowledge of histamine. In 1951 the author was privileged to join a team working on the problem of histamine release and took up the study of histamine releasing substances and their effects on acid gastric secretion and motility.

What conclusions may be drawn from the material which has been gathered from experiments on animals and human tissue over the years since then, and is set out in the foregoing chapters? In the first place the action of histamine liberators on gastrointestinal histamine indicates/

indicates that there is more than one physiological type of histamine. There would appear to be one type, a lesser fraction, of histamine in the gastrointestinal wall released by compound 48/80; in contrast, a large fraction remains resistant to the action of a substance such as this. This difference is suggested both indirectly, taking the acid secretory response following on close intraarterial injection of histamine liberator into various tissues as an indication of histamine release, and also by direct examination of any changes in tissue histamine. The histamine of the gastrointestinal tract is relatively resistant to the action of histamine liberators. The histamine which is most readily released is situated in the body of the stomach. At this site it has been shown that there are more mast cells than at other sites in the gastrointestinal tract. The hypothesis is advanced that labile histamine in such mast cells may be part of a mechanism for initiating gastric secretion, "priming, as it were, the pump".

What is the nature of the histamine which is resistant to the action of chemical releasers? The work of Schayer has established on a firm basis by means of radio-active isotopic techniques, that much of the gastrointestinal histamine is derived locally from histidine through the activity of histidine decarboxylase. (Schayer, 1954, 1955, 1956, 1957). His work strengthens the view that histamine in many organs is essentially elaborated and destroyed locally. One might envisage a balanced turn over of such histamine in intestinal

tissue/

tissue which is rich in histaminase. Yet the very fact that gastric tissue itself lacks the destroying enzyme suggests that any excess of histamine formed locally, or circulating there after general release from the tissues, may have to be dealt with by absorption of histamine or binding of it to the tissues. In experiments in which histamine entered the circulation in fairly large amounts, the one region where this trend could be observed was in the pyloric antrum, where in the absence of a tissue histaminase, the histamine values rose. The fact that the histamine values for the body of the stomach did not rise significantly indicates that during the acid secretion provoked by histamine some histamine is allowed to escape via the portal of the parietal cells. There is therefore a case for regarding some histamine as histamine absorbed into the gastrointestinal tract perhaps as a temporary measure before destruc-:tion or excretion.

It would support our view that histamine release may be an activating mechanism of acid gastric secretion were histamine liberators found also to initiate motor movements in gastrointestinal tissue, associated with local demonstrable changes in its histamine. Various chemical histamine releasers were tested for this and were shown to be capable to producing contractile, tonic and rhythmic effects. The case is argued for the initial contractile effect being related to the release of the extrinsic histamine of Dale (1948), while the/

the secondary rhythmic effects may be due to release of intrinsic histamine, and the subsequent increase in tone in plain muscle appears to be the result of the outward diffusion of histamine following the release of histamine; this was proved by the removal of the Tyrode solution from one preparation and testing it on another intestinal strip.

Histamine liberators may cause gastric ulceration; this was seen in the experiments in which compound 48/80 was injected repetitively in the cat. Since many sera and toxins have been shown to cause gastric ulceration, it seemed possible that an action involving histamine release - either in the manner of the chemical histamine releasers or after cellular damage - might explain the mechanisms underlying the action of a substance such as gastro toxin, which has in the past assumed an important role in the physiopathology of gastric ulceration. The importance of histamine release by gastro toxin was demonstrated by the fact that this substance lost its gastro cytotoxic effects after depletion of tissue histamine by chemical releasing substances - (providing the chemical releasing substances were applied to the organism deliberately in small quantities, often, so as not to induce gastric ulceration.) The converse was also shown that compound 48/80 and gastrotoxin would all the more readily produce gastric ulceration in animals also given injections of aminoguanidine which annuls the activity of histaminase and intensifies all the histamine release effects.

In/

In chapter 5 the paradox that antihistamines are also histamine releasing substances was further explored. The failure of antihist-:amine substances to antagonise gastric secretion has become an intellectual as well as a practical problem to the gastrointestinal investigator. If one could unravel the mystery of why antihistaminics omit the parietal cell from the many sites at which they annul the effects of histamine, an advance might be made in our knowledge of the essential relationship between histamine and the workings of the parietal cell. We have confirmed that antihistamines may lower tissue histamine (certain sites such as lung may be markedly affected) but it seems from a study of the distribution of histamine antagonists in various tissues, that one reason for the lack of antihistamine efficacy in the stomach is the much lower distribution of these substances in gastric tissue.

Lastly, a study has been made of human material to determine the layer of greatest histamine concentration and whether histamine was present in higher quantity in the acid secretory zone of the stomach than in the pyloric antrum. Histological techniques were employed to try to decide whether histamine was related to particular structures in the stomach wall, such as the parietal cells. It would appear to be in favour of histamine being concerned with the acid secretory process that more histamine was found in the mucosa of the stomach in its acid secreting portion and that the greatest amount/

amount was in the region of the parietal cells. Yet, when material was taken from stomachs secreting excessive amounts of hydrochloric acid and this was compared with material from stomachs secreting a low amount of acid, little or no difference was found. Nevertheless, it must be kept in mind that the rate of turnover of histamine may be more important than measurement of the actual concentration at any one time. When histamine liberators were applied to thin portions of material from these two types of stomach the histamine of the submucosa was found to be the most dynamic in yielding itself up. This may indicate a particularly labile type of histamine, and it is suggested once more that this may be mast cell histamine which acts in this layer to ensure vasodilatation of blood vessels and enrichment of blood supply via the submucosal vascular plexus when this is required. The histamine released, probably to this end, was far greater in the stomachs obtained from duodenal ulcer cases; if histamine metabolism is deranged in duodenal ulcer patients it would seem merely to be by way of local upset in tissue histamine in one site such as the submucosa; certainly it is not a general derangement since many observers have found that the urinary histamine is not elevated. All the evidence available today would appear to indicate a <u>local</u> role for histamine, probably as a "chemo-stimulator" of tissues, blood vessels and, in the stomach and intestinal tract, parietal cells, glands and plain muscle.

"The lyf so short, the craft so long to lerne,

Th' essay so hard, so sharp the conquering."

Chaucer, 'Parlement of Foules', i.

OBSERVATIONS ON SOME LOCAL AND GENERAL HORMONES IN THE ALIMENTARY TRACT OF IMPORTANCE TO GASTRIC SECRETION.

VOLUME TWO

VOLUME TWO.

CONTENTS.

PART THREE.

Introduction to studies on 5-hydroxytryptamine:

The history, distribution and physiological significance of 5-HT.

PART THREE.

THE HISTORY, DISTRIBUTION IN NATURE, AND PHYSIOLOGICAL SIGNIFICANCE OF 5-HYDROXYTRYPTAMINE.

History of 5-HT (enteramine; serotonin).

A vasoconstrictor substance has been known to be present in defibrinated blood or serum for a century (Ludwig and Schmidt, 1868; Stevens and Lee, 1884); indeed its presence impedes the attempts of physiologists to perfuse certain organs. It was only comparatively recently taken up by Irvine Page and his colleagues (Page, 1958) in Cleveland because of this very same nuisance value, as an intruding vasoactive substance impeding his work on angiotonin. Rapport, Green and Page in 1948 reported the isolation of a potant crystalline vasoactive substance from beef serum and in 1949 Rapport was able to demonstrate that the substance contained an indole base complexed with/

with creatinine sulphate. On the basis of chemical and physical tests he postulated that the substance, already named serotonin by Page, was more correctly 5-hydroxy-3 -aminoethylindole (5-hydroxytrypt-:amine; 5HT). The substance was later synthesised first by Hamlin and Fischer (1951) and by Speeter, Heinzelman and Weisblat (1951). Independently the Italian biologist Erspamer had been investigating over the past 30 years, pharmacologically active substances present in the gastrointestinal tract of various lower animals. Masson had earlier believed that the argentaffin cells (Masson, 1928) were of an endocrine nature possibly possessing some means whereby adjacent smooth muscle cells were stimulated. Erspamer therefore attempted to discover the properties of the substance in enterochromaffin and argentaffin cells which gave them their staining reaction. Vialli and Erspamer had in 1933 described the colour reactions and pharmacolo-:gical effects of a substance which they called enteramine. Its indolic nature was appreciated a decade later, and it was recognised as a powerful stimulant of the smooth muscle of the alimentary tract. The activity of extracts varied with the number of enterochromaffin-type argentaffin cells present in the parent tissue, and the idea arose that this was the hormone of the enterochromaffin cells, 'enteramine'. Erspamer and Boretti (1951) were able to separate enteramine from contaminants and it was identified by Erspamer and Asero in 1952 as 5-hydroxytryptamine.

Distribution in Nature.

5HT is widely distributed in nature. Its varied distribution encompasses plants, fungi and animals. Among vertebrates it has been described in mammals, birds, reptiles, amphibians and fish; for each class of the vertebrate phylum no constant distribution emerges such as might suggest one uniform function. It may be significant, however, that in most of the higher vertebrates 5HT finds its richest distrib-:ution in the alimentary tract.

In lower vertebrates, some arthropods and angiosperms, the role of 5HT would appear to be largely defensive. In amphibians, taking Bufo marinus, the giant toad, as an example, various secretory glands are often also venomous glands; in the case of the toad the venom gland situated in the shoulder exudes when the toad is handled and any noxious agent in the secretion may cause pain in the mouth or paws of an attacker if it penetrates the mucous membrane or skin. (It was shown by Armstrong et al. (1953) that 5HT produces pain on intracutaneous injection in humans).

Among the molluses 5HT has been found in the salivary glands of Octopus vulgaris, in various snails, and the edible mussel (Mytilus edulis, Twarog (1954)). In the posterior salivary glands of the octopus 5HT is released during secretion (Bacq and Ghiretti, 1951), but a poisonous role has been suggested for this gland by Phisalix (1922) since he found the gland juice to be toxic when injected into crabs.

Florey and Florey (1954) have found that the injection of as little as 1 ug into a crab's claw causes muscular spasms.

Arthopods of the class insecta, such as Vespa vulgaris (the wasp) contain 5HT in their sting fluid (Jacques and Schachter, 1954), as does the scorpion.

Similar mechanisms underly the skin lesions produced by the spicules of cowhage, Mucuna pruriens, (Bowden, Brown and Batty, 1954) and by the sting fluid of the common nettle, Urticaria dioica (Collier and Chesher, 1956) (dock leaves, long known to countrymen as an antidote to nettle stings have been shown to contain a powerful 5HT antagonist).

5HT, or a related indole, may well be the hallucogenic substance in the sacred fungus of the Atzecs, since it has recently been extracted from a hallucinogenic principle in a poisonous mushroom of the Paneolus species (Tyler, 1958). Turning to more innocuous foodstuffs, it has been found that bananas are a rich store; about 3.8 mgms. may be contained in the peel and as much again in the pulp, (Waalkes, Sjoerdsma, Creveling, Weissbach and Udenfriend, 1958). This intriguing fact was discovered out of an act of kindness (Anderson, Ziegler and Doeden, 1958). Monkeys were given a bunch of bananas as a reward and it was found that the urinary excretion of indolic substances,/

substances, which were being examined during the course of the experiment, rose, remained elevated and interfered with the satis-:factory completion of the experiment during the subsequent day. It is interesting to speculate that the 5HT in bananas may have contributed to its efficiency in some cases of coeliac disease by activating the motility of the gut, yet as much as 20 mgs. of 5HT must be taken orally before the effects of this substance are apparent.

In most animals the undoubted important sites of 5HT distribution are the brain (Amin, Crawford and Gaddum, 1953) and gastro-intestinal tract (Feldberg and Toh, 1953); in the former site it has been estimated that the turnover is rapid, but in the latter site occurs more slowly. There is also a considerable amount of 5HT attached to the platelets in most species (Zucker, Friedman and Rapport, 1954) but the hormone is merely being carried. 5HT is also found in the mast cells of the rat and the mouse (Parratt and West, 1957) but not of other species. It is also found in a trans-:plantable mast cell tumour (Sjoerdsma, Waalkes and Weissbach, 1957).

PHYSIOLOGY.

The physiological roles ascribed to 5HT are legion. The important claims are those which have been outlined in the introduction to/

to the thesis and ascribe to it:-

(a) Neurohumoral activity, because of its rich distribution in the brain (Crawford, 1958), its possible significance in autonomic and hypothalamic function, and the fact that many drugs which disturb brain function are also substances which potentiate or block the effects of 5HT e.g. iproniazid, reserpine and lysergic acid. (Udenfriend, 1958; Pletscher, 1957; Pletscher, Shore and Brodie, 1955; Shore, Silver and Brodie, 1955).

(b) Activity related to vasomotor function. The effects on the blood pressure are very varied (Page and McCubbin, 1953). The most constant effect in many species seems to be a transient fall in blood pressure; this may be succeeded by a rise in pressure, which may occasionally be the primary effect in the human subject (Spies and Stone, 1952). A great increase in cardiac output has been demonstrated in unanaesthetised patients using the Fick principle (Page, 1958). Serotonin is a powerful vasoconstrictor of the lung vessels; in the dog and cat its action at this site is more powerful than that of adrenaline and nor-adrenaline (Ginzel and Kottegoda, 1953). Released from platelet attachment, it may be important in the pulmonary hyper-:tension of certain cardiac anomalies, and it may play a part in the causation of some of the general circulatory effects of pulmonary embolism (Smith and Smith, 1955). Paradoxically, 5HT or serotonin, a name obviously chosen because of its vasoconstrictor properties, may/

Bulbring and Lin (1958) record that 5HT stimulates peristalsis, lowers the threshold for the peristaltic reflex, and increases the frequency of intestinal contractions and the volume of fluid transported along intestinal loops. Elevation of the intraluminal pressure caused release of 5HT from the isolated intestinal preparation; there was a good correlation between the rise in intraluminal pressure, the release of 5HT and the volume of fluid transported along the loops of bowel examined. The addition of the precursor, 5-HTP, increased the amount of 5HT in the effluent, and increased the peristaltic activity of the intestinal preparation. These experiments would appear to enhance greatly the physiological status of 5HT as a gastro-intestinal hormone.

(d) Erspamer and Ottolenghi advanced claims for 5HT as an antidiuretic factor; they found that it controlled water excretion in the rat (Erspamer and Ottolenghi, 1953) by reducing filtration, through vasoconstriction of afferent vessels. Erspamer's observations have been repeated successfully but with larger doses (1-3 mg/kg) (Sala and Castegnaro, 1953). On the contrary Abrahams and Pickford found that 5 HT produced minimal antidiuresis in conscious dogs. The antidiuretic effect was only present when vascular and respiratory side effects were present. They considered that 5HT did not meet the requirements of a specific renal hormone (Abrahams and Pickford, 1956).

Infusion/

may also be vasodilator at certain sites (Roddie, Shepherd and Whelan, 1955); it constricts major arterioles concerned with the peripheral resistance, but dilates the smaller vessels of the skin.

It is held that (Page and McCubbin, 1954) 5HT may act as a humoral agent stabilising vascular tone, acting as a chemical buffer to oppose neural effects. This might explain why 5HT has been shown to have so many conflicting dual effects; it may help to control function by opposing whichever is the predominant autonomic activity. Whether such a system is used in the body is not yet known, nor is the mechanism by which 5HT performs this action. It may do so by inhibiting transmission through ganglia (Marrazzi, 1953) or by potentiating it (Trendelenburg, 1956).

(c) Activity related to gastro-intestinal function. The work of Erspamer on the distribution of 5HT in the gastro-intestinal tract suggested that 5HT must have an important role at this site (Erspamer, 1955). Dalgliesh, Toh and Work (1953) identified 5HT in the dog's gastro-intestinal tract and Feldberg & Toh (1953) showed that it was principally distributed in the pyloric and duodenal mucosa. Toh (1954) later demonstrated release of 5HT into the portal blood stream and isolated a substance which released 5HT from the gastro-intestinal tract into the portal circulation (Toh, 1957). Interest has subsequently centred round the possibility that 5HT may affect the secretory and motor function of the gastro-intestinal tract. The effect of 5HT on acid secretion is discussed in Chapters 2, 3 and 4 of part 3 of this thesis.

Infusion of 5-HT leads to bilateral cortical necrosis of the kidney (Page and Glendinning, 1955).

(e) Reid (1952) studied the relationship of 5-HT to the platelets and felt that this substance might be of importance in haemostasis when releasing from disrupting platelets at sites of injury. This role of 5-HT is in doubt, since reserpine induced depletion (Shore, Pletscher, Tomick, Kuntzman, and Brodie, 1956) of platelets has been found to have no effect on the bleeding time.

(f) Armstrong, Dry, Keele and Markham (1953) have considered 5-HT as one of the many substances which may be responsible for the apparent transmission of pain sensation.

HUMAN PHYSIOLOGY OF 5-HT.

Only a few experiments are reported concerning the infusion of 5-HT in man; Page (1958) reports tingling and warmth in the extremities, tachycardia and blood pressure changes, substernal discomfort, and bladder or intestinal motor effects with 5-HT infusions. The precursor, 5-hydroxytryptophan, 5-HTP has also been given systemically to man (Bessman, Marlis and Borges, 1957), but with many of these untoward effects also. It is for this reason that it has seemed more desirable to investigate the human pharmacology of the carcinoid syndrome (hoping always to be able to do something for its relief) rather than to resort to infusions of an active substance, not easily controlled in the range of its effects.

PART THREE.

Introduction to the experimental observations recorded in Chapters 1 - 6.

PART THREE.

INTRODUCTION.

The author's interest in 5-hydroxytryptamine was aroused as far back as 1952. C.C. Toh had been working on a novel extract from the dog's gastrointestinal tract at The National Institute for Medical Research. It contracted the atropinised rat colon preparation in a manner not unlike that of any of the pharmacological substances which are active in high dilution. Professor Gaddum, seeing the acitvity of this substance demonstrated at a meeting of the Physiol-:ogical Society at Mill Hill in 1952, declared that it looked as if this might be Erspamer's enteramine (later characterised as 5-hydroxytryptamine). Dalgliesh, Toh and Work were able to confirm this and Feldberg and Toh thereafter published a description of the distribution of this substance in the alimentary tract of the dog. This distribution was largely a mucosal one, regions of high concentration being the pyloric antrum and the duodenum. Acting on the hypothesis that this substance might have gastrin like activity, the author and Toh infused this substance in varying dosage into cats, anaesthetised with chloralose and urethane, but were not able to produce gastric acid secretion; in similar negative experiments Schachter, Smith and Toh injected 5-hydroxytryptamine intravenously into 'pouch' dogs.

Six chapters are presented which are concerned with experiments on/

on 5-HT : three of these chapters, which are a continuation of this work on acid gastric secretion in the dog, are outlined as follows:-

Chapter 2 consists of an examination of inhibitory effects of 5-hydroxytryptamine on acid gastric secretion in anaesthetised dogs. The inhibitory effects of varied concentrations of 5-hydroxytryptamine have been examined and the effect of 5-HT on fully developed acid secretion and on the initiation of the process are compared. An estimate is made of how far the inhibitory effect is a local one or is induced reflexly.

Chapter 3 attempts to give greater physiological significance to the findings in the previous chapter by testing possible inhibitory actions of precursors of 5-HT. These substances were likewise examined for their effects on the initiation of the secretory process and an estimate was also made of whether their effect was locally or reflexly induced. A study was also made of how, as a result of conversion to 5-HT, the precursors in a diet rich in tryptophan can elevate the blood 5-hydroxytryptamine. The experiments in these two chapters were done in cooperation with Dr. J. W. Black and Mr. E. W. Fisher; the author was responsible for initiating these experiments, in that the work was taken up as a result of the observations begun at the National Institute for Medical Research (already mentioned), and described in Toh's thesis, "Histamine, Substance P and 5-hydroxytryptamine", for the Ph.D. degree of/

of the London University, 1953. The author personally conducted the experiments described in this Thesis, and although they have been published in conjunction with Black and Fisher, Black and Fisher were to have been concerned as their part with observations on the acid-base balance.

Chapter 4 consists of an examination of the possible supportive role of 5-HT for an inhibitory reflex of gastric secretion. Experi-:ments are described to show that the inhibitory effect of recent feeding is minimised by preventing acid spread on the surface of the pyloric antrum by placing a ligature at the mid-point of the stomach. The inhibitory effect of recent feeding, furthermore, was dependent on the presence of intact vagus nerves, and the case is argued for there being a mechanism for inhibiting acid secretion via vagal afferent nerve fibres. Evidence is presented as a result of pharmacol-:ogical activation of these fibres by phenyl-diguanide, that they may affect gastric secretion. The possibility exists therefore that 5-HT may serve on the afferent side as a transmittor of nervous activity of this sort, or that in some other way, perhaps centrally, it may support an inhibitory reflex controlling gastric secretion.

Secreting carcinoid tumours provide the clinical investigator with an opportunity of examining the human pharmacology of 5-HT if these cases/

cases present the functioning carcinoid syndrome. In Chapter 5 the subject of carcinoid tumours is reviewed on the personal experience of nine cases of the 'carcinoid syndrome" studied by the author. In this chapter and Chapter 6 the inter-relationships of 5-hydroxytryptamine and 5-HT have been studied in detail (much of this work provided evidence regarding the importance of 5HTP which, it is held by the author, may by formed as well as 5-HT by the argentaffin cells; this work was going on simultaneously with the work described in Chapters 2, 3 and 4, and influenced the arguments propounded in these chapters). In Chapter 6 the effects of 5HT on gastric secretion in the presence of high blood levels in human cases of the carcinoid syndrome have been studied. Various substances, such as histamine and alcohol which might activate secretion in these cases, have been shown to provoke a further secretion of 5HT. The effects of feeding a high protein diet and a high fat diet on 5HT release and formation have been compared. Lastly, the local 5HT concentrations have been increased by iproniazid and this substance has been given to determine whether the potentiation of 5HT tissue stores influences gastric secretion.

The experiments in Chapter 1 lead on, at this point, with a description of a "tissue role" shared by 5HT and tryptamine in certain species, namely the property of histamine release. It is shown that 5HT is an effective endogenous histamine liberator in cats and rats.

Why/

Why this property of histamine release does not lead to spontaneous acid gastric secretion remains a mystery, - it may be that the inhibitory effect of 5-HT overrides the secretory stimulant properties of the released histamine. It must be stressed once more that 5-HT has no prolonged stimulatory effect on acid secretion.

CHAPTER ONE.

Release of histamine by tryptamine and 5-HT.

CHAPTER ONE.

RELEASE OF HISTAMINE BY TRYPTAMINE AND 5-HYDROXYTRYPTAMINE.

MacIntosh and Paton (1949) have shown that many amines have in common the property of causing histamine release. In this chapter it will be shown that this applies to tryptamine and 5-hydroxytryptamine as well.

The following procedures were adopted for examining the histamine releasing properties of the tryptamine compounds. (1) Perfusion of isolated skin flaps and gastrocnemius muscles and assaying the histamine in the venous effluent after arterial injection of the tryptamine compounds. (2) Determining changes in histamine content of tissues after subcutaneous and intraperitoneal injections of tryptamine.

The release of histamine was examined in human cases of the carcinoid syndrome by estimating the urinary excretion of histaming using the method of Roberts and Adam (1950).*

METHODS.

The perfusion experiments were performed on skin flaps of the cat's and dog's hind leg and on the gastrocnemius muscle of the cat, according to the methods described by Feldberg and Paton (1951) and Feldberg and Schachter (1952).* The presence of the venous samples of tryptamine and 5-hydroxytryptamine collected after their injection had to be taken into account when assaying the samples for histamine on the atropinized guinea-pig's ileum preparation, because these substances/

(* See Appendix)

substances have themselves a contractile effect on this preparation. For this reason, the samples were first assayed for tryptamine or 5-hydroxytryptamine respectively on the atropinized rat's colon, according to the method described by Feldberg and Toh (1953). They were then assayed for histamine on the guinea-pig's ileum preparation by adding to the control solutions of histamine, tryptamine, or 5-hydroxytryptamine respectively. For instance, if a sample of perfusate was found to contain 100 ug. tryptamine per ml., and 0.05 ml. was required for producing a reasonable contraction of the guinea-pig's gut, the effect was compared with contractions produced by different amounts of histamine to each of which was added 5 ug. tryptamine.

Changes in histamine content of tissues after subcutaneous and intraperitoneal injections of tryptamine were studied in albino rats of about 100 g. body weight. The rats were killed by a blow on the head and samples of the tissue to be examined were removed, weighed, ground in 1/3 N H.Cl with saline solution, boiled and neutralized before testing. The method has been described elsewhere (Feldberg and Talesnik, 1953; Smith, 1953). Tissue samples from 4 to 6 rats were pooled and extracted together in order to minimize individual variations in the histamine content.

All values for histamine are expressed as base. Dr. R. K. Richards, Abbott Laboratories, kindly provided the sample of 5-hydroxytryptamine creatine sulphate. Its molecular weight is 355, of/

of which about half, i.e., 176, represents the base; all values given refer to the base. The tryptamine used was the hydrochloride. Its molecular weight is 196.5, of which about 80%, i.e. 160, represents the base; all values given refer to the salt. In some experiments the histamine releasing activity of tryptamine was compared with that of compound 48/80, kindly provided by the late Dr. C. H. Kellaway (Wellcome Research Laboratories).

RESULTS.

Perfused Skin Flap of the Cat.

Both tryptamine and 5-hydroxytryptamine caused a release of histamine from perfused skin flaps. There was, in addition, vasoconstriction and the development of oedema; the vasoconstriction, which was particularly strong after 5-hydroxytryptamine, was probably only to some degree due to the released histamine and was mainly a direct effect of the tryptamine compounds on the vessels. With the injection of either 2.5 mg. tryptamine or 5-hydroxytryptamine in 0.5 ml., the flow was either stopped or nearly stopped by a vasoconstriction which supervened about 2 min. after the injection. When the flow was not totally arrested, the vasoconstriction was found to reach a maximum in 5-7 min. and then very gradually subsided. The intensity of the oedema paralleled the output of histamine; it developed during the first 30 or 60 min. after the injection, was particularly pronounced in/

in the subcutaneous tissue, and could amount to about six times the weight of the skin flap.

As was to be expected, the first samples after the injections contained most of the tryptamine or 5-hydroxytryptamine. They were recovered in the venous effluent to between 50-70%. Since the first drops of venous effluent after the injection, which probably contained the highest concentration of the tryptamine compounds, were discarded, the actual amounts escaping into the venous effluent were probably greater. Further, some destruction and inactivation of these compounds may have occurred during the passage through the skin and some may have been retained, as evidenced by their presence in the oedema fluid collected after cessation of the perfusion.

Table I shows the amounts of tryptamine and 5-hydroxytryptamine from skin flaps of the same cat in successive samples of venous effluent, and the histamine content of these samples when assayed against known histamine solutions but incorporating the amounts of the tryptamine compounds present in them. (Table I).

Table II shows the amounts of histamine released in eleven experiments on injection of 0.5-2 mg. of the two tryptamine compounds. Doses under 0.5 mg. were ineffective, but with 2 mg. usually between 93 and 283 ug. histamine was released. The low value of 30 ug. in experiment 6 represents only the histamine of the sample collected during/

TABLE I.

ASSAY OF VENOUS EFFLUENT FOR TRYPTAMINE (T), 5-HYDROXYTRYPTAMINE (HT) AND HISTAMINE (Hi) FROM PERFUSED CAT'S SKIN FLAPS OF THE SAME CAT.

2 mg. T injected into the right, and 2 mg. HT into the left skin flap (same experiment as No. 9, Table II).

Samples after Injection	Minutes of Collection	Right Skin Flap			Left Skin Flap		
		ml. Collected	ug. T	ug. Hi	ml. Collected	ug. HT	ug. Hi
1	1½-2	3.0	525	36.5	2.2	660	79.2
2	8-8½	4.4	396	145.2	1.3	78	35.8
3	10	5.0	100	25.0	11.4	160	43.3
4	20	22.0	143	66.0	15.0	63	60.0
5	10	14.7	28	7.4	12.7	13	0.6
6	10	13.5	11	1.4	20.5	17	0.8
7	20	24.2	-	1.6	16.5	12	0.5
8	20	15.0	-	0.5	-	-	-
Oedema fluid		75.0	75	7.5	68.0	54	7.2
Total			1,273	291.1		1,057	227.4

during the first 5 min. after the injection; in this experiment the vasoconstriction was so intense that the flow stopped after this time and attempts to restart it by increasing the perfusion pressure led to leakage from the arterial side, so that the experiment had to be discontinued.

In experiments 4, 5, 8 and 9 of Table II, the skin flaps of both legs were perfused and tryptamine was examined on one side and the equivalent amount of 5-hydroxytryptamine on the other. It is evident that the two compounds have approximately the same histamine releasing ability; if there is any difference, tryptamine is slightly more active. Since we express 5-hydroxyptamine as base and tryptamine as salt, 2 mg. of tryptamine corresponds to 1.6 mg. of base only, and the difference between the activity of the two compounds is thereby accentuated.

A similar conclusion is reached from experiments in which the effects of repeated injections are compared on the same skin preparation. In the experiments of Fig. 1, 2 mg. of 5-hydroxytryptamine and 2 mg. of tryptamine were injected into the skin flaps of both legs of the same cat, but in reversed order. In both perfusions the first injection, whatever the tryptamine compound injected, produced a greater histamine output than the subsequent injection. It was possible, however, to increase the histamine output considerably by doubling the dose injected. This is seen for tryptamine at the end of/

TABLE II.

OUTPUT OF HISTAMINE AFTER TRYPTAMINE (T) AND 5-HYDROXYTRYPTAMINE (HT) FROM PERFUSED SKIN PREPARATIONS OF THE CAT.

Figures in brackets refer to the highest histamine concentration (ug./ml.) found in the initial samples after injection of the tryptamine compounds.

Expt. No.	mg. Injected	Output of Histamine in ug.			
		After (T)		After (HT)	
1	0.5	1	(0.09)	-	-
2	0.5	-	-	28.5	(2)
3	1.0	11.2	(1.05)	-	-
4	1.0	46.8	(14)	68	(20)
5	2.0	93	(4)	115	(8)
6	2.0	106	(5)	30 *	(6)
7	2.0	172	(20)	-	-
8	2.0	187	(32)	127	(28)
9	2.0	283	(33)	220	(36)
10	2.0	-	-	172	(20)
11	2.0	229	(22)	-	-

* Only one 5 min. sample collected.

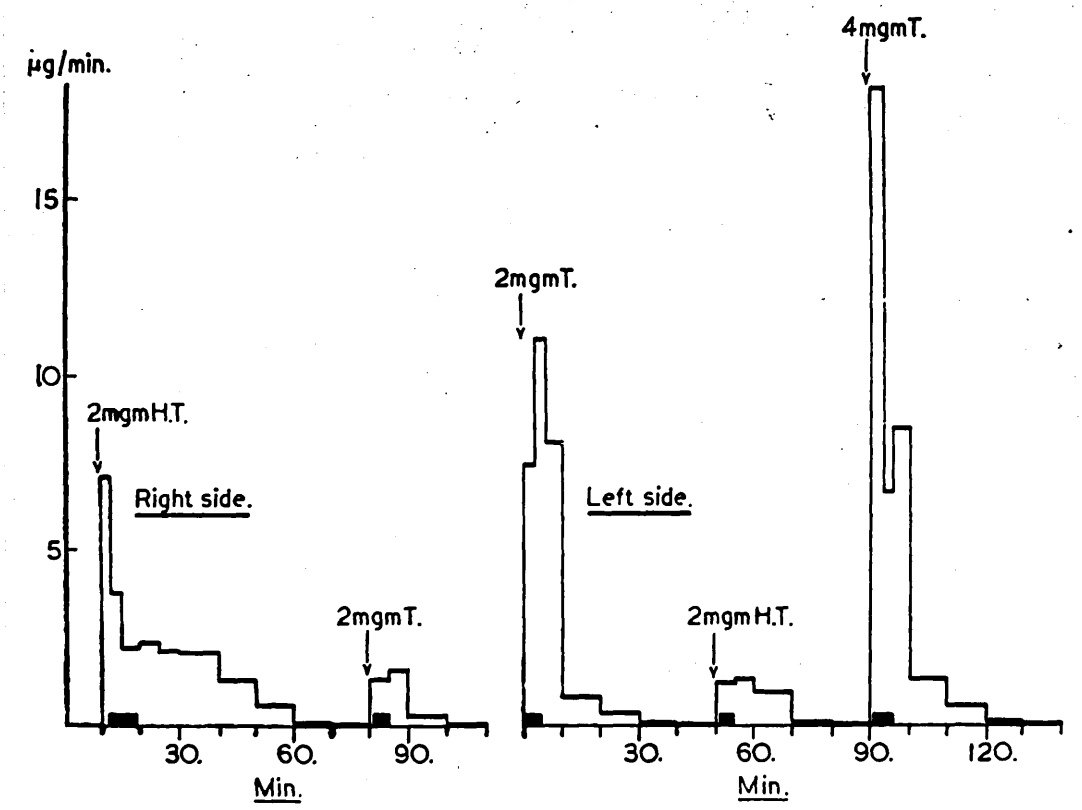


Fig. 1 - Histamine output from perfused skin preparations of the hind legs of the same cat after tyrptamine (T) and 5-hydroxytryptamine (HT.) The black areas above the base-line indicate periods of pronounced vasoconstriction.

of the perfusion of the left skin flap (Fig. 1).

The vasoconstriction interfered with the time course of the appearance of histamine in the venous outflow, and the trough seen in the record of the histamine output after 4 mg. tryptamine is explained by the fact that during the corresponding period the flow was greatly reduced and therefore only a small volume of venous effluent collected. The vasoconstriction interfered also with the output of histamine during some of the previous injections in the experiment of Fig. I, by delaying the maximal output per minute until the vasoconstriction had subsided. However, the actual "release" of histamine probably occurs as instantaneously as after the other known histamine liberators.

The histamine release was associated with a reduction in the histamine content of the central parts of the perfused skin. In one experiment a release of 173 ug. histamine after 2 mg. tryptamine reduced the histamine content in the central part of the skin flap by 75%, to 2.9 ug. /g.; in a corresponding experiment with 2 mg. 5-hydroxytryptamine a release of 171 ug. histamine caused a reduction by 88%, to 2.6 ug. /g.

When the histamine releasing property of the two tryptamine compounds is compared on the perfused cat's skin preparation with that of 48/80, it is found that, weight for weight, 48/80 is about 100 times more active. This is illustrated in the experiment of Fig. 2, in which the/

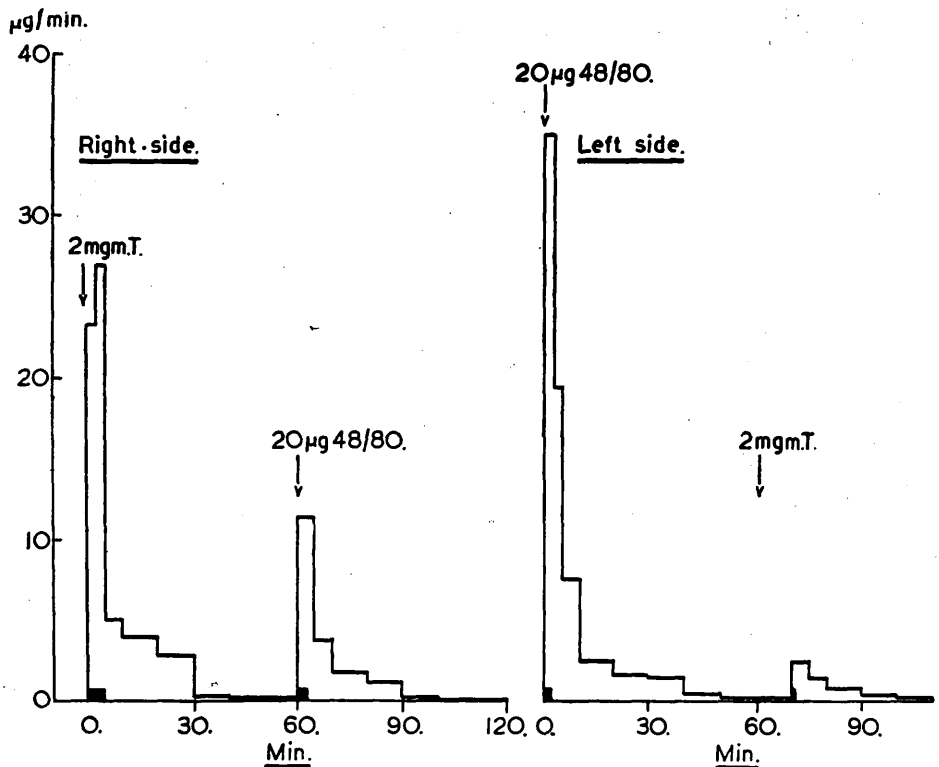


Fig. 2 - Comparison of the histamine output by 48/80 and tryptamine (T) from perfused skin preparations of the hind legs of the same cat. The black areas above the base-line indicate periods of pronounced vasoconstriction.

the effect of 20 ug. 48/80 is compared with that of 2 mg. tryptamine, on two skin preparations from the same cat in which the order of injection was reversed. The tryptamine compounds are therefore about as active as, or perhaps even slightly more active than, propamidine. They are certainly more active than the other amines and amidines examined by MacIntosh and Paton (1949), and also more active than the opium alkaloids and morphine derivatives examined by Feldberg and Paton (1951).

Perfused Gastrocnemius Muscle of the Cat.

The histamine releasing activity of tryptamine and 5-hydroxytrypt-:amine from the perfused gastrocnemius muscle is illustrated in the two experiments in Fig. 3, which are from different cats. Since the gastrocnemius has a low histamine content, the amounts released from this preparation are much smaller than those released from the cat's skin. In the first experiment, two successive injections of 2 mg. 5-hydroxytryptamine released 3.35 and 0.71 ug. histamine; in the second experiment 2 mg. tryptamine released 2.59 ug. histamine and a succeeding injection of 4 mg., 6.35 ug. Although the two experiments were from different cats, they suggest that for the perfused gastrocnemius muscle, as for the perfused skin of the cat, the mistamine releasing property of the two compounds is of the same order.

The amounts of histamine released in the two experiments of Fig. 3 represent only a fraction of the muscle histamine. In the experiments with/

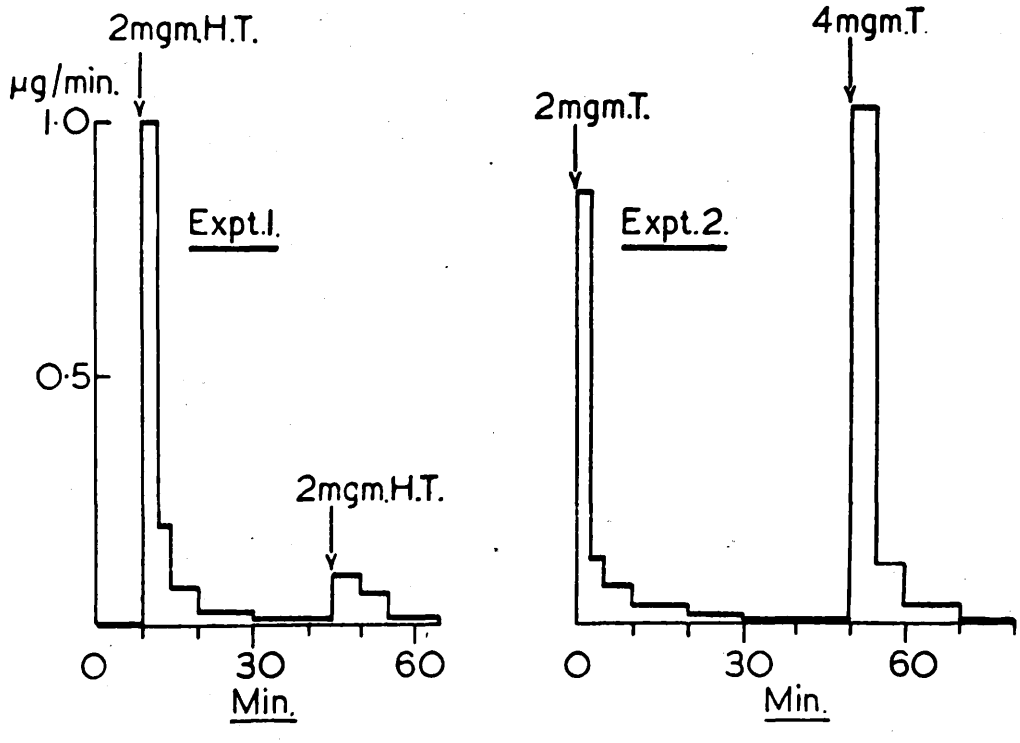


Fig. 3 - Histamine output from perfused gastrocnemius after 2 mg. 5-hydroxytryptamine (HT) in expt. 1 and after 2 and 4 mg. tryptamine (T) in expt. 2 which was from a different cat.

with 5-hydroxytryptamine, the gastrocnemius of the non-perfused leg weighed 30 g. and contained 1.38 ug./g. histamine; its total histamine content was therefore about 41 ug.; the amounts released by the two injections of 5-hydroxytryptamine correspond, therefore, to about 10% of this figure. In the other experiment, the weight of the non-perfused gastrocnemius was 28.5 g. and contained 1.64 ug./g. histamine; its total histamine content was therefore about 47 ug., of which a little over 20% was released by the two tryptamine injections.

Perfused Skin Flap of the Dog.

Tryptamine alone was examined, which produced some vasoconstriction, but to a less degree than on the cat's skin preparation. When assaying the effluent for tryptamine on the rat's colon, the values obtained for the combined first three samples, collected over a 10 min. period after the injection, were higher than the actual amounts of tryptamine injected. This may have resulted from the fact that these samples collected during the vasoconstriction were blood-stained, even if the effluent had become clear before the injection; and it had been found that samples collected at the beginning of the perfusion, when they were yet blood-stained and cloudy but no tryptamine had been injected, had some activity on the rat's colon. It was not examined whether this activity was due to tryptamine or 5-hydroxytryptamine. In order to avoid the error of exaggerating, by this activity, the histamine assayed in the effluent, two methods were adopted: one (method 1) by taking the excessively high/

high value actually assayed for tryptamine on the rat's colon as representing the tryptamine in the sample, and the other (method 2) by assuming that the first three samples contained the total amount of tryptamine injected. With both methods a histamine release could be demonstrated, but, as seen from Table III, the values

TABLE III

HISTAMINE OUTPUT BY TRYPTAMINE FROM PERFUSED SKIN PREPARATION OF THE DOG.

Tryptamine Injected	ug. Histamine Assayed	
	By Method 1	By Method 2
2 mg. in 0.5 ml.	7.0	-
4 " " 2 "	5.3	5.8
8 " " 2 "	6.8	9.6

obtained with the second method here higher; even these may be an underestimation of the actual amounts of histamine present in the samples, because it is unlikely that there was a 100% recovery of tryptamine.

Histamine Release from Rat's Tissue by Tryptamine.

Acid saline extracts from rat tissue produced contraction of the rat's colon; the activity corresponded to that of 10-20 ug. tryptamine per/

per gramme tissue. Somewhat higher values were obtained from the animals treated with tryptamine. Although such amounts of tryptamine would not greatly interfere in the histamine assay, they were taken into account by first assaying the extracts against tryptamine on the rat colon, and, in the subsequent histamine assay on the guinea-pig's ileum, adding tryptamine to the control solution of histamine, as in the corresponding assays of perfusate (see Methods).

Subcutaneous Injections.

As with 48/80, the subcutaneous injection of tryptamine leads to a reduction of skin histamine at the site of injection. For this purpose 2 mg. tryptamine was injected in 0.5 ml. in previously demarcated areas of the abdominal skin in eight rats. The injections caused locally a bluish cyanosed area surrounded by erythema which two hours later became cyanosed also. By this time a haemorrhagic weal had developed at the injection site. Half of the injected rats werekilled two hours after the injection; the skin at the site of the injection contained blood cells and plasma. The other half of the rats were killed 24 hours after the injection; the site of injection was no longer oedematous but contained a crust of cells and dried serum. The histamine content of the skin areas at the site of injection from these rats is given in Table IV and compared with the histamine content from normal rats and from skin areas of rats having received a control injection of 0.5 ml. saline solution. Tryptamine produces a great/

TABLE IV.

EFFECT OF SUBCUTANEOUS INJECTION OF 2 MG. TRYPTAMINE IN 0.5 ML. ON HISTAMINE CONTENT OF ABDOMINAL SKIN (POOLED SKIN SAMPLES) AT SITE OF INJECTION.

No. of Rats	Treatment	ug./g. Histamine	% Reduction
2	None	50	0
2	2 hr. after injection of saline	42	16
4	2 hr. after injection of tryptamine	14	72
4	24 hrs. after injection of tryptamine	12.8	74

great reduction in histamine content which persists after all traces of oedema have disappeared. The small reduction two hours after the saline injection must be accounted for by an increased fluid content of the skin due to the fact that the injected fluid was not completely absorbed.

There is this difference between the effects of tryptamine and those of corresponding experiments with 48/80 (see Feldberg and Talesnik, 1953) that the injection of tryptamine, probably through its intense vasoconstrictor properties, produces local damage to the tissue which may contribute to the observed reduction in histamine content.

Intraperitoneal Injections.

Eight rats were each given a total of 13 mg. of tryptamine intraperitoneally. The injections were made twice daily, starting with 1 mg. and going up to 4 mg., as shown in Table V.

After the injection of 1 mg., respiration became accelerated, and after 2 and 3 mg. was, in addition, laboured. The rats became cyanotic, their coat was ruffled, but later the skin became pink, and in some animals oedema developed round the mouth and on the paws. This occurred after 2 mg. in three, and after 3 mg. in five out of eight rats about 45 min. after the injections. With 4 mg. the animals were first prostrated, cyanosed, and cold, and oedema developed in half the animals when the began to recover.

Table/

Table V gives the histamine content of various tissues from these animals killed 24 hours after the last injection. The values from the pooled samples are compared with those obtained from six control rats. There is a definite reduction in skin histamine and also, to a lesser degree, in the histamine content of the diaphragm, but the histamine content of the viscera showed no consistent change. The difference of 10% or less cannot be regarded as sufficient proof of the tryptamine.

TABLE V

CHANGES IN HISTAMINE CONTENT OF RAT'S TISSUES AFTER INTRAPERITONEAL INJECTIONS OF TRYPTAMINE (FIRST DAY 1 MG. TWICE; SECOND DAY, 2 MG. TWICE; THIRD DAY, 3 MG ONCE AND 4 MG ONCE)

Area	ug/g. Histamine		% Change after Tryptamine
	Controls	After Pryptamine	
Skin of ear	50	35	-30
Skin of paw	55	30	-46
Skin of abdomen	32	18	-39
Diaphragm	11.5	8.5	-26
Liver	0.7	0.7	0
Lung	1.0	0.9	-10
Aorta	2.3	2.5	+9
Stomach	12.5	11.5	-8

Histamine release in the human subject.

The urinary histamine was measured in four instances of the carcinoid syndrome/

syndrome in patients secreting 5-HT endogenously (see Chapter 5). In each case of four examined the levels were raised (see Table 6), the results which are in agreement with those of Pernow and Waldenstrom (1956) and with Sandler and Snow (1958). It would appear that 5-HT releases histamine in the human case but that the concentration of 5-HT to which the tissues are exposed must be a very high one. It is of some interest that the histamine findings were highest in patients whose acid secretion was on the whole of a low order (see Chapter 6).

DISCUSSION.

The finding of tryptamine and 5-hydroxytryptamine having the ability to release histamine from living tissue brings them into line with the large groups of amines having this property. Although the activity of tryptamine and 5-hydroxytryptamine in releasing histamine is somewhat greater than that of the other amines and amidines examined by MacIntosh and Paton (1949), and is, in fact, slightly more active than that of propamidine, the most active compound examined by these authors, this action must, nevertheless, be regarded as a side-effect of their pharmacological properties. This does not eliminate the possibility that histamine release is at the root of certain phenomena, seen after injection of tryptamine compounds and resembling histamine effects; for instance, it may account for some of the depressor effects on the arterial blood pressure obtained with 5-hydrosytryptamine in cats and dogs (Erspamer, 1952; Page, 1952, Freyburger, Graham, Rapport, Seay, Govier/

TABLE 6.

THE URINARY EXCRETION OF HISTAMINE IN CARCINOID PATIENTS AS ESTIMATED BY THE METHODS OF ROBERTS AND ADAM (1950). (See Appendix: normal range 12.1- 41.1 ug/24 hr.)

Case No.	ug. per 24 hr. free histamine
3	1,100
2	1,800
4	2,400
18	3,600

of high concentration. The direct effects on smooth muscle have suggested to others, (Bulbring and Lin) (1958), that this substance may have a possible role in intestinal function as a motor hormone. Its localization to the mucosal layer, and in particular, its general distribution in the pylorus and duodenum, suggested a possible relation-:ship to secretory activity in this region of the alimentary tract, since these sites are closely similar to those described for gastrin and enterogastrone. It seemed important to determine the role of 5-hydroxytryptamine in acid gastric secretion for these reasons and also since in Chapter 1, part 2 we have discussed how histamine liberators such as d-tubocurarine, propamidine and compound 48/80 elicit acid gastric secretion.

It is of some interest that human cases of carcinoid tumours secreting large amounts of 5-HT into the bloodstream also excrete elevated amounts of histamine. A reasonable explanation would appear to be that histamine has been released in these patients from the tissues by 5-HT after the same manner as in the experimental animal. The fact that (see Chapter 6) the acid secretion in these patients was on the low side suggests that there may be some blocking effect of 5-HT on the activity of histamine on the parietal cell as well as a histamine-displacing action of 5-HT. For reasons such as this the direct effects of 5-HT on acid secretion in the experimental animal are examined in Chapter Two.

SUMMARY.

Tryptamine and 5-hydroxytryptamine release histamine from living tissue; this finding brings them into line with the large group of amines having this property. The histamine-releasing activity of tryptamine and 5-hydroxytryptamine is about 100 times less than that of compound 48/80. The release of histamine was demonstrated under the following experimental conditions:

(a) After arterial injection of either tryptamine or 5-hydroxy-:tryptamine into the perfused skin flap or gastrocnemius of the cat.

(b) After arterial injection of tryptamine into the perfused skin flap of the dog.

(c) After subcutaneous or repeated intraperitoneal injections of tryptamine to rats. The subcutaneous injection caused a local reduction in the histamine content of skin at the site of injection, the intraperitoneal injections a general reduction of the histamine content of skin and skeletal muscle.

(d) 5-HT secreted endogenously in patients with carcinoid tumours appears to provoke a high urinary excretion of free histamine. This may be one of the mechanisms underlying the flushing attacks of these patients. The relationship of the urinary excretion of histamine to acid secretion is discussed. (See also Chapter Six).

CHAPTER TWO.

The effect of 5-HT on gastric secretion.

of water balance (Erspamer & Ottolenghi, 1953) and Woolley & Shaw (1954) think it plays a part in cerebral function and in normal mental processes. Erspamer & Asero (1952) have put forward the idea that 5-HT in the specific hormone of the enterochromaffin cell system, or argentaffin cells: these are chiefly found in the alimentary tract. Toh (1954 showed that 5-HT is continuously produced from the stomach wall, presumably from the argentaffin cells at this site, when the stomach is perfused in situ: he found that in the intact animal the concentration of this substance in the portal blood is always higher than in any other part of the vascular system. It seems probable, therefore, that 5-HT has some relation to gastro-:intestinal function. We have examined further the possibility that 5-HT may influence gastric secretion, particularly in view of its mucosal distribution in the stomach, and also because as has been shown in Chapter 1, 5-hydroxytryptamine may act as a histamine releasing substance.

METHODS

The experiments were carried out on dogs anaesthetised with chloralose 2% (W/V) and urethane 20% (W/V), 2 ml./ug. being given intravenously. The animals were starved for 24-36 hours before start-:ing an experiment. An efficient airway was maintained by putting a McGill's No. 10 cuffed endotracheal tube into the trachea. Histamine and 5-hydroxytryptamine were given into the femoral veins by infusion controlled/

controlled by a Palmer's Slow Injection Apparatus. In a few experiments a fine ureteric catheter was passed up the femoral artery into the abdominal aorta for intra-arterial injections. The gastric cannula, made from glass tubing with a bore of 2.5 cm., was inserted into the most dependent part at the greater curvature. It had a flange near the inserted end over which a retained purse string suture was tied. The inserted end was covered by a perforated glass dome, designed to prevent prolapse of the mucous membrane or blockage by mucus (Fig. 1). The abdominal wall was closed round the flange and the animal placed on a frame to allow drainage of gastric juice. The stomach was washed out with warm isotonic saline, and the head placed low to allow external drainage of saliva. The secretion from the cannula was collected in a funnel where it was filtered through gauze. The gauze was changed every 15 minutes and by weighing it before and after, a rough estimate of mucus output was obtained. The filtrate was collected in a beaker and the volume measured every 15 minutes. A drop tube connected to a Thorp impulse counter was placed between the filter funnel and the beaker, and the rate of secretion was recorded continuously on a kymograph (Fig. 2). As shown in the figure, this method allowed examination of changes in secretory rate not detectable in 15 minute collections of juice. The juice was titrated with Topfer's reagent as indicator and the acidity expressed in m.equiv/l. The results in the tables are given as averages of consecutive samples, each sample having/

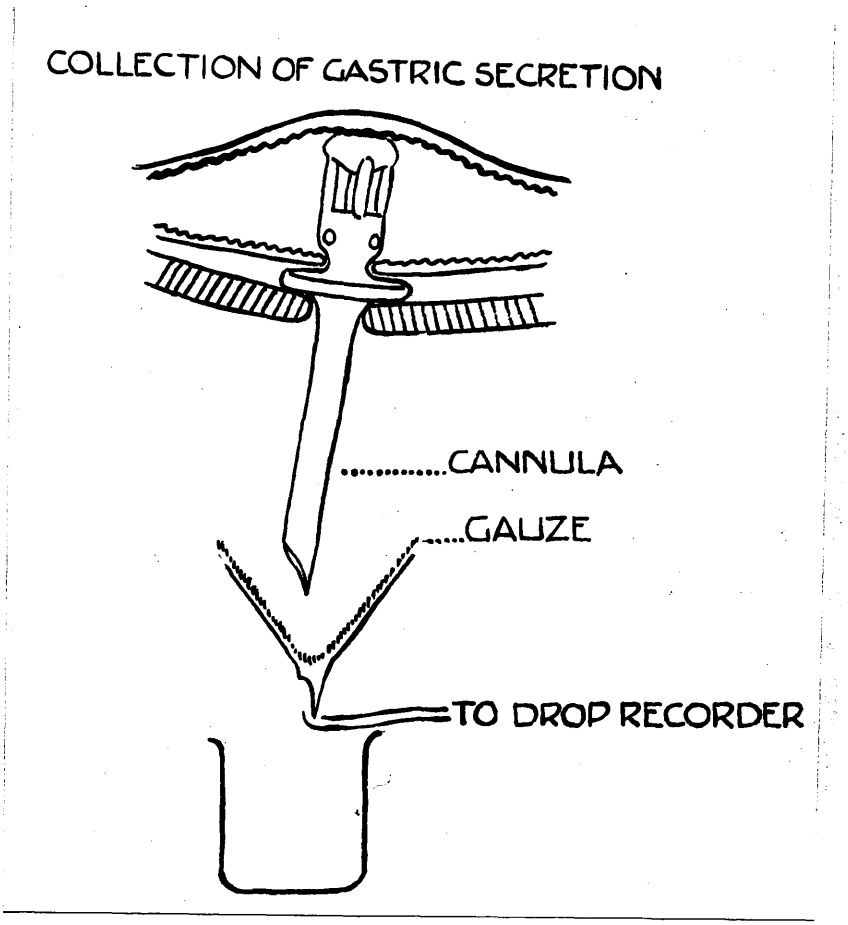


Fig. 1 - Arrangement for continuous recording of the rate of gastric secretion. The domed glass cannula allows free dependent drainage. The juice leaving the cannula is filtered through gauze before reaching the drop recorder electrodes. This prevents mucus sticking to the electrodes and when the gauze, dampened with saline, is changed regularly the difference in weight of the gauze gives a rough estimate of mucus output. The drops were recorded on a kymograph with a Thorp impulse counter.

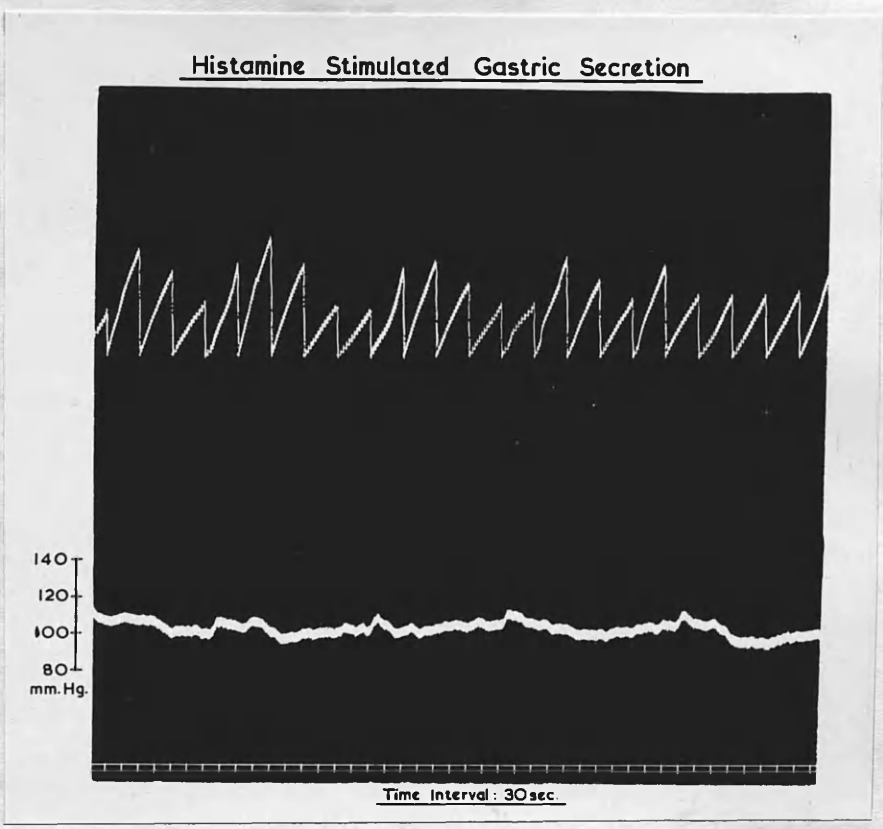


Fig. 2. Continuous record of the rate of gastric secretion. The top tracing is from the Thorp impulse counter set. to record the number of drops delivered at 1 min. intervals. The height of the vertical lines in mm on the original record equals the number of drops/min. The lower tracing is arterial B.P. recorded by a mercury manometer.

In this record, obtained during a continuous intravenous infusion of histamine, variations in secretory rate are seen to be associated with changes in arterial B.P. These changes would not be observed in 15 min collections of gastric juice.

having been calculated as total output in m. equiv. per 15 min. collection period.

RESULTS

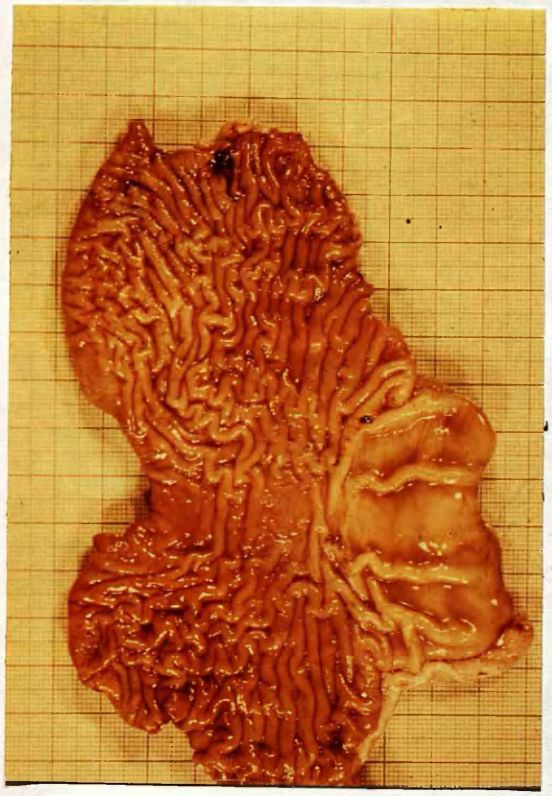
Effects of 5-HT alone on gastric secretion.

Intravenous infusions of 5-HT alone, for periods of 45-90 minutes, were given to four dogs. 5-HT did not stimulate an acid gastric secretion in any of the experiments. On the other hand, the infusion was accompanied by production of an alkaline juice rich in mucus. The amounts of this thick secretion expelled from the cannula varied; in one experiment it was as much as 47 ml. when 5-HT had been infused for 45 min. at a concentration of 10 ug/kg/min. When a smaller amount of mucoid secretion was expelled from the cannula, there was, at post-mortem, a collection of thick, clear, mucoid, secretion pooling over the area of the pyloric mucosa and duodenum. As much as 45 ml. of mucoid secretion has been collected from the pyloric region at the end of an experiment. The volume of the mucus secretion produced in these experiments was always greater than that found in 4 control dogs given anaesthesic alone, for a similar period.

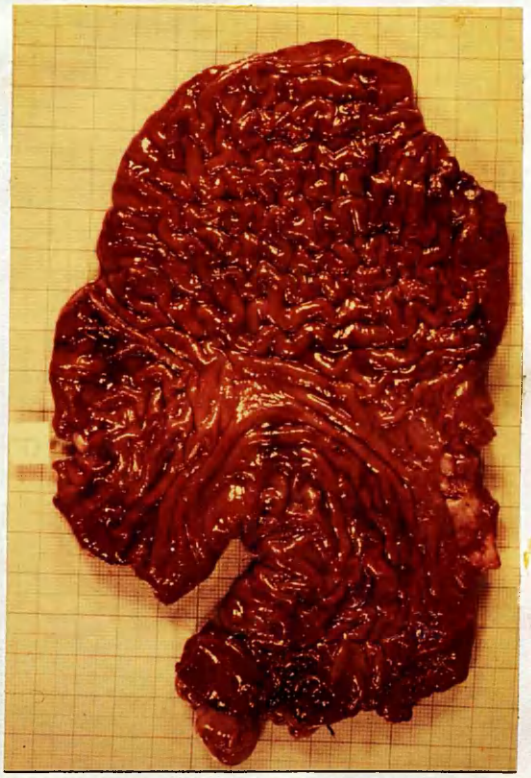
At post-mortem, after infusions of 5-HT alone, the mucosa of the stomach and intestines appeared to be very pale. (Fig. 3).

Effects of 5-HT on histamine-stimulated gastric secretion in starved dogs.

Infused histamine alone, in animals starved over a period of at least 24 hours, always/



a.



b.

Fig. 3a, which is a colour photograph, shows the excised stomach of a dog given infusions of 10 ug/kg/min 5-HT. *

Fig. 3b in contrast is from a dog given 5 ug/kg/min histamine and shows the stomach to be markedly engorged. *

* Appendix.

always elicited a rising curve of secretion, with the establishment of a secretory plateau approximately 1½ hours after onset of the infusion (Table 1). When 5-HT was given for a period of 30 min. during the continuous histamine-stimulated acid gastric secretion the volume and acidity of the juice decreased with a concurrent increase in the amount of mucus produced. A typical example is shown in Fig. 4. In this particular experiment 15 ug/kg/min of 5-HT given for 30 min. was associated with complete inhibition of the acid secretion and with an increased expulsion of mucus. In 12 experiments (Table 2), when the infusion of 5-HT was started 1½-2 hours after the onset of the histamine-stimulated secretion, and while the output of acid was still rising, inhibition of secretion was usually apparent in the first 15 min. sample after starting the infusion of 5-HT and continued throughout the infusion period. Recovery from the inhibition had usually begun within 60 min. of stopping the infusion, though in a number of experiments recovery was slower than this. The experiments in Table 3 show that two consecutive periods of inhibition can be produced by 5-HT.

The inhibition of gastric secretion by 5-HT was usually preceded by a short period of increased output of juice, a phase which in this group of experiments, rarely lasted long enough to mask the decrease in volume of the first 15 min. collection of juice after starting the infusion. This brief increase of output of juice was easily seen on the drop/

TABLE 1.

HISTAMINE-STIMULATED ACID GASTRIC SECRETION FROM 'STARVED' DOGS. THE FIGURES ARE AVERAGES OF THE TOTAL OUTPUT OF FREE ACID IN m-equiv/15 min FROM TWO CONSECUTIVE 15 min COLLECTION PERIODS. THE PERCENTAGE FALL IN ACID OUTPUT REFERS TO THE RELATIONSHIP BETWEEN THE LOWEST ACID OUTPUT REACHED AND THE PREVIOUS MAXIMUM VALUE. THE HALF-HOUR TIME INTERVALS REFER TO THE START OF THE HISTAMINE INFUSION AS ZERO.

Expt.	Dog wt.(kg)	Histamine 5 ug./kg./min. intravenously								Fall in acit output (%)
		Time after start of histamine (hr)								
		0.5	1	1.5	2	2.5	3	3.5	4	
1	15	0.03	0.42	0.95	1.11	1.20	1.28	1.16	-	9
2	24	0.92	3.84	4.49	4.46	4.48	-	-	-	1
3	11	0.04	1.09	5.01	5.45	6.08	5.10	-	-	15
4	15	0.39	2.34	3.03	2.46	2.64	2.75	2.76	2.75	18
5	12	0.17	1.14	1.41	1.55	1.60	-	-	-	0
6	9	0.02	0.32	0.58	0.68	0.94	0.99	0.78	-	21
7	17	0.03	1.25	2.41	2.42	2.42	-	-	-	0
8	18	0.04	1.08	2.18	3.88	4.90	3.80	-	-	22
9	5	0.05	0.56	0.59	1.03	1.21	1.00	-	-	17
10	13	0.05	0.63	1.50	1.66	1.31	-	-	-	21

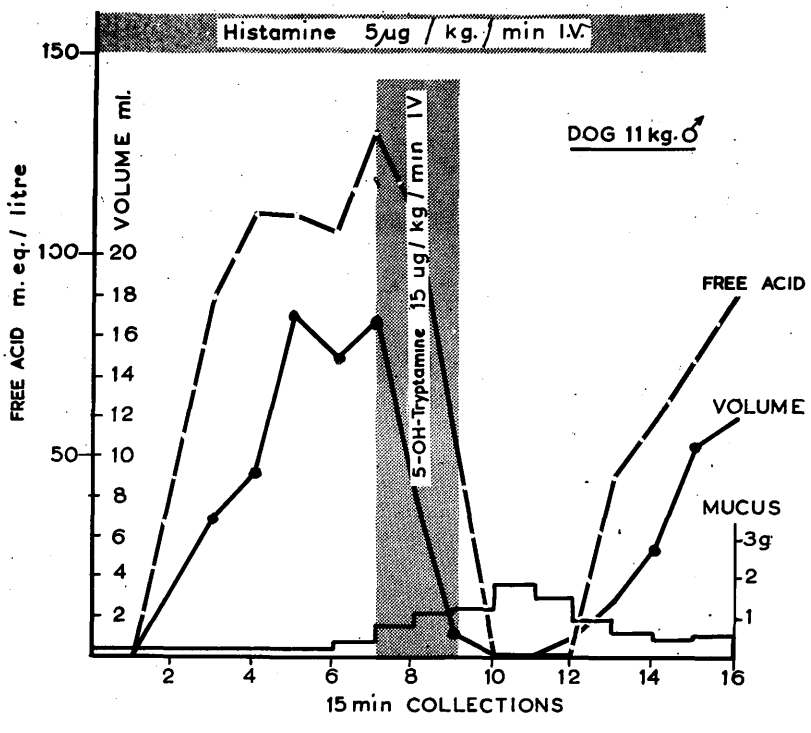


Fig. 4. Effects of infusing 5-HT for 30 min on histamine-stimulated gastric secretion. Ordinates: volume of juice secreted (ml/15 min, open circles) concn. of free acid (m-equiv/l., closed circles), and mucus output (g/15 min, histogram). Horizontal shaded area shows period of histamine infusion and vertical shaded area shows period of 5-HT infusion. It can be seen that the infusion of 5-HT was assoc-:iated with a fall in both the volume and acidity of gastric juice and a rise in the output of mucus.

TABLE 2.

EFFECTS OF INFUSING 5-HT FOR 30 Min ON ACID GASTRIC SECRETION STIMULATED BY A CONTINUOUS INTRAVENOUS INFUSION OF HISTAMINE. THE FIGURES ARE AVERAGES OF THE TOTAL OUTPUT OF FREE ACID IN m. equiv/15 min FROM TWO CONSECUTIVE 15 min COLLECTION PERIODS. THE COLUMNS ARE CONSECUTIVE $\frac{1}{2}$ hr INTERVALS. THE PERIODS OF 5-HT INFUSION HAVE BEEN SYNCHRONISED SO THAT THE SUCCESSIVE VALUES OF THE ACID OUTPUT FOR EACH EXPERIMENT ARE SHOWN AS + OR - WITH RESPECT TO THE $\frac{1}{2}$ hr OF THE 5-HT INFUSION AS ZERO TIME. THE PERCENTAGE INHIBITION REFERS TO THE RELATIONSHIP BETWEEN THE LOWEST ACID OUTPUT REACHED AFTER THE 5-HT INFUSION TO THE PREVIOUS MAXIMUM VALUE.

			Histamine 5 ug/kg/min intravenously									
			Time, in $\frac{1}{2}$ hr periods, after start of Histamine shown + or - with respect of 5-HT infusion as zero.									
Expt.	Dog wt. kg.	Dose of 5-HT ug/kg/min	-4	-3	-2	-1	5-HT 0	+1	+2	+3	+4	% inhib-:ition
1	10	5	0.00	0.10	0.80	0.80	0.28	0.02	0.16	0.57	-	75
2	16	10	-	0.40	0.91	3.07	1.02	0.85	1.32	0.68	-	73
3	25	10	-	0.20	2.03	3.18	2.12	2.35	1.98	2.31	1.80	33
4	17	10	-	1.40	1.16	1.19	0.94	0.20	0.00	0.72	0.72	100
5	10	10	-	0.00	0.78	1.73	0.75	0.48	0.72	-	-	72
6	11	5	-	0.02	0.18	0.78	0.12	0.14	0.48	0.57	-	85
7	6	5	-	0.01	0.09	0.28	0.21	0.18	0.15	0.18	-	47
8	10	20	0.02	0.26	0.46	0.78	0.76	0.30	0.06	0.02	0.00	100
9	12	10	3.17	3.64	5.38	4.36	2.50	1.06	0.93	0.92	-	79
10	23	10	4.44	4.50	4.66	4.65	4.37	2.90	0.92	1.28	2.82	81
11	11	15	-	0.03	1.90	1.91	0.49	0.00	0.13	0.66	0.88	100
12	14	10	0.50	2.05	1.52	2.30	1.91	1.07	0.47	0.21	-	91

TABLE 3.

THE EFFECTS OF TWO SUCCESSIVE INFUSIONS OF 5-HT ON HISTAMINE-STIMULATED ACID GASTRIC SECRETION. FIGURES AS IN TABLE 2. THE TIMES ARE $\frac{1}{2}$ HR INTERVALS IMMEDIATELY BEFORE, DURING AND AFTER EACH 5-HT INFUSION. INTERVALS BETWEEN $\frac{1}{2}$-1 HR SEPARATE THE TWO EXPERIMENTAL PERIODS

			Histamine 5 ug/kg/min intravenously							
			Time, in $\frac{1}{2}$ hr intervals before, during and after 5-HT infusion.							
			5-HT				5-HT			
Expt.	Dog wt. kg	Dose of 5-HT ug/kg/min	-1	0	+1	% inhibition	-1	0	+1	% inhibition
1	12	10	5.92	2.17	4.0	63	5.86	3.33	3.17	43
2	18	10	1.86	0.52	0.90	72	2.01	0.83	1.01	58

drop recorder record (Fig. 5). In a few experiments the stimulation was sufficiently marked to increase the volume of the first 15 min. collections of acid juice.

In these experiments, where 5-HT has been infused along with histamine, the stomach and intestines looked red and engorged at post-mortem. This appearance was indistinguishable from the control dogs which were given histamine along (Fig. 3). In some experiments, where large doses of 5-HT had been given along with histamine, there were small erosions in the gastric mucosa and the appearance of submucosal haemorrhages in the small intestine.

Effects of 5-HT given at the outset with histamine.

When 5-HT was given by slow infusion at, or near, the outset of the experiment with histamine for periods of 30-45 min 5-HT did not prevent the onset of secretion. In this group of 7 experiments (Table 4), there was initial stimulation of the acid secretion, with a fall in acid output occurring between 1-2 hr after the end of the 5-HT infusion in all but one experiment. Fig. 6 shows an experiment in which an initial 5-HT infusion did not prevent the onset of histamine-stimulated secretion but a subsequent infusion of 5-HT produced inhibition. Fig. 7 shows the findings for single injections of 5-HT given intra-arterially into the thoracic aorta so that the 5-HT, after injection into this site, was carried into the upper abdominal aorta, coeliac axis and then to the major/

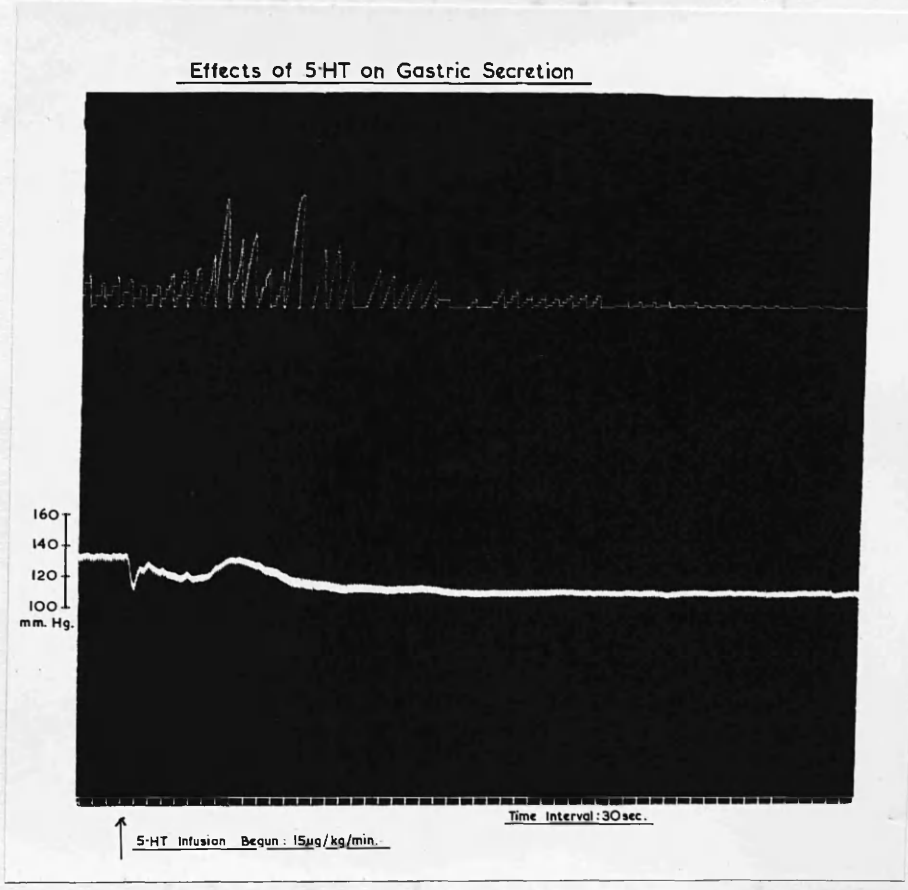


Fig. 5 is a continuous record of the rate of gastric secretion (conventions as in Fig. 2). The top tracing is recorded by the Thorp inpulse counter. In the lower tracing the arterial B.P. recorded on a mercury manometer.

5-HT was infused while secretion was stimulated by a continuous histamine infusion. The secretory rate was slightly increased for a short period and then fell almost to zero. Blood pressure was minimally affected.

TABLE 4.

EFFECTS OF 5-HT ON HISTAMINE-STIMULATED ACID GASTRIC SECRETION WHEN INFUSED FOR 45 min. ALONG WITH HISTAMINE AT THE START OF AN EXPERIMENT. FIGURES AS IN TABLE 2.

Expt.	Dog wt. kg.	Dose of 5-HT ug/kg/min	Histamine 5 ug/kg/min intravenously							
			Time, in ½ hr intervals, after start of Histamine							
			5-HT infusion for 45 min.							
			1	2	3	4	5	6	7	8
1	17	10	2.40	3.25	4.30	1.51	1.23	1.95	-	-
2	15	10	1.33	3.10	3.75	3.42	2.48	1.80	2.18	-
3	12	10	1.06	1.90	0.36	0.06	0.05	0.06	0.18	0.03
4	12	10	1.80	3.26	4.85	4.84	4.09	1.20	0.93	0.01
5	22	5	1.62	3.65	2.24	1.38	2.15	1.45	-	-
6	18	10	0.82	2.24	2.93	2.26	2.32	2.10	-	-

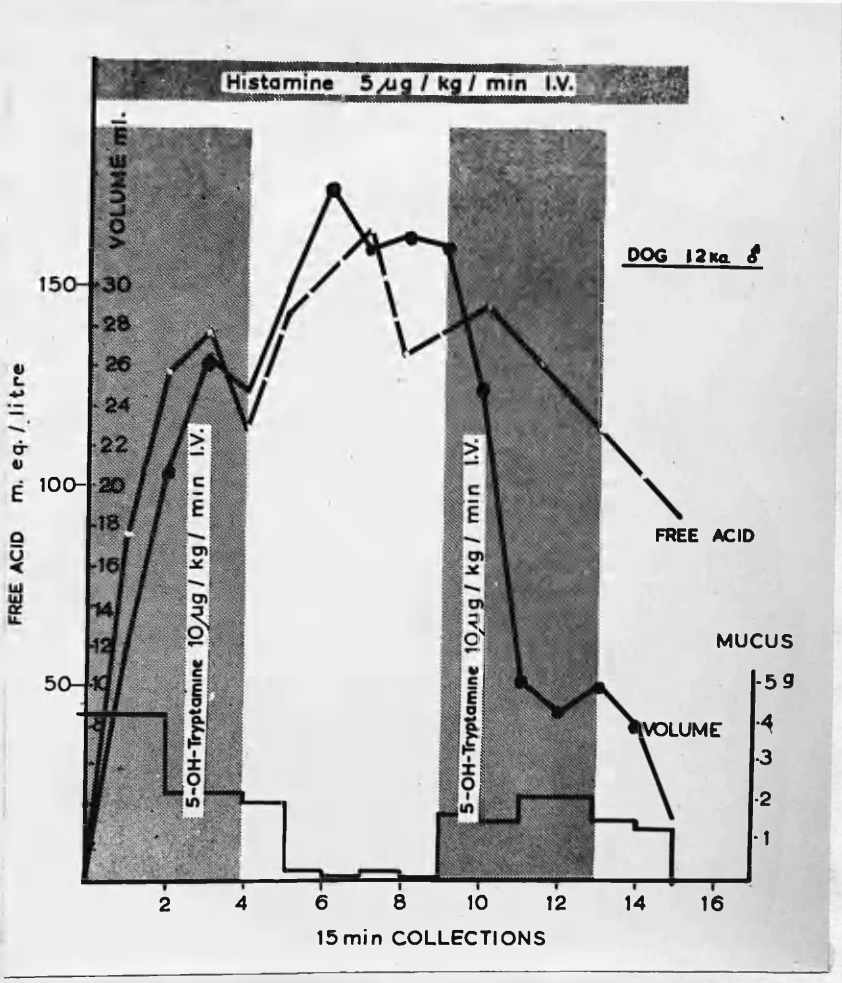


Fig. 6. Conventions in Fig. 1. The infusion of 5-HT given at the outset with histamine had little effect on acid gastric secretion though there were considerable amounts of mucus in the juice. The subsequent infusion of the same amount of 5-HT was associated with marked inhibition of both volume and acidity of secretion and with an increased output of mucus.

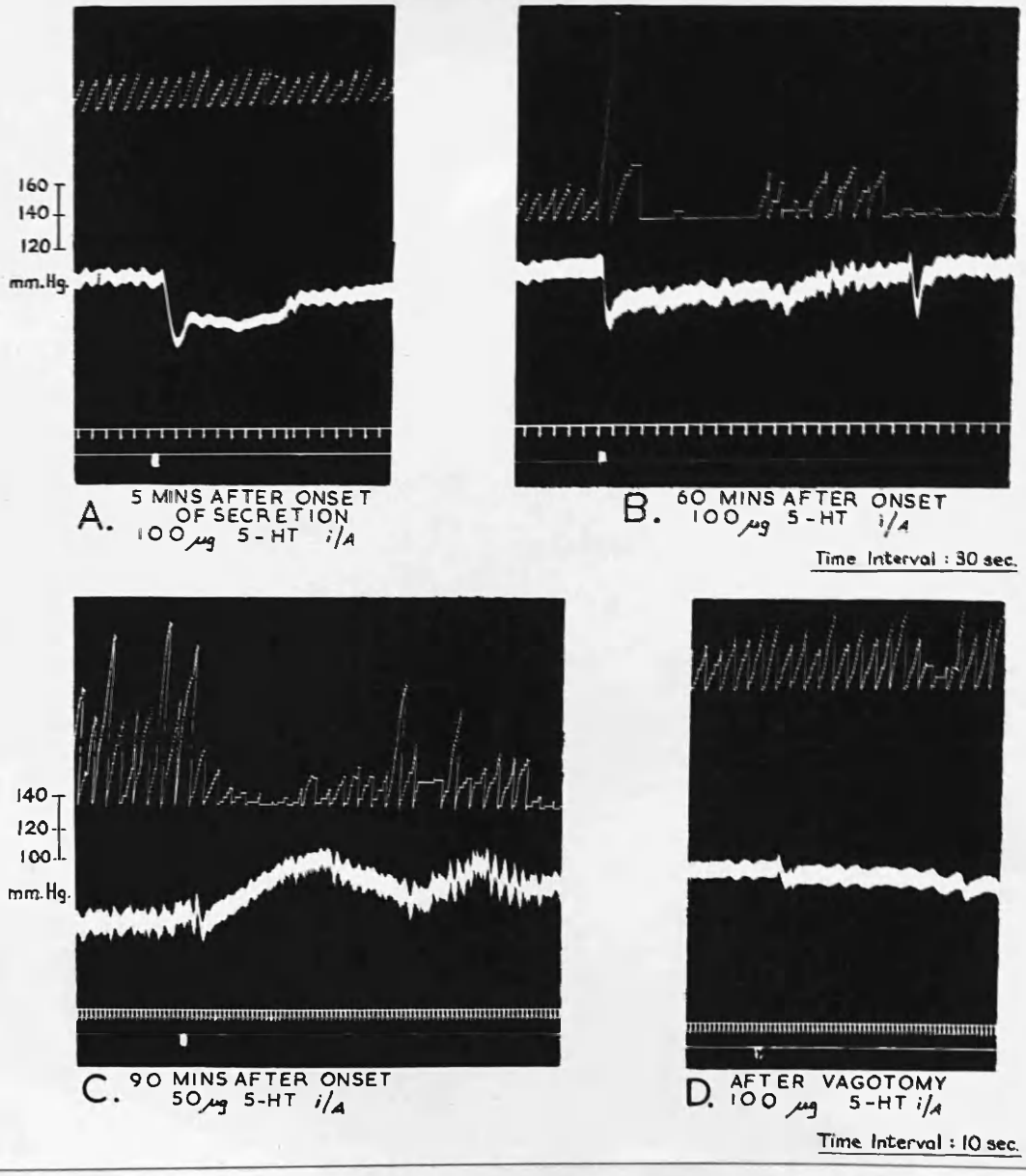
Fig. 7. As in Fig. 2. A,B,C, and D are records taken from the same experiment. 5-HT was given by single injections intra-arterially. Gastric secretion was being stimulated by a continuous intravenous infusion of histamine at the rate of 5 ug/kg/min.

A. single injection of 100 ug 5-HT given 5 min after the onset of secretion. This had no effect on the rate of gastric secretion.

B. 100 ug 5-HT given 60 min after the onset of secretion was associated with initial, brief, stimulation of secretory rate, followed by a longer period of inhibition.

C. 50 ug 5-HT given 90 min after onset of secretion also produced inhibition.

D. Between C and D both vagi were cut in the neck. 100 ug 5-HT given after vagotomy has very little effect on the rate of gastric secretion.


160
140
120
mm.Hg.
A. 5 MINS AFTER ONSET OF SECRETION 100 µg 5-HT i/A
B. 60 MINS AFTER ONSET 100 µg 5-HT i/A
Time Interval : 30 sec.
140
120
100
mm.Hg.
C. 90 MINS AFTER ONSET 50 µg 5-HT i/A
D. AFTER VAGOTOMY 100 µg 5-HT i/A
Time Interval : 10 sec.

major gastric arterial supply. Initially (Fig. 7A) a single injection of 100 ug 5-HT had no effect on the rate of secretion evoked by histamine. 60 min. after the onset of secretion evoked by histamine 100 ug. 5-HT then inhibited secretion (Fig. 7B) and 50 ug. 5-HT produced a pronounced effect 30 min. later (Fig. 7C).

Modification of the effects of 5-HT by bilateral cervical vagotomy.

In a number of experiments, when the secretion was increased again after a period of 5-HT induced inhibition, both vagi were cut in the neck and the infusion of 5-HT was repeated. Vagotomy invariably diminished, and in many cases prevented completely, the inhibitory effect of 5-HT on histamine-stimulated gastric secretion. A typical experiment is shown in Fig. 8 while Table 5 shows examples from nine other experiments. The administration of 0.6 mg. atropine also abolished the inhibitory effect of 5-HT. The effects of single intra-arterial injections of 5-HT were also abolished by vagotomy (Fig. 7D and 7D). The absence of the inhibitory effect after vagotomy could not be explained by tachyphylaxis since successive injections of 5-HT have similar inhibitory effects on acid gastric secretion stimulated by histamine.

DISCUSSION.

5-HT in these experiments failed to stimulate an acid gastric secretory response. On the contrary, it inhibited the acid secretion stimulated by histamine. It is well known that many non-specific factors may inhibit acid gastric secretion. Of these considerable importance has been attached/

TABLE 5.

EFFECTS OF 5-HT on HISTAMINE-STIMULATED ACID GASTRIC SECRETION WHEN GIVEN BEFORE AND AFTER BILATERAL CERVICAL VAGOTOMY. FIGURES AS IN TABLE 2., THE TIMES ARE ½ hr INTERVALS IMMEDIATELY BEFORE, DURING AND AFTER EACH 5-HT INFUSION. INTERVALS BETWEEN ½ - 1 hr SEPARATE THE TWO EXPERIMENTAL PERIODS. HISTAMINE WAS GIVEN CONTINUOUSLY THROUGHOUT.

			Histamine 5 ug/kg/min intravenously							
			Time, in ½ hr intervals, before, during and after 5-HT infusion.							
Expt.	Dog wt. kg.	Dose of 5-HT ug/kg/min	-1	5-HT 0	+1	% inhibition		-1	5-HT 0	+1	% inhibition
1	23	10	3.83	3.99	1.61	57		1.80	2.08	2.08	0
2	10	10	1.25	0.74	0.53	58		0.91	1.00	0.90	1
3	12	10	1.23	1.63	0.47	61		0.81	0.92	0.69	14
4	16	5	3.20	2.77	2.30	28		3.42	3.25	3.51	5
5	10	15	1.14	0.15	0.0	100		0.24	0.47	0.34	0
6	18	10	3.15	0.79	0.77	75		3.80	4.18	3.70	3
7	22	15	4.40	2.40	1.85	57		2.36	2.40	2.47	0
8	11	5	1.03	0.76	0.65	36		0.77	0.86	1.06	0
9	12	10	4.97	2.45	3.28	51		2.97	3.04	2.77	7
10	12	10	1.51	1.14	0.56	63		0.87	0.79	0.98	9

Bilateral Vagotomy

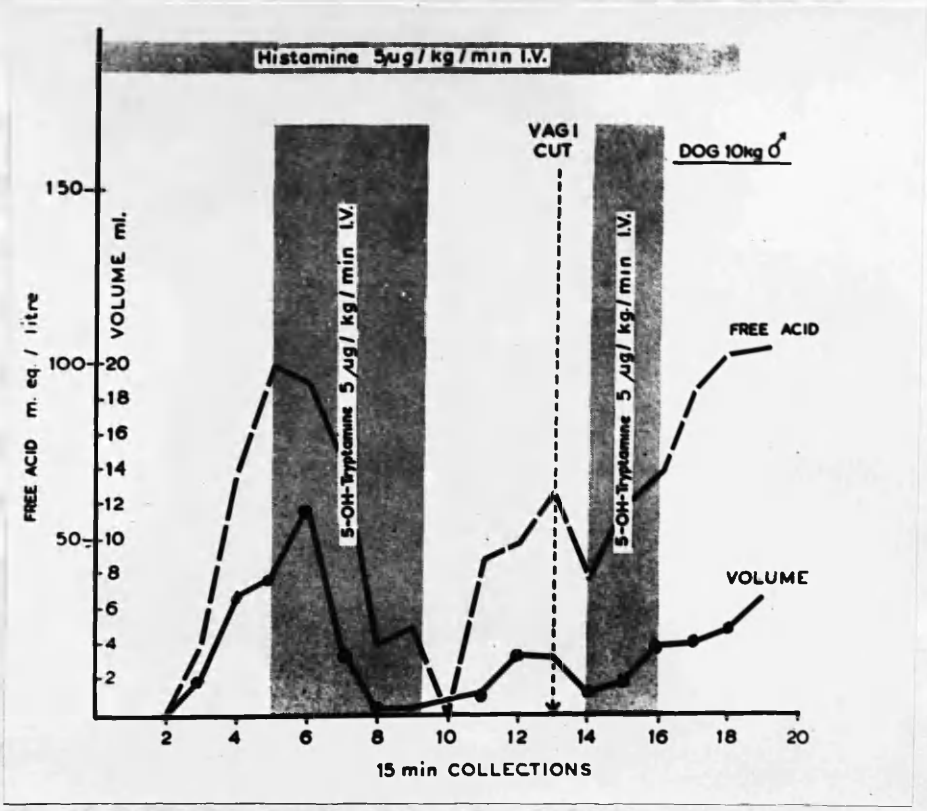


Fig. 8. Conventions as in Fig. 1. The first infusion of 5-HT produced nearly complete inhibition of both volume and acidity of gastric secretion. When a second infusion was given after bilateral cervical vagotomy there was no inhibition of secretion.

attached to nausea, pyrexia, and variations in gastric mucosal blood flow. In our experiments with anaesthetised dogs, there has been no evidence of pyrexia. It is difficult to know if inhibition of gastric secretion by nausea has any meaning in anaesthetised animals but, certainly, 5-HT did not produce any retching or vomiting movements.

Thompson & Vane (1953) have shows that the rate of histamine-stimulated gastric secretion can be varied by altering the rate of gastric blood flow. Of the several naturally occurring substances which have been shown to inhibit gastric secretion, noradrenaline, adrenalin and vasopressin probably act indirectly by reducing the blood flow through the stomach, though, on the other hand, urogastrone (Gregory, 1955) and enterogastrone (Gregory, 1956) have been shown to produce inhibition without blood pressure changes or changes in local gastric blood flow as estimated by mucosal temperature changes. Inhibitory effects of 5-HT when produced by slow infusion, are accompanied by quite small blood pressure changes until the dose rises above 20 ug./kg./min. (See Fig. 5). The inhibitory effects were produced in the absence of pathological changes in the gastric mucosa. The fact that 5-HT can produce inhibition of secretion without marked blood pressure changes, and in the absence of mucosal damage has been taken to mean that 5-HT probably does not act entirely by means of induced local vasoconstriction, and, in this respect, may be similar to urogastrone and/

and enterogastrone.

The inhibition of acid gastric secretion which has followed infusions of 5-HT in our experiments has developed partly on an intact vagal innervation. While the vagal pathway for excitation of acid gastric secretion is known, there may possibly be a similar pathway for inhibitory effects. Code & Watkinson (1955) have shown that an intact vagal innervation is necessary for acid applied to the duodenal mucosa to be able to inhibit histamine-stimulated gastric secretion. Schachter (1949), discussing the acid secretion elicited by certain anaesthetics, notes that the secretion was abolished by the traumatic procedure of preparing the gastric fistula and was prevented by manipulation of the vagi in the neck. He considers that his findings are in agreement with Pavlov (1902) who claimed that the vagi contained secretory-inhibitory fibres in its supply to the stomach and pancreas. In this respect it is of interest to note that 'mecholyl', a parasym-:pathomimetic agent, has been found to stimulate the production of an acid gastric juice for a brief period, to be followed by a later period of inhibition of acid secretory activity (Wener, Karp & Hoff, 1948).

It is an odd finding that when 5-HT is given, at the beginning, along with histamine it fails to prevent the onset of histamine-stimulated secretion. This suggests that there is some aspect of the acid secretory inhibition other than the mere infusion of 5-HT in adequate amounts. It may be that a critical pH on the surface of the gastric and pyloric mucosa is/

is a necessary preliminary for the inhibition to occur.

Another possible explanation may be that there are varying rates of enzymatic destruction of 5-HT. The fact that there is considerable individual variation in sensitivity to 5-HT in the animals we have used might be accounted for on the basis of variations in the rate of 5-HT break down. In this respect, it ought to be interesting to compare the effects of 5-HT with that of its pre-cursor, the amino acid 5-Hydroxytryptophan. This substance remains unaffected by amine-oxidase, which is of wide distribution. The administration of 5-HTP might lead to a general increase in the local stores of 5-HT and under these circumstances more intense effects on acid gastric secretion could be anticipated. The above experiments would then bear a more normal relationship to physiological events. 5-HT would be acting after local formation in the tissues rather than after intravenous infusion which is essentially a pharmacological method of approach.

SUMMARY

1. The effects of 5-HT on gastric secretion have been examined in dogs anaesthetised with chloralose and urethane and prepared with a stomach fistula and a ligature at the pyloro-duodenal junction.

2. Intravenous infusion of 5-HT alone for 45 - 90 min. did not/

not stimulate acid gastric secretion but appeared to increase the production of mucus.

3. When an acid gastric secretion was stimulated by a continuous intravenous infusion of histamine, infusion of 5-HT for 30 min. was found to inhibit the secretion.

4. Infusion of 5-HT at the start of histamine-stimulated gastric secretion was found to be less effective in producing inhibition than when given 1½ - 2 hr. later.

5. When 5-HT was given after bilateral cervical vagotomy it was found to be less effective in inhibiting histamine-stimulated secretion.

CHAPTER THREE.

The effect of precursors of 5-hydroxytryptamine and recent feeding on gastric secretion.

CHAPTER THREE.

THE EFFECT OF PRECURSORS OF 5-HYDROXYTRYPTAMINE AND RECENT FEEDING ON GASTRIC SECRETION.

It has been shown in the previous Chapter that 5-hydroxytryptamine (5-HT) may inhibit the acid gastric secretion stimulated by histamine. 5-HT is destroyed rapidly however, in the body by amine oxidase (Blaschko, 1952; Erspamer, 1955) and must be formed from precursors. The source of the precursors in the diet is the essential amine acid trypto-:phan and the pathway of formation follows a hydroxylation step to 5-hydroxytryptophan (5-HTP) and decarboxylation to 5-HT. (For review see Udenfriend, 1958).

This Chapter describes experiments on the effects of 5-HT precursors, 5-HTP and L-tryptophan, and of feeding on acid gastric secretion elicited by infusions of histamine.

METHODS

The experiments were carried out on dogs anaesthetised by a solution of chloralose 2% (w/v) and urethane 20% (w/v). This solution was given intravenously in the dose of 2.5 ml./kg. The animals in the main experimental group were starved for 24-36 hr. before starting an experiment unless stated otherwise. In a second group, the "fed dogs" the normal afternoon meal of meat was followed by a final meal of 1 pint of milk in the evening, about 12 hours before experiment, with water available. A gastric cannula was inserted into the most depend-:ent part/

part of the stomach and the contents allowed to drain spontaneously after abdominal closure. Secretion was collected in a beaker, the volume measured and the acidity determined by titration. The methods as described in the previous Chapter. Histamine was given by infusion into one femoral vein and 5-HTP into the other femoral vein by infusion controlled by a Palmer's Slow Injection Apparatus. L-tryptophan was fed orally or infused as an aqueous suspension directly into the second part of the duodenum through a cannula inserted at this site.

Biological assay of 5-HT was performed by the method of Dalgliesh, Toh & Work (1953). Extracts of portal blood were examined for 5-HT, the extraction procedure being that of Toh (1954); the blood samples were taken from the dogs which had been starved for 24-36 hr. and also from the dogs without preliminary starvation. The samples of portal blood were obtained by backflow through a polythene cannula inserted through the splenic vein.

RESULTS

Effects of 5-hydroxytryptophan on histamine-stimulated gastric secretion in anaesthetised dogs.

When 5-HTP was given intravenously for a period of 30 min. during continuous histamine-stimulated acid gastric secretion the most striking effect was fall in the volume and acidity of the guice.

Infusions of 5-HTP have been given on 22 occasions in 16 dogs (Tables 1 & 4). The first effect was often a short-lived stimulation of/

of acid gastric secretion. This is clearly seen in 7 or the 10 infusions listed in Tables 1 and 2, in which the values for the two or three collections, after commencement of 5-HTP infusion, may be raised. Inhibition of secretion was seen 30-45 min. after starting the infusions and lasted for 1-2 hr. (Tables 1 & 2 and Fig. 1).

In Table 1, 3 experiments are shows in which two successive infusions of 5-HTP have been given. The decrease in the histamine-induced gastric secretion associated with the second infusion of 5-HTP began more rapidly than after the first infusion in two of these experiments; this effect is also illustrated in the repeat infusion of Figure 2.

In four experiments (Table 2) the effects of 5-HTP were compared with effects of 5-HT. In two experiments the injection of 5-HTP preceded the injection of 5-HT and the order was reversed in the other two. There was a longer latency before the 5-HTP effects appeared; otherwise the effects of 5-HT and 5-HTP were similar. (Fig. 3).

When 5-HTP was given along with histamine for periods of 30 min, at the outset of three experiment (Table 3), it did not prevent the onset of secretion; a fall in acid output occurred between 1-2 hr. after the end of the 5-HTP infusion in the first two experiments recorded in this table. In the third experiment, the animal received an infusion of 15 ug/kg/min 5-HTP which was followed by no significant period/

TABLE 1.

EFFECTS OF INFUSING 5-HTP FOR 30 MINUTES ON ACID GASTRIC SECRETION STIMULATED BY A CONTINUOUS INTRAVENOUS INFUSION OF HISTAMINE. THE EFFECTS ARE SHOWN OF TWO SUCCESSIVE INFUSIONS SEPARATED BY AT LEAST 1 HOUR.

Expt. No.	Dog wt kg	Dose 5-HTP ug/kg/min	Histamine 5 ug/kg/min intravenously m. equiv of free acid in 15 minute collection periods						
					5-HTP				
1	14	5	(0.72	0.72	1.46	1.18	0.92	0.38	1.04
		5 *	(1.04	1.44	0.60	0.06	0.18	0.30	0.78
2	14	10	(1.08	1.38	2.00	2.10	0.46	0.30	0.70
		10 *	(0.70	0.70	0.62	0.12	0.15	0.15	0.78
3	20	15	(1.29	1.29	1.86	1.54	0.63	0.63	0.77
		15 *	(0.77	1.87	2.26	2.02	1.00	0.80	0.20

* Repeat infusion.

TABLE 2.

THE EFFECTS OF INFUSIONS OF 5-HTP AND 5-HT FOR 30 MINUTES ON HISTAMINE-STIMULATED ACID GASTRIC SECRETION; IN TWO EXPERIMENTS 5-HTP WAS GIVEN FIRST; IN THE OTHER TWO 5-HT WAS THE INITIAL SUBSTANCE INFUSED. CONVENTIONS AS IN TABLE I.

Expt. No.	Dog wt kg	Dose 5-HTP ug/kg/min	Histamine 5 ug/kg/min intravenously m. equiv of free acid in 15 minute collection periods									
			5-HTP									
1	22	10	2.40	2.60	2.86	1.40	0.70	0.66	0.03	0.03	0.62	-
			5-HT									
			0.66	0.66	0.88	0.30	0.12	0.00	0.48	0.48	-	-
2	18	15	5-HTP									
			0.95	0.95	1.08	0.70	0.54	0.10	0.10	0.00	0.02	0.41
			5-HT									
			0.25	0.30	0.14	0.00	0.02	0.02	0.88	0.88	-	-
3	8	10	5-HT									
			0.32	0.48	0.68	0.14	0.40	0.40	0.46	0.50	-	-
			5-HTP									
			0.50	0.62	0.66	0.66	0.34	0.32	0.22	0.07	0.06	0.06
4	12	15	5-HT									
			1.50	1.80	0.92	0.46	0.10	0.12	0.64	0.48	0.66	0.94
			5-HTP									
			1.00	1.00	1.30	0.94	0.80	0.22	0.09	0.16	0.60	0.72

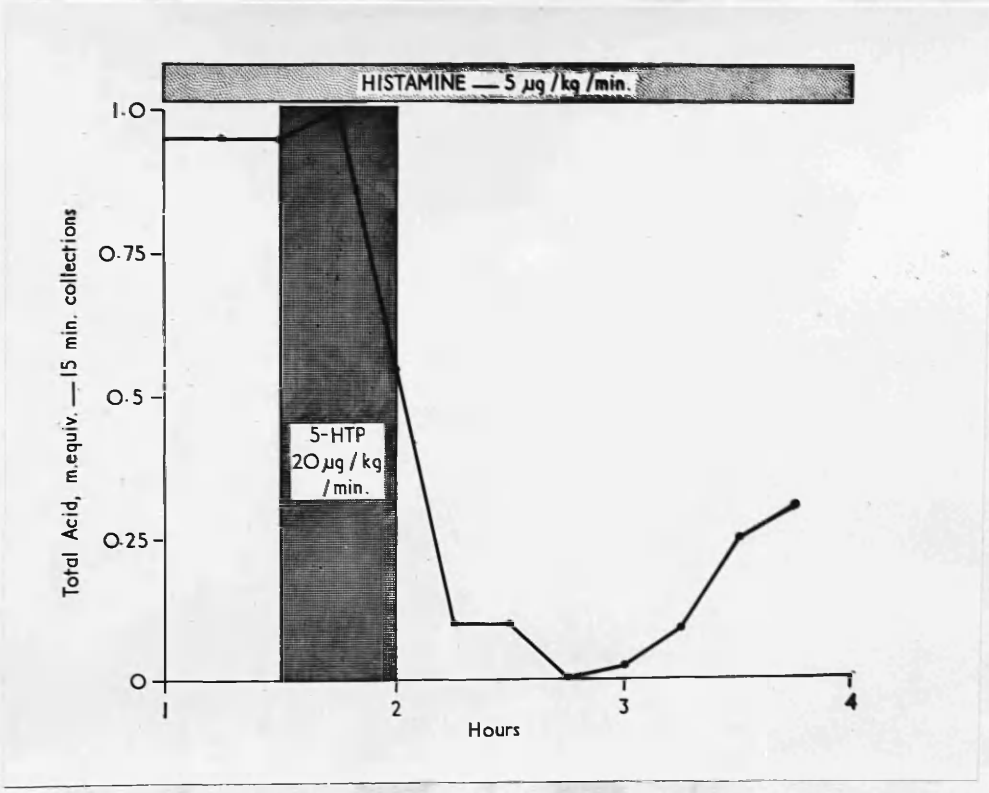


Fig. 1 shows the effect of infusing 5-HTP for 30 min. on histamine-stimulated gastric secretion. Ordinates: total acid in m.equiv. Abscissae: time in hours. Horizontal shaded area shows the period of histamine infusion and the vertical shaded area the period of 5-HTP infusion. The infusion of 5-HTP was associated with a fall in the acid secreted in response to histamine; it took place after 30 min and lasted for 1-2 hr.

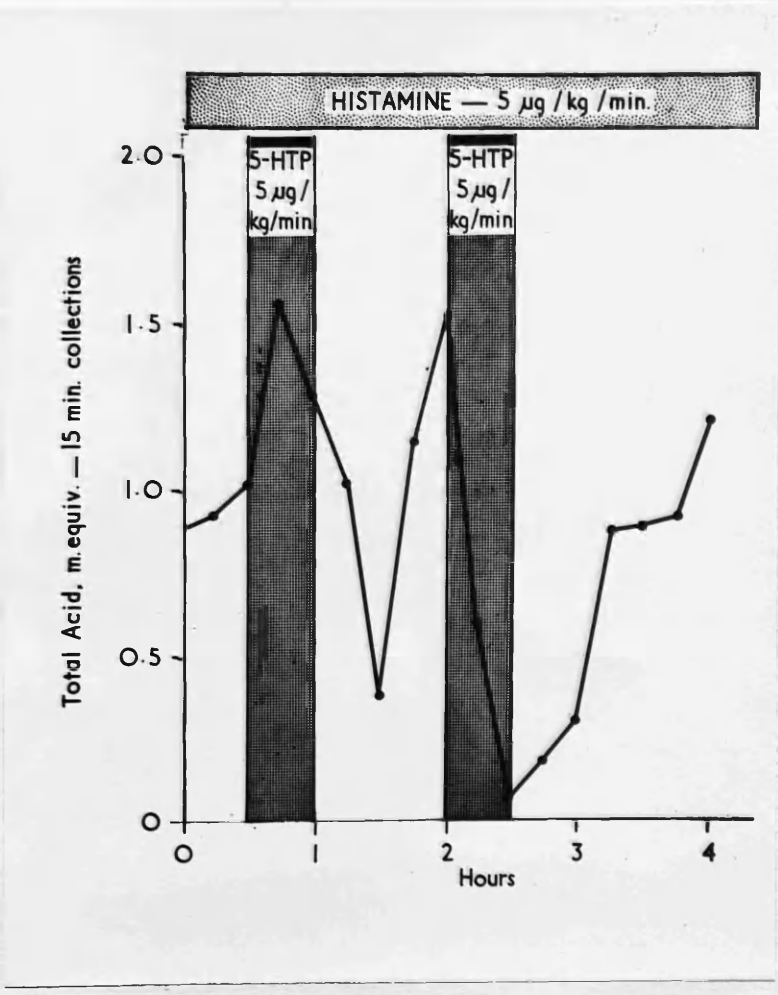


Fig. 2 shows the effects of two successive 30 min infusions of 5-HTP. (Same conventions as in Fig. 1). The second infusion of 5-HTP associated with a much more rapid inhibitory effect.

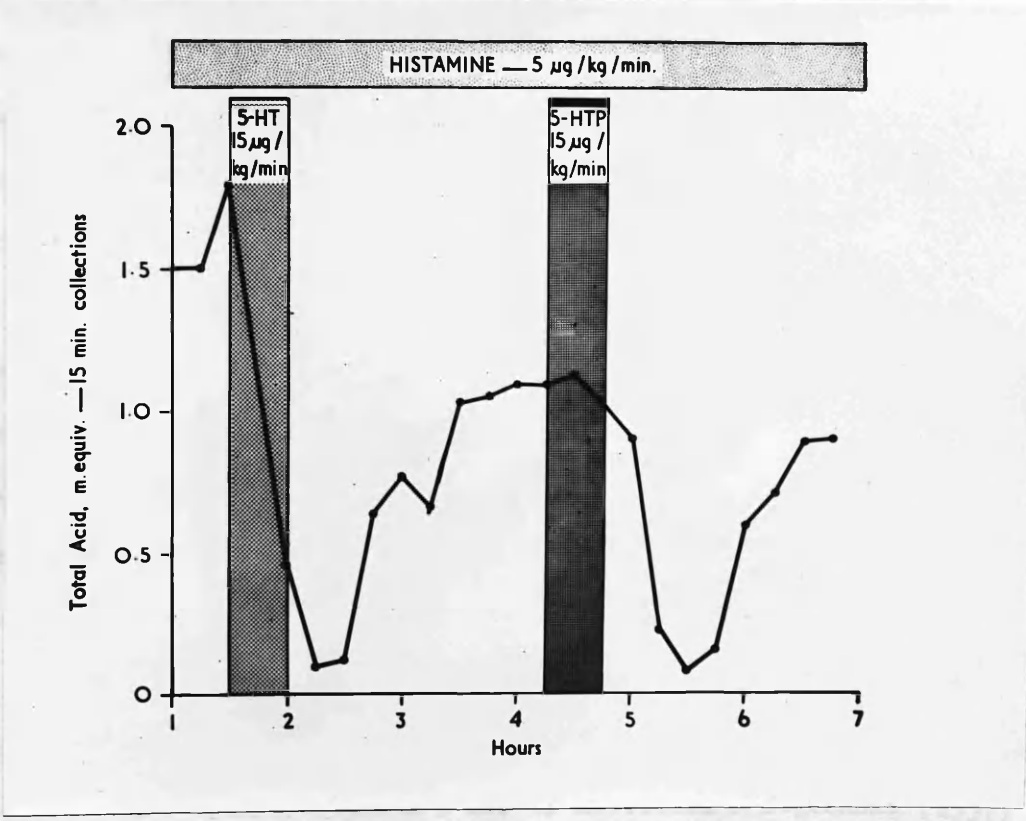


Fig. 3 gives a comparison of the effect of 5-HT and 5-HTP infused for 30 min in each case. The inhibition is of comparable extent but that of 5-HTP is slightly delayed (even although it is the second infusion).

period of inhibition; indeed the acid secretion rose from 0.28 to 1.8 mille equivalents during the course of the experiments. A later infusion given 1¼ hr after cessation of the first infusion, was followed by a fall in the secretory output (Table 3, No. 3); as is also shown in Fig. 4.

In six experiments the vagi were cut in the neck - either before starting the experiment, following the infusion of 5-HTP (Table 4)(in two instances a preliminary infusion (Table 4, Nos. 5 and 6) of 5-HTP came first.)

It can be seen that vagotomy diminished the inhibitory effect of 5-HTP on acid gastric secretion. These results are similar to those previously described for 5-HT (Fig. 5).

Following each experiment involving the infusion of 5-HTP the stomachs were examined post mortem. In each case there was an absence of retained secretion but there was usually a variable layer of mucus lining the stomach wall. Small areas of haemorrhage and erosion were often seen comparable to the pathological effects noted for 5-HT by Hedinger & Veraguth (1957) and for 5-HTP by Haverback & Bogdanski (1957) when these substances were administered systemically. Blood pressure changes during these experiments were similar to those produced by infusion of 5-HT, but the fall occurred after a variable latent period of several minutes; the levels in all instances remained above 100 mm. Hg.

Effects of tryptophan on histamine-stimulated gastric secretion.

Since the argentaffin cells of the alimentary tract might be the only/

TABLE 3.

EFFECTS OF 5-HTP WHEN ADMINISTERED FOR 30 MINUTES WITH HISTAMINE AT THE START OF AN EXPERIMENT. CONVENTIONS AS IN TABLE 1.

Expt. No.	Dog wt. kg.	Dose ug/kg/min	Histamine 5 ug/kg/min intravenously									
			m. equiv of free acid in 15 minute collection periods									
		5-HTP										
1	22	5	0.61	0.92	0.96	0.42	0.04	0.04	0.16	0.09	0.12	0.54	1.80
2	10	10	0.21	0.56	0.58	1.22	0.58	0.58	0.48	0.48	0.82	0.84	1.60
3	14	15	0.28	0.62	1.04	1.45	1.46	1.80	1.80	-	-	-	-
		15 *	1.80	1.96	1.82	0.40	0.75	0.86	0.96	-	-	-	-

* = repeat infusion.

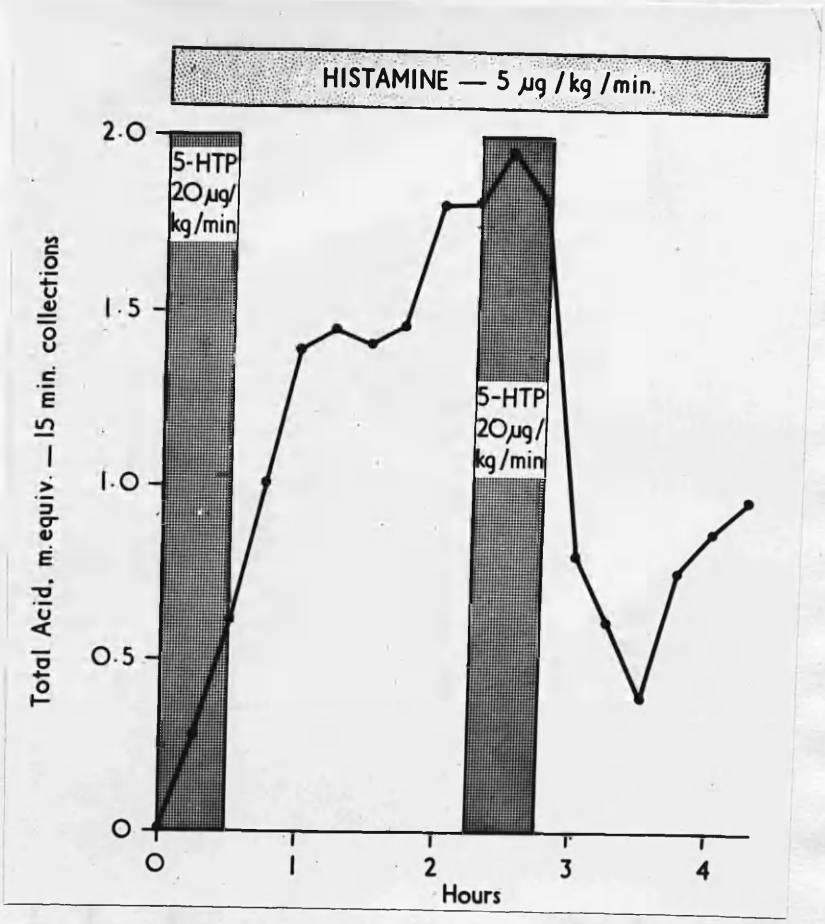


Fig. 4. Infusion of 5-HTP for 30 min at the commencement of the histamine infusion did not prevent the onset of acid secretion; a subsequent infusion of 5-HTP, however, produced the inhibitory effect. (Conventions as in Fig. 1).

TABLE 4.

EFFECT OF 5-HTP ON HISTAMINE-STIMULATED ACID GASTRIC SECRETION WHEN GIVEN BEFORE AND AFTER BILATERAL CERVICAL VAGOTOMY. CONVENTIONS AS IN TABLE 1. SECRETIONS WERE COLLECTED AT 15 MINUTE INTERVALS BEFORE, DURING AND AFTER THE 5-HTP INFUSIONS.

Expt. No.	Dose		Histamine 5 ug/kg/min								
			m. equiv of free acid in 15 minute collection periods								
					5-HTP						
1	5	Vagotomy	0.96	1.28	1.88	1.75	1.60	1.36	1.24	1.26	1.26
2	10	Vagotomy	0.88	0.92	0.80	0.64	0.64	0.48	0.70	0.86	0.86
3	5	intact vagi	0.15	0.17	0.17	0.17	0.12	0.12	0.16	0.20	0.24
4	10	intact vagi	0.78	0.84	0.88	0.82	0.60	0.75	0.79	0.81	0.81
5	5	intact vagi	0.56	0.92	1.06	1.20	0.86	0.31	0.48	0.96	1.00
6	10	intact vagi	0.43	1.16	1.48	1.50	1.00	0.66	0.10	0.28	0.88
*5	5	Vagotomy	1.20	1.00	0.80	0.82	0.60	0.64	0.62	0.64	0.64
*6	10	Vagotomy	1.00	0.94	0.90	0.92	0.60	0.70	0.68	0.66	0.66

* repeat infusions.

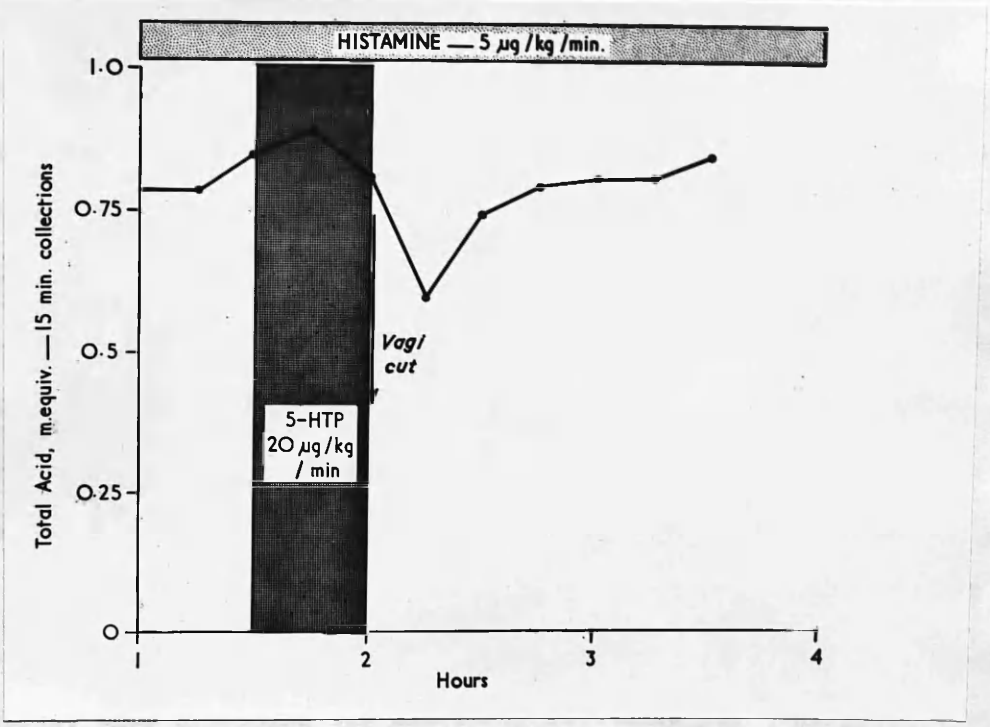


Fig. 5. The anticipated effect of 5-HTP should have been a marked secretory depression of the acid gastric secretion stimulated by histamine. A vagotomy was carried out after infusion of 5-HTP in this instance and the secretory inhibition induced by the infusion remained very small.

only source of 5-HTP (see Chapter Five) and since the intestinal mucosa contains very large amounts of 5-hydroxytryptophan decarboxylase, it seems likely that 5-HT is formed there and not merely accumulated and stored. The likelihood that the argentaffin cell fulfil the important role of forming 5-HTP can be deduced from the fact that, in a patient with a tumour of the argentaffin cells, 5-HTP as well as 5-HT was excreted in the urine (see Chapter 5). If, as far as 5-HT is concerned, argentaffin cells are occupied principally with decarboxylation the great increase alone of these cells in the carcinoid tumour state would not be expected to increase the 5-HT output: in the normal animal tissue, 5-hydroxytryptophan decarboxylase is greatly in excess and lack of available substrate rather than enzyme is the limiting factor to higher 5-HT production (Dalgliesh & Dutton, 1957). As 5-HT output increases grossly in the carcinoid cases it has to be presumed that 5-HTP, formed from the precursor L-tryptophan, is a principal product of the argentaffin cells, thus providing the necessary substance for 5-HT production (see Chapter 5).

On this assumption, that L-tryptophan may be converted to 5-HTP in the argentaffin cells, we have examined the effects of L-tryptophan on histamine-stimulated gastric secretion, and, because of its presumed conversion locally in argentaffin cells, the L-tryptophan was given into the alimentary tract. The L-tryptophan was given into the duodenum of starved dogs in three experiments about 3 hr before the establishment

of/

of secretion by histamine; in another two experiments the L-tryptophan was given orally in divided doses over the preceding 12 hr. The results of these experiments are shown in Table 5.

In the first three experiments the secretory output rose for 1 hr after the start of histamine infusion but failed to be maintained. The secretion fell between 1-2½ hr and began to show recovery between 2½ and 3 hr. In the fourth experiment the total acid output was never high but increased up to 1½ hr and then decreased until at 3 hr. there was no acid secretion at all. In the fifth experiment there was a marked delay in the secretory response which was not fully elicited till 2½ hrhad elapsed. We should have expected with starved dogs, receiving continuous intravenous infusions of histamine, continuous secretion for at least 4 hr. (see below).

The effect of recent feeding on (a) the acid gastric secretory response to histamine and (b) the concentration of 5-HT in the portal blood.

(a) It can be shown that in animals which have not been starved there is a fall in the acid secretory response to histamine. When histamine-stimulated acid gastric secretion is studied in dogs starved for 24-36 hr before experiment a typical secretory plateau is observed. This is illustrated in the results of 10 experiments, shown in Table 6.

It should be noted that between the end of the operative procedures and the start of the histamine infusion no acid gastric juice was secreted by any of the dogs. Once the histamine infusion was started, it was continued at a constant rate.

TABLE 5.

EFFECT OF ADMINISTRATION OF L-TRYPTOPHAN TO DOGS (STARVED 24-36 hr) ON HISTAMINE-STIMULATED ACID GASTRIC SECRETION. Nos. 1 to 3 HAD TRYPTOPHAN INFUSED INTO THE DUODENUM 4 hr BEFORE ONSET OF HISTAMINE STIMULATION. Nos. 4 and 5 WERE FED TRYPTOPHAN IN THE PRECEDING 12 hr. CONVENTIONS AS IN TABLE 1.

Expt. No.	Dog wt. kg	Dose of Tryptophan (mgm)	Histamine 5 ug/kg/min						
			m.equiv of free acid in 15 minute collection periods						
			½ hr	1 hr	1½ hr	2 hr	2½ hr	3 hr	Mucus
1	20	300	1.10	3.65	1.65	0.25	0.84	1.83	++
2	15	300	4.70	1.15	0.41	0.23	0.95	-	++
3	15	500	1.85	2.15	1.20	0.48	0.19	0.65	+
4	13	400	0.02	0.23	0.33	0.27	0.08	0.00	+
5	11	6 hourly for 18 hr	0.04	0.22	0.31	0.53	0.76	0.76	+
			0.72	0.58	0.45				

TABLE 6.

HISTAMINE-STIMULATED ACID GASTRIC SECRETION FROM DOGS STARVED FOR 24-36 hr BEFORE EXPERIMENT. THE FIGURES ARE AVERAGES OF THE TOTAL OUTPUT OF FREE ACID IN m.equiv/15 min FROM TWO CONSECUTIVE 15 MIN COLLECTION PERIODS. THE PERCENTAGE FALL IN ACID OUTPUT REFERS TO THE RELATIONSHIP BETWEEN THE LOWEST ACID OUTPUT REACHED AND THE PREVIOUS MAXIMUM VALUE. THE ½ hr TIME INTERVALS REFER TO THE START OF THE HISTAMINE INFUSION AS ZERO.

Expt.	Dog wt. kg.	Histamine 5 ug/kg/min intravenously								
		Time, in hr after start of histamine								% fall in acid output
		½	1	1½	2	2½	3	3½	4	
1	15	0.03	0.42	0.95	1.11	1.20	1.28	1.16	-	9
2	24	0.92	3.84	4.49	4.46	4.48	-	-	-	1
3	11	0.04	1.09	5.01	5.45	6.08	5.10	-	-	15
4	15	0.39	2.34	3.03	2.46	2.64	2.75	2.76	2.75	18
5	12	0.17	1.14	1.41	1.55	1.60	-	-	-	0
6	9	0.02	0.32	0.58	0.68	0.94	0.99	0.78	-	21
7	17	0.03	1.25	2.41	2.42	2.42	-	-	-	0
8	18	0.04	1.08	2.18	3.88	4.90	3.80	-	-	22
9	5	0.05	0.56	0.59	1.03	1.21	1.00	-	-	17
10	13	0.05	0.63	1.50	1.66	1.31	-	-	-	21

In all experiments acid gastric secretion began within ½ hr. of starting the infusion of histamine and the output of acid increased rapidly for 1½ hr. Between 1½-2 hr. after the start of the histamine infusion, the output of acid rose more slowly in the majority of the experiments; in experiment 2, a plateau was reached at 1. hr, and in experiment 10 a slight fall in secretion occurred at 2½ hr. Between 2½-3 hr, a fall in secretion was seen in most experiments. This fall was never more than 22% of the previous maximum output. It appears from these results that the output of histamine-stimulated acid gastric juice in previously starved, anaesthetised dogs, with intact vagi, approximates to a steady state between 1½-3½ hr. after the start of the histamine infusion.

When histamine-stimulated acid gastric secretion is studied in dogs fed in the 12 hr period before experiments, acid gastric secretion is not maintained. The results of 9 experiments are shown in Table 7. As in the previous series no acid gastric juice was secreted during the period before the start of the histamine infusion. Once started, the histamine infusion was continued at a steady rate.

In all experiments, acid gastric secretion began within ½ hr. of starting the histamine infusion and increased rapidly for 1 hr. Between 1-½ hr after the start of the histamine infusion, the output of acid fell/

TABLE 7.

HISTAMINE-STIMULATED ACID GASTRIC SECRETION FROM DOGS FED WITH MILK IN THE 12 hr PERIOD BEFORE EXPERIMENT. (Conventions as in Table .)

Expt.	Dog wt. kg.	Histamine 5 ug/kg/min intravenously								
		Time, in hr after start of histamine								
		½	1	1½	2	2½	3	3½	4	% fall in acid output
1	21	0.85	2.68	1.53	0.92	0.43	0.00	0.00	-	100
2	20	0.02	0.34	1.10	0.78	0.19	1.00	-	-	100
3	15	0.04	2.00	1.78	0.06	0.10	0.01	0.00	-	100
4	14	0.01	0.05	1.11	0.31	0.17	0.00	-	-	100
5	18	0.15	0.52	1.25	0.41	0.04	0.02	0.00	0.00	100
6	15	0.80	1.42	1.56	0.95	0.09	0.04	0.00	-	100
7	23	0.63	5.12	4.20	1.77	0.18	0.03	0.00	0.00	100
8	11	0.18	0.93	2.14	0.73	0.16	0.09	0.00	-	100
9	14	0.01	0.99	0.87	0.52	0.56	0.11	0.00	-	100

TABLE 8.

5-HT BLOOD LEVELS IN SAMPLES TAKEN FROM PORTAL VENOUS SYSTEM OF (a) STARVED DOGS (b) RECENTLY FED DOGS.

(a) Starved dogs ug/ml 5-HT	Starved dogs quoted from TOH (1954) ug/ml 5-HT
0.14	0.10
0.11	0.14
0.08	0.12
0.13	0.10
0.21	0.07
Mean = 0.13	0.12
	0.28
	0.08
	0.20
	0.16
	0.17
	Mean = 0.14

(b) ug/ml 5-HT in portal blood of recently fed dogs.

	0	1 hr	3 hr
	1.20	0.45	0.56
	0.15	0.12	0.12
	0.31	0.15	0.13
	0.18	0.21	0.26
	0.10	0.15	0.12
	0.40	0.38	0.32
	0.20	0.24	0.24
	0.60	-	0.56
Mean=	0.39	0.24	0.29

fell in all experiments. This fall in acid output became very marked between 2-3 hr, and no gastric juice was secreted in any of the experiments between 3-3½ hr after the start of the continuous histamine infusion. The secretion of an acid gastric juice did not return in two experiments which were continued for 4 hr.

(b) The difference that has been observed between fed and starved dogs in these experiments could be related to inhibition of histamine-stimulated acid secretion by 5-HT released locally. Stacey & Sullivan (1957) have shown that a high protein intake increases 5-HT stores in the wall of the intestinal tract. On the basis that 5-HT may be released from this site we have examined the 5-HT levels in the portal blood of fed dogs. The portal blood of fed dogs had more 5-HT than the portal blood from dogs starved 24-36 hr. The values given by Toh (1954) for starved dogs were also of a lower order (Table 8).

Levels of 5-HT were also examined in fed dogs given histamine by infusion. Repeated blood samples were usually taken, one at one hour when secretion had reached peak levels and a second at two hours, when, in fed dogs, considerable inhibition of acid secretion had been found. Examination of repeated portal blood samples showed no evidence of progressive change in 5-HT concentration.

DISCUSSION

The effects of 5-HTP on histamine-induced gastric secretion are similar to the effects of 5-HT except, 5-HTP occasionally appears to increase//

increase gastric secretion as its first, though transient action and that when it does eventually inhibit acid gastric secretion it does so after a longer interval. It may be inferred that 5-HTP mainly affects gastric secretion after conversion to 5-HT. The precursor of 5-HTP and of 5-HT, L-tryptophan itself, rarely produced an effect on the gastric secretion evoked by histamine comparable to the effect of 5-HT. It must be remembered that of administered tryptophan as little as 1% is converted to 5-HTP and 5-HT (Sjoerdsma, Weissbach & Udenfriend, 1956). Recent feeding caused in the experiments already recorded, a marked fall in the acid secretion elicited by histamine. Although this fall may be the result of many factors, it is tempting to speculate that the decline of acid gastric secretion evoked by infusion of histamine in recently fed dogs is due to release of 5-HT. Toh (1954) has described how 5-HT is continually being released into the portal blood stream. Furthermore, he has described a substance capable of releasing 5-HT (1957) from the wall of the gastrointestinal tract. In the present experiments in recently fed dogs the levels of 5-HT in portal blood were higher in fed than in starved dogs. Stacey & Sullivan (1957) have shown that feeding raised the level of 5-HT in the wall of the intestinal tract. It may be that it is this fraction, acquired from precursors in the diet, which is responsible for the added amounts of 5-HT in the portal blood of fed animals and for the fall of acid secretion in the same animals in response to infused histamine/

histamine; the fall in acid secretion in the fed animals occurs at about 2 hours, which is approximately the same time taken for the decline in acid secretion when 5-HTP is given at the same time as histamine.

The argentaffin cells may be the sole site of formation of 5-HTP (see Chapter Five). Activity of these cells in the gastro-:intestinal tract would locally increase the tissue stores of 5-HT; on the other hand, secretion of 5-HT from these cells into the blood-stream could be expected to influence 5-HT concentration in the tissues. Release of 5-HT in or from the argentaffin cell could influence gastric secretion either through a predominantly restricted local effect on 5-HT levels, or via a general effect on 5-HT levels in various tissues after general hormonal release of 5-HTP. One important site that might be influenced in this way is a central one, if there is a central inhibitory mechanism for the control of gastric secretion.

In conscious dogs equipped with Heidenhain (vagally denervated) pouches, (Haverback, Bogdanski & Hogben (1958) have found an inhibitory effect of 5-HTP on the spontaneous secretion, the secretion elicited by hypoglycemia and cholinergic substances, but not by histamine. In view of the fact that in all our experiments we have found that the inhibitory effects of 5-HTP and 5-HT were partly dependent on an intact vagal innervation it must be presumed that these substances were capable of influencing a central site for the control of gastric secretion.

This/

This could take place directly or via reflexes, possibly with ingoing fibres in the vagus and with inhibitory efferent fibres in the vagus as postulated by Pavlov (1902) or in the sympathetic nervous supply to the stomach itself.

SUMMARY

1. Intravenous infusions of 5-HTP were followed by a fall in the output of histamine-stimulated gastric secretion in anaesthetised dogs. Occasionally 5-HTP stimulated acid gastric secretion for a short time as its first action. The effects of 5-HTP were similar to those of 5-HT except that they occurred after a longer latent period.
2. In previously starved anaesthetised dogs feeding with tryptophan before the start of an experiment led to a fall in acid output between 1-3 hours after the onset of histamine-stimulated secretion.
3. The 5-HT levels in portal blood were found to be higher in fed than in starved dogs, but the levels did not increase after infusion of histamine. The acid secretion elicited by histamine infusions in recently fed dogs was less in amount than that of dogs which had been starved over 36 hours. Local increase in 5-HT concentration in the wall of gastrointestinal tract, with release of 5-HT into the portal blood stream, could account for these findings.

CHAPTER FOUR.

The mechanism of the inhibitory effect of 5-hydroxy-:tryptamine on acid gastric secretion.

CHAPTER FOUR.

The mechanism of the inhibitory effect of 5-Hydroxy-:tryptamine on acid gastric secretion.

In the previous two chapters we have described the effects of 5HT and 5HTP on the acid gastric secretion, stimulated, in the dog, by histamine. The central position in the inhibitory effects described in these chapters would appear to be occupied by 5HT, since it acts promptly; 5HTP acts only after a latency, suggesting interconversion to 5HT. Recent feeding and administration of L-tryptophan were also found to exert inhibitory effects on acid gastric secretion.

We have discussed the possibility that previous feeding and consumption of L-tryptophan and 5HTP may enrich the 5HT stores in the pyloroduodenal mucosa. Well known inhibitory mechanism of acid gastric secretion, such as the contact on the pyloric antral mucosal with acid, might involve release of 5HT in the mucosa. Code and Watkinson (1955) showed that application of acid to the duodenal mucosa inhibited histamine-stimulated acid gastric secretion; the vagus nerves had to be intact for these inhibitory effects. Since the inhibitory action of 5HT was somewhat diminished by division of the vagus nerve, it might be the case that a substance such as this could be released and by local activity stimulate vagal afferents when the acid concentration in the pyloroduodenal area rises.

Many/

Many of the vascular and respiratory effects of 5HT are known to be produced by stimulation of receptors in the heart and lungs and their vagal afferents (Dawes and Comroe, 1954). Stretch receptors sensitive to 5HT, have been described in the pyloric region by Paintal (1954), and Iggo (1957) has described vagal afferents in the same region activated by extremes of pH. It is conceivable that the 5HT effects on gastric secretion are the results of a reflex mechanism involving afferent activity in the vagus nerve, conducted to a central site from which inhibitory influences could be brought to bear on the stomach. On the outgoing side, these might be mediated via the splanchnic nerves and the sympathetic vasoconstrictor fibres to the stomach, or in vagal inhibitory fibres envisaged by Pavlov (1902).

It must be admitted that we have no precise anatomical knowledge of these pathways. Nevertheless the mechanism itself has been investigated as follows:-

Experiments have been performed

a) in which acid contact with the pylorus has been prevented by placing a ligature at the mid point of the stomach. The "inhibitory" effect of recent feeding on histamine stimulated acid gastric secretion has been examined under these conditions.

b) were the "inhibitory" effect of recent feeding dependent on a local mediator eliciting activity in afferent vagal fibres, the inhibitory effect might, like the 5HT effect, be reduced by vagal section.

c)/

c) Afferent activity in the vagus nerves can be elicited by the administration of phenyl-di-guanidine and by veratrine (Dawes, 1954); the administration of these compounds result in profound reflex changes in the circulation. Were the inhibitory actions of acid on the pylorus or of recent feeding dependent on afferent nervous activity, these compounds might have effects on acid gastric secretion such as may follow the administration of 5HT, which shares the property of stimul-:ating vagal afferents.

d) In view of the possible inhibitory fibres in the vagus nerves, and the fact that the inhibitory effect of 5HT was reduced on section of the vagus nerve, the effects of 5HT have been studied on gastric secretion produced by one of the substances which mimic vagal activity through their parasympathomimetic action.

METHODS.

All dogs, on admission to the animal house, were put on a standard diet of 1 pint of milk in the morning followed by a single meal of meat in the evening, with water available at all times. The dogs were divided into two groups. Group 1 : "starved" dogs. These animals were not given any food on the day before an experiment but water was available. Group 2 : "fed" dogs. These animals were not starved the day before an experiment. In addition to the normal daily diet of milk and meat, an extra pint of milk was given in the evening about 12 hr before an exper-:iment. These animals were used in the experiments exploring the effects of/

of acid spread on the pylorus and vagotomy on the inhibition produced by recent feeding.

Operative procedure. The dogs were anaesthetised with chloralose 2% (W/V) and urethane (W/V) given 2 ml/kg intravenously. The right femoral vein was cannulated and connected to a Palmer's Slow Injection apparatus for the constant intravenous infusion of histamine. An efficient airway was maintained and in some experiments both vagus nerves were exposed in the neck and prepared with loops of thread for subsequent section.

The abdomen was opened with a left, subcostal, muscle-splitting incision and the pyloro-duodenal junction was gently occluded with a ligature of white cotton tape. In several experiments the site of this ligature was varied (see Results (a)). A glass cannula was tied into the body of the stomach, near the greater curvature. In almost all dogs, including the "fed" dogs, the stomach was found to be completely empty: in one or two dogs, debris, such as masses of straw or wood chips, were found. The abdominal wound was closed around the cannula and the animal then suspended on a frame to allow free drainage through the cannula. The stomach was washed out with warm isotonic saline given by a stomach tube passed down the oesophagus. The head was kept very low to allow free external drainage of saliva and pharyngeal mucus. Lastly, a thermometer was put in the rectum and the animal kept warm with/

with radiant heat.

After the preparation just described the animal was left undis-:turbed for approximately an hour before the histamine infusion was begun. During this period, fluid from the cannula was collected and tested for the presence of free acid. Histamine acid phosphate was then given at the rate of 5 ug/kg/min, calculated in terms of the base, made up in sterile solution. Once the histamine infusion was begun, the gastric juice was collected every 15 min and its volume measured in a graduated cylinder. The concentration of free acid in the juice, expressed in m.equiv/l. was estimated by titration with N/20 NaOH, using Topfer's reagent as indicator and the output of free acid was expressed as m.equiv/15 min. The figures in the tables are averages of two consecutive collection periods each of 15 min. Veratrine was infused as the sulphate in a concentration of 20 ug/kg/min; phenyldiguanide was infused in the same concentration, as was the urecholine. (Dr. G. Dawes kindly provided the phenyldiguanide).

RESULTS.

a) Effects of variations in the site of the pyloric ligature on the inhibition of histamine-stimulated acid gastric secretion of recently fed anaesthetised dogs.

In the experiments described in chapters 2 and 3, a ligature was applied at the pyloro-duodenal junction to prevent contamination of the gastric/

gastric juice with regurgitated duodenal contents. This pyloric ligature, however, did not prevent the acid juice from the body of the stomach from reaching the mucosa of the pyloric antrum. To prevent this contact the tape ligature was tied at the junction between the pyloric antrum and the body of the stomach. (Site B Fig. 1). The position for the ligature could only be tentatively estimated at the start of the experiment: its position was checked post-mortem. As judged by the different appearances of antral and body mucosa (checked histologically in one experiment), the ligature had been applied very close to the antral-body junction in four experiments: in another four experiments the ligature had been applied between 2-4 cm on the antral side of the junction. (Site A, Fig. 1). The results of these eight experiments are shown in this figure.

In the four experiments where the ligature lay on the antral side of the antral-body junction, marked inhibition of acid secretion (shown on the graph by the downward curve of the heavy lines) occurred between $1\frac{1}{2}$-$2\frac{1}{2}$ hr. On the contrary, with the ligature close to the antral-body junction in the other three experiments, the secretion was maintained at relatively high levels between $1\frac{1}{2}$ - $2\frac{1}{2}$ hr. (shown by the graph of plateau appearance in dotted lines), although a fall in secretion can be seen in one experiment at $2\frac{1}{2}$ hr.

b) <u>Effects of bilateral cervical vagotomy on the inhibition of histamine-stimulated acid gastric secretion of recently fed, anaesthetised dogs.</u>

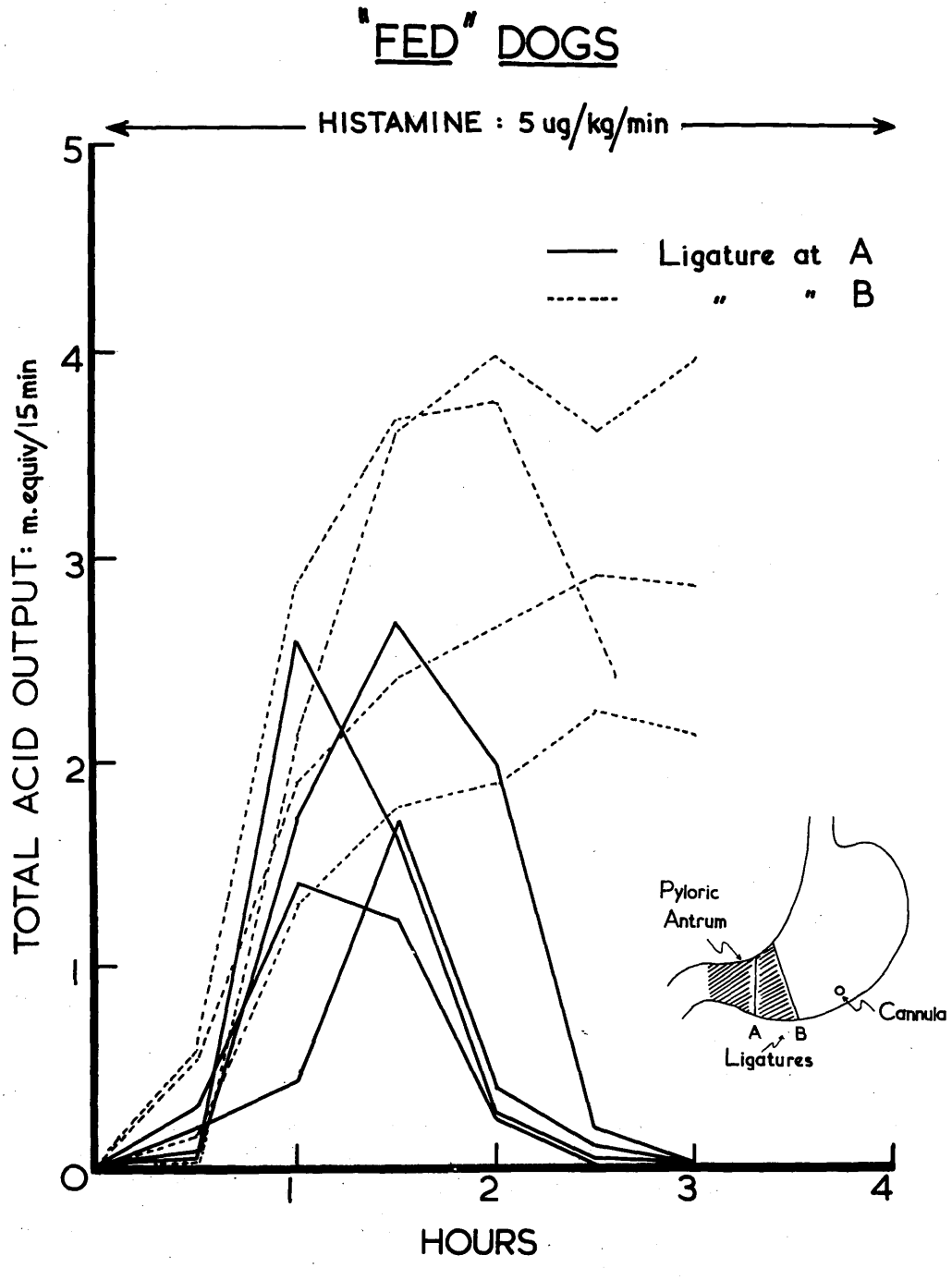
Bilateral/

Fig. 1. Acid secretion evoked by continuous infusion of 5 ug/kg/min histamine in fed dogs. Ordinates: total acid output m. equiv. in 15 min collection periods. Abscissae: duration of experiment in hours.

Inset shows position of ligatures: at A, a ligature was placed in the mid pyloric antrum.

At B a ligature was placed at the junction between the body of the stomach and the antrum.

When the ligature was at site A the acid secretion fell; when acid was prevented by ligature B from entering the antrum an undiminished plateau of acid secretion was maintained


"FED" DOGS
HISTAMINE : 5 ug/kg/min
Ligature at A
" " B
TOTAL ACID OUTPUT: m.equiv/15 min
0
1
2
3
4
5
HOURS
Pyloric Antrum
Cannula
A
B
Ligatures

Bilateral cervical vagotomy was carried out in four experiments as part of the operative procedures preceding the start of the histamine infusion. These dogs were fed with milk during the 12 hr pre-operative period, as in the previous group. The results are shown in Table 1.

In these experiments, the output of acid increased rapidly during the first 1½ hr. of the histamine infusion and then approximated to a steady state between 1½ - 2½ hr. The pattern of response to histamine in the fed, vagotomised dog appears to be similar to that in the starved dog with intact vagi, in that a "plateau" of secretion is elicited.

In another 6 experiments, shown in Table 2, bilateral cervical vagotomy was carried out during the inhibition of histamine-stimulated secretion seen in recently fed dogs. Complete inhibition of secretion had not occurred in the first three of these experiments when the vagi were cut. The acid secretion increased in all these experiments (No. 1,2, and 3, in Table 2) after the vagotomy. However, in another three experiments, (No. 4, 5 & 6, in Table 2) when vagotomy was done during complete inhibition, there was no significant return of secretion at the end of an hour.

c) Effects of phenyldiguanide and veratrine on acid secretion stimulated by histamine in starved dogs.

5HT in low concentration elicits chemoreflex activity (Dawes and Comroe, 1954). /

TABLE 1.

EFFECTS OF BILATERAL CERVICAL VAGOTOMY ON HISTAMINE-STIMULATED ACID GASTRIC SECRETION FROM DOGS FED IN THE 12 HR PERIOD BEFORE EXPERIMENT. THE READINGS REFER TO ACID CONCENTRATION IN m.equiv. FOR 30 MINUTE PERIODS. THE VAGI WERE CUT BEFORE THE START OF THE HISTAMINE INFUSION.

Expt.	Dog wt. kg	Histamine 5 ug/kg/min intravenously								
		Time, in hr after start of histamine								
		½	1	1½	2	2½	3	3½	4	% fall in acid output
1	12	0.39	0.48	1.03	1.23	1.20	1.20	-	-	1
2	14	0.22	0.95	2.3	3.05	3.06	-	-	-	0
3	20	0.75	4.55	5.1	4.97	4.90	-	-	-	4
4	11	0.155	0.47	0.51	0.44	0.46	-	-	-	13

TABLE 2.

EFFECTS OF BILATERAL CERVICAL VAGOTOMY ON HISTAMINE-STIMULATED ACID GASTRIC SECRETION FROM DOGS FED IN THE 12 hr PERIOD BEFORE EXPERIMENT. THE READINGS REFER TO ACID CONCENTRATIONS IN m.equiv/litre FOR 30 MINUTE PERIODS. THE VAGI WERE CUT DURING HISTAMINE INFUSION BETWEEN 2½ and 3 hr. AFTER THE START OF THE HISTAMINE INFUSION.

Expt.	Dog wt. kg.	Histamine 5 ug/kg/min intravenously Time, in hr after start of histamine. ½	1	1½	2	2½		3	3½	4	% fall in acid output before vagotomy
1	15	3.21	6.20	5.18	3.68	3.20	Before vagotomy	3.40	4.61	5.23	48
2	8	0.20	1.91	2.77	1.72	0.49		1.05	1.20	-	82
3	19	0.15	1.24	1.21	0.73	0.06		0.13	0.98	1.03	95
4	10	0.7	1.40	1.03	0.08	0.03		0.00	0.01	0.01	100
5	12	0.12	0.76	1.14	0.73	0.06	Before Vagotomy	0.00	0.00	0.00	100
6	18	0.02	0.82	0.86	0.36	0.21		0.01	0.02	0.04	96

Paintal (1954) has shown that 5HT stimulates afferent nervous discharge from receptors located in the stomach wall and that it is in this respect the most powerful substance of several tested. An attempt was made to determine whether other substances such as veratrine and phenyl-diguanide, as well as 5HT, all eliciting chemoreflex activity, would have inhibitory properties on acid gastric secretion.

In Table 3 the inhibitory effect of veratrine may be observed to last as long as the 30 minute infusion period in each of three experiments; a rapid recovery took place after cessation of the infusion. The inhibitory effect of this compound was greatly diminished by vagotomy. Figure 2 shows an experimental record obtained during the infusion of veratrine while recording the secretory rate by allowing the gastric juice to flow as drops across the points of a recording unit which is activated by each drop. The tracing recorded on the smoke drum shows a fall in the number of drops per minute when veratrine is infused during constant stimulation by histamine. It also shows that the inhibitory effect is diminished by vagotomy.

Similar falls of short duration in the acid secretion elicited by histamine were observed in two dogs following a thirty minute infusion of phenyl-diguanide; the diminution in secretion was not observed after vagotomy. (Table 4).

d) Effect of 5HT on parasympatho-mimetic stimulation of gastric secretion.

Urecholine was infused in four dogs to stimulate parasympathetic activity/

TABLE 3.

EFFECTS OF VERATRINE AND PHENYLDIGUANIDE ON GASTRIC SECRETION BEFORE AND AFTER VAGOTOMY. BOTH SUBSTANCES WERE INFUSED FOR 30 MINUTES DURING CONTINUOUS HISTAMINE INFUSION. THE ACID CONCENTRATION IN m.equiv./litre IN THE 30 MINUTES BEFORE AND AFTER THE INFUSION IS RECORDED, WITH THE PERCENTAGE INHIBITION IN EACH CASE.

Weight of dog	Before	Veratrine	After	% Inhibition		Before	Veratrine	After	% Inhibition
12	3.72	0.42	1.12	89		1.84	1.64	1.50	11%
13	2.78	1.22	1.97	56		3.62	3.70	3.70	+ 3%
10	2.97	1.86	1.89	37	VAGOTOMY.	1.2	1.0	1.0	17
		P.D.G.					P.D.G.		
22	6.98	4.98	5.96	29		4.6	4.1	4.1	11%
20	3.47	2.51	1.19	28		2.31	2.23	2.18	3%
12	4.38	2.71	3.68	38		1.39	1.01	1.28	27%

Fig. 2 shows the effect of veratrine infusion on the rate of secretion evoked by histamine infusion. The record has been taken before and after the performance of vagotomy in the neck.

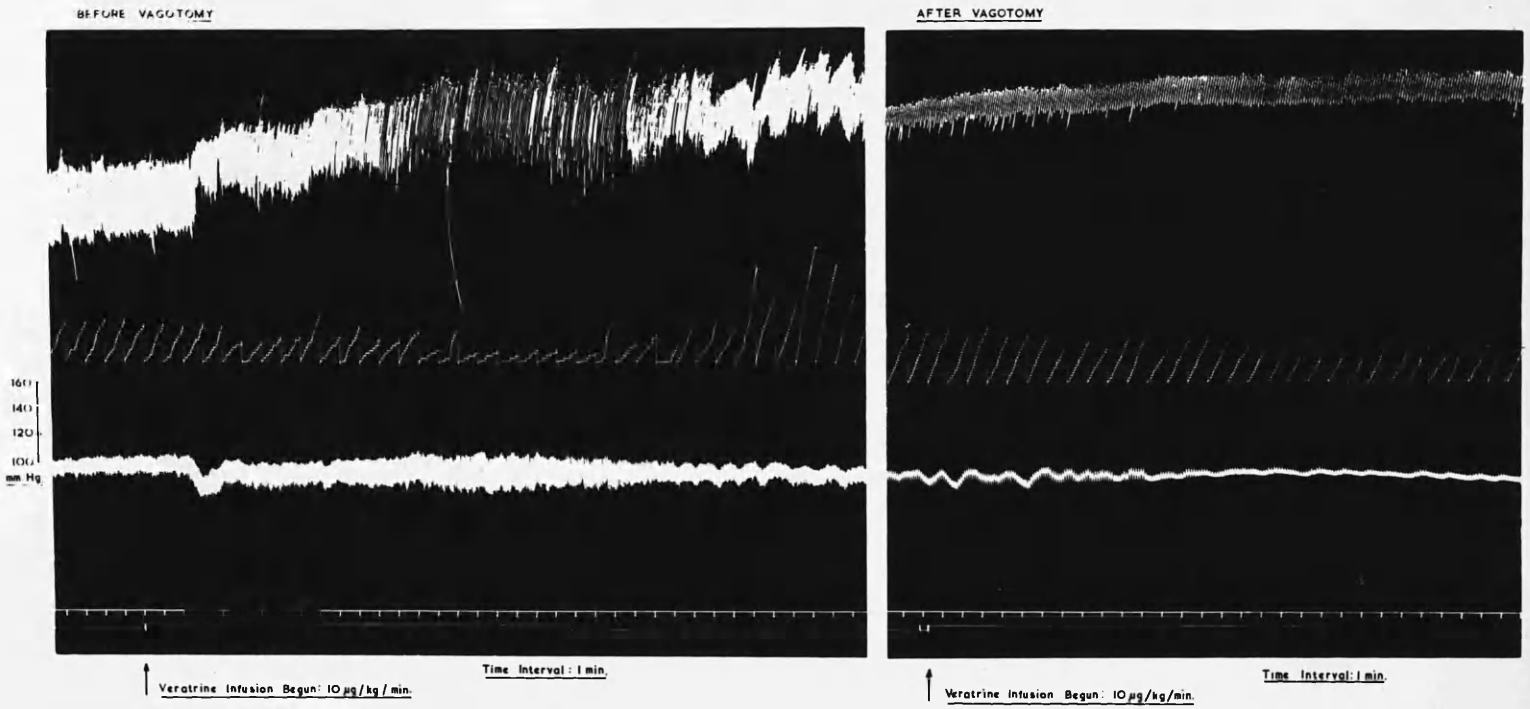
Before vagotomy: The upper tracing is a record of respiration obtained by means of a stethograph around the mid thorax operating a float recorder writing on a smoked drum.

The middle tracing shows a diminished secretory rate following the infusion of veratrine (note the period of respiratory stimulation at this point). There is an increase in secretion when the veratrine infusion ends.

The lower tracing illustrates the blood pressure levels during the experiment.

After vagotomy:

The infusion of veratrine is followed by little or nc change in respiration, secretory rate or blood pressure.


BEFORE VAGOTOMY
160
140
120
100
mm Hg
Time Interval : 1 min.
Veratrine Infusion Begun: 10 µg/kg/min.
AFTER VAGOTOMY
Time Interval: 1 min.
Veratrine Infusion Begun: 10 µg/kg/min.

activity peripherally. Continuous infusion of this substance (20 ug/kg/min) was found to stimulate an acid gastric secretion which soon reached plateau levels (Table 4). 5HT was infused for 30 minutes against this background leading to a fall in acid secretion lasting for about 1 hour, as was the case with histamine infusions.

DISCUSSION.

Increased sympathetico-adrenal activity, release of enterogastrone, and the presence of acid in the antral-duodenal area are well known mechanisms of gastric secretory inhibition. It is difficult to understand how shortening the starvation period could increase sympathetico-adrenal activity or the release of enterogastrone. However, the effects of vagotomy and of the site of the antral ligature suggest that an HCl-inhibitory mechanism is at work. Code & Watkinson (1955) have shown that the vagi must be intact if application of HCl to the duodenal mucosa is to inhibit histamine-stimulated acid gastric secretion. Acid applied to the mucosa of the pyloric antrum can inhibit acid gastric secretion elicited by liver homogenates (Woodward, Lyon, Landor, & Dragstedt, 1954) and by methacholine (Kim, 1955). It has been shown (Pincus, Thomas, & Rehfuss, 1942) that a critical intraduodenal pH is necessary to evoke inhibition and Sokolov (1904) showed that the animal's own gastric juice was effective. It is attractive to imagine that, in our experiments, the increasing acidity of the gastric juice brought about inhibition of secretion in spite of the continued infusion of histamine.

But/

TABLE 4.

EFFECT OF A 30 MINUTE INFUSION OF 5HT (15 ug/kg/min) ON ACID GASTRIC SECRETION STIMULATED BY URCHOLINE (20 ug/kg/min) CONTINUOUSLY IN FASTING DOGS.

Expt.	Dog wt. in kg.	Urecholine 20 ug/kg/min — Time in hr. after start of infusion									% inhibition
		½	1	1½	5HT 2	2½	3	3½	4	4½	
1	16	0.31	0.96	0.98	0.21	0.33	0.65	0.82	0.86	0.86	79
2	18	0.60	1.5	1.6	0.66	0.46	0.72	1.0	1.1	1.1	71
3	12	0.42	0.68	0.69	0.05	0.08	0.36	0.52	0.54	0.54	93
4	10	0.20	1.1	1.0	0.08	0.12	0.66	0.9	0.98	-	93

But such inhibition took place only in dogs which had been fed within 12 hours of the experiment. Dogs which had been starved for at least 24 hr do not show this inhibition.(See previous chapter). This may explain why there appears to be no previous description of the phenomenon. The usual diatary regime of dogs used for studies of gastric secretion is to give a single meal of meat per day. Most studies on gastric secretion in dogs have been done on animals starved for periods between 18 and 36 hr. In our experiments the dogs which showed inhibition were fed twice per day with meat and milk and were not without food for more than 12 hr. If the vagi are cut, as they not infrequently are, in experiments on histamine-evoked gastric secretion, the diminution in secretory response does not appear : in acute experiments using a washout technique the gastric juice is secreted with a large volume of saline and the acidity may not reach the critical value to evoke inhibition.

If vagotomy and exclusion of acid from the pyloric antrum are both factors which can reverse the diminution in acid secretion following recent feeding, it would be reasonable to look for a common mode of action in which they exert this effect. In the preceding chapters, recent feeding was shown to lead to increased levels of 5HT in the portal blood. Recent feeding, tryptophan, 5HTP and 5HT, all had comparable effects on acid gastric secretion. It has already been pointed out that 5HT may stimulate vagal afferents from pressure receptors/

receptors in the stomach. In a comparable way, nervous activity of this sort might influence cerebral centres controlling gastric secretion. Agostini, Chinnock, de Burgh Daly and Murray have described a high proportion of afferent fibres in the vagus. They estimate that less than 10% of the fibres in the abdominal vagus were efferent ones. It seemed to us that if veratrine and phenyldiguanide (both compounds exerting chemoreflex activity) influenced gastric secretion, they might do so via an afferent pathway, strengthening the case for this as a possible means through which control of gastric secretion could be exerted. Local release of 5HT in the gastric wall might activate this mechanism, as it has been shown to do in the case of vagal afferents from pressure receptors in the stomach (Paintal, 1954). (Fig. 3a).

Mere release of 5HT from the gastro-intestinal wall into the circulation would seem to be an unlikely mode of action for 5HT as an inhibitory hormone, because of the fact that it would be rapidly destroyed in circulation via the liver through the action of amine oxidase (see Chapter 5).

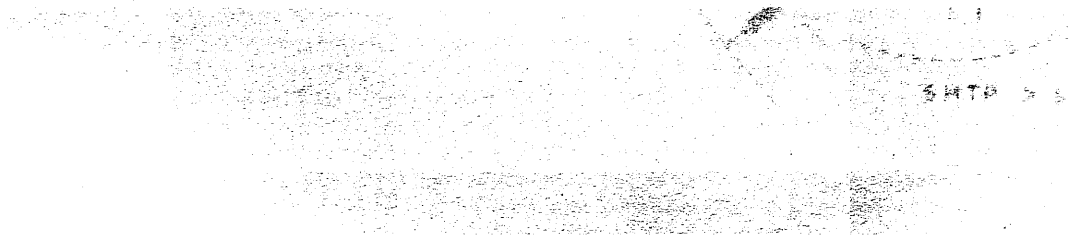
The hypothesis suggested for the action of 5HT (Figure 3) would of course, be dependent on an efferent mechanism such as might exist in inhibitory fibres in the vagus nerves or in the splanchnic nerves. 5HT has already been shown by Haverback, Bogdanski and Hogben (1958) and White and Magee (in the press) to exert a profound inhibitory effect

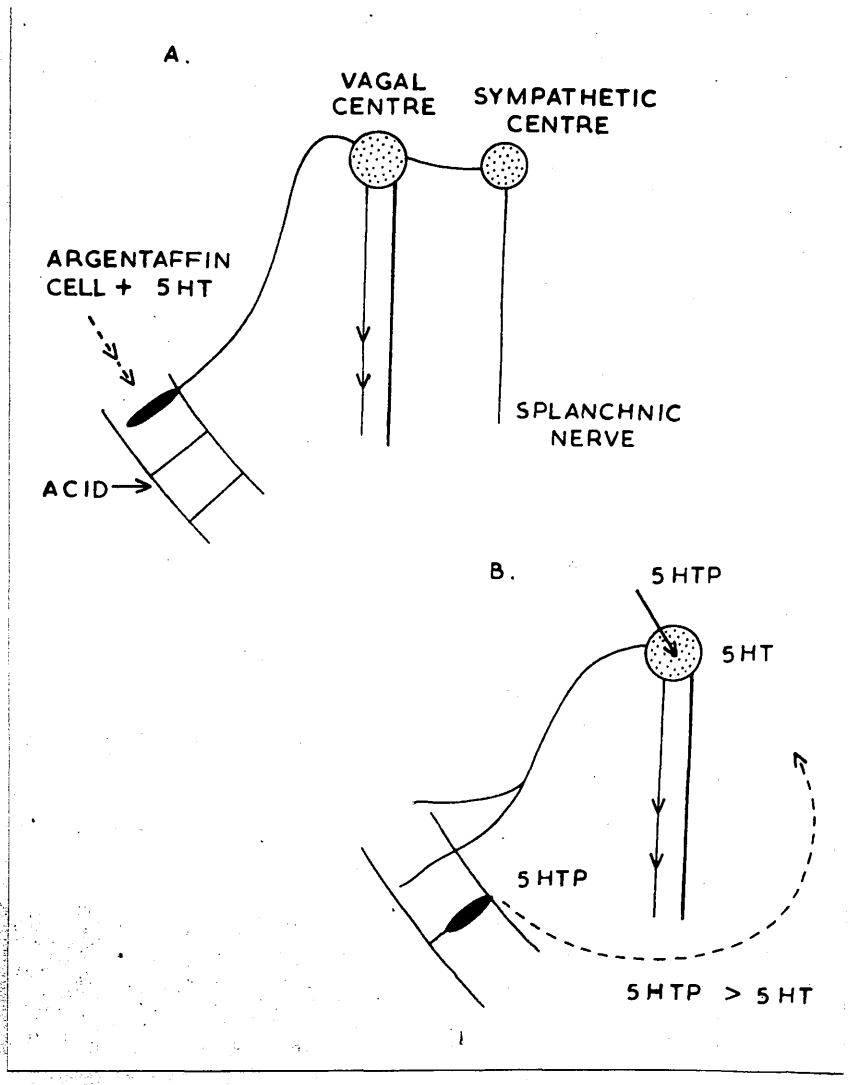
on/

Fig. 3 illustrates two hypotheses for the conjectured inhibitory mechanism of gastric secretion.

In A, acid in contact with the pyloric mucosa might stim-:ulate vagal afferent activity, via nerve fibres in close proximity with the argentaffin cell. 5-HT might serve as a neurohormone at this site. The inhibitory mechanism might have its outgoing side in the vagus nerve or in the sympathetic.

In B, the hypothesis is similar as regards the possibility of acid stimulating afferent activity, but the argentaffin cells are pictured as a source of 5-HTP and 5-HT, passing it on to central areas at which it might stimulate inhibitory activity.




A.
VAGAL CENTRE
SYMPATHETIC CENTRE
ARGENTAFFIN CELL + 5 HT
SPLANCHNIC NERVE
ACID
B.
5 HTP
5 HT
5 HTP
5 HTP > 5 HT

on the vagal juice obtained in Pavlov pouch dogs. 5HT in our experiments produced a fall in secretion elicited by a parasympatho-:mimetic agent. Undoubtedly the best means of testing this further is to examine the effects of 5HT on Pavlov (vagally innervated) and Heidenhain (vagally denervated). This experiment ought to be done since an alternative explanation of the facts so far described in these chapters is possible and is illustrated in Figure 3b. 5HTP might be the active substance released from the pylorus and would undergo conversion to 5HT at sites of decarboxylase activity; rapid conversion of 5HTP to 5HT takes place centrally and also at the nerve endings of the vagus nerve, both important sites of action. In this case, nervous afferent activity independent of 5HT release might be stimulated by spread of acid on the surface of the pyloric antrum. 5HT might in this instance be a central transmitter; brain tissue in the hypothalamic region is so active in 5HTP-5HT conversion that this seems a fruitful hypothesis to put to the test in future experiments.

SUMMARY.

1) It has already been shown that when dogs are starved for 24/36 hours histamine stimulated secretion reaches a steady state between $1\frac{1}{2}$ - $3\frac{1}{2}$ hours, after starting the histamine infusion. When the animals have been fed 12 hours before the experiments, inhibition of the histamine stimulated secretion began after 1-$1\frac{1}{2}$ hours and was completed by 3-$3\frac{1}{2}$ hours.

2) This inhibition of histamine stimulated secretion in recently fed dogs could be prevented by bilateral cervical vagotomy or by tying a ligature at the antral-body junction.

3) Compounds which like 5HT stimulate vagal afferent nerve activity have inhibitory effects on gastric secretion similar to those of 5HT; this would appear to be pharmacological evidence for afferent vagal nerve control, perhaps through a vago-vagal reflex, of acid secretion.

4) The efferent side of the mechanism controlling acid secretion outlined in these chapters has not been studied in detail. Two reports in the literature suggest that 5HT has a profound inhibitory effect on vagally stimulated secretion; it is possible that 5HT might produce this effect through fibres in the vagus nerve which are inhibitory to acid secretion, as were found by Pavlov (1902) and Schachter (1949).

CHAPTER FIVE.

Carcinoid tumours and 5-hydroxytryptamine.

CHAPTER FIVE.

CARCINOID TUMOURS AND 5-HYDROXYTRYPTAMINE.

It is as an example of the human pharmacology of 5HT that this tumour aroused the author's interest. The developing interest in the carcinoid syndrome from 1954 onwards brought several instances of it to the author's notice and nine cases have been personally investigated by him. This work was done in collaboration with Drs. MacFarlane and Lennox of the University Department of Pathology at the Western Infirmary, Glasgow, and with the co-operation of Dr. Dalgliesh and his assistant Dr. Dutton who devised the method used by the author for assay of 5-hydroxyindoleacetic acid and advised on biochemical aspects. The author was assisted in the clinical care of these patients by Dr. Lloyd Nyhus, Associate Professor of Surgery in the University of Seattle. The cases are described in the first place as a clinical entity, but in the subsequent chapter (Chapter 6) the author goes on to consider carcinoid tumours from a wider aspect, examining the effects of various substances which stimulate acid secretion on 5HT blood levels and considering the general argument for or against 5HT having a general or a local role in gastro-intestinal activity.

The present chapter opens with a historical description of the discovery of the carcinoid, continues with a review of the significant pathology. This/

This is followed by an assay on the clinical features and the treatment of the author's series of cases. The urinary excretion, the blood levels and the tumour tissue concentration are all listed.

HISTORICAL INTRODUCTION.

The carcinoid tumour, or argentaffinoma, is known to surgeons in a variety of ways. It is most commonly an incidental finding when situated in the vermiform appendix, and in the terminal ileum causes intestinal obstruction. In some cases, somewhat rarely however, it may cause the clinical manifestations of the "carcinoid syndrome", which has aroused great interest in surgery through its association with the internal secretion of the hormone, 5-hydroxytrypt-:amine. It is to the Swedish workers in Malmo that credit must go for the full recognition of this syndrome (Biorck, Axen and Thorson, 1952; Thorson, Biorck, Bjorkman and Waldenstrom, 1954; Waldenstrom and Ljungberg, 1955; Pernow and Waldenstrom, 1954; Thorson, 1958). Biorck and colleagues in 1952 had described the occurrence of cardiac lesions in a cyanosed young man of 19 years, who was also subject to flushing attacks and was found at autopsy to have a carcinoid or argentaffin tumour. With Thorson, Biorck and Bjorkman, Waldenstrom collected by 1954 seven closely similar cases, and outlined from this material a new clinical syndrome. It was tentatively suggested that the clinical effects might be the result of secretion of 5-hydroxytrypt-:amine from/

from the tumour. This idea had been made a convenient hypothesis by Lembeck's demonstration in 1953 that carcinoid tumour tissue was rich in 5-hydroxytryptamine (Lembeck, 1953). It had appeared probable from the classical work of Erspamer that a carcinoid tumour, in effect an aggregate of argentaffin cells, would possess rich stores of 5-hydroxytryptamine, since he had established in numerous papers (Erspamer, 1940; Erspamer, 1946; Erspamer, 1952; Erspamer, 1953; Erspamer, 1955; Erspamer and Asero, 1952; Erspamer and Boretti, 1951; Erspamer and Ottolenghi, 1953) that the argentaffin cells manufactured rich stores of a new hormone, enteramine, which had been shown to have the constitution, 5-hydroxytryptamine. Finally Pernow and Waldenstrom in 1954 established the hormonal nature of the carcinoid syndrome by showing that there was an elevated concentration of 5HT in blood of patients with a carcinoid tumour, extensive metastases and the systemic features; of these the cutaneous manifestations are the most striking, and are a reddish-blue cyanosis, telangiectasis and flushing attacks. These effects are often associated with diarrhoea, borborygmi and abdominal colic, "asthmatic attacks", occasional giddiness and the development of valvular lesions of the right side of the heart, often after a lapse of several years. These features are particularly associated with advanced metastasising carcinoids of the small intestine, having gross hepatic or glandular metastases (Sjoerdsma, Weissbach and Udenfriend, 1956).

PATHOLOGY OF CARCINOIDS OR ARGENTAFFIN TUMOURS.

Carcinoids/

Carcinoids have been described at most sites in the intestinal tract (Thorson, 1958; MacDonald, 1956; Morson, 1958; Mattingly, 1956; Olson and Gray, 1958) between the cardia and the ano-rectal junction; infrequently they occur in other organs, such as the lung, testis and ovary.

The most common site is the appendix (accounting for 65% of all cases) as the typical yellowish tumours of the appendix tip. They may be locally invasive in their histology and are potentially malignant tumours with a clinical course which is usually benign. Invasion near the appendicular base carries a sinister prognosis and is an indication, if discovered on routine examination, for radical treatment by a right hemi-colectomy with excision of related lymph nodes (Olson and Gray, 1958). The same is true if lymphatic invasion is detected; only 4%, however, of appendicular cases metast-:asise. The cases operated on for "appendicitis" may have had features akin to appendicular obstruction - appendicular colic may have been aided by secretion of 5HT, which is a powerful agent in initiating smooth muscle spasm. This in turn may aid the early recognition of these cases.

The most common extra-appendicular site is the terminal ileum (25% of all cases). Common features of these growths are that they are most often the functioning tumours, are a fairly constant yellow colour/

colour, show submucous spread with late ulceration into the bowel lumen, and may be multiple; marked hypertrophy of the adjacent bowel wall may be present. Histological examination reveals whorls of cells with a peripheral palisade arrangement; there may be fine dusting of the nuclear chromatin. (Fig. 1). Even in ordinary haemalin and eosin staining reddish granules are apparent. (Thorson, 1958; Smith, 1959).

Their special features are:-

a) strong affinity for silver salts (Gosset and Masson, 1914).

b) characteristic granules, a condensation product of 5HT and formalin, staining reddish-brown by the diazonium reaction (Barker and Pearse, 1955; Pearse, 1953). (Fig. 2.*)

c) fluorescence in the ultraviolet (with the same absorption spectrum as 5HT).

The number of carcinoids at other sites is small; 5% occur in the colon and rectum, where they may be relatively benign (Dukes, 1946) but show atypical histology in that they have poorer granulation and a tendency to form tubules and acini (Gabriel and Morson, 1956). Rectal biopsy specimens stained with the specific stains may help to establish the diagnosis.

Carcinoids develop rarely in the stomach, duodenum, gallbladder and pancreas. They also occur in the lung and ovarian, and presumably testicular/

* Appendix.

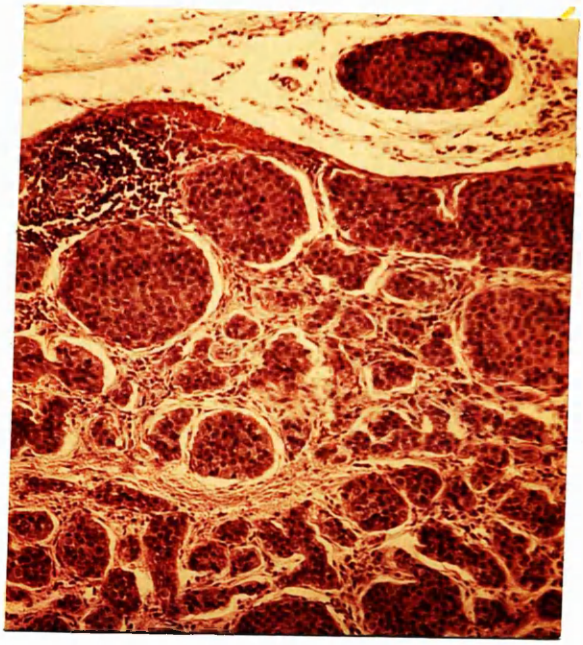


Fig. 1. The photomicrograph shows the histology of a typical argentaffinoma. Note the whorls or aggregates of cells, often with peripheral palisading, the fine dusting of the nuclear chromatin which has an open appearance, and the suggestion of granules in the cytoplasm of the cells at various sites (H. and E. x 300).

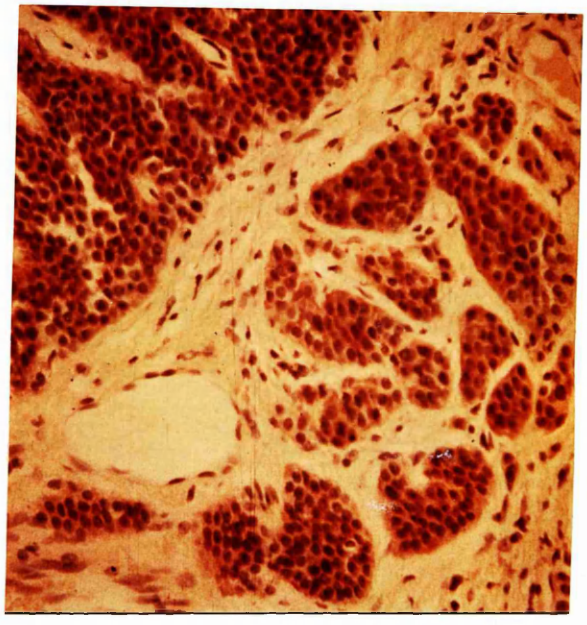


Fig. 2. The photomicrograph again shows the histology of the typical carcinoid or argentaffinoma; in this case the staining is with the diazo method outlined in the Appendix. The orange-brown granules represent a condensation product of 5-HT and formalin.

testicular teratoma (Thorson, 1958; MacFarlane, 1957; Warner and Southren, 1958; Sauer, Dearing, Flock, Waugh, Dockerty and Roth, 1958).

Metastasising tumours, usually with numerous secondaries, appear to be the sole examples of carcinoids capable of causing the clinical manifestations, presumably because the 5HT output of the primary tumour alone is inactive or insufficient in size at this stage to have any effect. Most cases of the syndrome have metastases in the liver and this has been stressed by some authors (Thorson, 1958; Sauer, Dearing, Flock, Waugh, Dockerty and Roth, 1958). It has been held that the development of the clinical manifestations of the syndrome is almost an indication that hepatic metastases are present; perhaps this is because metastatic tissue in the liver secrets almost directly the hepatic veins allowing more 5HT to escape oxidative deamination in the liver tissue. Estensive local spread of lymphatic glands and to the mesentery with gross intestinal adhesions (Dockerty and Ashburn, 1943; Hedinger and Gloor, 1954) is a feature of all late cases.

The histogenesis of the tumour is from the Kultschitzky or argentaffin cells of the crypts of Lieberkuhn which belong to the enterochromaffin cell system. These cells take up both silver and chrome salts (hence the alternative names argentaffinoma and chrom-:affinoma) and are present in the alimentary tract, bile ducts and pancreas,/

pancreas, and occur sparsely in the lung: one type of bronchial adenoma is, more correctly, thought to be an argentaffinoma (Olson and Gray, 1958). Since some patients have been found to have extensive carcinoid tumours which fail to show the typical yellow pigment or to take up the silver stain, it has been proposed that they may contain argyrophilic rather than argentaffin cells; this problem will be discussed later.

These tumours have not been produced experimentally, nor is there any evidence of a carcinogenic agent at work. Their origin has never been satisfactorily settled but the staining reactions suggest a developmental relationship with autonomic ganglia (Danisch, 1923).

CARCINOID SYNDROME.

The features of the syndrome (Biorck, Axen and Thorson, 1952; Thorson, Biorck, Bjorkman and Waldenstrom, 1954; Thorson, 1958) are:-

a) A malignant carcinoid of the small intestine, commonly with metastases to the abdominal lymph nodes and the liver.

b) Patchy flushing, a plethoric colour or cyanosis.

c) Frequent watery stools, borborygmi and abdominal pain.

d) Pulmonary stenosis of the valvular type and tricuspid stenosis or regurgitation.

e) Attacks of "bronchial asthma" of an unusual type.

f) Telangiectasis and dependent oedema.

The/

The main clinical effects present in nine cases of the syndrome examined by us are recorded in Table 1.* The main features, culled from these cases and those of others, are described in detail below.

CUTANEOUS MANIFESTATIONS.

A typical patient with this condition may flush transiently in "geographical" areas, or continuously more severely over the face (Fig. 3a) but the upper torso and extremities may also be affected; (Fig.3b) the part may feel warm with paraesthesia, but in a cold ambient temperature there is marked cyanosis, (Fig.3c), either diffuse or patchy. The skin temperature rises in the affected part during the flushing attack. Telangiectasis usually means that the condition is of some duration and cuts in the skin areas round these dilated vessels bleed with some severity. The sclerae are often reddened.

The flushing may be spontaneous, or may follow various stimuli - emotional, mechanical (prolonged standing, abdominal palpation, or compression of a testicular carcinoid, or following colonic irrigation), pharmacological (histamine or reserpine), or dietary (meals, tea, coffee or alcohol.) The flushing attacks are usually accompanied by vasomotor changes which have been described by Thorson (1956); there is a tachycardia and the systolic blood pressure, pulse pressure and cardiac output rise as the flush develops. On the contrary in the cold cyanotic state the pulse is weak, the blood pressure is low and the/

* For case reports see Appendix.

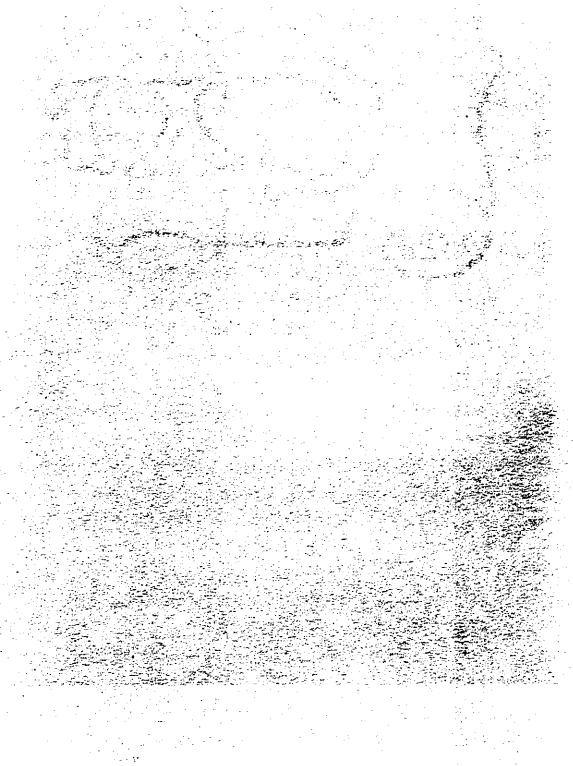
TABLE 1 GIVES THE CLINICAL PICTURE, HISTOLOGICAL DATA AND PATHOLOGICAL STATE OF NINE PATIENTS IN WHOM THE DIAGNOSIS HAS BEEN CLEARLY ESTABLISHED IN A GROUP OF PATIENTS EXAMINED BY THE AUTHOR; OTHER PATIENTS EXAMINED ARE QUOTED IN TABLES 2, 3 and 5 IN THIS CHAPTER. THE URINARY EXCRETIONS (in mg/24 hrs) AND 5-HIAA ARE LISTED. *

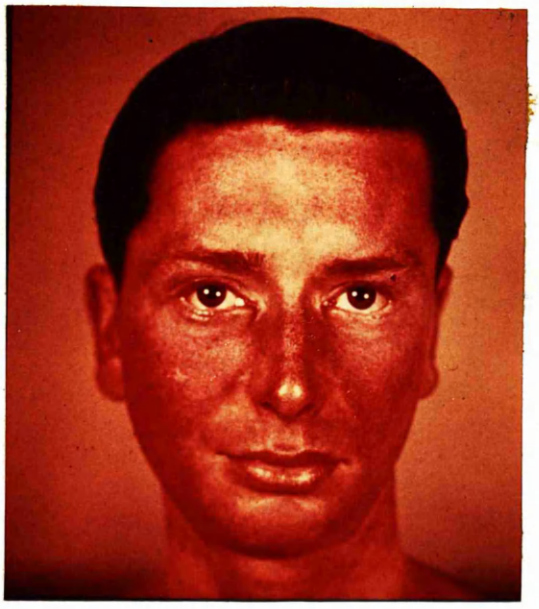
Case	CLINICAL			SITE	EXCRETION OF 5-HIAA (mg/24 hours)
	FLUSHING	PULMONARY STENOSIS	DIARRHOEA		
1	-	-	±	Small bowel and liver.	688 594
2	+	-	±	Ileum and mesentery. Not liver	65 90 84
3	+	-	-	Ileo-caecal and mesentery. Not liver	155
4	++	+	+	Ileum, mesentery and liver.	258 269
5	-	-	-	Ileo-caecal and mesentery. Not liver	43
17	±	-	++	Liver and glands, recurr-:ent after excisional operation.	17 20 40
18	++	?	+	? Ileum, liver bone kidney	160
19	+++	-	-	Ileum, liver.	200 to 300
24	+	-	+	Glands, recurrent + abd-:ominal mass ? liver, recurrent after R. Hemi-:colectomy.	53.2 63.4

* Appendix.

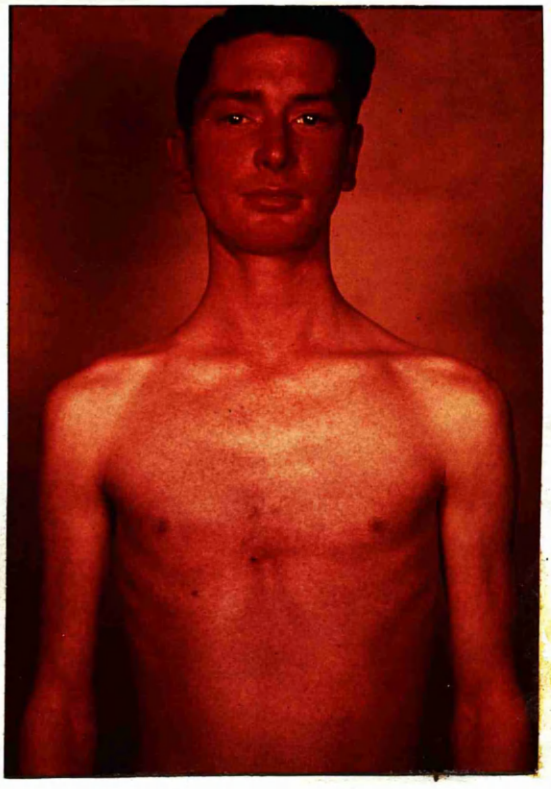
Fig. 3a. A common appearance of patients with the carcinoid syndrome is that of a bright red flush. This may be continuous or intermittent and may arise in one area as it fades in another.

It often extends from the face (Fig. 3b) over the neck and sternal region and macules may be present all over the body.





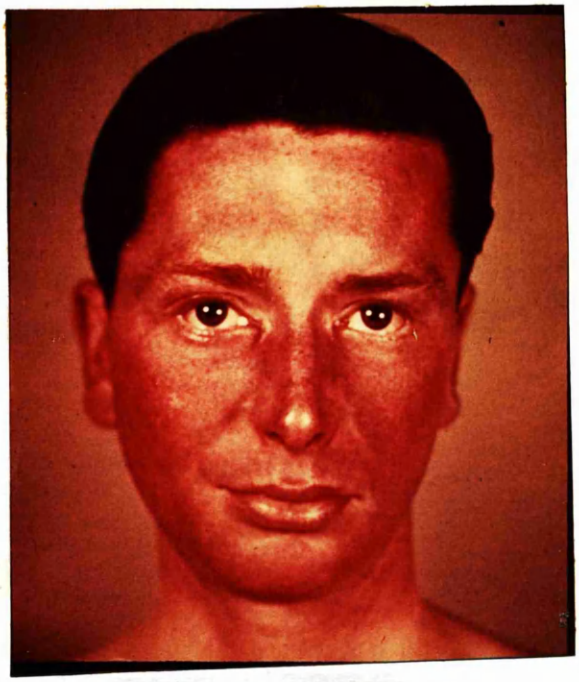
3a.



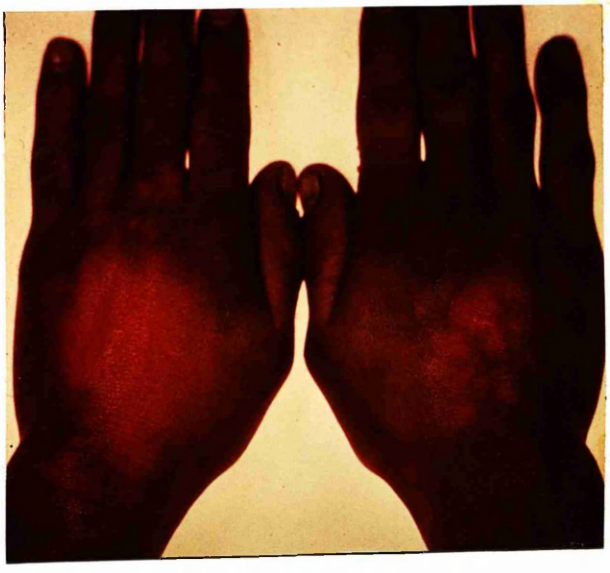
3b.

Fig. 3c. Illustrates the appearance of one of these patients in a cold environment. A dusky cyanosis spreads over the head and neck, the torso and the extremities.

Fig. 4. The appearance in the extremities may be that of intense cyanosis and there may be a Raynaud phenomenon.



3.c.



4.

the cardiac output is weak. A pilomotor "gooseflesh" appearance has been described (Thorson, 1958). There is no polycythaemia and full oxygenation does not lessen the cyanosis. A Raynaud-like phenomenon may be present at the extremities. (Fig. 4).

DIARRHŒA.

In certain patients this is the principal complaint; the bowels may move 20 times daily or more with watery stools and there may be abdominal discomfort, colic and borborygmi audible at some distance from the patient's bedside. Barium passes rapidly through the intest-:inal tract and may reach the ileocaecal valve in 1 hour, but not always - the incessant intestinal activity is audible with the steth-:oscope and may be recorded on an electromagnetic tape. The motility need not necessarily be of the propulsive type and may be of the segmentation variety. The increased motility is unrelated to the presence of obstruction and, in the presence of widespread metastases, continues after resection of the primary tumour. Increased borborygmi may be present without diarrhoea. Sinclair (1957) estimates that patients with an unusually active gut may be differentiated from true cases of the carcinoid syndrome with alimentary symptoms and signs by the use of ganglion blocking agents. In individuals with higher motility of the gut from various causes other than excess of the humoral agent 5-hydroxytryptamine, peristalsis is checked temporarily by doses which have no effect on peristalsis in patients with carcinoids secreting/

secreting 5-hydroxytryptamine. This is in keeping with the work of Robertson (1953) who showed that hexamethonium does not antagonise and may even augment the effect of 5-hydroxytryptamine on the guinea-pig ileum preparation suspended in vitro in Tyrode solution. Incessant activity of the gut, with diarrhoea and augmented bowel sound in the presence of an abdominal mass have led to the diagnosis of subacute obstruction of the small bowel. This may prove deceptive in that the small intestine may show evidence of dilatation and hypertrophy of its muscle coats without true obstruction of the intestinal lumen by the carcinoid tumour. That these effects may have been provoked by 5HT rather than by true obstruction was well demonstrated in one of our cases in which the carcinoid mass was outside the bowel wall in the mesentery.

ASTHMATIC-LIKE ATTACKS.

Respiratory stridor may be the result of direct 5HT constriction of the smooth muscle in the trachea and bronchi; and this may induce "asthmatic-like attacks", in cold weather principally. Difficulty may be experienced by the anaesthetist in inflating the chest to oxygenate the patient if bronchospasm supervenes during an operation for removal of a carcinoid.

OCCASIONAL FINDINGS.

These include oedema, which may variously arise from cardiac failure, obstruction of veins by a large abdominal mass, and as a result/

result of the antidiuretic effects of the hormone via its action in causing sodium retention (Hulet and Perera, 1956) oliguria, rarely amounting to anuria; lesions like rheumatoid-arthritis affecting the samller joints in some cases (Sjoerdsma, Weissbach and Udenfriend, 1956); scleroderma, a dark brown pigmentation with keratosis (Thorson, 1958) and a higher overall incidence of gastric and duodenal ulceration (Sjoerdsma, Weissbach and Udenfriend, 1956).

MENTAL SYMPTOMS.

It is surprising that few of these patients show psychological abnormalities, in spite of the important role of 5HT in cerebral function. This is almost certainly the result of poor penetration of 5HT through the blood brain barrier. 5HTP on the contrary penetrates the blood brain barrier readily.

ABDOMINAL EXAMINATION, reveals in most instances the scar of an oper-:ation undertaken often some years previously; there may be palpable, even visible, gross hepatomegaly or other metastases present. Osseous metastases (Fig. 5) while an uncommon occurrence, may be present terminally; they may mimic the osteogenic sarcoma yet are of a longer duration and are rich, on biopsy, in 5HT. 5HIAA is excreted in excess, but an anomaly exists in that these cases often have non-argentaffin argentaffinomata (Fig. 6). Any patient who shows this advanced state after an operation several years previously should be suspected of having /

Fig. 5 shows an osseous metastasis in the right humerus in a patient with a carcinoid tumour. On biopsy it was found to have 4 mg per g. 5-HT on biological assay.

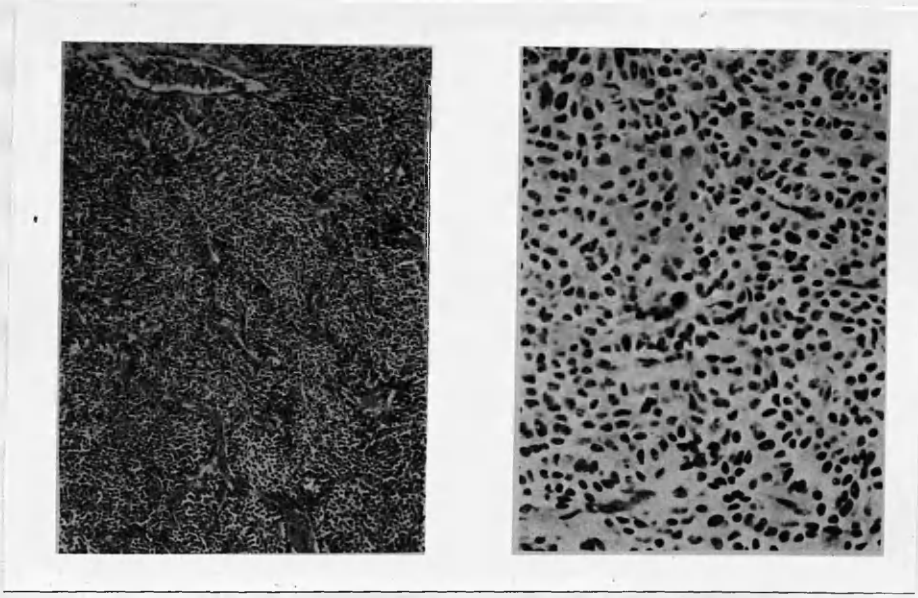


Fig. 6. Liver biopsy showing sheets of tumour cells irregularly arranged obtained from the patient with the osseous metastases. The left hand figure stained with H. & E, x 200: the right hand figure stained with H. & E., x 400. The tumour was a non- argentaffin argentaffinoma.

having had a carcinoid as these tumours usually grow very slowly with occasional cases surviving a decade or more.

THE DEVELOPMENT OF CARDIAC LESIONS.

The cardiac manifestations occur late in the natural history of the syndrome and appear almost exclusively in the right side of the heart unless in the rare instance of a patent foramen ovale, when they may occur in the left heart (Sjoerdsma, Weissbach and Udenfriend, 1956; McKusick, 1956). Pulmonary stenosis and tricuspid stenosis or regurgitation are the commonest abnormalities. It is possible that these changes are the direct effect of 5HT on the endothelium of the heart valves, (Waldenstrom and Ljungberg, 1955; Hedinger and Gloor, 1954) but it has been claimed that they follow damage induced by continuous fluctuations of pressure in the pulmonary vascular bed (Thorson, 1956). The concentration of 5HT, extremely high in the portal and hepatic venous blood, remains very high in the right heart, but falls to lower levels after passage of blood through the pulmonary circuit, where inactivation by amine oxidase rapidly occurs. (See Table 4).

The pulmonary and tricuspid valves have been shown at autopsy to be greyish-white and fibrotic, distorted and fused at the cusps and there is thickening of the chordae tendinae. The mural endocardium of the right atrium may be white and fibrous. Occasionally there may be

a/

a fibrous reaction in the pericardium and there may be a ventricular hypertrophy present greater than that which can be explained on the basis of the valvular lesions present (Jacobson, 1939). The heart is enlarged principally to the right as may be shown radiologically. There may be a small aorta and sufficient dilatation of the pulmonary artery to give the left border of the heart a "straight-line" edge; on the other hand a more conspicuous enlargement may result in the dilated pulmonary artery showing independently. (Fig. 7). Screening may show relatively avascular lung fields. The commonest indication that the pulmonary valve is affected is a loud "ejection-type" systolic murmur heart at the left sternal margin usually maximal at the 3rd left interspace. (Fig. 8). Systolic and diastolic murmurs have also been heard over the tricuspid region in many of these patients.

The E.C.G. commonly shows clockwise rotation of the heart. Cardiac catheterisation studies have shown a rise in the mean pressure in the right ventricle. There may be a low pressure in the pulmonary artery, distal to the valve, with a marked pressure gradient across it. There may also be a presystolic pressure gradient across the tricuspid valve if tricuspid stenosis is present. The cardiac output may be reduced.

LABORATORY STUDIES.

(a) Screening tests.

Several rapid tests for quick detection of increased urinary 5HIAA are now available. The most useful test depends on coupling of 5HIAA with -nitroso- naphthol to give a purple coloured substance

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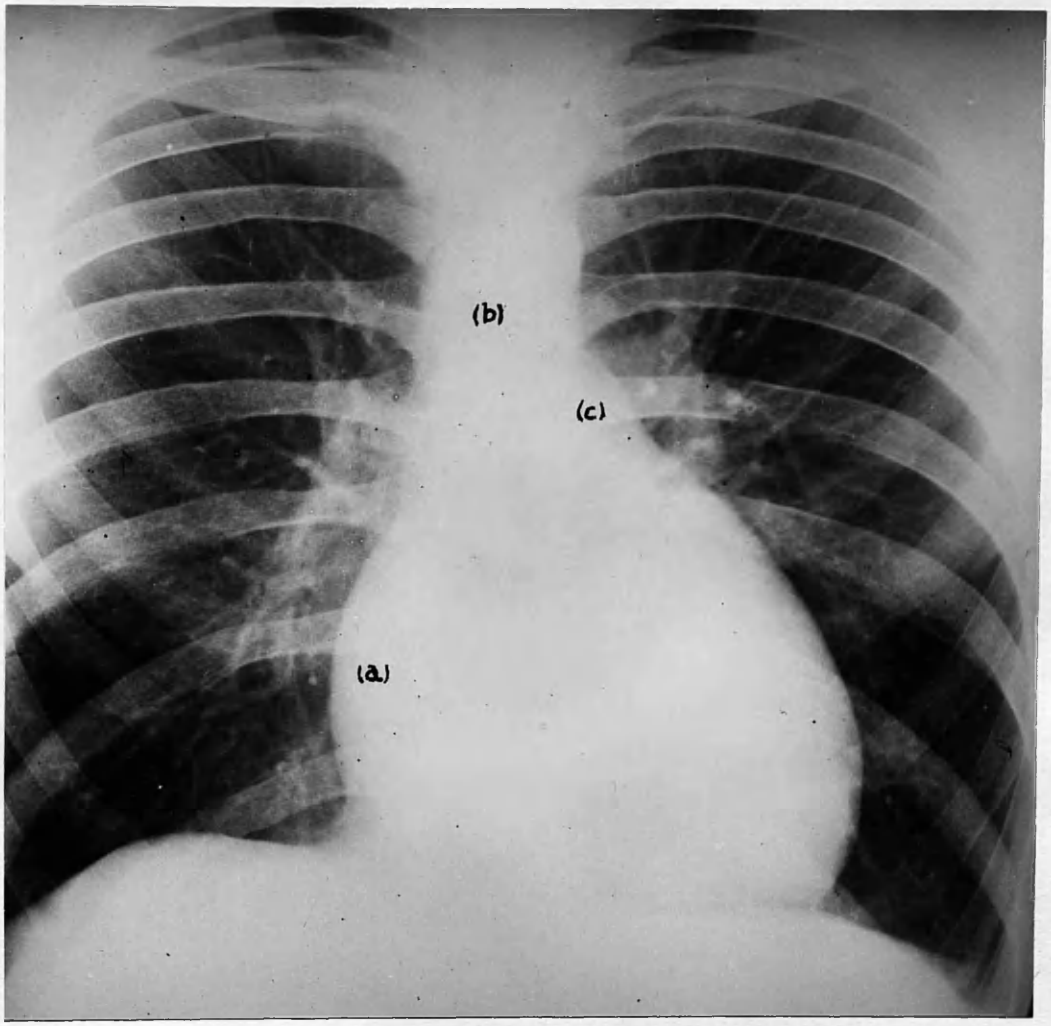

Fig. 7. The chest radiograph, in this case of the argentaffinoma syndrome, shows at (a) the enlargement of the right side of the heart, at (b) the small aortic knuckle, and at (c), the visibly dilated pulmonary conus.

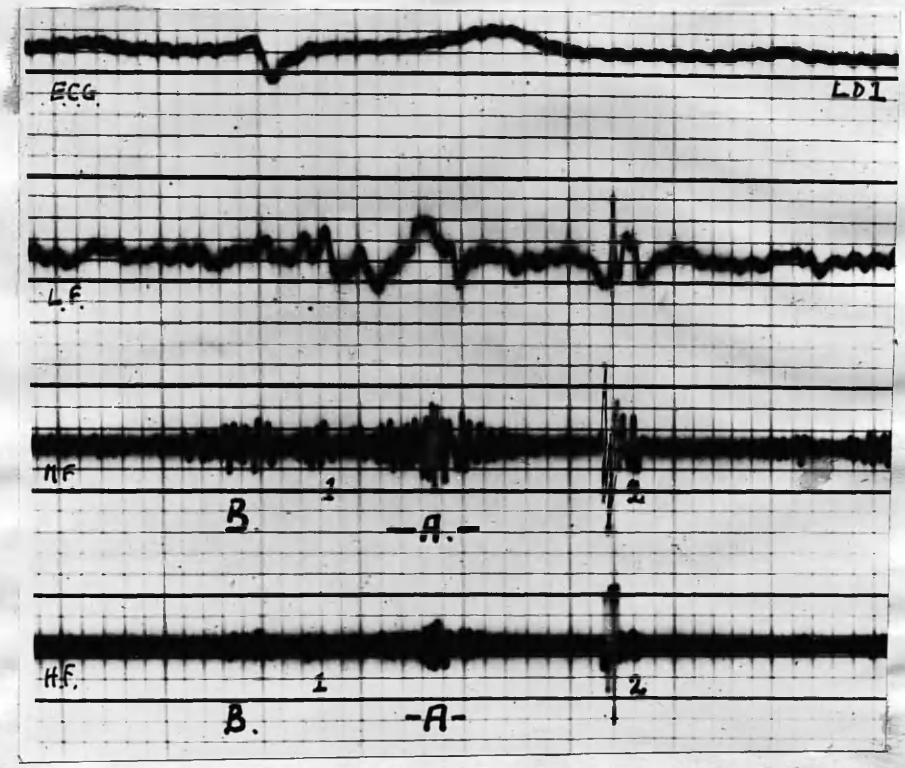

Fig. 8. A phonocardiogram was taken at the left sternal border (3rd left interspace) in the same case as Figure 7. The tracings recorded are the E.C.G. (LD_1) and low, medium and high frequencies respectively. Opposite the M/F and H/F records, the first and second sounds have been marked (1 and 2). The first sound is soft and the second sound is accentuated.

A "diamond-shaped" ejection type of systolic murmur is recorded, (A) which is characteristic of pulmonary stenosis. There is also a diastolic murmur at (B) which may indicate a stenosed and incompetent valve. The second sound (2) is highly accentuated.

(Sjoerdsma, Weissbach and Udenfriend, 1955). Ehrlich's aldehyde reagent may also be used combining with 5HIAA to give a blue colour.

(b) 5HT and 5HIAA in urine, blood and tumour tissue.

i) The urinary excretion of 5HT itself is low, and the excretion of 5HIAA, its oxidation product, (Fig. 9) is a more reliable index of the total production. An easily performed test using the reaction with nitroso- naphthol has been described by us.* The results for carcinoid cases are shown in Table 1. 2 - 10 mg. has been regarded by us as the normal range. The test may be used both in diagnosis and prognosis (Tables 2 and 3). A chromatographic technique of simplicity has been evolved (Curzon, 1955). High values of 5HIAA are not always associated with severe symptoms and vice versa; it has been suggested that there is an adaptive response of the enzyme mono-amine oxidase in the lungs which might account for this, yet Davison and Sandler failed to find evidence of this change. (Davison and Sandler, 1956). Although 5HIAA excretion should fall after resection of the tumour, a return to normal level of 5HIAA immediately after the operation should not be accepted as a test of cure since the sensitivity of the test may not be sufficient to detect the small quantities of 5HIAA which may be secreted by residual tumour tissue; it is difficult to determine whether values in the range 10-20 mgms. are abnormal or not (Smith, 1959). On the other hand the test may prove valuable in follow-up studies of the patients (Thorson, 1958; Sjoerdsma, Weissbach and Udenfriend, 1956).

ii)/

* Appendix.

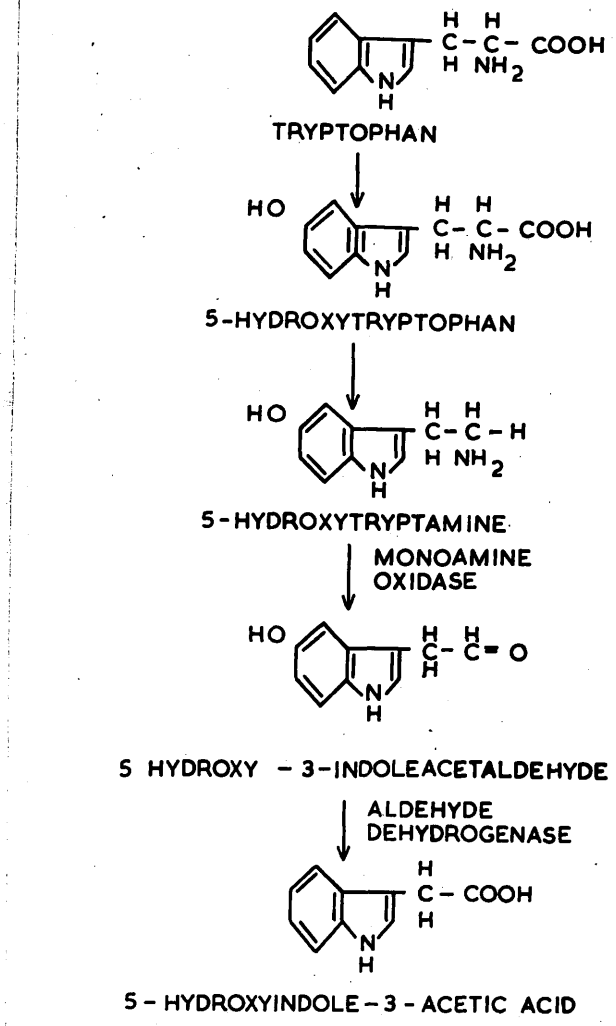

Fig. 9. 5-Hydroxytryptamine (5-HT) is derived in the tumour mass from amino-acid precursor, 5-hydroxytryptophan (5-HTP) which is in turn dependent on the dietary intake of the essential amino acid tryptophan.

5-HT is rapidly destroyed after release from the tumour by the amine oxidase in various tissues. The product 5-hydroxyindole-acetic acid (5-HIAA) is detectable in large quantity in the urine.

TABLE 2.

The diagnostic use of 5-HIAA excretion.

Case No.	Clinical Data	Pathology	Excretion of 5-HIAA (mg./24 hours)
6	Flushing ++ Secondary tumours in liver and bone. Primary not known.	Histology not clearly argentaff-:inoma. Specific stains negative.	140
7	Subacute intestinal obstruction: surgical diagnosis carcinoid.	Biopsy inadequate	11.2 9.1
8	Multiple colonic tumours.	3 adeno-carcinomata 1 - ? argentaffinoma Specific stains - negative	11.3
9 10 11	Cases of flushing and cyanosis of unknown origin.	-	less than 10
12	Spasmodic flushing: mass in right iliac fossa.	-	5.2

TABLE 3.

The use of 5-HIAA excretion in assessing prognosis.

Case No.	Clinical	Pathological	Excretion of 5-HIAA mg/24 hours
11	Appendicitis	Argentaffinoma invading local lymphatics	8.8
12	"	Argentaffinoma invading muscularis - not obvious in lymphatics.	6.1
13	"		2.9
14	"		7.1
15	"		3.8
16	Chronic ileo-ileal intus-:susception due to iliac tumour. Right hemi-colectomy 1953	Argentaffinoma with lymph nodes	11.1
17	Ileal tumour removed 1946. Liver palpable 1955 - Frequent diarrhoea. No flushing.	Argentaffinoma with lymph nodes	17 20

ii) The 5HT content of blood is substantially elevated in cases of the syndrome. (Table 4). The 5HT contents of the peripheral blood may rise during an attack (Table 4, A, B, C) but this is disputed (Sjoerdsma, Weissbach, Terry and Udenfriend, 1957). As already mentioned, the blood from the portal venous system, hepatic veins and right heart may have the highest concentrations of 5HT in the vascular system. (Table 4, D). In a case with osseous metastases in a limb the venous blood was found to have a higher concentration than was found in arterial samples (Table 4 E).

iii) Biological assay of samples of tumour or secondary deposits (Lembeck, 1954) have helped to establish the diagnosis and are particularly useful when the histological staining reactions are equivocal. (Table 5).

TREATMENT.

(1) Surgical management.

(a) For the patient who has had an argentaffin tumour of the appendix removed by appendicectomy there is no further problem.

(b) In those appendicular cases in which invasive histology is demonstrated near the base, right hemi-colectomy ought to be advised. Many such patients can expect a permanent cure.

(c) Right hemi-colectomy should also be advised for tumours of the terminal ileum, with ileotransverse colostomy as the palliative procedure in a case where there is a large fixed intra-abdominal mass with/

TABLE 4 LISTS THE VALUES FOR 5-HT IN PLASMA AND WHOLE BLOOD. NOTE THE ELEVATION, MAINLY IN PLASMA 5-HT, DURING FLUSHING ATTACKS IN 2 PATIENTS (A & B), AFTER RESERPINE (C) IN A THIRD, AND IN (E) THE VENOUS BLOOD SAMPLES FROM A LIMB GAVE HIGH VALUES BECAUSE OSSEOUS METASTASES WERE PRESENT IN THE LIMB: IN CASE (D) VALUES FOR PLATELET RICH PLASMA AND SERUM ARE COMPARED IN SAMPLES OBTAINED BY CARDIAC CATHETERISATION. NOTE THE EXCEPTIONALLY HIGH VALUES OBTAINED IN THE SAMPLES FROM THE PULMONARY ARTERY.

NORMAL VALUES FOR VENOUS BLOOD	PLASMA 5-HT ug/ml. MAXIMUM 0.02	WHOLE BLOOD 5-HT ug/ml. MAXIMUM 0.6
A NON-FLUSHING	0.12	2.8
FLUSHING	1.8	3.2
B NON-FLUSHING	0.08	1.6
FLUSHING	0.9	1.9
C NON-FLUSHING	0.04	2.1
FLUSHING (After Reserpine)	1.6	2.8
D* SUPERIOR AND INFERIOR VENA CAVA	1.3 and 2.5	2.5 and 2.25
PULMONARY ARTERY	6.2	5.6
BRACHIAL ARTERY	2.2	1.9
E FEMORAL ARTERY	Trace	0.78
FEMORAL VEIN	0.66	1.49

TABLE 5 LISTS THE 5-HT CONCENTRATION (in mg/G) IN BIOPSY SAMPLES FROM VARIOUS SITES, WITH THE HISTOLOGICAL ASSESSMENT AND 5-HIAA EXCRETION IN EACH CASE. THE HISTOLOGY WAS ROUGHLY GRADED ON THE BASIS OF THE DEGREE OF GRANULATION DISCLOSED BY THE USE OF THE SPECIFIC DIAZO STAINING METHOD.

NOTE THE LOW VALUES FOR THE APPENDICULAR CARCINOID IN (A), IN CONTRAST TO (B) AND (C) WHICH WERE CLASSICAL CASES OF THE SYNDROME. (D) WAS FROM A RECURRENT CASE, WITH REAPPEARANCE OF SYMPTOMS. (E) WAS A NON-ARGENTAFFIN ARGENTAFFINOMA WITH PROVED PRIMARY AND SECONDARY TUMOUR TISSUE: THIS HISTOLOGICAL ANOMALY OCCASIONALLY OCCURS.

SITE	HISTOLOGY (granulation)	BIOPSY mg/G	URINARY 5-HIAA mg/24 hrs.
A APPENDIX	++	0.2	4.9
B ILEUM	+++	4.1	210
C DUODENUM & 2 IN ILEUM	+++	1.3, 0.9, 1.5	82
D ILEUM. RECURRENT CASE	++	2.1	44
E METASTASIS (OSSEUS)	- (also non-argentaffin)	4	160

with involved nodes and secondaries in the liver.

(d) It should be remembered that any reduction of the secretory mass, even through local excision, may help to reduce the intensity of flushes and other systemic effects; it may on occasion, for instance, be possible to remove some of the secondaries from the liver, but this should not be entered into lightly, since death may follow handling of the tumour due to sudden release of 5HT. The operation should be done with the minimum of trauma, with the anaesthetist forwearned of possible hypotension and bronchospasm, using possible antidotes to these effects.

(e) Rectal carcinoids may be treated by local excision, but if invasive may require anterior resection when situated in the upper rectum, or abdomino-perineal excision when situated in lower rectum.

(f) For the patient who has had a laparotomy performed for an inoperable tumour, yet survives 3-5 years after this, general medical measures aimed at minimising the flushing attacks, reducing bronchospasm, diarrhoea and oedema must not be overlooked. X-ray therapy probably is ineffective (Foreman, 1952).

(2) Medical management.

Various pharmacological antagonists to 5HT may be tried clinically, among which are chlorpromazine (Gyermek, 1955), brom-ortholysergic acid diethylamine (B.O.L.) (Cerletti and Rothlin, 1955; Solero, Page, Salmoir-:aghi, 1956), /

1-benzyl -2, 5 dimethyl serotonin hydrochloride (B.A.S.) (Shaw and Woolley, 1956). On the whole, usage of these substances has produced disappointing results (Schneckloth, Page, Del Greco and Corcoran, 1957). Antihistamine drugs (Snow, Lennard-Jones, Curzon and Stacey, 1955) have been suggested on the assumption that the flushing attacks might have been provoked by the release of histamine in the tissues and have been reported to be of benefit in some cases (Davison and Sandler, 1956). Few of these agents have proved beneficial. The administration of radioactive gold (198 Au.) intravenously may produce a short-lived benefit (Daugherty, Manger, Roth, Flock, Childs and Waugh, 1955; Goble, Hay and Sandler, 1955; Stacey, 1957). Agents which antagonise adrenaline such as ergot alkaloids may be given a trial (Shaw and Woolley, 1953). Woolley and Shaw have prepared numerous antimetabolites of 5HT and it seems reasonable to expect benefit eventually from antagonists of this type (Schneckloth, Page, Del Greco and Corcoran; Shaw and Woolley, 1957).

In patients with flushing attacks provoked by excitement or embarrassment, benefit may follow the administration of phenobarbitone, 30 mg. ($\frac{1}{2}$ gr.) or of a tranquillising agent. Probanthine 50 mg. orally or intramuscularly may lessen the diarrhoea, which may also be reduced in severity by chalk and opium powder. Aminophylline may be beneficial if respiratory distress is a feature of an antihistamine such as piriton may help in giving temporary relief from this. The unpleasant cyanosed appearance/

appearance in the cold may force many patients indoors; it is an impression that bronchospasm occurs more severely and most often in cold weather. Pellagra and other vitamin deficiencies should be avoided by the administration of nicotinic acid parenterally. If the plasma proteins are low, general improvement may follow blood transfusion. Little improvement follows restriction of protein intake in the diet, since the tumour draws heavily, (see Chapter six), on the available tryptophan to form an unvarying amount of 5HT. Occasionally the consumption of tea, coffee and fats may trigger the flushing attacks and these may have to be omitted from the diet; alcohol almost always provokes intense flushing. It is unlikely that many of these patients will ever be considered fit for operative treatment of the heart valves because the lesions occur so late in the natural history of the syndrome and because the operative risk is a high one.

DISCUSSION.

The growth of knowledge concerning the physiology and pharmacology of 5-hydroxytryptamine has been a fast one but the precise role of this substance has not yet been elucidated. The carcinoid syndrome provides the clinician investigator with fruitful material in the manner that the study of Addison's disease aroused interest in and paved the way for our modern knowledge of the hormones of the adrenal gland, so may the study of this syndrome help elucidate our knowledge of mental and gastrointestinal physiology, vascular and respiratory physiology, and the like. It seems strange that the syndrome has remained undetected, in

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in the modern sense, for so long; in this respect it is worth recalling that the clinical features had been well documented almost thirty years ago, though unfortunately ascribed to carcinomatosis and before the role of 5HT (serotin enteramine) had been elucidated (Cassidy, 1930).

A review of the features of argentaffin tumours or carcinoids is therefore justified by the interest which has been aroused by the discovery that they secrete an important new hormone, 5-Hydroxytryptamine. The study of patients with this condition may enable the investigator to elucidate the normal physiological role of the new hormone; on the other hand a knowledge of the complex human actions of 5HT, its form-:ation from parent substances, and its degradation pathways and products may enable one to establish new features of the syndrome and plan effective treatment accordingly.

The features of the syndrome may be diverse in their manifest-:ations since there may be not only 5HT effects present, but those of a more general disturbance of tryptophan metabolism. This will be discussed in detail in the next chapter.

SUMMARY.

The incidence, aetiology, pathology, histogenesis and histology of argentaffinoma is firstly surveyed.

This account continues with:

i) a review of nine cases of metastasing argentaffinoma and the features attributable to 5HT secretion; blood levels of 5HT have been/

been examined in each case.

ii) the application of a urinary test for 5HIAA, the oxidation product of 5HT, which has provided a useful way of detecting over-production of 5HT by the tumour. Various applications of the test have been made, in particular to check whether all the tumour has been eradicated, and in this way to assess prognosis.

iii) Therapeutic measures should be planned so as to remove the tumour or ameliorate the effects of 5HT on the major systems of the body and in particular on the heart. This may be possible by the use of specific antagonists and by radiotherapy, including the use of isotopes, in advanced cases where surgical removal may not be possible.

CHAPTER SIX.

The interrelationships of tryptophan, 5-HTP and 5-HT in carcinoid patients; the acid gastric secretion in these cases and the effects of alcohol, histamine, reserpine and iproniazid.

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The interrelationships of tryptophan, 5HTP and 5HT in carcinoid patients; the acid gastric secretion in these cases and the effects of alcohol, histamine, reserpine and iproniazid.

In Chapter Six the degradation of 5HT is further discussed, as is its formation from precursors. Evidence is presented to show that 5HTP may have to be considered as much the hormone of the argentaffin cell as is 5HT. Evidence for this was already in our hands when the experiments described in Chapter Three were being performed and, as has already been related, this influenced the experimental emphasis considerably.

Although it is accepted from biological and biochemical evidence that 5HT owes its origin to L-tryptophan, this has only recently been confirmed by ourselves and Udenfriend for the human subject. This has been done by showing that L-tryptophan is converted to 5HT, but another striking discovery has been the isolation of 5HTP from human carcinoid from one of our patients with metastatic carcinoid tissue in the kidney. This implies that 5HTP was made by the tumour cells in this secondary deposit and is a function of them. It also implies that these cells must be capable of hydroxylation of tryptophan.

The effect of various diets on 5HT blood levels has also been studied in these cases; it had been reported that fatty meals precipitated flushing/

flushing attacks and it could have been the case that fat released inhibitory hormones such as enterogastrone, which in turn require 5HT for their action on the stomach.

The effect of acid secretagogues such as alcohol and histamine has been examined to see whether they release 5HT as part of their secretory stimulant action or in response to it. Acid gastric secretion has been examined in several secreting cases, and in some non-secreting cases given reserpine to deplete 5HT or iproniazid which potentiates it.

1. Metabolism of 5HT.

5HT is derived from the precursor 5-hydroxytryptophan which is in turn derived from the essential dietary aminoacid tryptophan. The first step in the production of these compounds from tryptophan is a hydroxylation process by an enzyme, tryptophan hydroxylase, reported by Udenfriend (Udenfriend, Clark and Titus, 1953) in liver and kidney. 5-hydroxytryptophan (5HTP) is then converted to 5HT (Fig. 1) by means of a specific decarboxylase. Carcinoid tissue contains a rich store of this enzyme. 5HT is rapidly oxidised by amine oxidase which is widely distributed in the lung, liver and kidney (Bradley et al, 1950; Blaschko and Philpot, 1953; Gaddum and Giarmin, 1956). The principal product of the oxidative process is 5-hydroxyindole acetic acid, 5HIAA, which is excreted in the urine. Other hydroxyindoles are formed, as well as 5HIAA and the further breakdown products, hydroxyindoleacetic (HIAA); we have described/

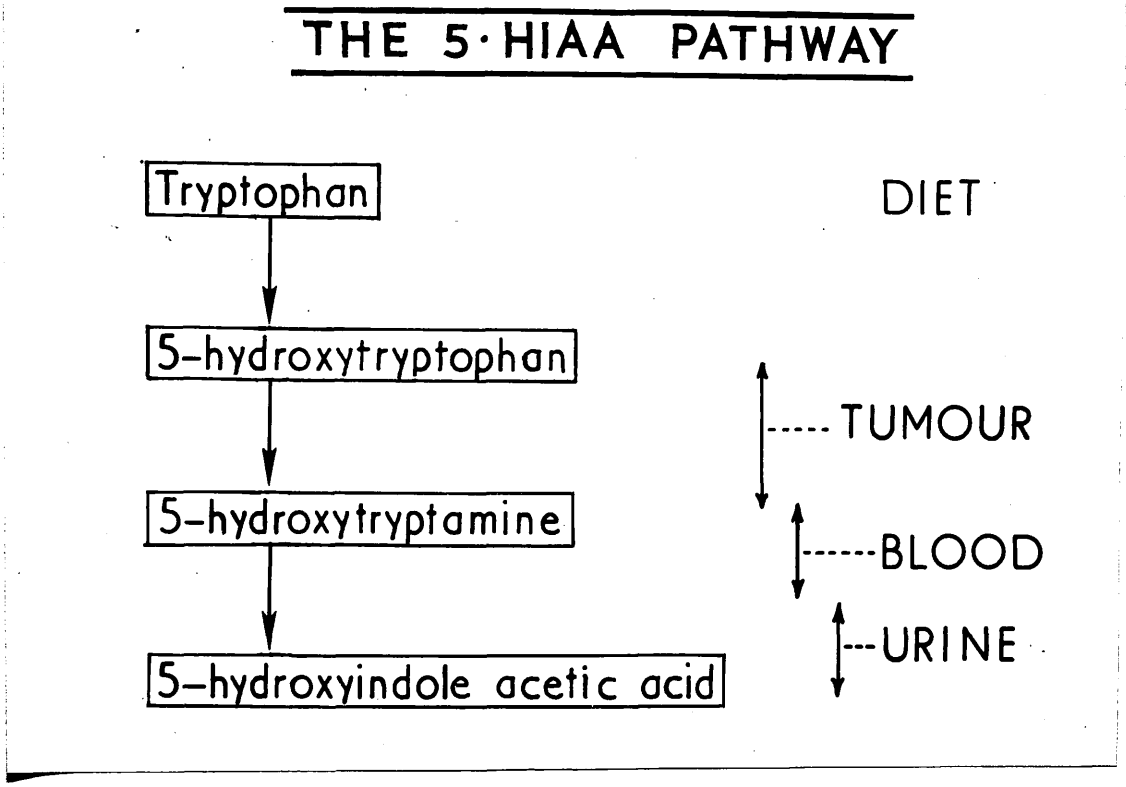

Fig. 1 illustrates the 5-HIAA pathway - for comparison with Fig. 9 in the previous chapter. The figure illustrates the sites at which the precursors of 5-HT are formed, and of 5-HT and 5-HIAA formation themselves.

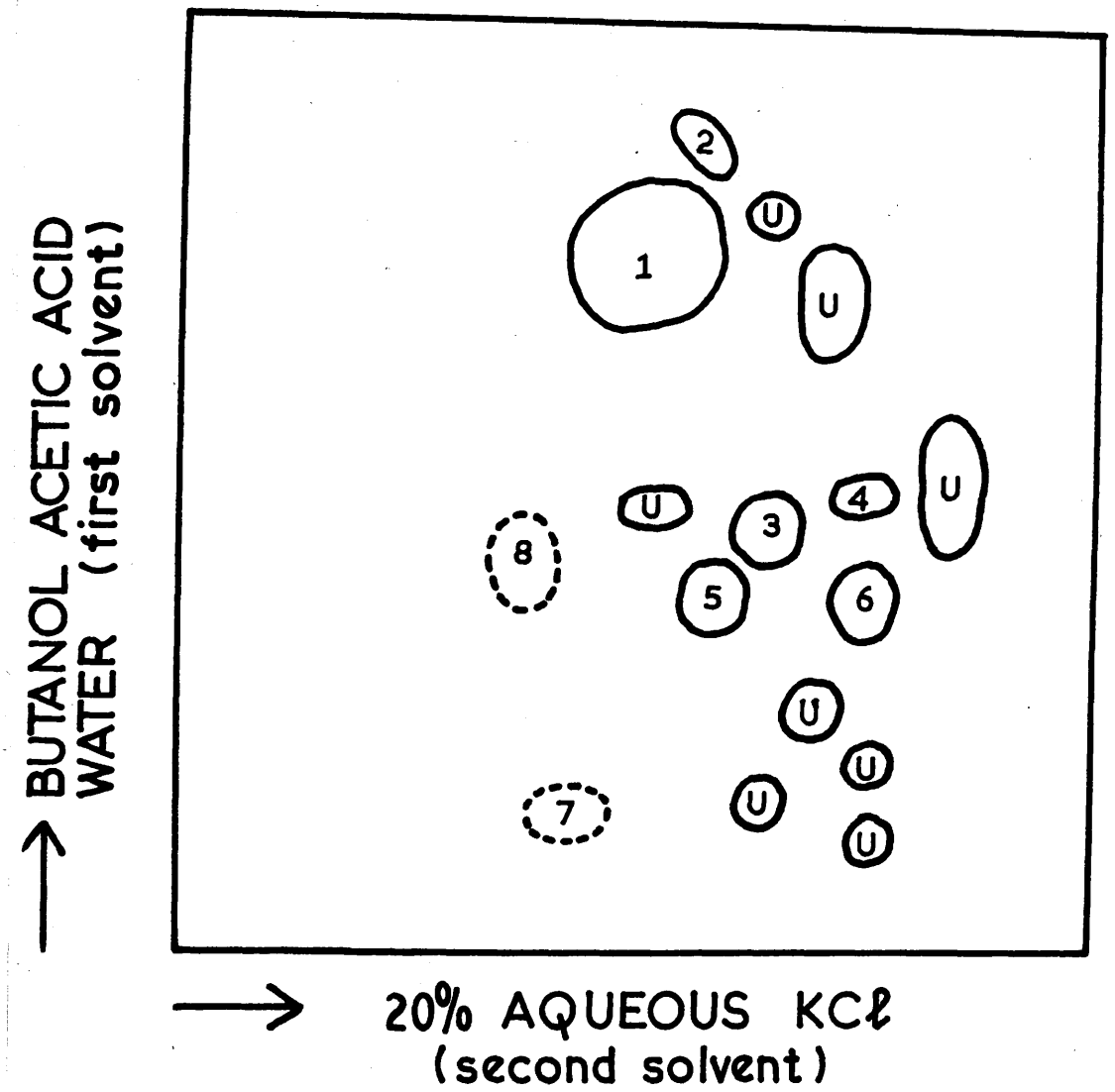

Fig. 2. Diagram of the 2-dimensional paper chromatographic pattern given by the urinary indoles and related substances excreted by Case 19. Spots marked U, unidentified; I, 5-HIAA; 2, indole-acetic acid; 3, indican; 4, 2-amino-3hydroxyaceto-:phenone O-sulphate; 5, tryptophan; 5, probably 5-hydroxy-indoleacetic acid O-sulphate. The dotted spots, 7 and 8, represent the positions occupied by 5-hydroxytryptophan and 5-hydroxytryptamine respectively; neither of these substances was present in this urine.

described 14 indoles in the urine of carcinoid patients (Fig. 2). Sjoerdsma et al. were able to recover 80% of administered 5HT as 5HIAA, the residual amount presumably being converted to the other hydroxyindoles (a small portion of normal urinary 5HIAA may be derived from other sources).

2. Role of 5HTP.

Since tryptophan decarboxylase was held to be absent from the tumour by Udenfriend, it was assumed that tryptophan underwent conversion in the liver and kidney to 5HTP, with later conversion of 5HTP to 5HT after uptake of 5 HTP by the tumour, or in other tissues containing decarboxylase. We have described a case of some importance in which 5HTP as well as 5HT was secreted in the urine (Fig. 3).

In this exceptional case with multiple metastases (Fig. 4), 5HT and 5HTP were both present in the urine in considerable amounts (Fig. 5). This confirms the postulated route of formation of 5HT for the human case since, in this subject's urine the precursor, active substance and breakdown product were present together. It can be deduced that the tumour, and so presumably the normal argentaffin cells from which the tumour has arisen, must be capable of 5-hydroxylation of tryptophan (Fig. 6) according to the scheme outlined. It follows that the major function of the argentaffin cells may not only be the local release of 5HT but the manufacturers of its precursor, 5HTP for general circulation to various other cells which decarboxylate it and turn it into the active hormone locally. This would account for the fact that though 5HT is produced by different kinds of cells, no other kind of tumour

produces/

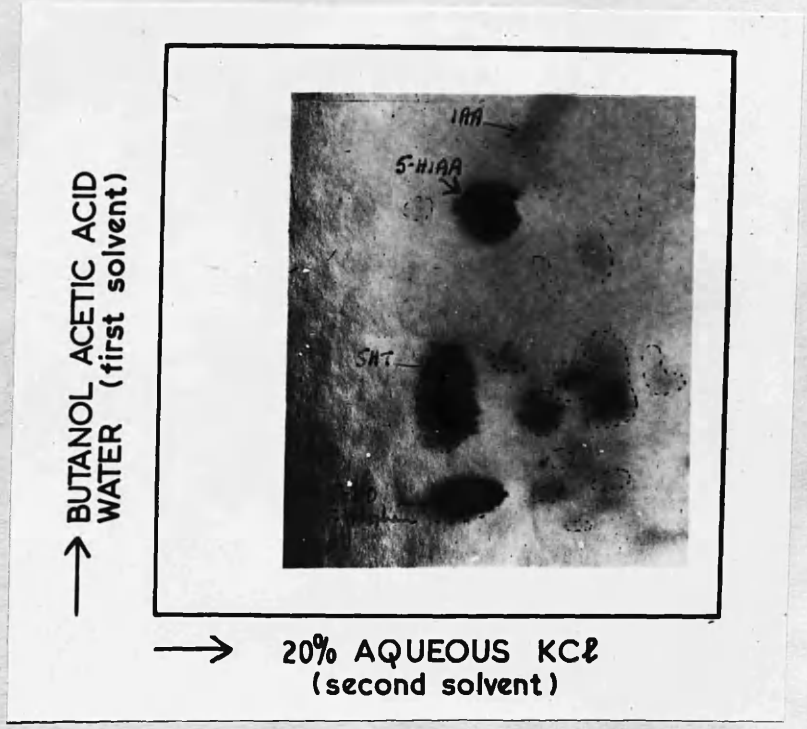

Fig. 3 shows a photograph of the developed chromatogram in a patient secreting 5-HTP and 5-HT. Two dimensional chromatograms, descending technique, Whatman No. 1 paper, using the solvents indicated. Indolic substances were located using Ehrlich's reagent.

Fig. 4 is a photograph of an intravenous pyelogram obtained in this patient and shows the extreme deformity resulting from renal metastasis in this case.

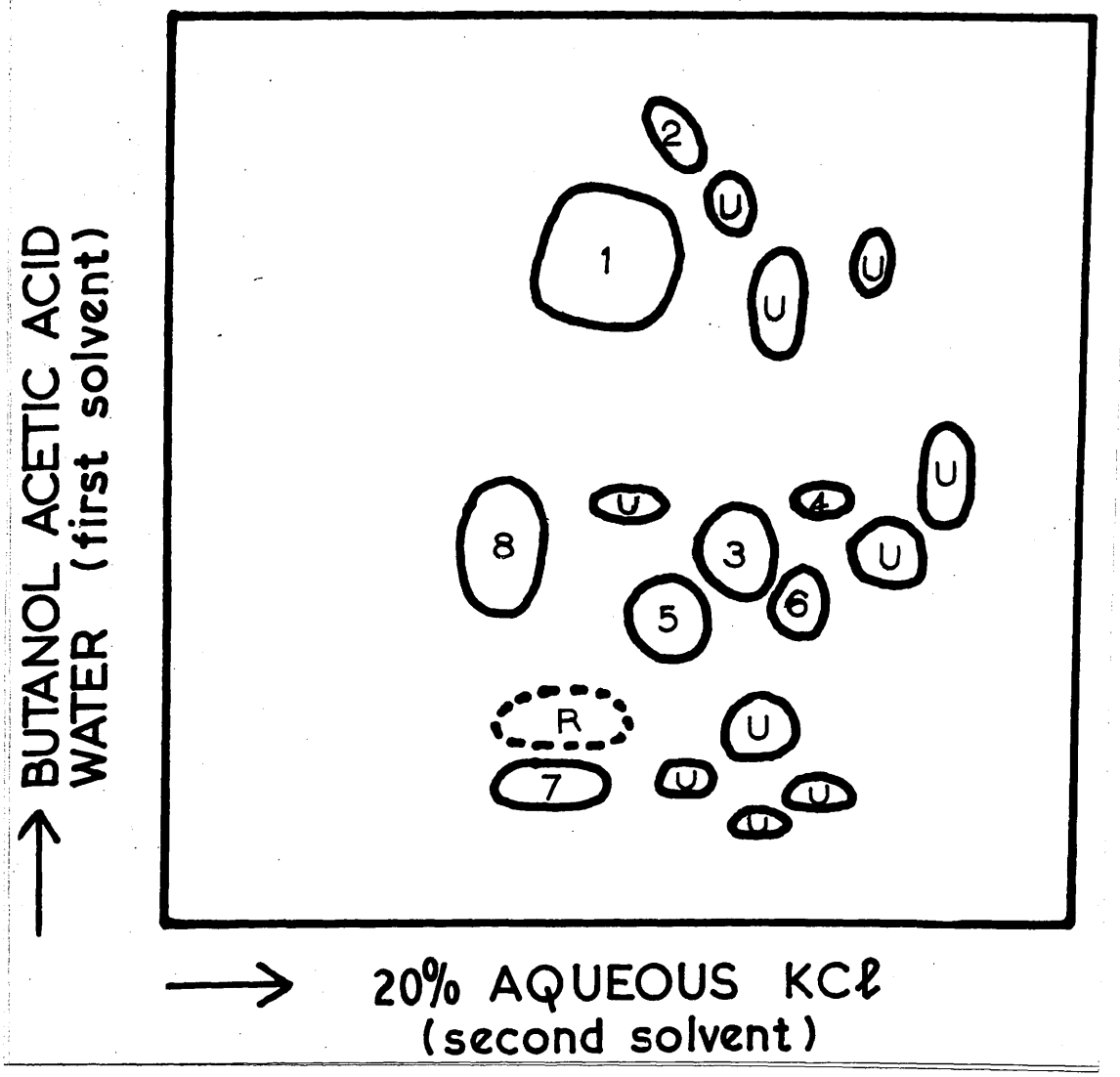

Fig. 5. Diagram of the 2-dimensional paper chromatographic pattern of urinary indoles and related substances excreted by Case 18. The numbers have the same significance as in Fig. 2. The dotted spot, R, shows the position of riboflavin, detected by its fluorescence and useful as a marker.

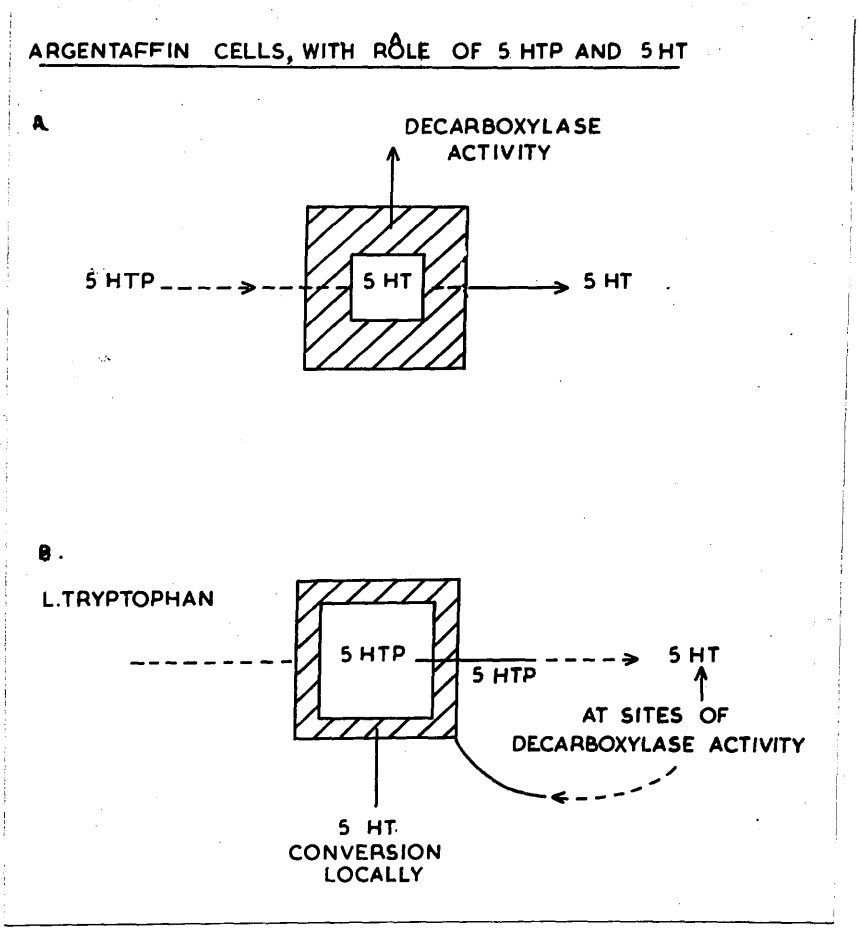

Fig. 6a. The most simple hypothesis is that 5-HTP already formed in the liver or elsewhere is converted to 5-HT in the argentaffin cell, whence it is released.

Fig. 6b. An alternative hypothesis, advanced after recognition that carcinoid tissue contains 5-HTP, envisages it as the local hormone of the argentaffin cell. 5-HTP may be converted locally into 5-HT, but the greater part probably acts as a source of supply for other tissues.

produces excess of it.

Atypical cases, such as this one secreting 5HTP, may owe their unusual features to an unusual cellular composition; it has been suggested that some tumours are argyrophil rather than argentaffin in type (Sandler and Snow, 1958). This unusual type of carcinoid may have osseous metastases, a brick red flush, 5HTP and 5HT in the urine, and a high urinary histamine. The flushing attacks in this type of case may be the result of histamine liberation in the tissues (see Chapter 1) since they could be attenuated by treatment with an antihistamine.

3. The effects of various diets on 5HT blood levels.

(a) Relationship of 5HT to tryptophan and protein intake.

The tryptophan requirement for normal 5HT production is small compared with tryptophan intake (500-1000 mg/day). However, when argentaffinomata are present 5HT production can be enormously increased, e.g., in the cases reported 5HIAA excretions up to 600 mg./day were encountered. In such cases a major part of tryptophan intake must be utilized for 5HT production.

This matter was investigated in some detail and more cursorily in three others. In the first case the daily output of 5HIAA showed two clearly defined phases. In the first phase the daily output was remarkably constant. The values shown in Figure 7a were obtained while the patient was on an unrestricted ward diet, immediately prior to the dietary experiments. After the dietary experiments, described below,

the/

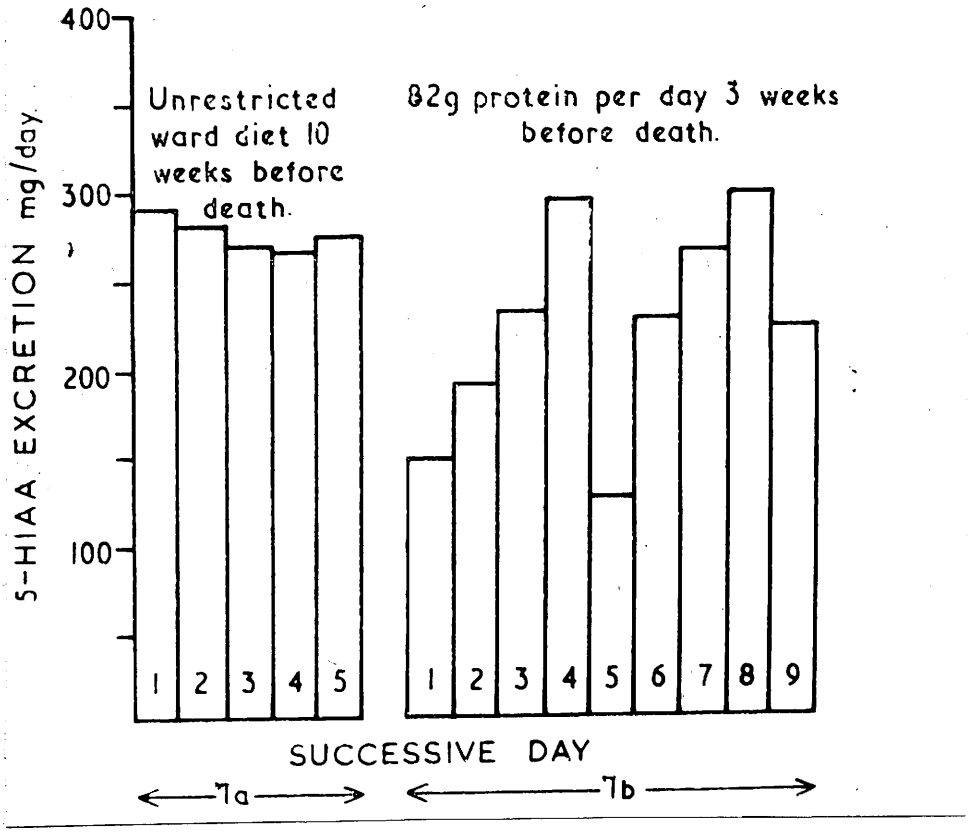

Fig. 7. 5-HIAA excretion in Case 19. Showing the constant excretion during the earlier part of his stay in hospital (7a) and the irregularity of excretion produced later, presumably by necrosis of the tumour (7b).

the patient entered a phase of marked variations in daily 5HIAA output, even on a constant protein intake, and this prevented further balance studies. This phase continued till the patient's death. Typical variations in 5HIAA output during this phase are illustrated in Fig. 1b. It is plausible to associate this change with the onset of the extensive recent necrosis found at post-mortem in the major metastasis in the left lobe of the liver.

On a protein intake of 82 g/day the patient was in positive nitrogen balance. Nitrogen balance became negative on reducing protein intake to 31 g./day; it was not restored by adding 500 mg. L-tryptophan/day to the 31 g. protein diet, but returned to its previous positive value on returning to the 82 g. protein diet (Fig. 8). The negative nitrogen balance was therefore not due to a simple tryptophan deficiency. On the 82 g./day protein diet, mean 5HIAA excretion was 312 mg./day (Fig. 8a). This dropped to a mean of 224 mg/day on the 31 g./day protein diet (Fig. 8b), rose to a mean of 301 mg./day on supplementing the diet with 500 mg. L-tryptophan (Fig. 8c) and returned to a mean of 222 mg./day on returning to the low protein diet (Fig. 8d). The 500 mg. L-tryptophan/day added to the diet in stage 8 corresponded to the estimated tryptophan content of the 51 g./day drop in protein content of the diet between stages 8a and 8b. Of this 500 mg. tryptophan, approximately one-fifth appeared as urinary 5HIAA. It follows that a larger amount than this was probably metabolized by the 5HT pathway (cf. discussion above). On the low protein diet the tryptophan intake was only of the order of 300-400 mg./day and/

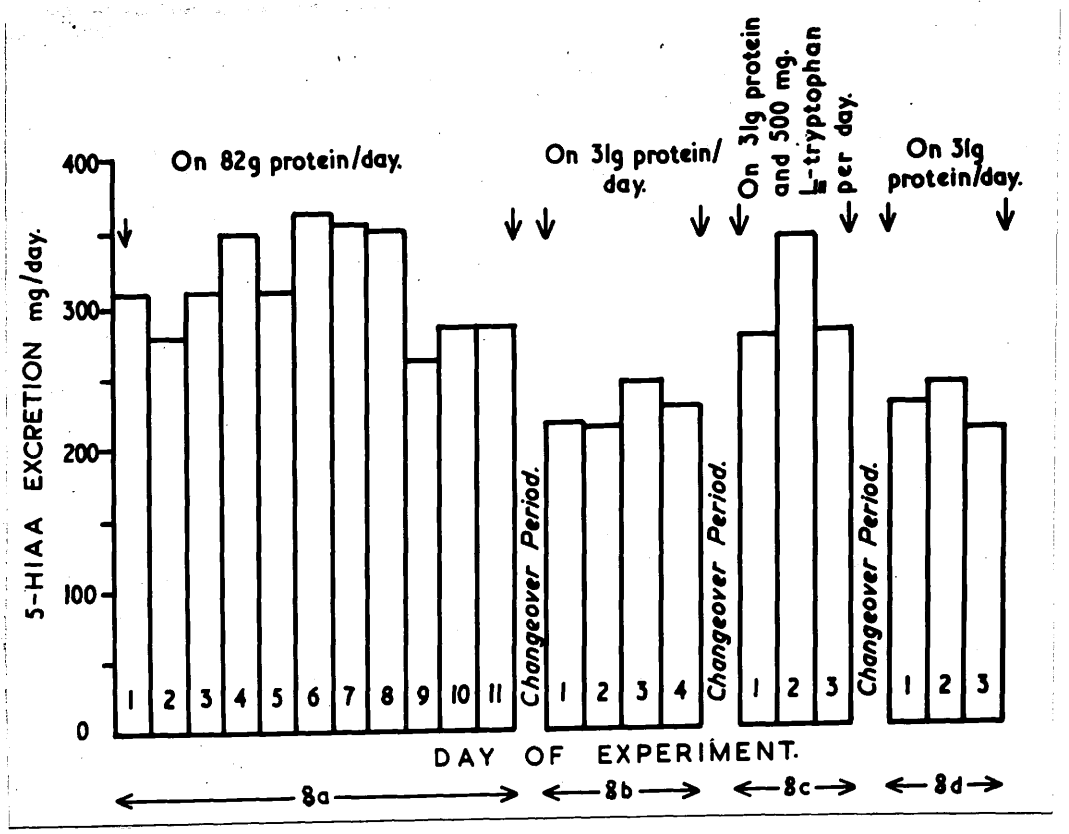

Fig. 8. 5-HIAA excretion in Case 19: the effects of the level of protein and tryptophan intake. A low protein intake reduced the 5-HIAA output slightly unless the tryptophan intake is maintained.

and probably more than two-thirds of this was metabolized by the 5HT pathway.

In the three other cases observations were limited to the demonstration of a small rise in 5HIAA excretion on a high protein diet (Table 1) and a significant fall in 5HIAA excretion on a low protein, high fat diet (Fig. 9). No nitrogen balance studies were done in these cases nor was tryptophan given during the low protein stage of the test but the results confirm the findings already given.

These findings are best explained by assuming that the tumour has a basic rate of production of 5HT, and will draw on circulating plasma tryptophan till this basic production is satisfied. Increase in available tryptophan may increase the rate of production of 5HT by the tumour slightly, but not in proportion to the increased availability. Until the basic tumour production of 5HT is satisfied the tumour may exert priority for available circulating tryptophan over protein biosynthesis and at low levels of protein intake an argentaffinoma may seriously impair the nutritional status of a patient.

Most inhabitants of this country achieve a daily intake of 1 g. of tryptophan, which should suffice for not only the tryptophan requirements of the tumour but also the needs of normal protein synthesis, which are comparatively small (under 250 mg. a day, in all adults investigated by Rose et al., 1954). The present results also suggest that if nutritional deficiency is suspected, the protein content of the diet could be increased/

TABLE 1.

EFFECT OF HIGH PROTEIN DIET ON DAILY URINARY EXCRETION OF 5HIAA.

	Mean level on normal hospital diet (mg.)	Levels on high protein diet (mg.)
Case 2	41	44; 47.6; 41
Case 4	250	286; 295; 292

Fig. 9. 5-HIAA excretion in 2 patients.
There is no significant increase
diet, and it is followed after

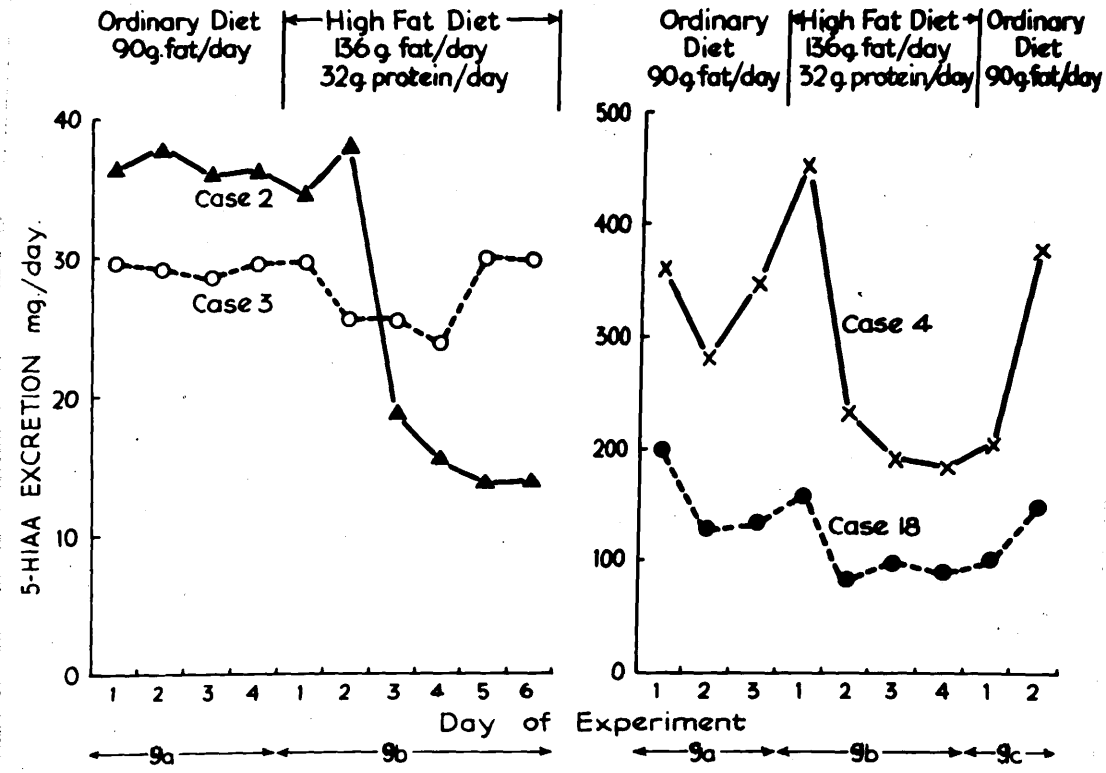

Fig. 9. 5-HIAA excretion in 4 patients; effect of a high fat diet. There is no significant initial rise after starting the fat diet, and it is followed after a day or two by a fall due to the low protein content of the diet.

increased without causing an undue increase in 5HT production by the tumour. It should, however, be noted that diarrhoea frequently accompanies disseminated argentaffinoma and might unfavourably influence the nutritional picture.

The classical carcinoid syndrome may be modified in yet another way. It has been established in the manner just recorded that the source of 5HT is the dietary tryptophan; in some cases a very large proportion of the normal intake of tryptophan is diverted to meet the requirement of the tumour in the formation of 5HT and this, combined with the diarrhoea often present, leads to a considerable risk of tryptophan deficiency. Since tryptophan is the precursor of nicotinic acid, this becomes apparent as nicotinic acid deficiency or pellagra.

It would also appear from the above studies that the tumour has a basic rate of production of 5HT; we have shown that increases in available tryptophan on a high protein may increase the rate of production of 5HT slightly but not in proportion to the increased availability. The tumour draws on all available circulating plasma tryptophan till 5HT production is satisfied. Sjoerdsma et al. (1957) have examined some of these points in detail; they found that the plasma tryptophan was low in carcinoids even on a tryptophan intake exceeding 1000 mgms. and at a time when there had been diarrhoea or change in weight. They/

They also found that the urinary excretion of N-methyl nicotinamide was lowest in the cases with the highest urinary hydroxyindole excretion. Studies with radioactive 5HTP enabled them to estimate that some of those cases had a tumour pool of over 2000 mgms. of 5HT, which is all the more striking when one recollects that serious pharmacological changes may follow the systemic injection of 1-2 milligrammes.

(b) Relationship of 5HT to fat intake.

In one patient investigated by Bleehen (1955) the flushing attacks occurred regularly within a few minutes of eating a fatty meal. To explain such a phenomenon it was suggested that fat might release 5HT from its tumour depots either directly, or via an intermediate mechanism such as the release of hormones (e.g. secretin, enterogastrone) (Lancet Editorial, 1955; Sjoerdsma, Weissbach, Terry and Udenfriend, 1957). Direct examination of patients on high fat diet failed to detect an increase in indoles excreted (in fact, the reverse was present). (Fig. 9).

4. Release of 5HT by drugs which are reputed to be acid secretagogues.

Effect of alcohol and antabuse on 5HIAA excretion. Flushing is often excited by the intake of alcohol in argentaffinomatosis (Snow et al., 1955). We have been unable to demonstrate any consistent rise in 5-HIAA excretion after taking 50 ml. of alcohol (30% U.P.) in any of our patients, whether measured in 24-hour periods or in 4-hour periods (Table III), though all showed marked flushing in response to the dose.

Since antabuse (tetraethylthiuram disulphide) acts by preventing the/

TABLE 2.

Effect of a single dose of alcohol (50 ml. 30% U.P.) on 5-HIAA excretion (mg.) in (a) 4-hour periods and (b) 24-hour periods.

	(a)			(b)		
	Control 4 hrs. before alcohol	4 hours after alcohol	Subsequent 4 hrs.	Control days before alcohol	Day with alcohol	Control days after alcohol
Case 2	-	-	-	45 47	19	30 41
Case 3	9	7	13	45 34	34	37 35
Case 4	44	32	55	286 370	262	401
(Repeat)	80	41	30	-	-	-
Case 18	25	18	-	159 158	114	-

the oxidation of acetaldehyde formed from ethyl alcohol and since the oxidation of 5HT to 5HIAA proceeds through the stage of 5-hydroxyindole-acetaldehyde, it seemed possible that antabuse might block the subsequent oxidation of this aldehyde to 5HIAA and lead to an accumulation of the aldehyde in the blood. The drug was given in doses of 0.5 g./day to two patients. In Case 3 it had no effect and no change in 5HIAA excretion could be observed. In Case 18 flushing, nausea and vomiting occurred soon after it was started, and a moderate but probably significant increase in excretion of both 5HIAA and 5HT occurred in the following days. These experiments require repetition.

Reserpine. Since one of the main actions of reserpine in the normal subject is to liberate 5HT at various sites (Shore et al., 1955), it seemed worth while observing the effects of the drug in argentaffinoma cases. In each case the substance was given for one day only. In the normal subject 1-3 mg. reserpine produced flushing of the face, a characteristic suffusion of the conjunctivae and slight falls in blood pressure. The urinary excretion of 5HIAA was increased on the day of administration (Fig. 10) and in one case on the succeeding day also. In one of the argentaffinoma cases the cautious exhibition of reserpine provoked an overall increase in frequency and intensity of flushes and in another an increase in bowel movements (this latter patient was on BOL therapy at the time - see below.) In each case the administration of/

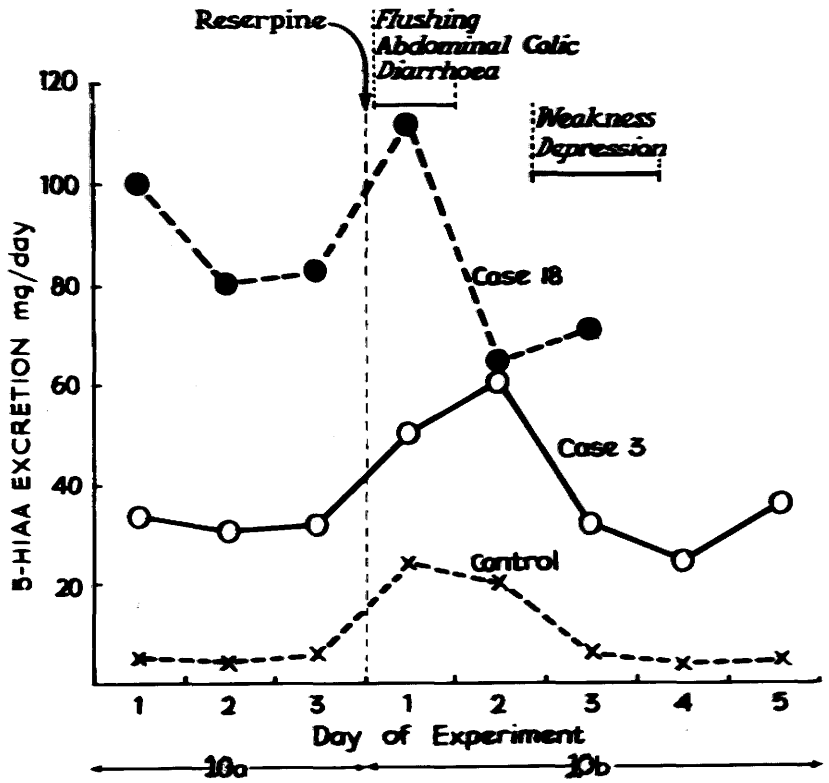

Fig. 10. 5-HIAA excretion after injection of reserpine in 2 cases (1 mg.) and a control (3 mg.).

of reserpine was followed by a rise in 5HIAA output. The subsequent fall in 5HIAA excretion could be interpreted as indicating a period of restorage of 5HT in the tissues (Fig. 10).

Our results, therefore, indirectly confirm the action of reserpine in facilitating release of 5HT in the human subject and they point to a strong parallelism between the flush induced by the drug in the normal subject and the characteristic facial flush of the argentaffinoma syndrome. These findings suggest that during the prolonged use of this drug a careful watch should be kept for the development of the later cardio-vascular complications.

Histamine. In view of the report from the Mayo Clinic (Daugherty et al., 1955) of increased secretion of its hormone by a carcinoid tumour after injection of histamine, in a manner similar to that induced in phaeochromocytomata, this substance was injected into four of our patients. It produced flushing with headache in every case and a significant rise in 5HIAA excretion in Case 4 suggested that an increased amount of 5HT had been liberated: this was not, however, so strikingly demonstrated in the other cases (Table 3).

5. Gastric function in carcinoid cases.

(a) Secretory tumours: Four cases were secretory, as far as could be judged from the clinical appearances of flushing, almost continuously. They have been examined after histamine stimulation but histamine tests were not in themselves conclusive; achlorhydria in 2 out of 4 patients (both/

TABLE 3.

Effect of a single injection of 0.5 mg. of histamine acid phosphate on 5-HIAA excretion (mg.).

	2 hours before injection	2 hours after injection	24 hours before injection	24 hours after injection
Case 2	-	-	45	49
Case 3	4.4	5.5	32	38.4
Case 4	29	53	262	401
Case 18	14.8	15.9	146	178

(both elderly women) being set against a normal acid output in the two men. The urinary excretion of pepsinogen by these cases is quite remarkable, however (Fig. 11). By the method used (Sircus, 1954) most normal subjects gave figures for daily excretion in the region of 400 units and figures over 600 are rarely seen except in the presence of duodenal ulcers; patients with histamine fast achlorhydria usually excrete negligible or undetectable amounts of uropepsin, and in most normal subjects uropepsin excretion and acid levels tend to run parallel. The discrepancy between the results of the two tests in the argentaffinoma cases is obvious. The figures for acid production are normal or low; the figures for uropepsin are normal in one case, high in two and exceptionally high in one. In Case 2 the presence of a histamine fast achlorhydria associated with a level of uropepsin excretion of such magnitude that it would ordinarily be suggestive of duodenal ulcer, is specially noteworthy.

On the basis of experimental work on gastric secretion, a marked depression of acid secretion might have been expected, but the very high levels of uropepsin were a surprise; possibly the high levels of pepsin have something to do with the gastric erosions and ulceration which follows 5HT infusion and occurs occasionally in the carcinoid cases. Normally the ratio of pepsin secreted into the stomach to the uropepsin passed in the urine is fairly constant, something like 1 unit appearing in the urine for each 100 units secreted into the stomach. We have no

figures/

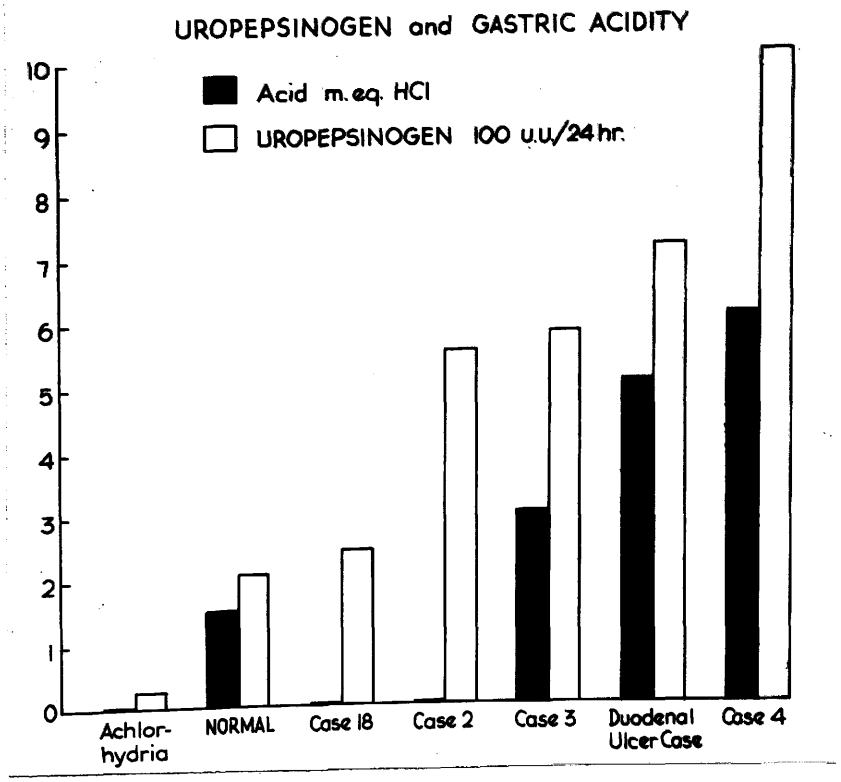

Fig. 11. Comparison of acid and peptic secretory activity of stomach. The acid is measured as milliequivalents per hour after 0.5 mg. of histamine: the peptic activity by the daily output of uropepsinogen.

figures for intragastric pepsin secretion in our cases and it is therefore not yet possible to say whether the results indicate an abnormally high secretion of pepsin with a normal ratio to urinary excretion, or an alternative in the process of pepsin secretion, leading to an abnormally high proportion of the enzyme being lost into the blood stream and ultimately in the urine.

(b) Non-secreting tumours: Two non-secreting cases were each given 1 mg. reserpine twice daily to act as a provocative test and the acid secretion in response to histamine was measured. The same two cases were on a subsequent occasion each given 25 mg. of iproniazid three times per day. The acid secretory values in both experiments were compared with two normal controls given identical treatment. After reserpine the carcinoid cases secreted the same amount of acid as on a day when no reserpine was administered. The acid secretory values were comparable to the control. After ipronizid the acid secretory values were very much greater in both groups (Table 4).

DISCUSSION.

The conversion of tryptophan to 5HT depends on the oxidation of tryptophan to produce 5 hydroxytryptophan; loss of CO_2 from the carboxl group then produces the corresponding amine, 5HT. The presence of a decarboxylase which accomplishes the formation of the amine is now well authenticated. The tryptophan oxidase that would produce the 5-hydroxytryptophan has never yet been demonstrated in the various tissues/

TABLE 4.

Acid output in mille-equivalents per hour after 0.5 ug. histamine in normal controls and non-secreting carcinoids given (a) Reserpine; (b) Iproniazid.

Case No.	(a) Reserpine Before	(a) Reserpine After	(b) Iproniazid Before	(b) Iproniazid After
1	2.6	2.9	2.4	1.6
2	1.8	1.3	1.0	0.6
3 *	1.2	1.6	1.5	0.6
4 *	1.8	1.8	1.6	0.5

* Non-secreting carcinoid.

tissues examined. Tryptophan incubated with liver slices, for instance, shows no 5-hydroxylation. Our case with 5 hydroxytryptophan in the urine led to the deduction that the tumour, so presumably the argentaffin cells, must be capable of the 5-hydroxylation of tryptophan. It follows that one of the major functions of the argentaffin cells may be not only the local release of 5-HT, but also the manufacture of its precursor for circulation to other cells which decarboxylate it. Among other things this would account for the fact that 5HT is produced by various cells but no other tumour produces excess of it. The body's total production of 5HT is presumably limited by the availability of the precursor.

Metabolic studies using L-tryptophan have confirmed that in humans the 5HT production is dependent on the intake of tryptophan in the diet. They have also disclosed the autonomous nature of the tumour, in that it can be shown to utilise the same amount of tryptophan whatever the level of protein intake.

As a background to therapy, various diets have been given to the patients. It had been claimed that fat specifically influenced 5HT release. No confirmation of this has been found.

At low levels of protein intake, with low tryptophan intake, and in the presence of lowered absorption of nicotinic acid from the intestinal tract, the parasitism by the tumour of tryptophan stores may be/

be so great that tryptophan deficiency, seen as pellagra-like symptoms, might be expected.

Having examined the effects of protein intake and a high fat diet our attention turned to other forms of gastric stimulation. Blushing attacks have been reported quite commonly after meals, after alcohol and after histamine. Little increase in 5HT concentrations were found after any of these substances. Whether 5HT is released under these circumstances by the secretagogue substance or in response to it, is difficult to assess. The fact that no striking increase of 5HT occurs in the plasma suggests that the action in this case is predominantly in the tissues rather than on circulating 5HT.

Turning to the acid secretion in secreting cases of this tumour, it is disappointing to be able to report that only two out of four of these cases had a drop in acid values - and these two, even at that, might be explicable on the basis of age. Reserpine, releasing more 5HT into the circulation, did not markedly affect gastric secretion. On the other hand iproniazid which acts by antagonising the enzyme amine oxidase elevates in consequence the 5HT levels in the tissue and causes the acid secretion level to fall. These facts tend to suggest that it is as a tissue hormone that 5HT will act, rather than as a diffuse circulatory one.

SUMMARY.

(1) The normal metabolism of 5HT is again discussed and the first isolation/

isolation of 5HTP in carcinoids is described.

(2) Reasons are advanced for considering that 5HTP rather than 5HT is the hormone of the argentaffin cell.

(3) The effects of high protein feeding and L-tryptophan have been shown to confirm in humans the accepted biochemical pathways of 5HT formation. The feeding of fat did not influence 5HT release.

(4) The effects of alcohol, reserpine and histamine on 5HT blood levels are described.

(5) The acid secretion in response to histamine has been examined in four secreting carcinoid cases and the effects in two other cases provoked by reserpine and iproniazid. Acid gastric secretion was most markedly changed in the latter instance, probably because iproniazid affects the level of 5HT in the tissues rather than in the blood stream.

CONCLUDING DISCUSSION.

CONCLUDING DISCUSSION.

The experiments reported in these six chapters stress the varied biological activity of 5-hydroxytryptamine. A study of its actions and those of its precursors on the acid secretion evoked by histamine discloses, not so much a picture of an inhibitory substance, as of a local hormone or pharmacologically active substance triggering off an inhibitory mechanism of acid secretion, or subserving it. Had this substance a significant role as an inhibitory hormone in its own right, one would have expected in the case of functioning carcinoid tumours that a marked rise in circulating 5-HT would have annulled the acid secretory response - in these human cases it was low but not consistently affected. It is also the case that substances activating secretion, such as alcohol, histamine and reserpine, evoked symptoms without greatly altering the blood level of 5-HT. This again suggests that the flushing and other manifestations of the carcinoid syndrome can be induced by local changes of 5-HT in tissues and thus elicit vaso-motor reflexes and one could extend this argument to the gastric secretion - that 5-HT would have to be similarly released in certain areas of the gastrointestinal tract, with emphasis on the known inhibitory areas, before gastric secretion is affected.

Our experiments illustrate this same bias in the approach to the analysis of the effects of 5-HT on gastric secretion. Although we have been able to get an inhibitory effect with infusions of 5-HT, there is

no/

no doubt that the experimental objective was more readily achieved under certain local conditions, namely the allowance of acid contact with the pyloric mucosal region, fairly recent feeding and the presence of an intact vagal innervation. This suggests to us that in many of these experiments endogenously formed 5-HT could be acting in the same way as the exogenous, and one envisages this substance on the afferent side of an inhibitory reflex for gastric secretion, or supporting it, as was illustrated in Chapter 4. The fact that in fed animals the portal levels of 5-HT were higher than those of starved dogs, but that the levels did not rise further during the development of secretory inhibition, again suggests an intrinsic action of 5-HT in the tissue rather than as a released hormone; the higher portal blood level could be regarded as a spill over of 5-HT from a site of high concentration. Stacey and Sullivan have already been quoted as finding that feeding elevates the 5-HT concentration in the gastro-:intestinal wall. The case for any general humoral role of 5-HT is weakened by two other points, the first of which is that there would be many circulatory disturbances following any significant rise of 5-HT in the circulation and the second is that rapid destruction by amine oxidase in the liver follows the release of any 5-HT into the portal blood stream, greatly diminishing its effects.

Much of the work done in Chapters 2, 3 and 4 could, however, be constructed as based on pharmacological methods - the infusion of a highly/

highly active chemical agent and the recording of its effect on a particular organ. To enter the physiological domain, one would have to correlate some change in the functioning of the stomach - be it towards stimulating or inhibiting of acid secretion or motility - with a change in its production or utilisation of 5-HT. Nevertheless, an attempt has been made to give physiological significance to the experiments recorded by showing that, not only does 5-HT affect acid secretion, but that other members of the tryptophan family act likewise. Professor Garry has related to the author how in his work on colonic motility he has often observed that previous feeding altered, in his experience, the motor responses which would have been expected in a fasting animal; it is interesting that a similar physiological event, feeding and the digestion of proteins and absorption of amino acids of this particular family, affect secretory processes too.

Reverting once more to the human carcinoid syndrome, it is interesting to find that the argentaffin cells, even when they have multiplied beyond all the normal boundaries, divert from the protein intake all the tryptophan that they require - indeed, when they overdo this, there is a consequent diminution in the tryptophan which serves in the body normally as the precursor of nicotinic acid; then pellagra-like symptoms may be precipitated. The great utilisation of tryptophan, plus the finding of its direct derivative 5-hydroxytryptophan in tumour tissue being secreted from it, leads us to suggest that this substance may be the/

the hormone of the argentaffin cells. Were 5-HTP released from these cells it might usefully serve the inhibitory process by boosting local 5-HT stores in many tissues such as the central nervous system or, having passed round the circulation, in the stomach wall itself.

5-HT was shown in Chapter 1 to be a histamine liberator, para-:doxically one that does not stimulate acid gastric secretion. One might argue that this type of histamine release occurs only in certain species and is demonstrated as somewhat of an artefact in isolated perfused tissues. This was the author's suspicion till he and two other groups of workers in Sweden and in this country verified histamine release in the human case also by reporting the finding of large amounts of histamine in the urine of carcinoid patients. One has to assume therefore that 5-HT can release histamine but that in view of other pharmacological activity manages to block the effects of histamine on the parietal cell. Three findings therefore become consilient with one idea; firstly that 5-HT itself in the animal does not stimulate acid secretion; secondly that this may be related to the fact that 5-HT goes far to antagonising the action of histamine on gastric secretion; and thirdly that in the carcinoid syndrome, even in the presence of released histamine, the acid secretion, tested by stimulants such as histamine, though not abolished remains very low.

It seems to us that histidine and tryptophan, both producing aromatic/

aromatic amines by decarboxylase activity stand in biological opposition to one another.

Histidine has been found to be absorbed in inverse proportion to L- tryptophan (Pinsky and Geiger, 1952). This strange antagonism repeats itself in the case of the derivatives histamine and 5-hydrox-:ytryptamine: the latter is destroyed by monoamine oxidase and the former by diamine oxidase; one stimulates acid secretion and the other inhibits it; the former releases the latter from cell storage and vice versa. One is in the ascendancy in the proximal part of the stomach, while the other is in the distal end. Each has its special-:ised cell, the oxyntic or argentaffin cell, but share a common embassy in the mast cell. One was discovered over 40 years ago and its precise significance yet eludes us; the biographical details of the others are accumulating at a rapid rate and physiologists or pharmacologists may yet discover their functions together.

"Rational, industrious human beings are divided into two classes: first, those whose work is work, and whose pleasure is pleasure; and secondly those whose work and pleasure are one. Of these the former are the majority ..... but Fortune's favoured children belong to the second class. Their life is a natural harmony. For them the working hours are never long enough. Each day is a holiday, and ordinary holidays when they come are grudged as enforced interruptions in an absorbing vocation."

Sir Winston Churchill in
"Painting as a Pastime."

OBSERVATIONS ON SOME LOCAL AND GENERAL HORMONES

THE ALIMENTARY TRACT OF IMPORTANCE TO GASTRIC SECRETION.

VOLUME THREE

VOLUME THREE.

CONTENTS

PART FOUR.

Experiments on Substance P.

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Experiments on Substance P.

The relevant history of this substance has already been reviewed in the general introduction to this thesis. The subject is admirably reviewed by Pernow in "Studies on Substance P" (published as a supplement to the Acta Physiologica Scandinavica), and also in C.C. Toh's thesis, "Histamine, Substance P and 5-Hydroxytryptamine".

Euler and Gaddum had observed as early as 1931 that alcoholic extracts of the gastrointestinal tract and of brain tissue stimulated the movements of an isolated preparation of the rabbit's intestine. The contractions differed qualitatively from those of acetylcholine; moreover they were not inhibited by atropine. This substance was thereafter extensively studied by Gaddum and Schild and Von Euler as has already been described, in Volume 1.

Two groups of workers have concentrated on the occurrence of Substance P in the gastrointestinal tract. Pernow, (1951, 1953) has described the distribution of Substance P in the stomach, duodenum and ileum in several species. The concentration of Substance P was consistently two to three times greater in the mucosa than in the external muscle coat. The highest concentration of Substance P was in the muscularis mucosae, a relatively high value was obtained from the/

the submucosa and a somewhat lower one from the glandular structure. Douglas, Feldberg, Paton and Schachter (1951) also studied the distribution of Substance P in the digestive tract and their results in dogs agreed with those of Pernow.

Various investigations have already established that Substance P is most likely to constitute a physiological stimulus for spontaneous activity of the gastrointestinal tract; Gaddum in his book "Polypeptides which Stimulate Smooth Muscle" goes as far as suggesting that Substance P may be a normal stimulating smooth muscle factor in the intestinal wall.

All parts of the central and peripheral nervous system have been found by Gaddum to contain activity attributable to Substance P, the largest amounts being found in the hypothalamus and vagal ganglia. Substance P is also present in many types of nerves and peripheral ganglia and it has been argued that the Substance P in the submucosa of the alimentary tract must contain its Substance P in nerve cells and nerve fibres in that layer, since in this region unlike the mucosa, a glandular function must be discounted; unlike the outer muscle coats, a muscle-stimulating role seems unlikely. In view of the possible release of glandular or neural Substance P in the stimulation or inhibition of gastric secretion, it was decided to determine whether an extract of this substance would affect hydrochloric acid secretion from/

from the stomach, particularly since Vogt (1949) considers this substance possibly to be of importance in vagal transmission to the stomach.

METHODS.

Substance P was prepared as outlined in the appendix.* Cats were anaesthetised with chloralose and urethane as described in Part 2, Chapter 1, (Volume 1). Substance P was infused in a concen-:tration of 50 units per kg/minute for 15 minutes; the preparation had been tested for biological activity immediately beforehand on a guinea-pig's ileum preparation and 1 unit was arbitrarily chosen as comparable to 1 ug acetylcholine. Each infusion of Substance P was given slowly over 15 minutes and the blood pressure was recorded throughout each experiment. The acid secretion, obtained by a washout technique, was titrated using Topfer's reagent. In several experiments to stimulate acid secretion one of the vagus nerves was dissected free in the vago-sympathetic trunk in the neck, cut and placed across the points of a platinum electrode and the moistened nerve stimulated with a voltage of 5 volts using shocks of 0.5 millesecond duration and frequency of 20 per sec. (using discontinuous shocks in bursts so as to avoid as far as possible circulatory effects on gastric secretion from the cardiac slowing which follows vagal stimulation). Histamine was also infused in concentrations of 5 ug/kgs/minute to stimulate acid secretion.

RESULTS.

Substance P did not stimulate acid gastric secretion in 4 cats (see Table 1). Substance/

* Appendix.

Substance P did not affect the acid secretory output provoked by stimulation of the vagus nerve, nor did it influence the acid secretion evoked by histamine. It was observed that intestinal motility had been markedly enhanced in these animals when a terminal laparotomy was performed.

DISCUSSION.

The effects of Substance P on acid gastric secretion were examined because of the localisation of this substance in the gastrointestinal tract. The distribution is mainly a mucosal one and a considerable quantity is present in the stomach of most species described in the relevant literature, although the stomach is by no means the region of highest concentration. Substance P was found to have no gastrin-like effect on the spontaneous secretion of acid or inhibitory action on such secretion as was elicited by vagal stimulation and histamine.

The occurrence of a high Substance P concentration in the submucosal tissue suggested a relationship to ganglion cells occurring in Meissner's plexus, but if this substance is formed in or around those cells its relationship would seem to be more closely related to smooth muscle fibres than to the adjacent glandular structures. Both histamine and Substance P have been found in rich concentration in the muscularis mucosae. Walder (1953) has shown that the muscularis mucosae is insensitive to histamine. Substance P might be the local transmitter for/

for producing contractions in this smooth muscle layer. It has always been a difficult question to assign a specific function to the muscularis mucosae, but it seems to the author that one function which might be investigated is the role of the muscularis mucosae in controll-:ing the blood supply to the adjacent glandular structures - the vessels which penetrate the muscularis mucosa to reach the glandular mucosa are surrounded by a lattice work of plain muscle which on contraction must surely render the mucosa somewhat deficient in blood supply.

SUMMARY.

(1) Substance P did not evoke secretion of hydrochloric acid from the stomach of cats.

(2) Substance P failed to diminish the acid secretion produced by vagal stimulation and by histamine, nor did it enhance it which seems important in view of the idea propounded by Vogt (1949) that this substance might be engaged in transmission at vagal nerve endings in the upper gastro-intestinal tract.

TABLE 1.

THE ACID SECRETED IN 30 MINUTE COLLECTION PERIODS - WHICH WERE (a) CONTROL SECRETION AND SUBSTANCE P ALONE; (b) DURING HISTAMINE ALONE AND HISTAMINE PLUS SUBSTANCE P; (c) VAGAL STIMULATION ALONE AND VAGAL STIMULATION PLUS SUBSTANCE P. HISTAMINE WAS GIVEN AS 5 ug/kg/min. THE VAGAL STIMULATION WAS 5 VOLTS, DURATION 0.5 MILLISECONDS, FREQUENCY 20/SEC.

Weight of Cat in kg.	Substance P units/kg for 15 mins.	a	a + Substance P	b	b + Substance P	c	c + Substance P
3.2	20	0	0	0.30	0.36	0.65	0.62
2.4	30	0	0	0.24	0.21	0.36	0.31
2.8	50	0	0	0.12	0.10	0.18	0.16
3.3	50	0.10	0.02	0.42	0.46	0.42	0.48

PART FIVE.

The properties and mode of action of gastrin.

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The properties and mode of action of gastrin.

Introduction.

It has been clearly established (see introduction to Thesis) that there is a humoral phase of gastric secretion and that the main source of the hormone is in the glandular structures of the distal part of the stomach and the upper intestinal tract. While glands of mesodermal origin appear mainly to be activated by steroid hormones, the work of Bayliss and Starling (1902) on pancreatic secretion and of Edkins (1905), Komarov (1938), Uvnas (1944 & 45), and Harper (1946) on gastrin indicates that the hormones of glands of ectodermal or endodermal origin may be expected to be mainly proteins or nitrogenous substances.

There are two main approaches to work in this field. One method is to devise experiments which prove, in outline, that substances secreted from, say, the stomach may activate acid gastric secretory tissue transplanted to another site. One difficulty inherent in this approach is that there remains the difficult problem of isolating the hormones, or pharmacologically active substance, and identifying their nature. The other method consists of attempting to analyse the nature, distribution and mode of action of the humoral substances by tackling its extraction from sites of rich distribution. Most of the techniques required depend on a knowledge of protein chemistry and it should be obvious/

obvious that the higher degree of refinement of the extraction procedure, the less is the experimental yield with which to investigate the biological actions of the extract.

Notwithstanding these difficulties, it was decided in 1951 when the author joined the Division of Physiology at Mill Hill, that the time was ripe for re-investigating the properties and mode of action of gastrin, especially in view of the fact that this substance, be it protein, polypeptide or nitrogenous base, might act via such a mechanism as that of liberation of tissue histamine, and owe its acid secretory action to this.

Part V gives an account of work done in this field since then, using gastrin prepared by the method of Jorpes, Jalling and Mutt, (1952); a similar sample also was prepared for comparison by the method of Uvnas (1945). (See Appendix).

Part V also includes an addendum on the inhibitory gastric hormone enterogastrone on which, however, no original work has been included - this topic is merely added for completeness.

Methods.

The main preparation was made according to the method of Jorpes, Jalling and Mutt, (1952).

1. Preparation of the hog pyloric mucosa.

The 'maw' or stomach was removed not later than one hour after slaughter, emptied of its contents, and everted. The pyloric part, making up a fourth of the stomach, was cut off, rinsed with cold water and immersed for 10 min. in boiling water in order to destroy the enzymes. After cooling, the mucosa was carefully wiped clean of the slimy layer of mucus covering it and dissected free from the underlying muscular tissue. It made up about 20% of the pyloric part of the stomach. The dissected mucosa was then minced, frozen and stored at - 40°, a procedure which did not affect the yield of gastrin. About forty hogs yielded 1 kg. of mucosa.

2. Extraction of gastrin.

Deeply frozen mucosa (20 kg.) from about 800 animals was minced and suspended in 80 l. 0.1 N-HCl in 95% (v/v) methanol). This and all subsequent operations were carried out at room temperature. After stirring for 2 hours the suspension was strained through gauze and the cloudy liquor filtered through fluted paper. The clear filtrate was brought to pH with N-NaOH. (All pH measurements were made with a glass electrode). A heavy precipitate formed at this/

this stage. 10 g. of Hyflo Super-Cel were mixed with each litre of the suspension and the mixture filtered with suction. The filter cake contained about one-tenth of the total gastrin activity. The clear filtrate was brought to pH 5.5, when a slight precipitate formed which was filtered off with the aid of Hyflo, and discarded. The filtrate was brought to pH 7 and the precipitate formed allowed to settle for half an hour. The supernatant was then decanted and discarded. This rather voluminous precipitate was filtered with the aid of Hyflo Super-Cel. The mother liquor was discarded and the precipitate dissolved in, or extracted with, about 4 l. of 0.1 N-HCl in 99% (v/v) methanol (solution II: 1 ml. 10 N-HCl + 99 ml. methanol). The dark-brown methanolic solution, which contained about three-fourths of the gastrin activity extracted from the mucosa with methanol was stored for part of a week at - 20° without any loss of activity. This extraction was handled in bulk by the large scale plant of Burroughs and Wellcome - an arrangement which had been made through the generosity of the late Dr. Trevan, who placed the skilled assistance of several chemists at the author's disposal for several days work on this project at Dartford. The material was later transferred, in solution now reduced to 5 litres, to the National Institute for Medical Research, where Dr. J. Walker, F.R.S., of the Division of Chemistry handled and supervised the penultimate stages.

3. Preparation of purified gastrin.

Solution II (5 l., containing extract from about 30 kg. of mucous membrane and 800 animals) was precipitated by the addition of 20 l. of ethyl ether. The precipitate was removed with suction and the filter cake washed with dry ethyl ether. The ether was allowed to evaporate and the filter cake, about 15 g., half of which consisted of inorganic salts, was dissolved in 500 - 1000 ml. of distilled water. The pH of the solution, about 2.5, was brought to 6.8 - 7.4 with 0.1 N-NaOH. The precipitate was collected by centrifugation, dissolved in about 200 ml. of 0.05 N-HCl and reprecipitated at pH 6.8 (our own finding;* Jorpes in his original description said pH7). The precipitate was dissolved in 50 ml. or less or 0.05 N-Hcl, 1 l. of methanol was added and the gastrin reprecipitated by the addition of 1 l. of ethyl ether to the clear solution. The precipitate, which contained most of the gastrin, was filtered with suction and dissolved in the smallest possible amount of distilled water (solution A).

The addition of 3 l. more of ether to the mother liquor precipitated some more material, which also contained gastrin. The amount was about one-third of that in the first precipitate and the activity per unit wt. was likewise about one-third. Solution A was dialysed for 12 hr. at 5° under toluene against distilled water

* (Professor Harper of Newcastle in a personal communication has related a similar experience.)

through which carbon dioxide was passed during the dialysis to prevent isoelectric precipitation of the gastrin. The inner fluid was freeze-dried. The dry powder weighed about 1 g. The substance was readily soluble in distilled water and dilute acids. It was fairly stable in the dry state at room temperature, although some loss of activity did occur when it was stored for some months.

I wish to record my indebtedness to the late Dr. Trevan, F.R.S. and to Dr. J. Walker F.R.S. for their very great help with this task.

A much smaller sample of gastrin was prepared by the method of Uvnas (1945) and was used for comparison (See appendix). A sample of his own original gastrin was sent to me from the Karolinska Institutet by Dr. Jorpes when he heard that work was proceeding on this topic at Mill Hill, and I wish to thank him for this kindly gesture.

The techniques for collecting and estimating the hydrochloric acid present in gastric juice were performed in the unanaesthetised cat and did not vary from those outlined in 'Methods', Chapter 1 (Part 2 of this thesis). Jorpes estimated that 1 mg given to a vagotomised cat caused the secretion of 10 ml. of 0.1 N. HCl; and arbitrarily converts this standard amount to "10 secretory units" - we prefer to recognise the amount of gastrin used by the direct weight (mg/kg) (in view of variable response an estimate of secretory potency seems invalid).

Footnote.

In a letter to the author, Jorpes states 'I am sending you 100 mg.

of/

of our gastrin preparation. 1 mg. gives 5 cc. of N/10 HCl in a cat' (which must be a weaker preparation than that described). 'Almost every second injection gives in spite of vagotomy a much weaker response, many of them none at all'.

Dated Stockholm, November, 21, 1951.

Signed Erik Jorpes, Kemiska Institutionen,

Kalolinska, Institutet.

TABLE 1.

PROPERTIES OF GASTRIN.

Features	Jorpes et al.	This preparation	Uvnas
Solubility	In water, if acidified precipitates at pH 5.5 also at 7.	Soluble in water. Precipitates at pH 6.8.	Soluble in water, varies with pH.
Precipitation	Acetone and ethyl ether.	Acetone, benzene and ether.	Same plus absolute and 80% alcohol.
Stability	-	Resists boiling in acid.	Same, destroy-:ed by alkali.
Inactivation	-	-	Pepsin, tryp-:sin.
Dialysis	Not dialysable.	Not dialysable.	Dialyses through cellophane.

Results.

(a) General Properties.

The main preparation was made according to the method Jorpes, Jalling and Mutt (1952), which dissolved sparingly in saline, freely, however, on the addition of a few drops of N/10 HCl. The extract was not dialysable through cellophane; it was insoluble in such solvents as ethanol, acetone, benzene and ether, and precipitated from solution by saturated NaCl solution, saturated ammonium sulphate, trichloracetic acid and 5% tannic acid. (See Table 1).

Each extract was examined for histamine contamination, the presence of histamine being assessed by the ability of the extract to contract the atropinised guinea-pig's ileum suspended in Tyrode solution. The extracts had histamine-like activity of 0.01 - 0.02 ug/mg; that the contaminant present in such low concentration was in fact histamine could be demonstrated by the ease with which the contractile effect was abolished by mepyramine. It is unlikely that histamine, present as a contaminant, would affect gastric secretion (though it might produce blood pressure changes). Although histamine produces abundant acid secretion when injected subcutaneously and infused slowly intravenously, it has little or no action when injected intra-:venously - this was one of the earliest observations on histamine (Rothlin and Gundlach, 1921; Koskowski, 1922; Gutowski, 1924 - and reported once again in modern times by Schofield, 1957).

(b)/

(b) Acid secretion evoked by injection or infusion of gastrin.

When given intravenously by slow infusion the extract elicited the secretion of an acid juice (pH1-2), free of mucus and poor in pepsin content (less than 10 Mett units). Infusion of gastrin almost always, but not invariably, stimulated acid secretory activity; in about 10% of all experiments no acid secretion could be obtained, a finding which was commoner with second or third injections. This is illustrated in Fig. 1 in which infusions of gastrin and of histamine have been alternated. The effect of gastrin is decreased on repetition of it, but the effect of histamine is roughly comparable on both occasions.

The acid secretion evoked by intravenous infusion was greater than that elicited by subcutaneous or intramuscular injection; this had to be assessed in different animals because of the diminishing secretory effect of gastrin, by comparison with the acid secretory response to histamine. (Table 2). The greater efficacy of the intravenous injections could be shown in another way: repeated subcutaneous or intramuscular injections were followed by diminishing secretory effects similar to that described for intravenous injections. Intravenous injections, given after repeated subcutaneous or intramuscular injections, are as efficacious as, or even more so, than the subcutaneous or intramuscular ones, from which it may be deduced that/

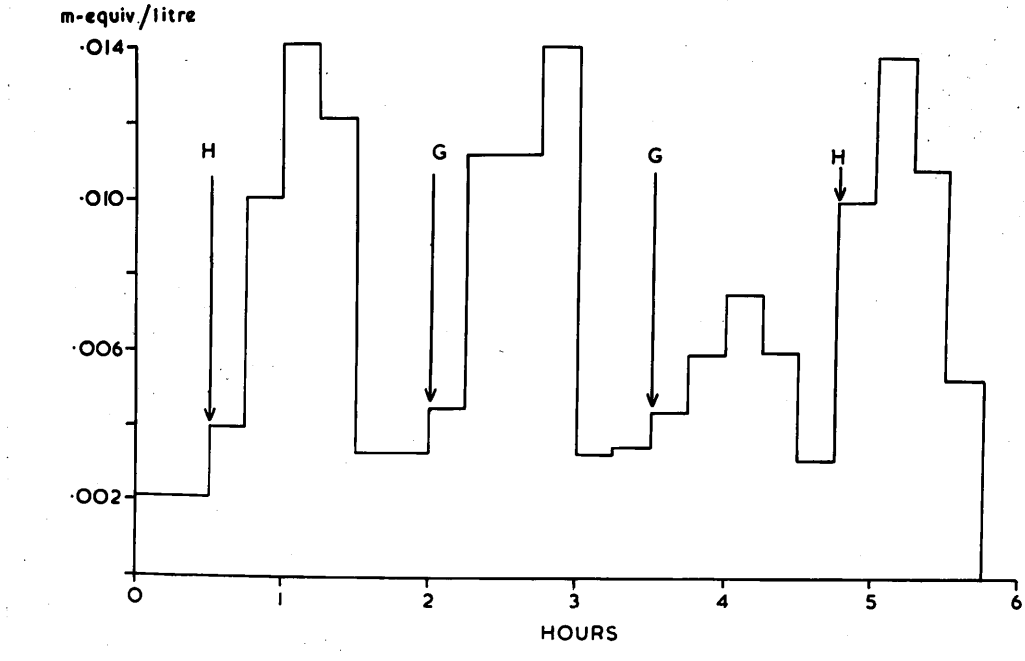


Fig. 1 shows the effects of

left to right (H) Histamine 5 ug/kg/min for 10 minutes.
(G) Gastrin 5 mg/kg.
(G) Repeated dose of gastrin.
(H) Repeated infusion of histamine. (Note the more prompt response to histamine.)

The ordinates give the m. equiv. HCl secreted and the abscissae the duration of the experiment in hours. The second administration of gastrin was followed by a markedly reduced response; to histamine the response was comparable on both occasions.

TABLE 2.

INJECTIONS OF GASTRIN MADE SUBCUTANEOUSLY (S), INTRAMUSCULARLY (M) AND INTRAVENOUSLY (V) ARE COMPARED AGAINST A STANDARD INFUSION OF HISTAMINE (H).

TWO PREPARATIONS WERE USED INDICATE J (JORPES et al.) AND U (UVNAS).

IN THE LAST FOUR EXPERIMENTS REPEATED INJECTIONS WERE GIVEN. IN THREE OF THESE INJECTIONS (V) OF THESE IS GREATER THAN (S) OR (M); YET IF (V) IS GIVEN REPEATEDLY THE EFFECT FALLS OFF. (SEE ALSO FIGURE 1). 5 mg/kg GASTRIN GIVEN IN EACH CASE.

Preparation	Wt. of cat in kg.	Injections of gastrin or infusion of histamine, with m. equiv. HCl in brackets S, M, V = Gastrin. H = Histamine.		
J	3.5	S (0.23)	H (0.68)	-
J	2.0	S (0.21)	H (0.81)	-
U	2.8	S (0.08)	H (0.65)	-
J	3.6	M (0.56)	H (0.68)	-
U	3.1	M (0.42)	H (0.78)	-
J	3.8	V (0.82)	H (0.79)	-
U	3.6	V (0.51)	H (0.75)	-
J	3.8	S (0.21)	M (0.31)	V (0.53)
J	3.2	S (0.16)	M (0.12)	V (0.31)
J	4.0	S (0.21)	M (0.21)	V (0.22)
J	4.0	V (0.85)	V (0.28)	V (0.05)

TABLE 3.

THE ACID SECRETORY EFFECTS OF GASTRIN INJECTED INTRA-:VENOUSLY (IV) AND INTRA-ARTERIALLY (IA) INTO THE COELIAC ARTERY ARE COMPARED, USING EQUAL AMOUNTS FOR EACH INJECTION (10 mg/kg).

Wt. of cat	Injections of gastrin (IV and IA) with m. equiv. of HCl resulting.			
	IV	IA	IV	IA
3.2	1.08	1.86	0.26	0.73
3.6	1.10	1.92	0.35	0.58
3.8	1.6	2.1	0.38	0.62

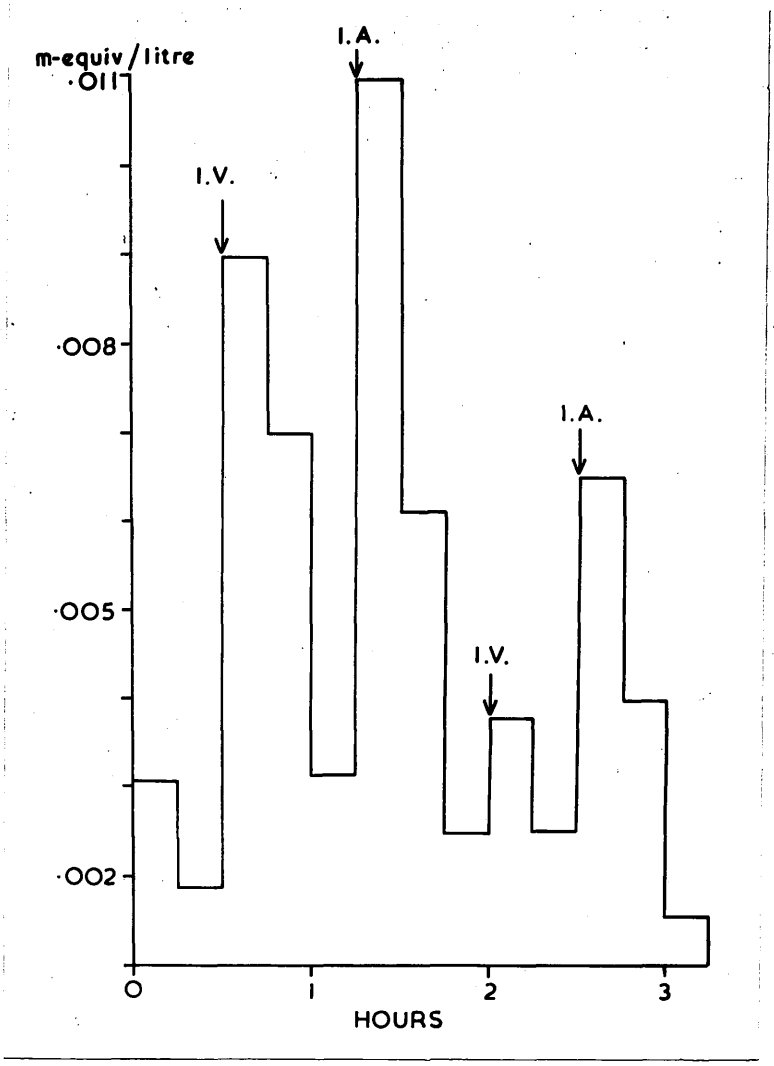


Fig. 2. The acid secretory effects of gastrin intravenously and intra-arterially into the coeliac artery are compared (conventions as in Fig. 1.)
Left to right; Intravenous gastrin 5mg/kg.
Intra-arterial gastrin 1mg/kg.
Intravenous gastrin repeated.
Intra-arterial gastrin repeated.
The small dose intra-arterially is at least as effective or even more so than the larger one intravenously.
Second injections are reduced, but the greater reduction is in that following the intravenous injection.

that the greater effect is exerted by the intravenous injections.

(c) Close intra-arterial injection of gastrin into the coeliac artery.

In four experiments recorded in Table 3, two intravenous injections of gastrin were followed by an intra-arterial one. The table shows also the phenomenon observed after intravenous injection: if the same dose is injected twice the secretory response to the second injection is greatly reduced. In spite of this trend the intra-arterial injections were more efficacious than the intravenous ones. In the experiment illustrated in Fig. 2, unequal amounts of gastrin were given both intra-arterially and intravenously, much smaller doses being given intra-arterially. The results illustrate the greater efficacy of the intra-arterial route of administration as well as the diminution of secretion on repeated injections, whether given intra-arterially or intravenously. When injections of gastrin were repeated three or four times by either route there was a progressive reduction in the secretory effect, but this was more marked with intravenous or intra-arterial injections.

(d) Injection of gastrin into other parts of the circulation.

In the above experiments the secretory response after local intra-arterial injections was mainly due to a local effect on the gastric mucosa. This site of action is also suggested by the fact that gastrin injected into the coeliac artery is more active than when injected into the portal vein (Fig. 3). But in both groups of experiments of Fig. 2 and Fig. 3 some part of the secretory response must be attributed to a general/

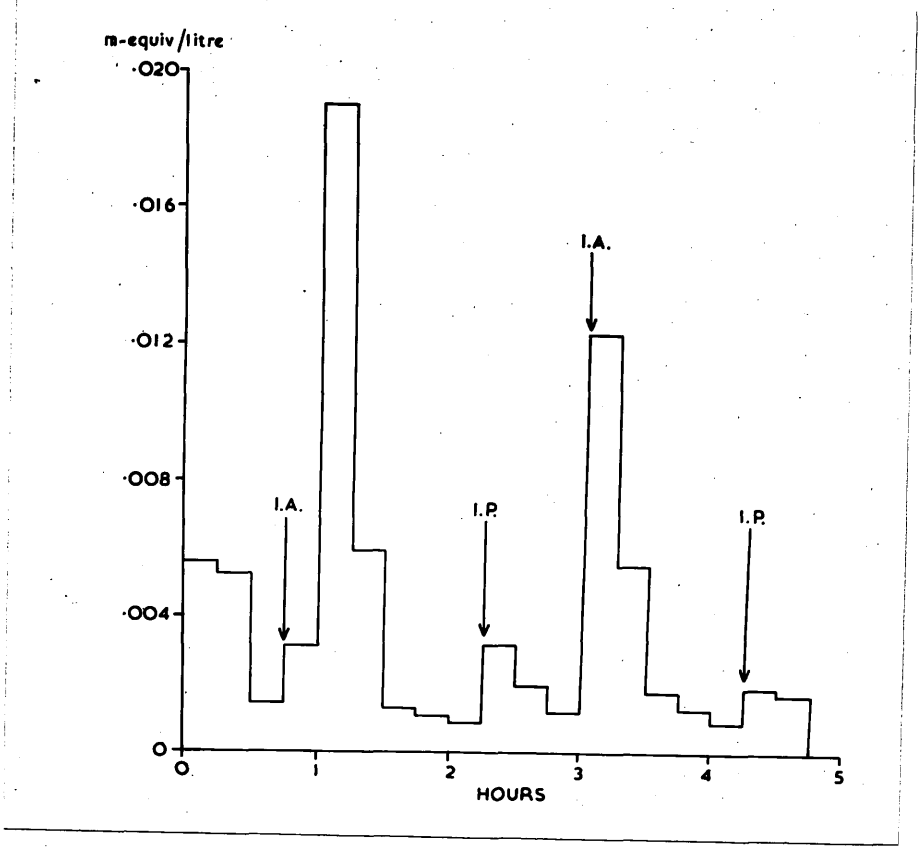


Fig. 3. The acid secretory effects of gastrin into the coeliac artery and into the portal venous system are compared (conventions as in Fig. 1).
Left to right; Intra-arterial gastrin 1 mg/kg.
Intraportal gastrin 1 mg/kg.
Repeated intra-arterial dose.
Repeated intraportal dose.

The acid secretory effect of the intraportal injection is much smaller than that of the intra-arterial ones.

general effect of gastrin on the other tissues. This conclusion was arrived at as follows: When gastrin in injected into the portal circulation it must first circulate to the liver. At this site according to Lim and Ammon (1923) gastrin is taken up and very largely inactivated. Any acid secretion resulting from the intraportal injections would appear to be related to histamine release in the liver since this organ in the cat has a low histamine content (see Chapter 2, Part 2). The secretion produced in Fig. 3 by the intra-:portal injection could result more from an action on lung, skin and skeletal muscle histamine rather than from any other sites. It could be shown (Fig. 4) that gastrin exerts an action in the peripheral tissues of this type of injecting it into the stump of the inferior mesenteric artery in a preparation eviscerate of all but the stomach. The secretory agent passed distally to the inferior extrem-:ities of the cat and elicited a secretion which was almost, but not quite, as great as the amount following injection into the coeliac artery.

(e) Blood pressure.

On slow infusion of gastrin 5 mg/kg there was no disturbance of the arterial blood pressure (Fig. 5). On intravenous injection of the same amount of gastrin in rapid manner, however, there was a depressor response which appeared characteristically 10-20 seconds after the injection, and suggested (as such a "delayed depressor response" had/

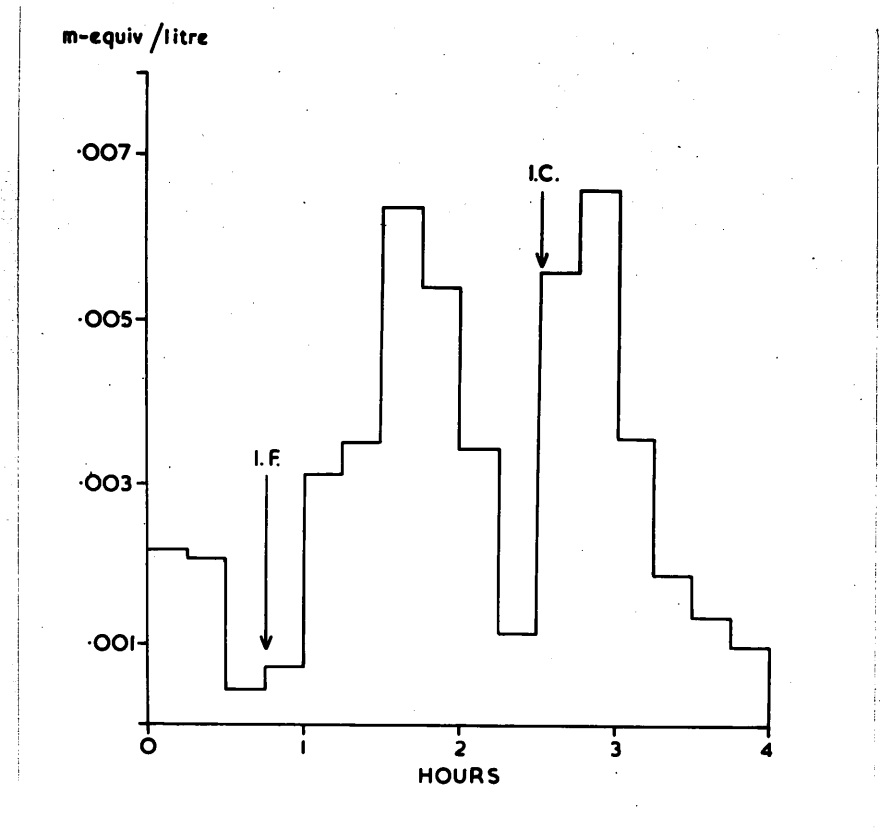


Fig. 4. The acid secretory effect of gastrin given by distal injection into the stump of the inferior mesenteric artery (via a poly-:thene catheter threaded down as far as the common femoral artery) is compared with the effect of close intra-arterial injections into the coeliac artery.

Left to right; Intra-femoral gastrin 1 mg/kg.
Intra-arterial gastrin 1 mg/kg.

The acid secretory effect of gastrin given into the peripheral tissues is quite marked and is slower to develop than when gastrin is given by close intra-arterial injection into the stomach wall.

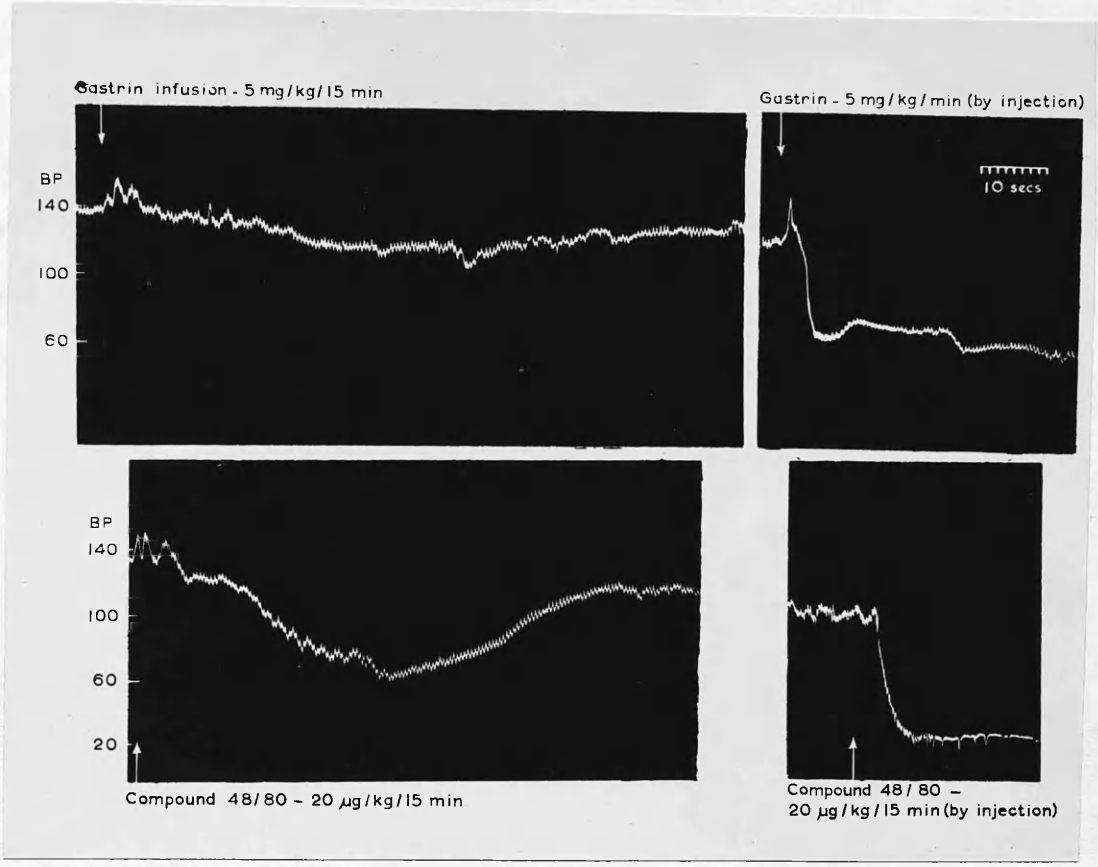


Fig. 5 shows the arterial blood pressure record in a 4 kg cat.
Upper tracing; a) Left: Infusion of gastrin 5 mg/kg, begun at the point indicated by the arrow.
b) Right: Injection of gastrin 5 mg/kg, begun at point indicated.

Lower tracing; c) Left: Infusion of 20 ug/kg/15 min 48/80 begun at point indicated by arrow.
d) Right: Injection of same amount of 48/80.

The effect on the blood pressure is very much less following slow intravenous infusion; rapid injections even when performed after a preliminary infusion can cause a considerable fall in blood pressure (very much greater, however, in the case of 48/80).

had done for "histamine liberators" to McIntosh and Paton), that gastrin might be acting secondarily via released histamine; this effect brought about by approximately 20 mg. gastrin is shown in Fig. 5b and has induced a fall of arterial blood pressure of 30 mm. Hg. Repeated injection led to diminution, then to a disappearance of the depressor effect. Direct injection or infusion of a histamine liberator, such as Compound 48/80, in amounts required to give comparable rates of acid secretion, reduced the arterial blood pressure in a manner not unlike that of gastrin (Fig. 5 c, d). After slow intravenous infusion the histamine liberator had a small depressor effect (Fig. 5c) but with rapid intravenous injection of the same substance there was an almost instantaneous fall in blood pressure, (Fig. 5d), this however being far greater than that produced by gastrin.

The depressor effect of gastrin was not affected by the atropinis-:ation but was greatly lessened by an antihistamine such as mepyramine. (Fig. 6a and b).

Intra-arterial injections of gastrin into the coeliac artery with the main vessel momentarily occluded, so as to direct all the injected gastrin to the stomach wall led to a slight fall in blood pressure overshadowed, however, by a strong pressor response. That the rise in blood pressure was probably due to outpouring of adrenaline from the adrenals could be shown by the fact that it was no longer obtained after/

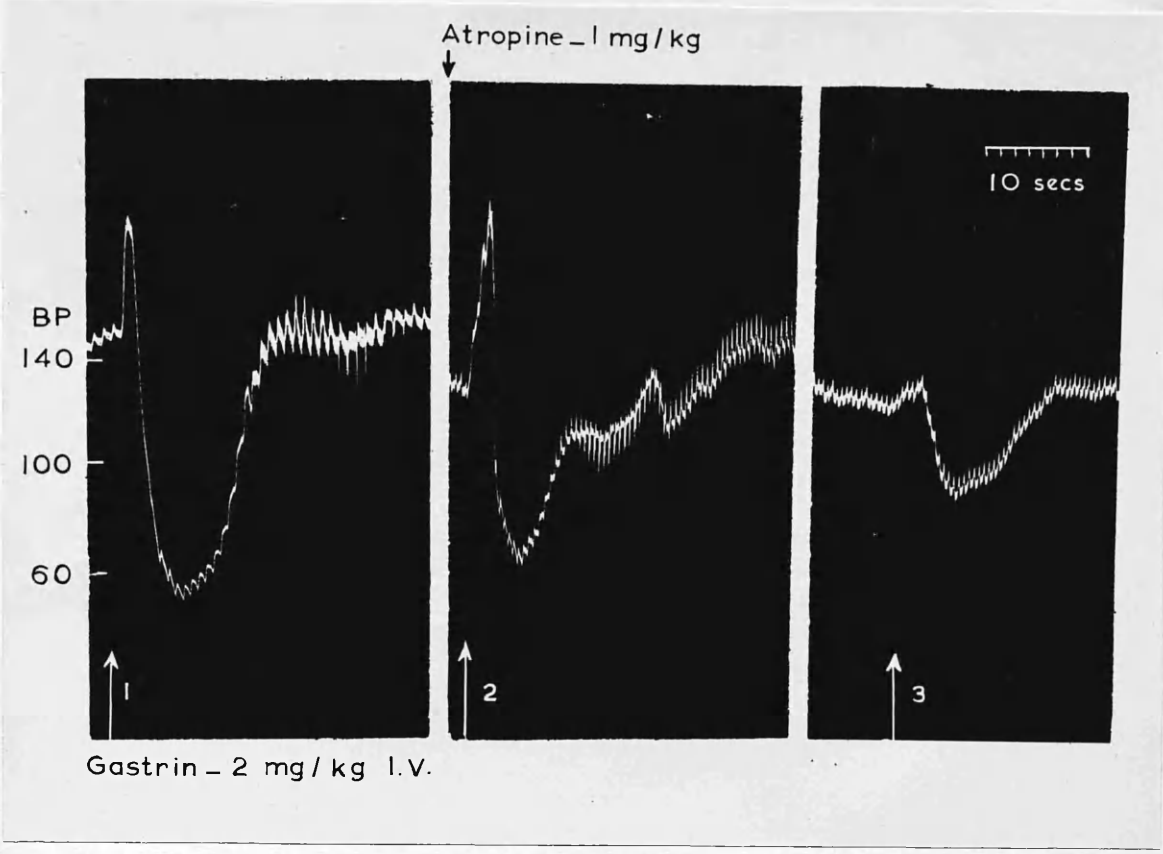


Fig. 6 a) The effect of 1 mg/kg atropine on the depressor response to injections of gastrin. Note that the depressor response is of the "delayed" type which is a typical action of histamine-releasing substances; the fall in blood pressure is not prevented by the atropine. (Successive injections tend to elicit smaller effects which also is a characteristic of "histamine liberators").

after administration of an anti-adrenaline substance (Fig. 7). This type of activity might explain, in some animals, why gastrin fails to elicit its customary response even with a known, active preparation. This may also account for the finding by Uvnas that his preparation was more effective if he cut the splanchnic nerves (1945).

Release of histamine into the portal blood stream after close antra-arterial injection of gastrin into the stomach wall could not be demonstrated by the methods of extraction and assay used in Chapters 1 and 2, Part 2 - the amounts may be too small when diffused in the portal blood stream, may escape rapidly, or may be lost into the gastric juice if, as seems likely, the histamine release takes place from the parietal cell.

That release of histamine appears likely, however, may be shown by applying the following procedure. Donomae and Feldberg (1934) diverted portal blood directly into the systemic circulation by connecting the splenic vein after splenectomy to the external iliac vein; when they clamped the portal vein below its entrance into the liver, the portal blood flow shortcircuited the latter part of its course and passed directly from the splenic vein through tubing into the systemic circulation. Donomae and Feldberg found that this procedure was followed by a spontaneous fall in arterial blood pressure in several animals.

We have observed similar spontaneous depressor effects which, however, are/

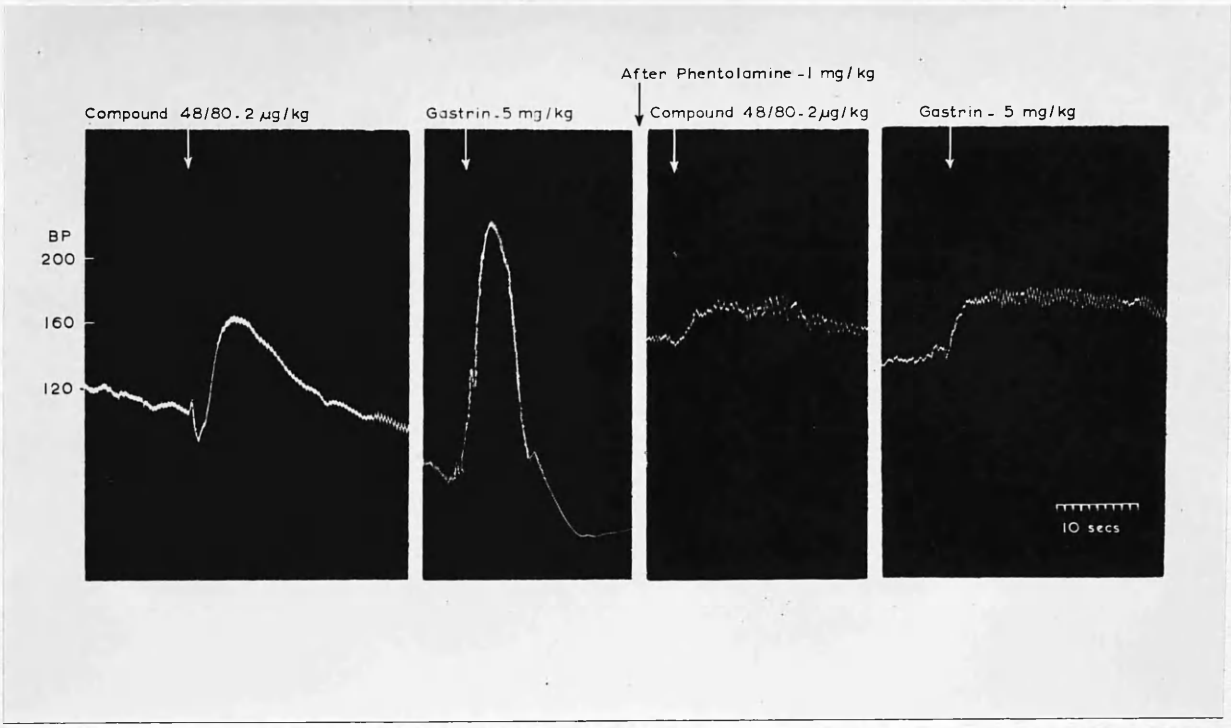


Fig. 7. Effect on arterial blood pressure of injections of gastrin given into the coeliac artery and washed in retrograde manner into the aorta, which was momentarily occluded above the superior mesenteric artery so that this substance passed into the blood supply of the suprarenal glands. Compound 48/80 and gastrin cause a sharp elevation of blood pressure which may be the result of adrenaline release. Since the effects are antagonised by phentolamine, which is anti-adrenaline in nature, this seems likely.

are consistently present after the arterial injection of gastrin into the stomach wall (see Fig. 8); these are made greater by such an injection if the depressor effect is occurring spontaneously. The fall in blood pressure is resistant to the action of atropine, but is abolished by mepyramine (Fig. 9c). An action due to the slow circulation of gastrin itself through the tubing to the iliac vein and beyond is unlikely since the injection of gastrin directly into the tubing, so that it circulates throughout its length before entering the systemic circulation, produces little effect on blood pressure (Fig. 8c).

(f) Histamine-release by gastrin studied in perfused tissues.

Gastrin caused the release of histamine from perfused skin flaps isolated from the hind limbs of cats; in addition vasoconstriction and considerable oedema were concomitant features. Vasoconstriction varied with the intensity of the histamine release and was detected by a marked drop in the rate of perfusion for the first two minutes after the injection of gastrin. (See also Chapter 1, Part III). Oedema was a later development, most marked towards the end of the first hour and caused great swelling of the connective tissue adherent to the subcutaneous surface of the skin flap, so much so that an average amount of 116 ml. of fluid could be expressed from the perfused tissue at the end of six experiments; this increased the weight of the skin flap/

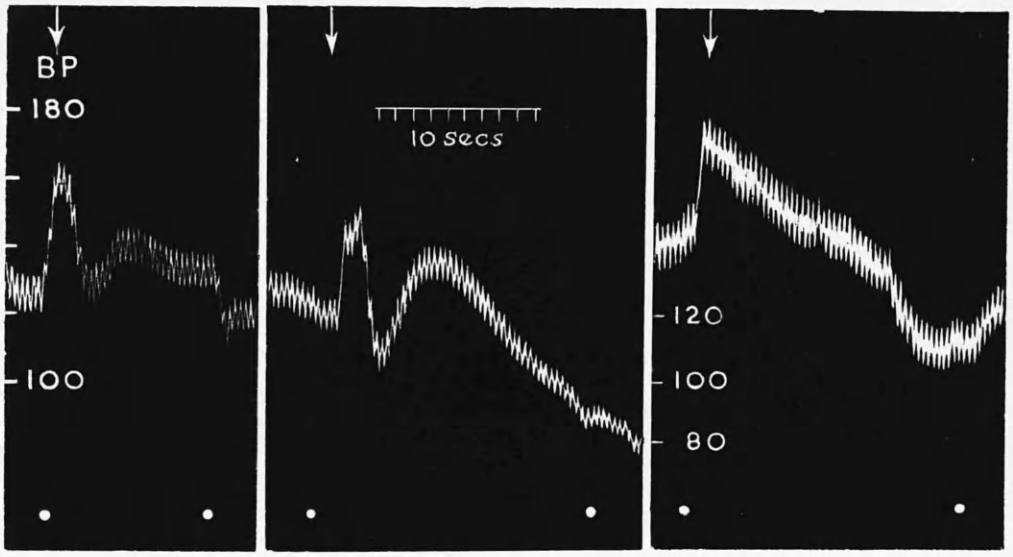
Fig. 8 illustrates the "Donomae-Feldberg" experiment. The small white circles below the record of the arterial blood pressure indicate the points at which a shunt was opened and closed from the portal venous system (by back-flow from the splenic vein while the porta hepatis was compressed by a clamp placed lightly on the portal vein) to the external iliac vein.

The arrows mark the injections given.

Left to right; Panel 1: Shunt open, injection of 0.25 mg/kg gastrin which is followed by a small depressor effect.

Panel 2: Shunt open, injection of 0.5 mg/kg gastrin which is followed by a more marked fall in B.P., plus a compensatory rise with a further fall in B.P. to 80 mm. Hg.

Panel 3: Shunt open, injection of 0.5 mg/kg. gastrin into the tubing as a control. There is no sharp fall in B.P. after the preliminary deflection which is the result of the clamping of the portal vein and takes place as the shunt begins to function. The B.P. tends to fall to about 100 mm. Hg. before becoming stable.


BP
180
100
10 secs
120
100
80

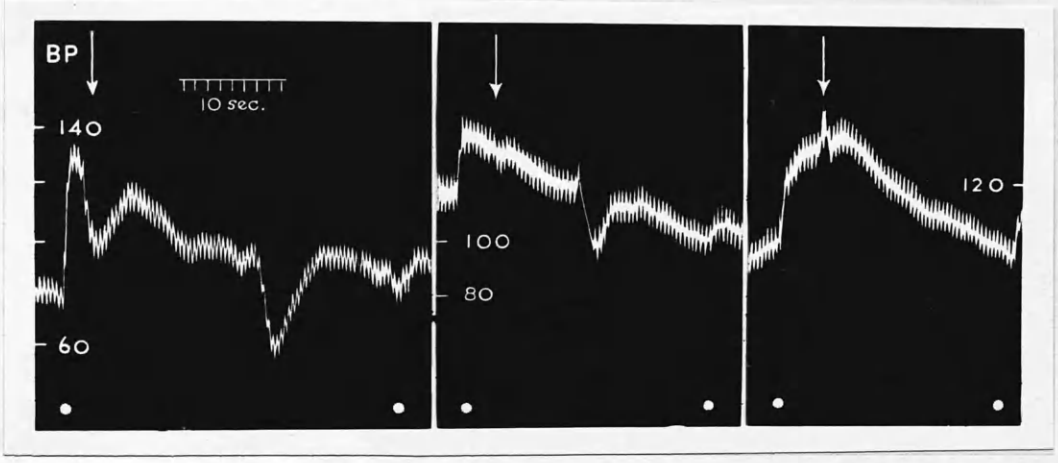


Fig. 9. Same animal (to demonstrate reproducibility of 0.5 mg/kg gastrin intra-arterially).

Left to right; Panel 1 shows the delayed depressor response.
Panel 2 shows the effect of mepyramine 1 mg/kg which markedly reduces the effect.
Panel 3 shows the effect of atropine which finally abolished it.

Feldberg and Domomae had concluded that this factor blocked by atropine was probably small amounts of choline or acetyl choline.

flap by about three times, from over 58 g. to over 170 g. Gastrin preparations were injected in six experiments in amounts varying from 0.25 - 5 mg. gastrin. Doses under 0.5 mg. were ineffective, but with 2 mg. between 15 and 37 ug. histamine were released; with 5 mg. there was a release of 47.6 ug. histamine. The histamine release was associated in the latter experiment with a fall in the histamine of the central part of the skin flap by 37% to 8.3 ug./gm. When the histamine releasing property is compared with that of a potent histamine liberator, such as 48/80 (see values given in Fig. Chapter 1, Part III) it was found to be at least 250 times less active than this particular histamine releasing agent. The histamine release from perfused gastrocnemius muscle was small; in two exper-:iments 5 mg. of gastrin released 1.2 and 1.6 ug. histamine; the muscles weighed 30.5 and 27 gm. and contained 1.28 and 1.46 ug./gm. histamine respectively. (Fig. 10).

Perfusion of the stomach also yielded very small amounts of histamine, vasoconstriction again temporarily reduced the flow of effluent and oedema of the gastric wall developed after 30-60 minutes. We thus record the histamine output, if any, after 0.1 - 10 mgms. gastrin; doses below 1 mg. were ineffective; 1.6 ug. and 2.8 ug. followed the injection of 1 mgm. of gastrin while 4.6 and 7.5 ug. followed the injections of 2-5 mgms. 8.2 ug. histamine was released following the injection of 10 mgms. The histamine releasing property of gastrin was again/

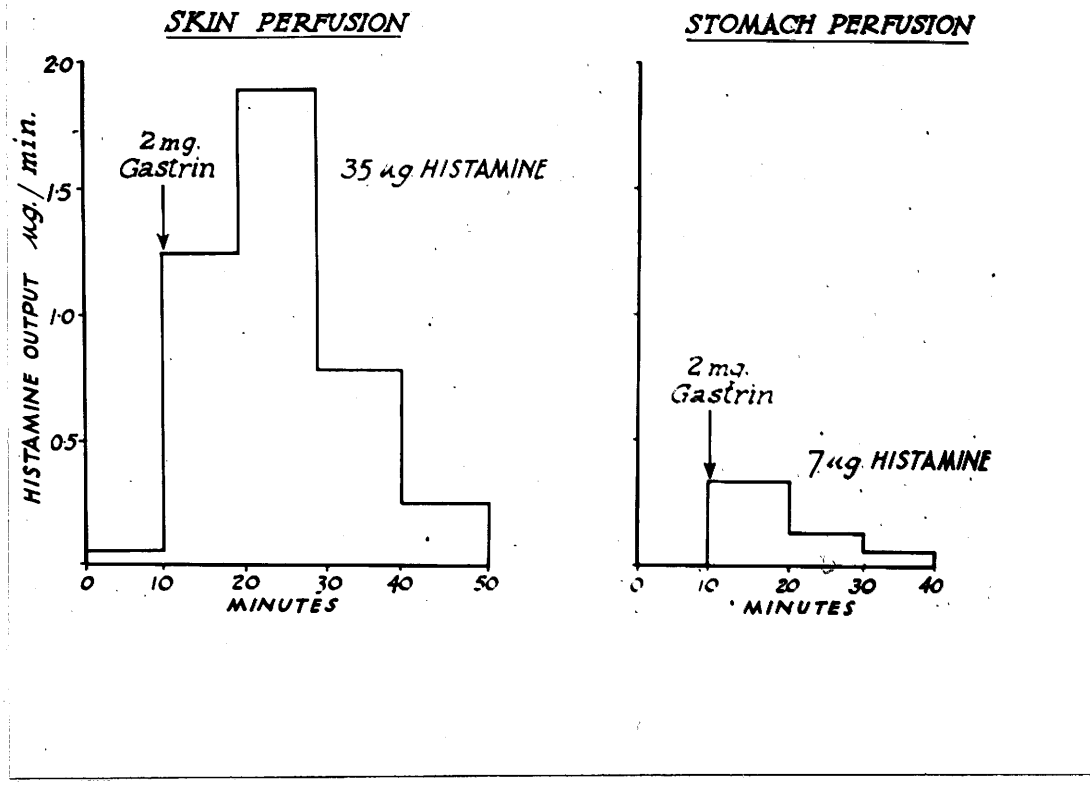


Fig. 10 illustrates the appearance of histamine in the effluent from:

(a) the perfused skin flap preparation.
(b) the perfused stomach preparation.

The perfusate was examined after 2 mg gastrin in each case. A much higher concentration of histamine was produced from the skin flap preparation and it was noted that it appeared later than it usually does after injections of compound 48/80.

again compared with that of Compound 48/80; it was found that in this tissue 0.5 mgm. released 15.8 ug. histamine, and 1 mgm. 48/80, 18.7 ug. histamine. For this tissue, on the basis of these results, 48/80 would appear to be only 10 times more active than gastrin. Perhaps gastrin would be even more effective but for the following experimental difficulty; during the course of these perfusion experiments on the stomach, it was observed that when Indian ink was injected with the perfusion fluid that the mucosa was both irregularly and poorly stained; indeed, in some of the experiments it remained quite free from discolouration by the dye. It could be that following the operative trauma involved in excising and perfusing the isolated stomach that the perfusate was being deviated away from the mucosa (which is the region of rich histamine storage) through shunts present in the muscle coats of this organ.

(g) Histamine release and its relevance to the secretory action of gastrin.

The experiments quoted above show, by different methods, that gastrin may release histamine in small amounts from many tissues of the body. How far is the release of histamine relevant to the acid secretory action of gastrin? This has been examined as follows:

(i) By testing the acid secretagogue effect in animals which have been depleted as far as possible of their tissue histamine (see Chapter 2 or Part 2 of this thesis). Cats were treated with Compound/

Compound 48/80 till the tissue histamine had been lowered as far as was possible. This entailed injecting the animals with amounts as much as 5 mg/kg. The tissue histamine was rendered comparable with the figures in Table 4 of Chapter 2 (Part 2). The extent of the histamine depletion was verified by checking that the response to Compound 48/80 was almost lost. There is presumably so little residual histamine of the type able to be released that relatively large doses are required for its release. In Table 4 the amounts of hydrochloric acid secreted in normal cats in response to 20-50 ug/kg Compound 48/80 are given; after prolonged treatment with Compound 48/80 the intravenous injections of greater doses of Compound 48/80 produced very much smaller secretory effects.

It was against this background of histamine depletion (as far as is possible with Compound 48/80) that the acid secretory effect of gastrin was examined. It was found to be present, though reduced to about one third of the secretion obtained in normal cats (Tables 4 and 5). The acid secretagogue effect of histamine itself was markedly lower in these animals indicating some general drop in the sensitivity of the parietal cells to both stimuli. When both gastrin and histamine were administered together the acid secretory effect was almost completely restored to that of a normal histamine effect which may indicate some synergistic action of both compounds (Table 5).

TABLE 4.

EFFECT OF INTRAVENOUS INJECTION OF COMPOUND 48/80 ON ACID GASTRIC SECRETION IN NORMAL AND 48/80-TREATED CATS GIVEN AMOUNTS OF COMPOUND 48/80 UP TO 5 mg/kg DAY. THE ACID SECRETED IS EXPRESSED IN m-equivalents.

Normal			Compound 48/80-treated		
Wt. of Cat (kg)	Compound 48/80 (ug/kg)	Acid secretion (m-equiv. N-HC1)	Wt. of Cat (kg)	Compound 48/80 (ug/kg)	Acid secretion (m-equiv. N-HC1
2.8	20	1.25	3.2	50	0
3.2	20	1.39	4.0	100	0.10
4.0	20	1.20	2.8	300	0.19
4.0	30	1.40	3.8	500	0.20
3.8	30	1.47	3.1	2000	0.42
3.6	50	1.68	3.1	5000	1.01

TABLE 5.

EFFECT OF GASTRIN AND HISTAMINE ON ACID GASTRIC SECRETION IN NORMAL AND COMPOUND 48/80-TREATED CATS. THE FIGURES REPRESENT m. equiv. HCl IN INDIVIDUAL EXPERIMENTS. CATS WEIGHED 3.6 to 4.1 kg.

Secretory stimulus	Normal cats	Compound 48/80-treated
Intravenous injection of gastrin 10 mg./kg.	1.86, 0.92, 1.2 mean = 1.33	0.40, 0.28, 0.39 mean = 0.36
Intravenous infusion of histamine 5 ug/kg/min. for 15 min.	0.66, 0.68, 0.78, 0.80, 0.75 mean = 0.73	0.16, 0.19, 0.21, 0.22, 0.26 mean = 0.21
Intravenous infusion of histamine and gastrin.	1.92, 1.86, 1.69 mean = 1.82	0.62, 0.68, 0.78, 0.59 mean = 0.67

(ii) By giving a preliminary injection of Compound 48/80 before the administration of gastrin. The effect of gastrin was still present though reduced to a considerable extent if a large dose of the histamine liberator, Compound 48/80 preceded it. (Table 6). Indeed gastrin had this action even after multiple successive doses of Compound 48/80, and could be said to have a degree of secretory potency at a stage when the multiple injections of 48/80 had little or no effect presumably when they had become ineffective by reason of utilisation of all available histamine.

DISCUSSION.

It is now scarcely in question that a hormone is responsible for the second or gastric phase of digestion, but the identity of this substance has been strongly debated. Histamine has been considered to be identical with gastrin, since Sachs, Ivy, Burgess and Vandolah demonstrated a parallelism between the secretory activity and depressor activity of a gastrin extract.

MacIntosh felt that any general circulatory role for histamine should be revealed by an increase in the blood levels of this substance during acid secretory activity, as, for instance, during a meal. But in his experiments the blood level was not increased - this was left open to re-examination, however, since he had measured histamine in whole blood which gives values representing histamine mainly in the corpuscles. (MacIntosh's findings were confirmed by Emmelin, Kahlson and Wicksell, (1941)) In humans, Addam,/

TABLE 6.

THE SECRETORY EFFECT, IN MILLE-EQUIVALENTS HCl, OF GASTRIN 10 mg/kg AFTER A FIRST AND SECOND INJECTION OF COMPOUND 48/80 (20 ug/kg).

Wt. of cat in kg.	Compound 48/80	Compound 48/80	Gastrin
3.9	1.06	0.26	0.98
3.6	1.58	0.21	1.62
3.4	1.21	0.46	1.32

Addam, Card, Riddell, Roberts and Strong took up the question of the plasma level, but failed to find any increase in plasma histamine content during secretory activity, even when the extracts were concentraded three to five fold. The negative blood findings may merely represent our inability, by the present techniques available, to detect small increments of histamine which might activate the parietal cells without systemic signs of histaminaemia.

The concept of histamine as a blood borne agent active in the gastric phase was furthered by the isolation of a gastrin preparation from the pyloric mucosa, as a protein-like substance, apparently free from histamine which on intravenous injection causes a profound secretion of acid from the fundic glands (Komarov, 1938; Komarov, 1942; Uvnas, 1942, 43; Unvas, Munch-Petersen, Ronnow, 1944; Uvnas, 1945; Harper, A.A., 1946 and Jorpes, Jalling and Mutt, 1952).

It has been held by some that one solution to the identity of the true secretory hormone might lie in an examination of whether gastrin acted as a histamine liberator (Gaddum, 1959). Emmelin and Kahlson (1944) and Kahlson (1948) have also suggested that whatever the action of gastrin, it seems to involve histamine too, since histamine is present richly in gastric juice after gastrin injection. It was suggested that gastrin might thereafter act indirectly, after a preliminary release of histamine as an intermediate step. Does gastrin therefore release histamine?

We have accumulated in this work some evidence that it does so.

Gastrin/

Gastrin prepared after the manner of Jorpes, Jalling and Mutt (1952) and Uvnas (1945) released small quantities of histamine from stomach skin and skeletal muscle. The fact that second injections had much weaker effects than the first ones is reminiscent of an acid secretory effect dependent on histamine release; this, for example, was the same type of result as found for chemical liberators, taking Compound 48/80 as a typical example. (See Chapter 1, Part 1). It was the constantly diminishing effect of injections of gastrin which sttracted the author's attention to the possibility that they might act via histamine release. Uvnas (1956) has referred to this as his own experience in this as being the result of 'tachyphylaxis': he described to the XXth International Physiological Congress a reduced response after a first injection of gastrin and told how the secretory response frequently ceased after 2-3 consecutive injections. It is our view that this must be something more than tachyphylaxis since the diminished effect has been seen as much as 6 hours later, and it is a curious fact that the same observation, uncommented upon, was made by Jalling and Jorpes (1947). More direct evidence for gastrin extracts being involved in histamine releasing activity is that they may produce "delayed" depressor actions on quite intravenous injection (this is masked on slow intravenous injection), they release histamine from perfused skin, skeletal muscle and stomach wall and they cause an acid gastric/

gastric secretion when injected into the acral regions. There are certain criticisms that one must make, however, of each part of this evidence. Firstly, the delayed depressor response may represent in the cat at least a reaction to foreign protein; swine gastrin might not have this effect on the pig itself. Secondly, the release of histamine in isolated perfused preparations is a small one, and, since a small release of histamine can also be demonstrated in similar preparations with bile salts which do not cause an acid secretion, the action may be something incidental to the true action. Thirdly, the demonstration of gastric secretion after injection into the lower extremities could in part be explained by the continued circulation of gastrin from the arterial to the venous side of the circulation.

What is the evidence for a more direct action of gastrin on the gastric tissue? Firstly its secretory action is far greater on gastric mucosa than on any other tissue. When injected into animals which have been depleted of much of their tissue histamine there is a secretory action though this is markedly diminished. Gastrin, though its own effects diminish rapidly on repeated injection thus suggesting some action in common with histamine liberators, cannot be regarded as being of the same type as Compound 48/80, since a previous injection of Compound 48/80 does not detract from its secretory action. Histamine liberators are now known which exert the full force of their action at

different/

different sites, for instance, Feldberg and Mongar have contrasted the actions of octylamine and Compound 48/80 which act principally in the former case in the lung and in the latter in the skin.

We have considered the evidence for and against extracts of gastrin stimulating the parietal cell, directly or via the intermediary action of histamine. One must be very wary of applying these results to an interpretation of the natural role of the gastric hormone; great care must be taken to avoid experimental artefacts, especially if, as a result of these, histamine is released from the tissue and evokes acid secretion, leading to the mistaken belief that one is handling gastrin - organic solvents unphysiological pH, osmotic pressure effects and potassium contamination are hazards for the unwary. Again, acid hydrolysis may yield active polypeptides from inert proteins 'in vitro' and the true functioning role of these polypeptides 'in vitro' can scarcely be evaluated till the final factors controlling their formation and release have been worked out.

The experiments on these extracts of gastrin therefore tell us little of the true secretory principle. How far might they go towards 'suggesting' mechanisms for its actions? The most simple mechanism might be the direct type of action. Alternatively gastrin might release histamine locally in the region of the parietal cell - this theory has been supported most strongly by those who see in it as has been mentioned, a convenient way of bringing together and linking up these two/

two substances, for both of which the role of gastric hormone has been claimed by the respective proponents.

Our preparation of gastrin serves to illustrate a possible fallacy in work in this field - that a histamine liberator could be extracted from the pyloric mucosa and elaborated on as a gastric hormone on the basis of pharmacological evidence and the ability of it to cause gastric secretion without a true physiological function. Nevertheless, this might also be a pointer towards the other possible role of gastrin - that just as in the histamine depleted animal gastrin and histamine act synergistically, both substances may be necessary for full secretory activity. Gastrin may indicate changes in histamine metabolism fairly widely all over the body leading to release of histamine and may, at the same time, directly or via a local release of histamine open the portal of secretion of histamine via the parietal cell. The surface tension reducing activity of gastrin may allow it to change the permeability of cell walls leading to migration outwards from the cell of histamine at some sites rather than a true release of histamine; the released histamine may then act synergistically with gastrin, since it is already known that histamine requires a co-factor in the blood before it will cause acid secretion. This was shown by Thompson and Vane (1953) who had great difficulty in eliciting secretion with histamine directly injected into the perfused stomach and found that preliminary mixing of histamine with blood greatly potentiated the action of histamine when it was injected into the vascular supply of the stomach.

SUMMARY.

1. Gastrin prepared by the method of Jorpes, Jalling and Mutt (1952) had properties comparable to a smaller sample prepared by the method of Uvnas (1945) and both preparations had many characteristics in common with the accounts of gastrin given by Komarov (1942) and Harper (1946).

2. Gastrin evoked acid gastric secretion on intravenous, intramuscular and subcutaneous injection. On repeated injection, the secretory effect diminished though the parietal cells remained sensitive to histamine.

3. Preparations of gastrin are thought to release histamine; the evidence is that:

(a) they may produce on quick intravenous injection, a 'delayed' depressor which is typical of the action of a histamine liberator (this is not so evidence on slow infusion intravenously of this substance).

(b) gastrin releases histamine from skin, skeletal muscle and the stomach wall.

(c) it also provokes an acid secretion when injected as remotely as from the stomach as possible into the acral tissues, which suggests, but does not prove that it may act by a release of histamine taking place in the tissues of the extremities.

(d)/

(d) systemic injections of gastrin cause a depressor response which is probably due to histamine. When the portal blood stream is linked to the systemic one temporarily and a close intra-arterial injection of gastrin is given into the stomach wall, a depressor effect is brought about which is annualled by a histamine antagonist.

4. Gastrin, on the other hand, acts in some fairly specific way on the gastric tissues since:

(a) its secretory action is far greater on gastric mucosa than on any other tissue.

(b) when injected into histamine-depleted animals, there is a secretory action, though this is diminished (the secretory action of histamine itself is lessened in histamine depleted state).

(c) the action of gastrin is maintained, even if an injection of Compound 48/80 precedes it.

5. It is postulated that extracts of gastrin may release histamine locally; if such preparations are related closely to the "true" hormone it too may do something of this sort, acting as a "permissive" agent allowing any generally released histamine to escape via the parietal cell as well as releasing histamine locally, or having a more specific local action.

Addendum on Enterogastrone.

Inhibitory hormone of gastric secretion.

An inhibitory hormone of gastric secretion, known as entero-:gastrone, is distributed in the small intestine, and it has been reviewed by Grossman (1950), Code (1951) and Gregory (1952). Its discovery stemmed from the fact that ingested fat inhibited both the motor and secretory activity of the stomach. Ivy and Farrell (1925) showed that the inhibitory effect on motility was induced by a hormonal mechanism in autotransplanted pouches. Similar studies were done for acid secretion by Feng, Hou and Lim (1929). Rich extracts were obtained from intestinal mucosa previously treated with fat by Kosaka and Lim (1930) who obtained a substance whose properties would conform with those of a postulated hormone. Gregory (1956) has attempted to provide further physiological evidence for the existence of a humoral agent, or hormone, of intestinal origin by perfusion in pouch dogs emulsified olive oil and other substances into the duodenum, and the results confirm previous findings that acid secretion may be inhibited by a humoral agent of intestinal origin. There has been little development of the relatively crude inhibitory extracts obtained by him and in view of the relatively low potency on a weight basis, together with the fact that gastric secretion may be intensified by such reactions as nausea, the response to proteins and to pyrogens, it appears doubtful whether the inhibitory responses attributed to the enterogastrone preparations so far described are due to the presence of a hormone.

GENERAL DISCUSSION.

GENERAL DISCUSSION OF THESIS.

This thesis deals with substances which are elaborated by the cells of the gastrointestinal tract and affect the same cells or other cells, in some circumstances after transport via the blood stream. Local hormones have been the main topic for discussion. By 'local hormones' we imply 'hormones' inside cells, and Dale (1933) has been regarded as the authority in establishing their definition (Feldberg and Schilf in 1930 touched on the topic of the possibility of 'tissue hormones' and Gaddum had reiterated this theme in his book). It is at present difficult to demarcate the point at which substances such as adrenaline and acetylcholine change from being humoral transmittors to become local hormones, but it can be stated that the latter role is engaging more and more attention (see Burn, 1950).

Turning to the question of the 'general hormones', the same semantic problem exists: one must differentiate 'hormones' from humoral agents and secretagogues, reserving the first term for the most specific group of substances elaborated by the gastrointestinal tract, the second for the non-specific substances and the last term to those substances which affect gastric secretion as digestion-products. (Gregory, 1952).

Considering these chemical substances, we have to envisage something/

something after the nature of a spectrum: they range from substances like acetylcholine and adrenaline, known neural transmitters which may also be local hormones, substances like histamine and 5-hydroxytryptamine and its precursor 5-hydroxytryptophan, which are almost certainly local hormones but may have a general hormonal role as well, to the general hormones gastrin and enterogastrone; then there are the secretagogues to be considered; perhaps, for the future, we may hear something of this activity for bradykinin, which has been shown to be an essential humoral substance acting as a link for parasympathetic transmission to the submandibular salivary gland and to play a part in its secretory hyperaemia (Hilton and Lewis, 1955, a, b, c).

We have concentrated our attention for the present on four substances, histamine, 5-hydroxytryptamine, Substance P and gastrin because of the fact that all four are extractable from gastric tissue.

The studies on histamine described in this thesis have been planned round an attempt to influence the distribution of histamine and to provoke effects by it, whether normal ones such as gastric secretion, or abnormal ones such as gastric ulceration. The changes in distribution of histamine following the administration of a histamine liberator and a commonly-used type of antihistamine have been studied, and the distribution of histamine and its release in the gastric wall of the abnormal /

abnormal stomach of duodenal ulceration have all been studied. Perhaps the emphasis must now be placed for good on this type of experiment, involving a study of histamine in the tissue: this must surely follow the demonstration, by Lindell and Schayer (1958), that a great deal of histamine can be formed locally in the kidney of the dog from the precursor histamine so that any experiments using the urinary method and measuring the output of free histamine developed by Roberts and Adam now seem on insecure ground, at least for this species. Results based on the urinary excretion of histamine in the post digestive phase will bear little relationship to the acid secretion and histamine production by the gastric mucosa if in fact the greater quantity of histamine is formed within the kidney itself.

Gregory in 1956 reviewed the evidence that histamine may be concerned as a local agent in the excitation of the parietal cell and pointed out that the presence of histamine in the region of the parietal cells, in the absence of histaminase, together with its occurrence in gastric juice, whatever the stimulus, suggests that it may be concerned in the excitation of the parietal cell, but does not constitute a proof of this; in his cautionary own words "It must be admitted that the liberation of histamine in the parietal cell might well be an effect rather than the cause of its response".

When dealing with highly active pharmacological substances it has/

has been agreed that some kind of order must be accepted in their classification; one has also to agree on some classification on their relation to the cell. For instance, histamine has already been discussed on the basis of its local and general hormonal roles. One should remember that it may have one type of activity locally in the cells, and another when secreted from the cell or released from it by injury. So active may some of the substances be that they may stimulate hormones of great potency if administered intravenously and yet their role may be intended to be an intracellular one or at least one confined locally to the tissues. Such may be the case for 5-hydroxytryptamine; although the functioning human carcinoid tumour secretes amazingly rich amounts of 5-HT and also illustrates the metabolism of it and other indole derivatives, it tells us little about the precise physiological significance of 5-HT. One would have imagined that studying the human pharmacology of the carcinoid syndrome, one particular hormonal action of 5-HT would have dominated the clinical picture. The paradox exists that the carcinoid syndrome serves to reinforce one's belief in the local rather than the general role of this substance: the very platelet-absorption and amineoxidase mechanisms seem to exist for the prevention of the small amounts of 5-HT which normally may leak from the tissues into the blood stream from doing any harm there.

The experiments on 5-HT and acid gastric secretion also suggest

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a local role for this substance since it has been our experience that 5-HT infused at the outset of an experiment does not affect gastric secretion. We believe that a more pronounced inhibition of gastric secretion than was achieved by 5-HT itself infused exogenously, can be obtained by enriching the local tissue stores through the previous administration of 5-HT precursors. We envisage an inhibitory mechanism which depends on acid stimulation of the pyloric mucose and which acts via a neural mechanism in which the vagus nerve seems to play a part, either on the ingoing or outgoing side of a reflex arc, with 5-HT or its precursor closely associated with its functioning. It has long been known to invest-:igators of gastric secretion that manipulation of the pyloric region in the cat or dog inhibits gastric secretion: if one performs this simple experiment, and records blood pressure and respiration, it is interesting to note that the blood pressure changes are closely similar to those of 5-hydroxytryptamine (the pylorus is an area of rich distribution of this substance) and that the animal shows respiratory stimulation. It seems to have been neglected that the vagus nerve, which one commonly thinks of as the secretomotor nerve to the alimentary tract, is a predominantly afferent one; it has been estimated for instance by Agostini et al (1957) that the vagus nerve may be as much as 80% afferent below the diaphragm. It has long been

a/

a practice in gastric investigation to section the vagus nerves when working with a humoral substance such as histamine in anaes-:thetised cats - the grounds for this are often stated to be that one must not confuse the responses to neurogenic and humoral secretion. It may be that in performing this manoeuvre one is also guaranteeing for oneself, in the performance of the experiment, a plateau of acid secretion which will not be interfered with by inhibitory nervous reflexes. A high acid concentration, stimulating the pyloric zone, may also be obviated by the wash out techniques which are used which may involve placing as much as 20 mls. of warm saline in the cat's stomach. The same general principles may also apply to the construction of gastric pouches; Heidenhain pouches secrete more freely than is customary with Pavlov pouches, and it may also be the case that in the construction of these pouches little acid secreting mucosa is left in the main stomach for the activation of the pyloric inhibitory mechanism for which it is widely accepted that the pH must fall. (Wilhelmj, O'Brien and Hill, 1936; Woodward et al., 1954). On the other hand, the action of acid secretion on the pyloric region, while apparently inhibiting secretion, may be more directly concerned in suppressing the release of the excitatory hormone. And, although other workers such as Code and Watkinson have had similar findings of inhibitory effects on acid secretion arrived at through the vagus nerve, these experiments do not tell us whether these are "true" inhibitory/

inhibitory fibres or whether they act through a vasoconstrictor supply to the stomach, or by reflexly stimulating respiration and disturbing the acid base balance of the body.

Substance P was investigated because of its distribution in the stomach wall, as well as its wider distribution in the central nervous system and in autonomic ganglia - which seemed to imply some functional connection among all three. No action was discovered either in stimulating or inhibiting acid gastric secretion.

Lastly an examination has been made of the preparation of the substance which is thought to be the chemical hormone of gastric secretion, gastrin. It should be quite clear from all that has been said before that, in making these extracts, one has no knowledge that this is the specific gastric hormone, other than by the fact that it causes gastric secretion. It seemed to us that any extract, quite apart from the true hormone, gastrin, might be acting as the histamine liberator, and that this might be one of the experimental pitfalls - a claim might be established that a substance was gastrin, the specific hormone, when the substance might be merely an extract of large molecular size, having the apporpriate prosthetic group, or being an amine, all of which have a marked tendency to induce histamine release and consequently acid gastric secretion. Our experiments demonstrate that gastrin does release histamine, but that, quite clearly/

clearly, other factors are at work which differentiate it from 'histamine liberators'. These have already been discussed, the selective action on gastric mucosa where histamine release does not readily take place, the significant (though greatly reduced) acid secretion when gastrin is given to histamine depleted cats, and the fact that gastric secretion can still be evoked by injections of gastrin (which is a weak histamine liberator) after injections of Compound 48/80 (which is a strong histamine liberator). If both act as histamine releasing substances it may be that their sites of action are different. Compound 48/80 has its major site of action on such peripheral tissues as skin and skeletal muscle, with the gastro-intestinal tract, other than stomach, little affected; the reverse picture may be true for gastrin, that the release of histamine takes place predominantly as local histamine release in the stomach wall. Perhaps gastrin has a permissive role initiating the secretory action of and allowing the escape of histamine from the parietal cells and any generally released histamine besides.

From the description of this protein hormone, resistant to boiling acids and organic solvents, we must regard it as composed of rigid units of fixed internal structure of such size as would enable it to penetrate cellular membranes to the appropriate site of action. It seems strange that pyloric tissue should have as its target organ, not the same organ, but another part of it, the proximal part of the stomach. We/

We know that thyrotrophic hormone is 'bound' by thyroid cells and adrenocorticotrophic hormone by cells of the adrenal cortex. We do not know of special groups for these protein hormones, but anti-thyrotrophic groupings are known to which thyrotropic hormone becomes fixed and likewise for gonadotrophins. To solve the question of whether preparations of gastrin are physiological in type (and therefore to close the gap between the physiological experiments on the 'hormone' and the biochemical experiments on the 'extract'), it may soon be possible to examine gastrin preparations to see whether they are true hormones by examining their possible combination with cells of the organ where they are supposed to act, using techniques currently being applied to diseases in which auto-antibodies are being investigated. Perhaps, till then it would be wiser to remind ourselves, quoting again from Sir Henry Dale's Dohme Lectures, 1933, that, for the meantime "the discovery in artificial extract from an organ or tissue, of a substance which produces a pharmacodynamic effect, provides only a first item of presumptive evidence that the action of this substance plays a part in normal physiology. Much more evidence is required before we can attribute clearly defined functions to such a substance ..... but even where this is possible, we have still no evidence to justify the assumption that the substance comes naturally into action in the body of the free condition in which we isolate and identify it".

SUMMARY.

SUMMARIES OF VOLUMES 1, 2 and 3.

Summary of Volume 1.

Summary of Volume 2.

Summary of Volume 3.

VOLUME ONE.

SUMMARY OF CHAPTER ONE.

1. Compound 48/80, when injected intravenously in doses of 5 ug/kg or more into cats, causes acid gastric secretion. Repeated injections lessen the secretory response.

2. The secretory response to compound 48/80 when injected intrvenously is not due to an action of the histamine liberator on the gastric mucosa, because injections into the coeliac artery are less effective than intravenous ones. It is probably accounted for almost entirely by release of histamine from such as skin and skeletal muscle.

3. The secretory response to compound 48/80 on injection into the coeliac artery, however, is due to a local effect on the mucosa, since secretion elicited in this way is greater than that obtained with the same dose injected intraportally.

4. Compound 48/80 injected into the coeliac artery acts by release of a small fraction of the mucosal histamine, since it may lead to temporarily increased plasma histamine levels in the venous effluent. A small fraction of the histamine in gastric tissue, such as is released by histamine liberators, may be released to activate the acid secretory process.

SUMMARY OF CHAPTER TWO.

1. The histamine content of the skin of the cat shows regional differences similar to those found in other species. In is possible, with single intravenous or intra-injections of Compound 48/80, to reduce the skin histamine particularly in regions of highest histamine content. Injections of Compound 48/80 into the coeliac artery lead, in addition, to a slight reduction in the histamine of the mucosa of the corpus region of the stomach. In all other tissues examined the histamine content is unchanged. The release of histamine from the skin leads to a transient rise of plasma histamine.

2. Intraperitoneal injections of Compound 48/80 in cats are followed initially by severe symptoms of prostration and vascular effects. On recovery, erythema and facial oedema are noticeable. With repeated intraperitoneal injection of Compound 48/80, the symptoms decrease in intensity and higher doses have to be given to be effective. This refractoriness is mainly accounted for by lack of labile tissue histamine. By these injections it is possible to reduce the histamine in the skin by over 80%. Lung, skeletal muscle and gastric mucosa of the corpus release small amounts. The histamine of the other tissues examined is resistant to release by Compound 48/80.

3. In cats treated with repeated intraperitoneal injections of Compound 48/80 and in cats treated with massive doses of histamine in beeswax, an intravenous infusion of histamine produces a much smaller rise in plasma histamine than in untreated cats. This may account in part for the

greater/

greater resistance of the Compound 48/80-treated cats to histamine. Means for this disposal of released histamine are discussed, among which may be absorption into the gastro-intestinal tissues.

SUMMARY OF CHAPTER THREE.

1. The effect of compound 48/80 on the guinea-pig's ileum preparation suspended in 15 ml. magnesium-free Tyrode solution was examined. When added to the bath in a dose of 2 mg, it produced a strong transient contraction, followed by increased motor activity and development of tone after washing out the compound 48/80; the sensitivity of the preparation to histamine and acetylcholine decreased.

2. The periods of increased motor activity induced by compound 48/80 were associated with the diffusion of histamine from the intestinal wall into the bath fluid.

3. The motor effects of compound 48/80 could be explained by the release of histamine; a distinction might be made between the action of the histamine diffusing into the bath fluid (extrinsic histamine) and the histamine acting within the muscular coat at the site of its release (intrinsic histamine).

4. Other histamine liberators, like propamidine, D-tubocurarine and tryptamine, produce motor effects comparable to those produced by compound 48/80, and also associated with the appearance of histamine in the bath fluid; but histamine liberators may have motor effects on the intestine independent of histamine release. This appears to be so with tryptamine.

5. Small doses of mepyramine, which is known to be a potent histamine liberator,/

liberator, sometimes produce, in the guinea-pig's ileum preparation, before the antihistamine effect develops, a transient period of increased sensitivity to histamine and the appearance of small, rhythmic movements. In preparation previously treated with compound 48/80, small doses of mepyramine may occasionally even cause contraction with superimposed rhythmicity. These effects are attributable to histamine release. (See Chapter Five).

6. The possibility is discussed that in some species histamine plays a role in 'spontaneous' motor activity of the intestine.

SUMMARY OF CHAPTER FOUR.

1. Intravenous and intraperitoneal injections of Compound 48/80 in guinea-pigs and rats cause gastric erosions and ulceration which are the result of histamine release.

2. Prolonged treatment with Compound 48/80 effected considerable reduction in the tissue histamine of rats; the reduction of tissue histamine of guinea-pigs was by comparison slight. More thorough release of histamine was prevented by the outstanding sensitivity of guinea-pigs to small amounts of released histamine. Measures to antagonize or counteract the intensity of the histamine-like effects were adopted, but these in turn influenced the process of histamine release. In the case of mepyramine, the histamine liberation from the tissues was even accentuated.

3. Horse serum produces the same effects as a histamine liberator in rats; the gastric effects are intensified with the antihistaminase, aminoguanidine. The effect of horse serum is invalidated by prior histamine release effected by small doses of Compound 48/80 given over a prolonged period.

4. Release of histamine accounts for the action of gastrotoxin or Bolton toxin in guinea-pigs. Comparable effects on the stomach are produced by Compound 48/80 and anaphylactic shock. Aminoguanidine acting as an antihistaminase intensifies these effects. Prior treatment with Compound 48/80 in small doses over a prolonged period invalidates the effects of gastrotoxin given subsequently.

SUMMARY OF CHAPTER FIVE.

1. A method of assaying an antihistamine substance such as mepyramine has been devised; it has been adapted to the assay of histamine and antihistamine in mixtures.

2. The amount of antihistamine in guinea-pig tissue has been measured; it was highest in the brain and lowest in the alimentary tract. The antihistamine appears to release histamine mostly from skin and lung, but little change occurs in the gastrointestinal tissues.

3. Tissue from human subjects injected with mepyramine shows higher levels of antihistamine in skin and skeletal muscle than in gastric tissue.

SUMMARY OF CHAPTER SIX.

1. Histamine was present to a greater extent in tissue form from the body of the human stomach than from the pyloric antrum.

2. Histamine was present in higher concentration in the mucosa than in the submucosa or tunica muscularis: on construction of histamine profiles it seemed likely that much of the histamine of the body of the stomach was in the same region as the parietal cells.

3. The amount of histamine present in the stomachs with high acid secretion from duodenal ulcer cases was not greater than in stomachs with a low acid secretion obtained from cancer cases.

4. The release of histamine, following application of a histamine liberator to the tissues, was greater in the mucosal and submucosal layers of the body of the stomach than in the corresponding layers in the pyloric antrum; the release of histamine was highest in the submucosæ, and was also greater in duodenal ulcer cases than in cancer ones.

VOLUME TWO.

CHAPTER ONE.

SUMMARY.

Tryptamine and 5-hydroxytryptamine release histamine from living tissue; this finding brings them into line with the large group of amines having this property. The histamine-releasing activity of tryptamine and 5-hydroxytryptamine is about 100 times less than that of compound 48/80. The release of histamine was demonstrated under the following experimental conditions:

(a) After arterial injection of either tryptamine or 5-hydroxy-:tryptamine into the perfused skin flap or gastrocnemius of the cat.

(b) After arterial injection of tryptamine into the perfused skin flap of the dog.

(c) After subcutaneous or repeated intraperitoneal injections of tryptamine to rats. The subcutaneous injection caused a local reduction in the histamine content of skin at the site of injection, the intraperitoneal injections a general reduction of the histamine content of skin and skeletal muscle.

(d) 5-HT secreted endogenously in patients with carcinoid tumours appears to provoke a high urinary excretion of free histamine. This may be one of the mechanisms underlying the flushing attacks of these patients The relationship of the urinary excretion of histamine to acid secretion is discussed. (See also Chapter Six).

CHAPTER TWO.

SUMMARY.

1. The effects of 5-HT on gastric secretion have been examined in dogs anaesthetised with chloralose and urethane and prepared with a stomach fistula and a ligature at the pyloro-duodenal junction.

2. Intravenous infusion of 5-HT for 45 - 90 min. did not stimulate acid gastric secretion but appeared to increase the production of mucus.

3. When an acid gastric secretion was stimulated by a continuous intravenous infusion or histamine, infusion of 5-HT for 30 min. was found to inhibit the secretion.

4. Infusion of 5-HT at the start of histamine-stimulated gastric secretion was found to be less effective in producing inhibition than when given 1. - 2 hr. later.

5. When 5-HT was given after bilateral cervical vagotomy it was found to be less effective in inhibiting histamine-stimulated secretion.

CHAPTER THREE.

SUMMARY.

1. Intravenous infusions of 5-HTP were followed by a fall in the output of histamine-stimulated gastric secretion in anaesthetised dogs. Occasionally 5-HTP stimulated acid gastric secretion for a short time as its first action. The effects of 5-HTP were similar to those of 5-HT except that they occurred after a longer latent period.

2. In previously starved anaesthetised dogs feeding with tryptophan before the start of an experiment led to a fall in acid output between 1 - 3 hours after the onset of histamine-stimulated secretion.

3. The 5-HT levels in portal blood were found to be higher in fed than in starved dogs, but the levels did not increase after infusion of histamine. The acid secretion elicited by histamine infusions in recently fed dogs was less in amount than that of dogs which had been starved over 36 hours. Local increase in 5-HT concentration in the wall of gastrointestinal tract, with release of 5-HT into the portal blood stream, could account for these findings.

CHAPTER FOUR.

SUMMARY.

1. It has already been shown that when dogs are starved for 24/36 hours histamine stimulated secretion reaches a steady state between $1\frac{1}{2}$ - $3\frac{1}{2}$ hours, after starting the histamine infusion. When the animals have been fed 12 hours before the experiments, inhibition of the histamine stimulated secretion began after 1-$1\frac{1}{2}$ hours and was complete by 3-$3\frac{1}{2}$ hours.

2. This inhibition of histamine stimulated secretion in recently fed dogs could be prevented by bilateral cervical vagotomy or by tying a ligature at the antral-body junction.

3. Compounds such as phenyl di-guanide and veratrine, which like 5-HT stimulate vagal afferent nerve activity, have inhibitory effects on gastric secretion similar to those of 5-HT; this would appear to be pharmacological evidence for afferent vagal nerve control, perhaps through a vago-vagal reflex, of acid secretion. The pyloric zone might act as a reception area for this; its mucosa being stimulated by contact with a high concentration of acid. 5-HT could act as an afferent transmitter for this mechanism; recent feeding would tend to enrich the local stores of this substance via its precursors.

4. The efferent side of the mechanism controlling acid secretion outlined in these chapters has not been studied in detail. It is possible that 5-HT might produce its effect through fibres in the vagus nerve which are inhibitory to acid secretion such as were found by Pavlov (1902) and Schachter (1949); it is known that 5-HTP penetrates the blood brain barrier and may lead to local increases of 5-HT in important centres. In neural tissue the conversion to 5-HT is extremely rapid.

CHAPTER FIVE.

SUMMARY.

The incidence, aetiology, pathology, histogenesis and histology of argentaffinoma is firstly surveyed.

The account continues with:

i) a review of nine cases of metastasing argentaffinoma and the features attributable to 5HT secretion; blood levels of 5HT have been examined in each case.

ii) the application of a urinary test for 5HIAA, the oxidation product of 5HT, which has provided a useful way of detecting over-production of 5-HT by the tumour. Various applications of the test have been made, in particular to check whether all the tumour has been eradicated, and in this way to assess prognosis.

iii) Therapeutic measures should be planned so as to remove the tumour or ameliorate the effects of 5-HT on the major systems of the body and in particular on the heart. This may be possibly by the use of specific antagonists and by radiotherapy, including the use of isotopes, in advanced cases where surgical removal may not be possible.

CHAPTER SIX.

SUMMARY.

1. The normal metabolism of 5-HT is again discussed and the first isolation of 5-HTP in carcinoids is described.

2. Reasons are advanced for considering that 5-HTP rather than 5-HT is the hormone of the argentaffin cell.

3. The effects of high protein feeding and L-tryptophan have been shown to confirm in humans the accepted biochemical pathways of 5-HT formation. The feeding of fat did not influence 5-HT release.

4. The effects of alcohol, reserpine and histamine of 5HT blood levels are described.

5. The acid secretion in response to histamine has been examined in four secreting carcinoid cases and the effects in two other cases provoked by reserpine and iproniazid. Acid gastric secretion was most markedly changed in the latter instance, probably because iproniazid affects the level of 5-HT in the tissues rather than in the blood stream.

VOLUME THREE.

PART FOUR.

1. Substance P did not evoke secretion of hydrochloric acid from the stomach of cats.

2. Substance P failed to diminish the acid secretion produced by vagal stimulation and by histamine, nor did it enhance it which seems important in view of the idea propounded by Vogt (1949) that this substance might be engaged in transmission at vagal nerve endings in the upper gastro-intestinal tract.

PART FIVE.

(1) Gastrin prepared by the method of Jorpes, Jalling and Mutt (1952) had properties comparable to a smaller sample prepared by the method of Uvnas (1945) and both preparations had many characteristics in common with the accounts of gastrin given by Komarov (1942) and Harper (1946).

(2) Gastrin evoked acid gastric secretion on intravenous intramuscular and subcutaneous injection. On repeated injection, the secretory effect diminished though the parietal cells remained sensitive to histamine.

(3) Preparations of gastrin are thought to release histamine; the evidence is that

(a) they may produce on quick intravenous injection, a 'delayed' depressor which is typical of the action of a histamine liberator (this is not so evident on slow infusion intravenously of this substance).

(b) gastrin releases histamine from skin, skeletal muscle and the stomach wall.

(c) it also provokes an acid secretion when injected as remotely from the stomach as possible into the acral tissues, which suggests, but does not prove that it may act by a release of histamine taking place in the tissues of the extremities.

(d) systemic injection of gastrin causes a depressor response which/

which is probably due to histamine. When the portal blood stream is linked to the systemic one temporarily and a close intra-arterial injection of gastrin is given into the stomach wall, a depressor effect is brought about which is annulled by a histamine antagonist.

(4) Gastrin on the other hand acts in some fairly specific way on the gastric tissues since

(a) its secretory action is far greater on gastric mucosa than on any other tissue.

(b) when injected into histamine depleted animals, there is a secretory action, though this is diminished (the secretory action of histamine itself is lessened in histamine depleted state.)

(c) the action of gastrin is maintained even if an injection of Compound 48/80 precedes it.

(5) It is postulated that extracts of gastrin may release histamine locally; if such preparations are related to the "true" hormone they too may do something of this sort acting as "permissive" agents, allowing any generally released histamine to escape via the parietal cell, but they must also exert some more specific role, which may be a local release of tissue histamine, on the gastric mucosa.

APPENDIX.

APPENDIX.

1. Pharmacology of Compound 48/80.
2. Biological assay of histamine and 5-hydroxytryptamine.
3. Estimation of histamine in the tissues.
4. Experimental production of gastric ulcer by the injection of gastrotoxin (Bolton toxin).
5. Perfusion of skin flaps by the method of Feldberg and Paton; perfusion of stomach.
6. Estimation of free histamine in the urine.
7. Pathological effects of 5-hydroxytryptamine and histamine.
8. Histological methods.
9. Assay procedure for 5-hydroxyindoleacetic acid.
10. Case reports.
11. Substance P.
12. Gastrin (extraction method of Uvnas).

APPENDIX.

1. PHARMACOLOGY OF COMPOUND 48/80.

At the present the most potent histamine liberator described is the long acting depressor agent Compound 48/80. Baltzly, Buck, de Beer and Webb (1949) who first described the family to which this drug belongs, obtained it by condensation of alkoxyphenylkylamine with formaldehyde in the presence of acid. The compound is prepared by heating N-methylhomoanisylamine with an exact equivalent of formaldehyde for four hours in the presence of 6 N HCl. After evap-:oration to dryness the residue is taken up in ethanol. On the addition of ethylacetate and ether, a solid separates which is filtered off and dried. Compound 48/80 is a fine, white non-crystalline powder, very soluble in water and apparently quite stable. It has been found easy to prepare reproducible batches of identical toxicity and pharmacological properties. Baltzly and co-workers (1949) have given reasons for considering it to be a mixture of dimers, trimers and tetramers, with some admixture of higher polymers.

A preliminary report of the pharmacological properties of compound 48/80 was given before the Federation of American Societies for Exper-:imental Biology (Dews and de Beer, 1949). At that time it was observed that some of the effects of the compound resembled those produced by histamine. Feldberg and Paton (1951), Paton (1951), and Paton and Schachter/

Schachter (1951) later emphasised that the drug belongs to that class of substances which have been named "histamine liberators"; it produces the characteristic delayed depressor response on intravenous injection which is typical of histamine releasing agents (MacIntosh and Paton, 1949).

When plasma samples were withdrawn one or two minutes after the injection, the histamine equivalent of the plasma was markedly elevated (Paton, 1951). A substance then appeared in the latter samples of plasma which caused a slow contraction of the guinea-pig's ileum even in the presence of a histamine antagonist such as mepyramine.

Compound 48/80, in a dose of 10 ug. injected intra-arterially into the perfused skin preparation of the cat caused the appearance of 160 g. histamine in the venous effluent (Feldberg and Paton, 1951). Detectable histamine release can be obtained with even smaller doses of compound 48/80, e.g. 1 ug. Compound 48/80 was injected, and 7.7 ug. histamine base was collected in the venous effluent.

APPENDIX.

2. Biological assay of histamine and 5-hydroxytryptamine.

The apparatus used was the isolated organ bath (Palmer catalogue 1953) outlined in Fig. 1.

i) The test object in the case of histamine was a portion of the terminal ileum obtained from a guinea-pig. The test object for 5-hydroxytryptamine was the distal rat colon washed out.

ii) For the guinea-pig's ileum preparation the temperature of the bath was kept at 35°C; for the rat colon preparation the bath fluid was at 24°C.

iii) Tyrode solution was used as follows for the guinea-pig ileum preparation:

Sodium chloride	8.0 g.
Potassium chloride	0.2 g.
Calcium chloride	0.2 g.
Dextrose	1.0 g.
Sodium phosphate	0.05 g.
Sodium bicarbonate	1.0 g.
Distilled water to	1000 ml.

For the rat colon preparation Gaddum's solution was used as follows:

Sodium chloride	9.0 g.
Potassium chloride	0.42 g.
Calcium chloride	0.03 g.
Dextrose	1.0 g.
Sodium bicarbonate	0.2 g.
Distilled water to	1000 ml.

iv) In each case atropine was added to the inner bath fluid when performing assays. 0.2 ml 1:500,000 was the amount commonly used

v) Histamine and 5-hydroxytryptamine were added by means of a tuberculin type syringe in dilutions of one in 5, 10 and 20 million dilution.

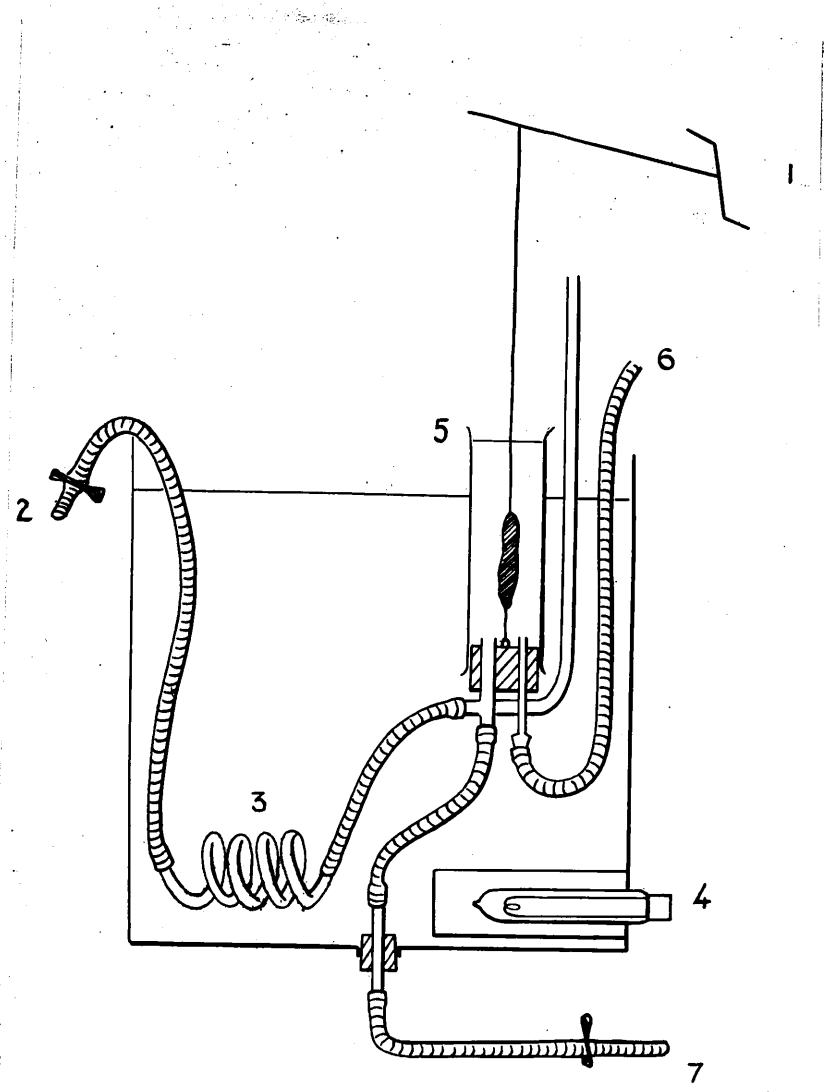

Fig. 1 illustrates the outline of the method for biological assay of histamine. The guinea-pig ileum is suspended in Tyrode solution to which the drug is added. The smooth muscle of the intestinal strip contracts and activates a lever carrying a writing point, which records the contraction as a vertical stroke on smoked drum.

1 - frontal writing lever: 2,3,7 - inlet, coil, and outlet for Tyrode solution: 4 - heater: 5 - 18 ml. bath: 6 - O_2 and CO_2 supply. The guinea-pig's ileum is attached by means of an anchoring thread to the bath and pulls on the frontal writing lever.

APPENDIX.

3. Estimation of histamine in the tissues (Feldberg & Paton, 1951); (Feldberg & Talesnik, 1953).

The histamine content of the injected portion is compared with a corresponding area on the other limb or other side of the abdomen or in another animal.

The skin was freed from the underlying subcutaneous tissue, and after weighing, cut up in 2 ml. N-HCl/g. skin, and ground in a mortar with sand. This process does not fully macerate the tissue. This is achieved as follows: The partly macerated tissue is transferred into another mortar, again cut up and reground after the addition of distilled water (about 10 ml./g. tissue). The contents are returned to the original mortar, again ground and brought into a flask. The mortars are washed several times with saline solution and the washings added to the flask, which is boiled for a minute or two. The flasks are then stored in the cold until the assay is carried out (usually after 24 hours). For this purpose they are centrifuged or filtered, the residue washed twice with saline solution, the supernatant or the filtrate respectively and the washings mixed, neutralised with N+NaOH, made up to a given, suitable volume and then assayed for histamine on the guinea-pig's ileum preparation.

APPENDIX.

4. The experimental production of gastric ulcer by the injection of gastro-toxin. (Bolton, 1904, 1908).

Guinea-pigs starved for 24 hours were killed and the thoracic viscera cut out. A cannula was inserted into the thoracic aorta and saline was perfused to wash out the blood. The stomach was removed, opened and washed. The mucosa was scraped off onto a sterile plate and was then ground down to an emulsion with sterile saline.

A fresh filtered extract was then injected into the rabbit's peritoneal cavity, 4-5 injections being given at 7-10 day intervals. At the end of this time 30-40 ml. blood were obtained from the rabbit's ear vein, whipped, centrifuged, and used the same day. 10 ml. of this serum were injected intraperitoneally. Within 24 hours gastric lesions could be identified at post mortem. There was patchy mucosal necrosis at first with the appearance of punched out ulcers at 24 hours. As a control experiment it could be shown that this gastro toxic serum had no effect in the rabbit but acted only in the guinea-pig.

APPENDIX.

5.(a) Perfusion of skin flaps by the method of Feldberg and Paton (1951).

An area of skin from the right hind leg, supplied by the saphenous artery was perfused in the anaesthetised cat with Locke solution. After shaving and marking out the area of skin to be isolated, the skin was divided with a thermocautery by two incisions round the right leg, one about 2 cm. below the inguinal ligament, the other about 2 cm. above the ankle. The two incisions were joined by a further incision along the lateral aspect. At the border of the lower circular incision, the saphenous vein was tied and cut. The skin was then dissected off from the underlying muscles and fascia, starting from the longitudinal incision and working from both sides to the centre of the piece of skin where the saphenous artery and vein enter it and leave it. Anastomostic vessels from the popliteal space, the gluteal region, and the lateral aspect of the ankle were ligatured and cut. In addition a few smaller vessels penetrating the muscles required ligature.

In order to prevent spasm of the saphenous artery, the prepar-:ation throughout the dissection was kept warm by radiant heat from a bowl fire, and drying of the inner surface of the dissection skin was prevented by repeated flooding with warmed liquid paraffin.

The saphenous artery and vein up to the femoral vessels were then/

then carefully isolated. Because of the length of this vascular trunk, handling the saphenous vessels was avoided to prevent spasm, and the saphenous nerve was left undissected with the vessels so that it gave additional support and protection. A length of femoral artery and vein, with the saphenous vessels, was dissected free by ligating and cutting all side vessels. (Fig. 2). The dissected patch of skin with its vessels still attached to the animal was packed in warm saline swabs and kept warm by radiant heat for 30-60 minutes to allow any arterial spasm to pass before perfusion was started.

For the perfusion the Locke solution passes from a reservoir whose height can be adjusted, through a hot water jacket near the injection cannula, to which it is connected by a thick-walled rubber tubing through which the injections of the histamine liberators are made. A small vertical side arm on the injection cannula serves to trap any bubbles in the perfusion liquid. The femoral artery below the origin of the saphenous is ligated and cut, the cannula is tied into the femoral artery above the saphenous, perfusion at once started, and the femoral artery divided above the cannula. Another cannula is tied into the femoral artery divided above the cannula. Another cannula is tied into the femoral vein in the same way above the saphenous for the collection of the venous effluent.

The/

The entire isolated patch of skin with its cannulated vessels is transferred to a paraffin bath warmed up a water jacket. The skin was spread out with its inner surface upwards on a perforated immersed celluloid platform, so that it is possible to observe the washing out of the blood from the perfused parts of the skin, as well as the formation of oedema if it should occur. The paraffin bath used is funnel-shaped, with an outlet at the bottom so that any fluid escaping from the skin can be collected. It usually amounts to a few ml. only. Warm water is kept circulating through the water jacket near the injection cannula, and through that surrounding the paraffin bath by an 'air lift' (see Fig. 3). By this means the temperature of the fluid perfusing the skin and of the paraffin surrounding it is kept at about 37 C.

The perfused pressure is adjusted so as to maintain a venous outflow of 2-4 ml./min. For this purpose the pressure required is usually about 100 cm.

(b) Perfusion of stomach 'in situ' by the author's technique.

The stomach was also perfused with Locke's solution. A fine polythene or needle cannula was inserted into the largest available branch of the coeliac axis and positioned so that the direction of flow took place down the left gastric artery into the body of the stomach. The coeliac axis was then tied proximal to the site of cannulation and the splenic vessels interrupted in their course

by/

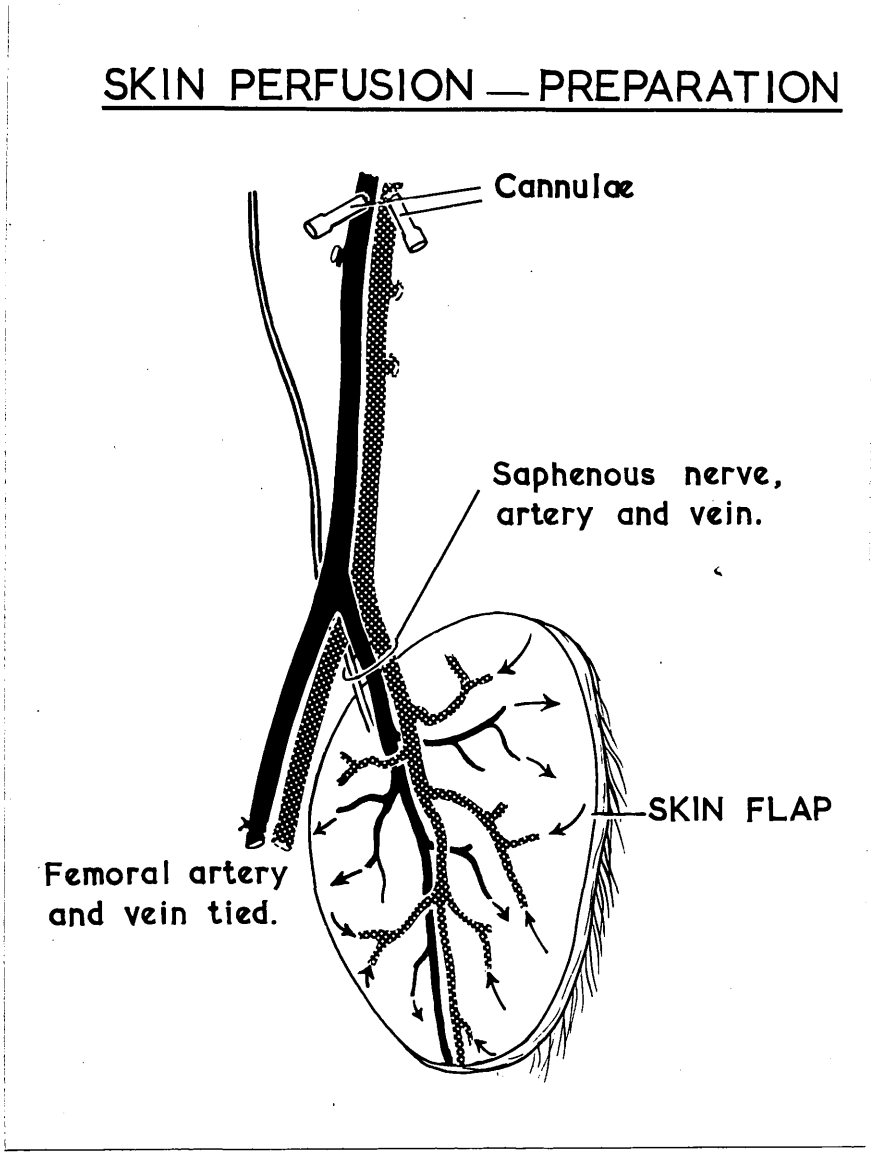

Fig. 2 shows the isolated skin falp which is perfused via its arterial supply, the effluent being collected on the venous side.

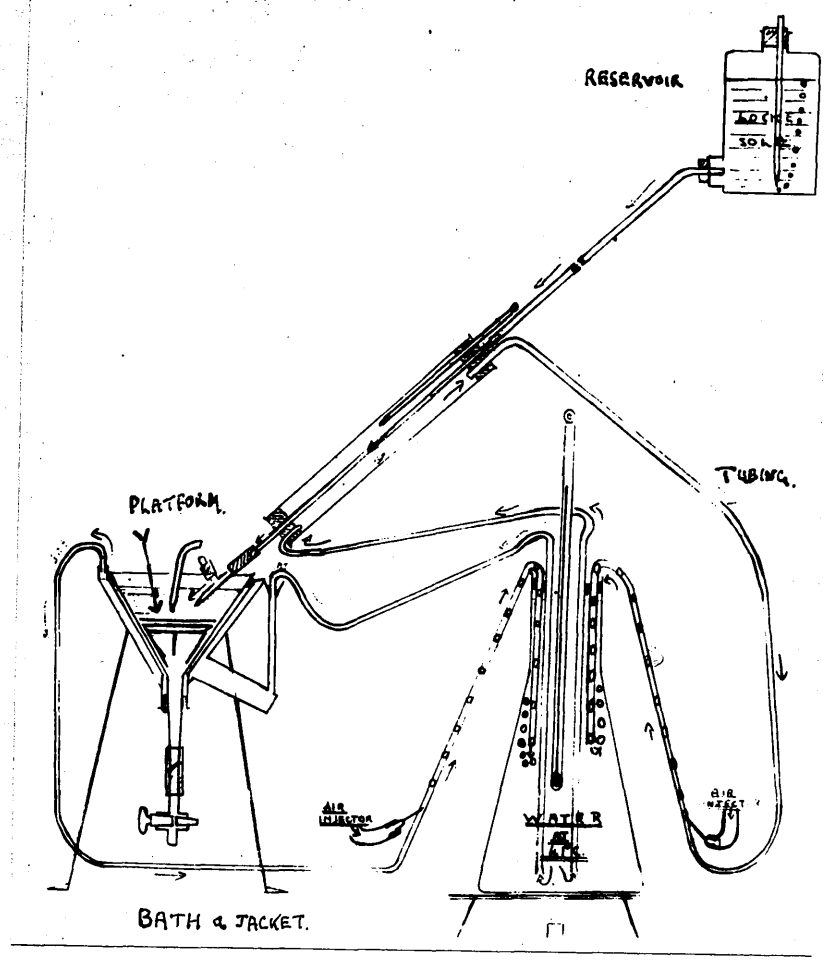

Fig. 3 illustrates the surrounding medium in which the skin flap was maintained and shows how an 'air-lift' technique was employed to maintain the fluid circulating in the jacket of the water bath used in these experiments.

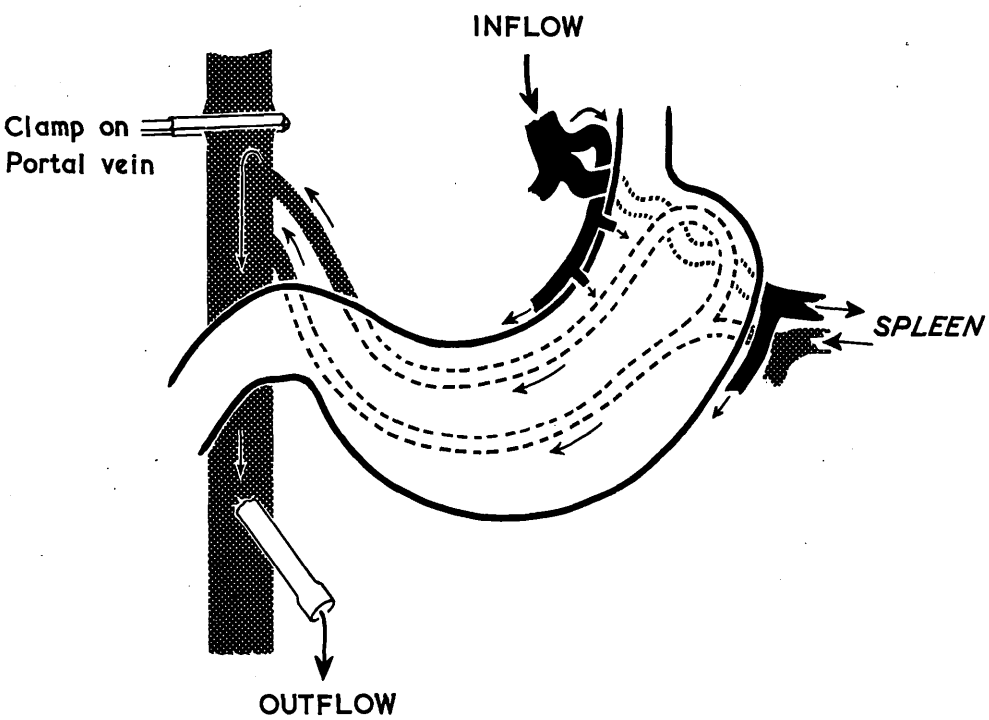

Fig. 4. illustrates the method of perfusion of the stomach. (It was more usual to do a preliminary removal of the spleen).

by removal of the spleen; the hepatic vessel was also tied after it had given off its gastroduodenal branch. The pylorus was ligated and divided between ligatures with a thermocautery between ligatures. The portal venous drainage was now found to be undergoing marked dilution with the perfusing saline solution. The animal was then eviscerated distal to the pylorus, the superior and inferior mesenteric arteries being tied. A polythene or find glass cannula was inserted into the superior mesenteric vein and the portal vein was surrounded by a ligature or spring clamp operated by a remote control photographic release cable. By compressing the portal vein the perfusate collected as venous effluent from the stomach flowed in reverse from the cannula in the superior mesenteric vein where it could be collected. (Fig. 4).

Some experiments with Indian ink verified that the mucosal region of the body of the stomach was being perfused satisfactorily. The stomach could also, in some instances, be perfused after separation from surrounding tissues, division of the oesophagus and interruption of the vessels mentioned above. Venous perfusate was collected from a polythene cannula and inserted in the main gastric vein. The stomach was maintained in a similar background to that described in (a) above.

APPENDIX.

6. Estimation of free histamine in the urine.

The Decalso method described by Roberts and Adam was used to separate the free histamine from the urine. Such adsorbents act by cation-exchange and are capable of taking up the ions of organic bases from very dilute solutions. The synthetic zeolite known as Decalso adsorbs only free histamine in this pH range 8 - 10. Glass columns containing Decalso were prepared, each column containing about 3 gms. of the zeolite packed into place with a glass plunger to achieve a density allowing 50 ml. of urine to percolate through the column in not less than 1 hour. Each hourly collection of urine was adjusted to a pH of 8, filtered and 50 ml. placed on a Decalso column which had previously been moistened by 10 ml. distilled water. Following the urine, each column was washed with 25 ml. of normal saline and most of the water removed by 15 ml. absolute alcohol. The free histamine was eluted with 3.5 ml. AnalaR ammonium hydroxide, followed by 50 ml. of ammoniated chloroform, this organic solvent carrying the liberated histamine through the column and into a 300 ml. pressure flask. The chloroform ammonia eluate in each flast was evaporated to dryness in a water bath at 40°C under reduced pressure. The slight residue was then dried off with 10 ml. absolute alcohol containing 3% (V/V) concentrated HCl to neutralise traces of alkali, and with 10 ml. of absolute alcohol. Finally extracts were taken up in known volumes of saline, their pH adjusted to 7.5 and the histamine content estimated by bio-assay.

APPENDIX.

7. Pathological effects of 5-hydroxytryptamine.

Following the infusion of 5-hydroxytryptamine using concentr-:ations in the range 5 to 20 ug/min. various changes in the internal organs of the dogs were observed. The morbid anatomy is as follows:- The opened stomach was found to be pale in its mucosal aspect and there was pooling of mucus on its superficial surface. There were occasional small erosions.

The kidneys when cut across were markedly pale, but there was no evidence of ischaemia particularly located in the cortex.

The jejunum and ileum also had a pallid mucosal surface, a characteristic feature was the presence of bile staining as far as the terminal ileum, a finding which indicates strong propulsive activity of the alimentary tract.

The brain showed fairly consistently engorgement or even discrete haemorrhages in the region of the pituitary stalk and the floor of the third ventricle. The heart occasionally showed sub-endocardial haemorrhages - these were more numerous on the right side of the heart. (See Fig. 5).

These effects are in marked contrast to those obtained with histamine in which (Fig. 6) the general tendency is in engorgement of blood vessels.

Fig. 5 shows the ischaemic changes after infusions of 5-hydroxytryptamine; the photograph illustrates these for the stomach, intestine, kidneys and brain; the spleen is included to show how it may occasionally be found to be engorged. (Compare histamine effects).

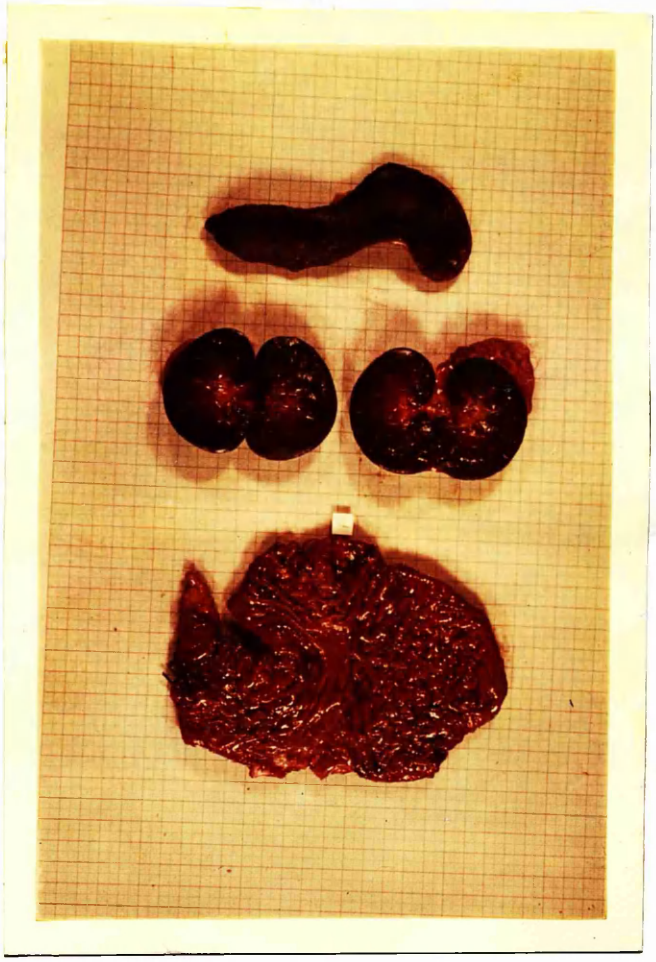

Fig. 6 shows the effects produced by infusion of histamine (for comparison with Fig. 5). The photograph illustrates the engorged state of the stomach and the marked vascularity of the kidneys. The spleen is smaller and shrunken.

APPENDIX.

8. Histological methods.

The surgical material was dealt with in the following manner. Formalin (10% followed by corrosive-formol, or corrosive formol alone were the fixatives used, followed by dehydration and paraffin embedding (Lendrum, 1951)). Sections were stained by haemalum and eosin, by the Masson-Fontana silver impregnation method and by the alkaline diazo method (Pearse, 1953) using Nuclear Fast Red Salt B (Gurr). The latter stain was found to be particularly useful. The diagnosis of argentaffinoma was made both on morphological grounds and on the specific staining properties of the cells.

APPENDIX.

9. Assay Procedure for 5-hydroxyindoleacetic Acid.

Twenty-four-hour specimens of urine are collected into a bottle containing 25 ml. of glacial acetic acid and 3 ml. of toluene (the 5-HIAA disappears rapidly from alkaline urine). Aliquots of the urine collected in this way can be transmitted through the post or kept in the ordinary refrigerator without marked loss of 5-HIAA.

2 g. sodium chloride and 25 ml. ether are added to 5 ml. urine (which should already be at pH of about 3) in a 70 ml. stoppered tube. The mixture is shaken for 1 minute and the two phases are then separated by centrifugation. 20 ml. of the ether layer is transferred to a 100 ml. Quickfit flask and evaporated under reduced pressure. The residue is taken up in 4 ml. of distilled water and a measured volume (up to 3 ml.) transferred to a test tube for colorimetric estimation. 1 ml. each of the 1-nitroso-2-naphthol and nitrite reagents are added and the tubes warmed to 55° for 5 minutes. The solution is then extracted with 10 ml. ethyl acetate to remove excess nitroso-naphthol and interfering colour given by indoleacetic acid. The optical density of the lower layer is read (preferably after ½ hr.) at 540 mu. The results are read from a standard curve prepared at the same time from amounts of the more readily available 5-hydroxytryptamine creatinine sulphate equivalent (on a 5-hydroxyindole basis) to 0.90 ug. 5-HIAA.

The/

The recovery of 5-HIAA is almost quantitative.

Reagents. 1-nitroso-2-naphthol and nitrous acid as described by Udenfriend, Weissbach and Clark (1955).

The above is a modification of Udenfriend's method (loc. cit.) for the estimation of 5-hydroxytryptamine. It differs from the author's method for 5-hydroxyindoleacetic acid (Udenfriend, Titus and Weissbach, 1955) in two respects. In Udenfriend's method keto acids are removed by 2:4-dinitro=phenylhydrazine and indoleacetic acid by extraction with chloroform. As keto acids are rarely encountered, this step is normally unnecessary. It has been found that the interfering pink colour formed by a reaction between indoleacetic acid and the nitrite reagent is quantitatively removed by the one extraction with ethyl acetate. As all the extractions are done in acid solution (in contrast to Udenfriend), in which 5-hydroxyindoleacetic acid is relatively stable, it is unnecessary to remove peroxides from the ether to prevent destruction of 5-hydroxyindoleacetic acid. The time taken for the procedure is less than half that required for Udenfriend's. The mean excretion of 5-HIAA for 11 normal controls was 7 mg./24 hr., over the range 3.2-13.7 mg. This agrees well with the normal values quoted by Udenfriend. (See Fig. 7).

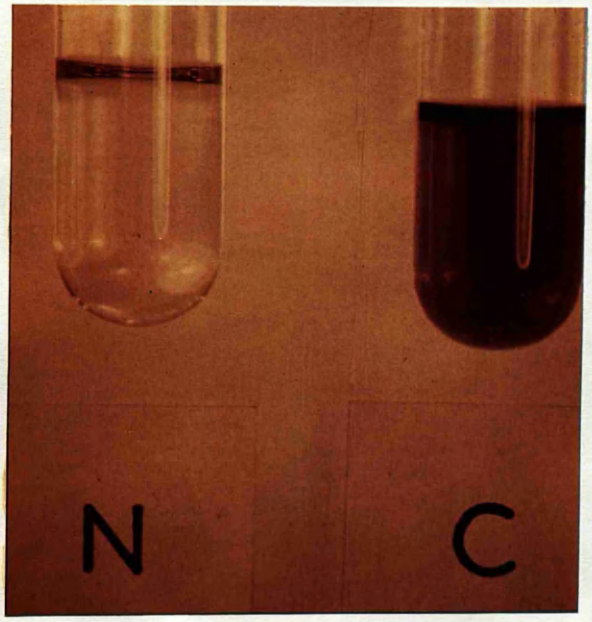

Fig. 7 shows the fully developed test.

N = normal

C = carcinoid.

TABLE 1.

Percentage recoveries of authentic 5-HIAA added to water or urine.

Concn. of added 5-HIAA ug/ml.	% recovery from aqueous solution	% recovery from urine
240	103.5	99
80	100	101
70	100	97
40	99,98,98,98,98	-
24	97.5	101
8	88	94.5

APPENDIX.

10. CASE REPORTS.

Case 1. A male, aged 53 years, admitted to hospital complaining of pain in the rectum and breathlessness on exertion of 9 months' duration. The rectal pain was apparently due to a fissure-in-ano which had been previously diagnosed and treated elsewhere. Up till two weeks before admission there had been moderate constipation for which he had used liquid paraffin. Occasional diarrhoea had occurred during the two weeks prior to admission. Loss of appetite and loss of weight (2 stones in 9 months) were definite features. There was no history of subjective or objective flushing attacks.

Examination showed a well-coloured middle-aged male. No skin rash was evident. There was marked oedema of the ankles, but no cyanosis or lymph node enlargement. The liver, hard and nodular, was grossly enlarged, reaching the iliac crest in the mid-axillary line. The pulse, 80/min., was regular. B.P. 130/100 mm. Hg. Clinical assessment of the heart, including radiography revealed no abnormality. An E.C.G. showed right axis deviation. There was no clinical evidence of pulmonary stenosis. Barium meal and enema showed rapid progress through the bowel and failure of the terminal ileum to fill.

At laparotomy (August, 1955 - Mr. A. B. Kerr) the liver was displayed/

displayed riddled with small, pale, secondary tumours. No gross primary tumour could be felt throughout the length of the alimentary tract, gall bladder or pancreas. The only major lesion noted, other than the liver tumours, was in the mesentery of the small bowel near its root. This was thought to be a lymphatic secondary though it was possible that it might represent a primary in a fixed loop, the anatomy of which had not been displayed. Histological examination of a small portion of the liver tumour showed an argentaffin carcinoma (silver and diazo stains were positive).

The urinary 5-HIAA was 688 and 594 mg./24 hrs. on two occasions in the post-operative period.

Case 2. A female, aged 60 years, admitted to hospital complaining of upper abdominal discomfort for the previous 8 months and looseness of the bowels for 3 months. In addition, she had experienced 'hot flushes' of the face and neck for 2 years. The appetite had always been good and the weight had been constant. Bowel function and micturition had been normal. At the menopause 12 years previously there had been flushing attacks but these had not been in evidence for many years.

Examination showed a well-coloured elderly female. The pulse was regular and of normal rate. B.P. 190/98 mm. Hg. The apex beat was rather diffuse, but the heart sounds were pure. No abnormality of the respiratory system was found. A mobile firm non-tender mass was palpable/

palpable in the lower right abdominal quadrant.

At laparotomy (Prof. I. Donald and Mr. R. A. Jamieson in January, 1955) the pelvic organs were entirely normal: small post-menopausal uterus and ovaries. Loops of gut which presented were firmly adherent to and surrounding a hard tumour occupying the small bowel mesentery and about the size of a tennis ball. About 1 foot from the caecum there was a small, firm tumour of the ileum causing marked stricture of the bowel. This was considered to be a carcinoid tumour. The liver and spleen were normal. There was no other secondary involvement of the abdominal contents: no free fluid was present. The main tumour was considered not suitable for removal without excising too much gut. Side-to-side small bowel anastomosis was carried out to short circuit the tumour and about 18 inches of small bowel. A small biopsy was taken from the mesentery.

Histology. This wedge of material was composed largely of hyaline fibrous tissue infiltrated by round cells. At one point there were foci of closely packed, rounded cells containing granules which give a positive staining reaction with silver salts and the diazo stain. The appearances were those of an argentaffin carcinoma (carcinoid).

Progress. Convalescence was uneventful and the patient proceeded to live a normal existence, doing housework and shopping as usual. The flushing attacks continued as before. They seemed to be brought on by drinking tea, but this mechanism was by no means certain. The tumour mass/

mass was palpably larger in June and again in October, 1955. Clinical examination, including phonocardiography and electrocardiography, revealed no evidence of pulmonary stenosis. Urine samples taken between October, 1955, and January, 1956, contained 65, 90, 84, 63 and 69 mg. 5-HIAA/24 hrs.

Case 3. A male, aged 73 years, admitted to hospital complaining of colicky pains across the mid-abdomen for the past 7 years. These pains, accompanied by borborygmi starting on the right side and passing across the umbilicus to the left flank and back, came on about half-an-hour after a meal and were associated with flushing of the face and conjunctiva and sometimes with a feeling of faintness. The appetite had been good and the bowels regular. A loss of 2 stones in weight had occurred in the last year and a half. There had been no diarrhoea or vomiting and apart from occasional nocturia no upset of micturition had occurred.

Examination showed an elderly male of normal complexion. A large firm mass was palpable in the right iliac fossa. There was slight overlying tenderness. The spleen and liver were not palpable. The sigmoid colon was full of faeces. There was a small, right, indirect, inguinal hernia. Rectal examination shows moderate prostatic enlargement and ballooning of the terminal rectum. The pulse, 80/min., was regular and of good volume. B.P. 185/80 mm. Hg. A blowing systolic murmur was present/

present at the apex and the second aortic sound was accentuated. There were numerous sibilant rhonchi over both lower lung lobes. Barium meal with follow through and barium enema showed marked narrowing of the caecum. The ileum appeared normal.

At laparotomy (Mr. E. G. Gerstenberg in September, 1955) a tumour was found at the ileo-caecal junction incorporating the base of the appendix. The terminal ileum was dilated and hypertrophied. Enlarged firm glands were present in the adjacent mesentery, and the greater omentum was extensively infiltrated by firm, white tumour deposits varying from 1 to 20 mm. in diameter. There was definitely no evidence of tumour in the liver. Resection was not possible and an ileo-transverse-colostomy was performed.

Histology. Examination of specimen obtained from omentum shows infiltration of the tissue by argentaffin carcinoma (silver and diazo stain positive).

Urinary 5-HIAA five days after operation was 155 mg./24 hrs. Acute retention of urine developed before another specimen was collected. A specimen collected by indwelling catheter was heavily infected. Trans-:urethral pouch prostatectomy was performed (Mr. W. S. Mack in October, 1955). The 5-HIAA excretion was 24 mg./24 hrs. The 5-HIAA excretion remained between 20 and 30 mg./24 hrs. during the next month. The urine specimens throughout were heavily infected and it was thought that 5-HIAA was/

was being destroyed due to bacterial action since the flushing attacks continued as before. However, urine specimens taken 6 and 7 weeks after prostatectomy were clear of infection and the 5-HIAA excretion did not return to the initially high value. We are not at the moment able to explain this drop in 5-HIAA excretion, but the case is being further investigated.

Case 4. This case has been fully described by Duncan et al (1955). He is a man of 23 years, now under surveillance (Dr. C. D. Anderson) in Glasgow. He is continuing at his work as an electrician and although the objective signs of pulmonary stenosis and right heart strain are increasing he remains remarkably well. The flush of the face and hands is now permanent. Excretion of 5-HIAA on two occasions in December, 1955, was 258 and 269 mg./24 hrs.

Case 5. A female, aged 64 years, was admitted to hospital complaining of colicky pains across the abdomen. There were no signs or symptoms which, in retrospect, could be attributed to excess 5-HT production.

At laparotomy (Mr. G. Russell Thomson in November, 1952) the clinical diagnosis of subacute intestinal obstruction was confirmed. A tumour was constricting the ileo-caecal region and invasion of the local lymph nodes and mesentery, more distantly, was evident. There was no evidence of tumour in the liver. Resection was not possible

and/

and an ileo-colostomy was performed. A small biopsy was taken.

Histology. This was a small lymph node showing invasion by an argentaffin carcinoma (silver and diazo stains were strongly positive).

Recovery was excellent and the patient has remained in good health since. There have been no symptoms suggestive of increased 5-HT production. Urinary 5-HIAA estimated in January, 1956, was 43 mg./ 24 hrs.

Case 16. A male, aged 54 years, presented as a subacute intestinal obstruction with a mass in the right iliac fossa. No symptoms refer-:able to excessive 5-HT production had been present.

At laparotomy (Mr. R. G. Main in February, 1953) a chronic ileo-ileal intussusception with a yellow submucous tumour at the apex was found. Local lymph nodes were obviously invaded. A right hemi-colectomy was performed.

Histology. The specimen consisted of 10 cm. of terminal ileum and 14 cm. of caecum and colon. A 2 cm. diameter broad based polypoidal golden-yellow tumour was present in the ileum near the ileo-caecal junction. Penetration of the bowel wall had occurred. The appendix was not remarkable. Microscopy shows a morphologically typical argentaffin carcinoma (silver and diazo stains are positive) penetrating the bowel wall and present in the local lymph nodes.

Recovery/

Recovery was good and progress uneventful since then except for anginal attacks. E.C.Gs suggest myocardial ischaemia. There is no evidence of pulmonary stenosis. Excretion of 5-HIAA in December, 1955, was 11.1 mg./24 hrs.

Case 17. This patient, a woman of 64 years, first reported to hospital complaining of colicky abdominal pain of 8 months' duration. She presented as a subacute intestinal obstruction. There were no symptoms which might be considered due to excess production of 5-HT at that time.

At laparotomy (Mr. J. Scoular Buchanan in August, 1946) there was a firm tumour constricting the ileum about a foot from the junction with the caecum. Numerous firm glands extended along the mesenteric vessels to the origin of the superior mesenteric artery. There was considerable hypertrophy and distension of the proximal bowel and the tumour had obviously been causing subacute obstruction. Resection of the terminal ileum and caecum was carried out, and end-to-end anastomosis between the ileum and ascending colon effected. It was uncertain whether all the invaded glands had been removed. There were no obvious liver metastases.

Histology. The tumour proved to be a typical argentaffin carcinoma penetrating the muscularis of the bowel and present in the local lymph nodes. Silver and diazo stains were positive.

Progress: The patient has been seen once a year since the oper-:ation. She continued in good health although, in 1951, the liver was palpable/

palpable but apparently smooth. In 1953 she complained of constipation but was otherwise well. The liver remained as before. In April, 1955, slight weight loss was evident and a mass was now definitely palpable in the right hypochondrium. She complained of constipation associated latterly with nocturnal diarrhoea. Ba. enema and meal were negative, but radiography of the left hip showed osteo-arthritic changes and the left femur showed gross changes of Paget's disease. Since April, 1955 she complains of being weaker than before, but objective assessment reveals little change. Estimations of 5-HIAA were done in December, 1955, and January, 1956. The following results were obtained - 17 mg. and 20 mg./24 hrs.

Case 18. This patient, a female, aged 50 years, was first seen at the Out-patient Department of the Southern General Hospital, Glasgow, in October, 1953. Her main complaints were anorexia, nausea and facial flushing. Examination revealed marked weight loss and a rounded mass in the left upper abdomen. At laparotomy (Mr. W. Sillar, in November, 1953) the liver was seen to be grossly enlarged to a massive yellowish white tumour in the right lobe and numerous smaller similar tumours scattered throughout the parenchyma, which was otherwise of normal texture. No primary tumour was found and a biopsy was taken from the liver. This specimen was reported as an anaplastic carcinoma.

The expected deterioration in the patient's condition did not take/

take place. On the contrary, she apparently improved and gained a little weight.

In December, 1954, radiological examination showed a metastasis in the right humerus and the facial colouration was seen to be more intense.

In December, 1955, she sustained fracture of the neck of the left femur; this was apparently a traumatic lesion and healing has been slow but progressive after nailing. During her stay in hospital a diagnosis of metastasizing carcinoid was suggested. The previous histology was reviewed but no specific granules could be demonstrated. On this occasion the urinary 5-HIAA was shown to be 160 mg./24 hr. and the whole blood 5-HT content was 0.48 mg./ml. Biopsy of the metastasis in the humerus was done and it was shown to contain 4 mg. 5-HT per g. wet tissue.

The full clinical details of this case, which presents many points of interest, have been published elsewhere.

Case 19. This patient, a man, aged 58 years, complained of cough with shortness of breath on exertion and attacks of flushing. About 14 years earlier he had an attack of coughing which continued with a production of some purulent sputum. He was off work for three weeks and then felt well again. The cough continued sporadically but never became more severe. Shortness of breath on exertion began just over two years ago, and/

and from this time on he also noticed attacks of flushing. The shortness of breath was noticeable when he walked upstairs and had become more severe in the last few months. He never had attacks at rest. The flushing attacks were particularly noticed in the morning after his breakfast but came at any time during the day and he would even awake at night aware of a flush. The increase in frequency was only gradual and in the period of three months before his death he was getting between six and twelve flushes a day. On observation there were even more minor flushes which he did not himself notice. While at first the flush was confined to his head and neck, in the last two months, it would spread over his shoulders and chest and down both arms in a patchy red fashion. The flush was bright red with sometimes a bluish tint. At no time was there any red and blue mottling. A major flush lasted about 10 minutes. There was no definite history of diarrhoea at any time. On examination the striking features were a perpetually pink to red face and neck, slight oedema of both ankles and brown freckles on both shins up to three or four mm. in diameter (said to have been present only in the last two years) with pulsatile venous congestion in the neck, up to 3" above the sternal angle (this was extremely variable but did not run parallel with his attacks of flushing). The heart size was difficult to determine clin-

:ically, but/

but there was no movement of the chest wall. The left border percussed 4" out in the 5th space. The heart sounds were noted to be presystolic gallop rhythm to the left of the sternum at the bottom, and a systolic murmur loudest at the apex. The blood pressure varied between 160/110 and 210/130.

An x-ray of chest showed that the diameter of the heart was at the upper limit of normal (20/10/55). Chest screening did not suggest the presence of cor pulmonale. Barium meal with follow through showed that a considerable portion of the barium had filled the transverse colon in 45 minutes; no organic abnormality seen. Occult blood in stools was negative on six occasions. The haemoglobin value varied between 90 per cent and 100 per cent, and there were no significant changes in the white cell count at any time during this period of observation. On 16th December, 1955, the plasma proteins were 6.5 g. per 100 ml., albumin 4.1, globulin 2.4, with a normal electrophoretic pattern.

The diagnosis of a carcinoid of the ileum with metastases to the liver was made by demonstrating an excess of 5-hydroxyindoleacetic acid in the urine: when followed daily for some weeks, it varied usually from 200 to 300 mg. per 24 hours.

Observation during an attack showed no change in the pulse rate or blood pressure, but a definite increase in the rate of respiration accompanied the flush. It was decided to attempt a partial hepatectomy when/

when a first laparotomy (Professor C. G. Rob) indicated that the liver metastases seemed to be confined to the left lobe. During the first operation there was difficulty with the respiration; in view of later events this was probably significant. The bronchospasm then encountered was controlled by aminophylline 0.75 g. intravenously. The anaesthetic was nitrous oxide and air.

At a second operation (Professor C. G. Rob) ascites was found to be present and metastases in the right lobe, as well as the large mass in the left lobe of the liver. Just after removing a primary tumour from the ileum the patient developed severe bronchospasm and resisted all attempts at manual inflation. Deep cyanosis appeared and the heart stopped. Cardiac massage restarted the heart. Despite BOL (2 brom-d-lysergic acid diethylamide) the bronchospasm persisted for at least 5 minutes. The patient was returned to the ward with assisted respiration but succumbed 3 days later.

Resume of post-mortem findings: Ileum. Four yellowish primary sessile carcinoid tumours were found on the antimesenteric border of the ileum. No notable lymph nodes were present in the mesentery. Liver. One metastasis was found in the left lobe, about 100 mm. in diameter, necrotic in the centre. Two or three scattered metastases up to 10 mm. in diameter were found in the right lobe, but no other metastases elsewhere. Heart. There was a mild degree of right ventricular hypertrophy, with/

with moderate tricuspid stenosis and thickening of the chordae. Pulmonary valve. The cusp closest to the aorta was thickened and had a rolled edge. This valve, unlike the aortic, was incompetent at post-mortem. No other valve or cardiac defects noted.

Case 22. This patient, a woman aged 53, was admitted to the Southern General Hospital in 1951 (Mr. R. B. Wright). She had a visible and palpable swelling in the right iliac fossa. A right hemi-colectomy was performed removing the tumour but glands were noted which were too extensively involved to be excised.

The patient remained well until 1957 when she began to have attacks of flushing and intermittent diarrhoea. She was admitted to hospital and her urinary 5HIAA was found to be 31.3 mg. Six months later it had risen to 39.7. She received symptomatic treatment. Further readings in 1958 showed levels of 53.2 mg and 63.4. There are no signs of cardiac involvement.

APPENDIX.

11. EXTRACTION OF SUBSTANCE P.

The material used for the preparation of substance P was obtained from the University Veterinary Pathology Department and consisted of small intestine and brain from cattle. While the intestine was available in large enough quantities to allow immediate preparation, it was necessary to collect the brains in a frozen state until the requisite quantity had been obtained. The frozen brains were immersed in boiling water, since experience had shown that thawing at room temperature led to a rapid decrease of activity. The active substances from brain and intestine tissue were treated separately throughout the purification process.

Extraction of the active substance and the initial purification by precipitation with ammonium sulphate were done, in principle, according to the method reported by Euler (1942).

The tissue was freed from fat, and cut in pieces; the intestines were also rinsed in cold water. Extraction was then done at 100°C with 2 volumes of distilled water, acidified to pH 4 with hydrochloric acid. The extraction, which lasted ten minutes, was repeated once. The pH level was carefully checked during boiling. After filtration the filtrates were combined and concentrated in vacuo at 25°C until 1 ml was/

was equivalent to 10 gm tissue. To the concentrated extract was then added 2 volumes of 95 per cent ethanol. After 12 hours at +3°C, a large quantity of inactive proteins had precipitated and was filtered off. The filtrate was concentrated again in vacuo, this time to 1 ml per 20 gm tissue and the procedure repeated with 3 volumes of 95 per cent ethanol which precipitated further inactive material. The filtrate was again concentrated in vacuo. To the clear aqueous extract was added, after adjusting the pH to 4, ammonium sulphate up to 70 per cent saturation under stirring. Previously the ammonium sulphate had been cooled down to about 0°C. The precipitate was left to develop over night at +3°C, then filtered off, carefully pressed between filter paper, and further dried in vacuo.

This mode of preparation yielded 900,000 units in 300 gm dry powder per 100 kg intestine (3 units per mg). Twenty kilograms of brain tissue yielded 140,000 units in 55 gm dried powder(2.5 units pr mg.).

saturated ammonium chloride solution a concentration of about 50 per cent was obtained. A precipitate formed. After 1 - 2 hours the material was centrifuged at high speed for about 15 minutes. The supernatant fluid

APPENDIX.

12. PREPARATION OF GASTRIN (UVNAS, 1945).

I. Extraction with HCl. The pyloric portions of stomachs from recently killed pigs were kept on ice and carried from the slaughter-house to the laboratory within 1 - 2 hours. The stomachs were washed under running tap-water and the mucosa removed. It was ground in a mincing-machine and then thrown down into boiling N/10 HCl. 200 ml. HCl were used per stomach. After boiling for 15 - 20 minutes the material was left at room temperature over night.

II. Precipitation of inert material at pH 3 - 4. The following day the mucosal fragments were removed by filtering through gauze. By adding N NaOH the mixture was brought to an acidity of pH 3 - 4. A precipitate containing inert material was removed by centrifuging for 10 minutes. Care must be taken not to exceed pH 4, active material then being removed with the inactive. The centrifuge was filtered through cotton wool to remove fatty substances floating on the fluid.

III. Precipitation with NaCl. Sodium chloride was dissolved in the filtrate to give a concentration of 10%. By adding the same volume of saturated sodium chloride solution a concentration of about 20 per cent was obtained. A precipitate formed. After 1 - 2 hours the material was centrifuged at high speed for about 10 minutes. The supernatant fluid/

fluid was removed and the sediment collected in a glass vessel. To remove as much water as possible the precipitate was centrifuged once more for 30 minutes. It was then washed several times with acetone in great excess. During this procedure the precipitate acquired a tenacious consistency and stuck to the vessel walls. The washing had to be repeated until this consistency had quite disappeared and the material had become quite dry and crackled like sand against the vessel walls.

IV. Removal of inert material by dissolving in N/10 HCl at 60°C.
The dried NaCl-precipitate was suspended in N/10 HCl, 700 ml being used for a quantity of dry material corresponding to 15 stomachs. During frequent stirring for 30 minutes at 60°C. considerable amounts of inactive material went into solution. The active material remained undissolved. It was separated from the solution by centrifugation and dissolved in 1,000 ml N/10 HCl at 100°. The solution was cooled to room temperature and was then brought to an acidity of pH 3 - 4 by adding N NaOH. A precipitate formed containing inert material, which was removed by centrifugation.

V. Precipitation with tannic acid. 5 ml. of a 5 per cent tannic acid solution per stomach were added to the centrifugate. A heavy precipitate appeared, which was separated by centrifugation during five minutes. The precipitate was very difficult to get dry in acetone. The washing had to be repeated numerous times. The material was finally washed twice in ether.

VI/

VI. Precipitation of inert material at pH 8. The dried tannic acid precipitate was dissolved in a 0.9% solution of NaCl at 40°C. 1,000 ml. saline were used for an amount of dry material corresponding to 15 stomachs. The saline had to be added successively under frequent stirring or otherwise the material did not go into solution. N NaOH was added until a pH of 8 was reached. A precipitate formed, which after about an hour was removed by centrifuging.

VII. Isoelectrical precipitation of active material. To the centri-:fugate obtained as described above N HCl was added. Between a pH of 4.0 - 5.5 a precipitate formed containing active material. After adjusting the pH to the desired value the acid solution was allowed to stand at a temperature of 8 - 10°C. for 24 hours. The precipitate was then centrifuged off, washed several times in acetone, twice in ether, and dried in air. A complete flocculation of the active material was obtained at a pH of about 4.4. A more selective precipitation was obtained at a pH of about 5.0, the preparations being more active. At this pH, however, a loss of active material up to 50 per cent occurred. By adding small amounts of $CuSO_4$, 1 - 2 mg per 100 ml, most of the remaining active material could be precipitated.

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"When a man hath finished then he is but at the beginning; and when he ceaseth then shall he be in perplexity."

Ecclesiasticus XVIII, 7.