PHARMACOLOGICAL ACTIONS OF URINE EXTRACTS

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PHARMACOLOGICAL INVESTIGATIONS OF BIOLOGICALLY ACTIVE URINE EXTRACTS

by

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INTRODUCTION

The presence of a substance(s) in extracts of eosinophil leucocyte suspensions with antihistamine-like action was first reported in 1950 (Kovacs, 1950).

In the past 16 years these findings have been confirmed and considerably extended (Vercauteren, 1953; Archer, 1963; Francis *et al.*, 1963 and Kovacs *et al.* 1963). Recently it was observed that extracts of horse eosinophils can antagonize serotonin and bradykinin-induced wheal formation as well as histamine-induced bronchoconstriction (Archer, 1963). It has also been reported (Kovacs and Melville, 1962, 1963) that extracts of human and animal urine exert similar activities and can antagonize the effects of histamine, serotonin and bradykinin not only *in vitro* but also *in vivo*.

This wide range of activity suggested that the biological actions of tissue and urine extracts differ basically from actions exerted by synthetic antihistamines. In the light of these observations it was therefore of interest to investigate the possible action of urine extract under experimental conditions in which synthetic antihistamines are ineffective, for example, histamine-induced gastric acid hyper-secretion.

The experiments described in this work deal with the effects of partially purified urine extracts, obtained from fractions of pregnant

mare urine, on histamine and gastrin-induced gastric acid hypersecretion. Furthermore, the action of the extract was investigated on experimentally-induced ulcer formation.

In the course of these experiments it became evident that the partially purified urine extract is capable of inducing definite actions on the central nervous system and the latter part of the results, presented in this thesis describe these effects. During these studies drugs eliciting a central effect were also used in an effort to establish the possible site of action of the urine extract.

HISTORICAL REVIEW

A. GENERAL INTRODUCTION TO GASTRIC PHYSIOLOGY

The two main functions of the stomach are muscular and secretory. The muscular function allows the stomach to act as a reservoir to retain the ingested food long enough for digestive activity and then to expel the resulting chyme in small amounts through the pyloric canal into the duodenum.

The digestive power of the stomach is based upon its secretory products, which form the gastric juice. The chemical composition of gastric juice is rather complex, containing most of the time free hydrochloric acid (further referred to as HCl), a mixture of all the inorganic ions, found in other body fluids and a number of organic constituents. Included among the latter are several enzymes and their precursors, mucin and intrinsic factor.

There are two different types of glands in the stomach, the gastric glands and the pyloric glands. The gastric glands are located in the corpus of the stomach, while the pyloric glands are located in the antral portion of the stomach. A few cardiac glands, which are almost identical with the pyloric glands, are located immediately beneath the cardia.

Different cell types are found at the surface of the mucosa and in the neck portion and body of a gastric gland (Myren, 1963). The mucosal surface and the neck portion of the glands are covered by mucous-producing cells. The surface cells produce the unsoluble mucous, which covers and protects the mucosal surface. The neck cells produce a fluid mucous containing muco- and glyco-proteins. In contrast to the surface cells they respond to histamine stimulation.

In the body of gastric glands are the chief cells which secrete

.pepsinogen, the inactive precursor of pepsin.

Acid is secreted by the parietal cells, which are located both in the body and neck of these glands (Hollander, 1952; Myren, 1963). The pyloric and cardiac glands do not produce acid.

The stomach is the only organ which produces large quantities of a strong acid. The acidity of the gastric juice varies; the higher the secretory rate the greater the acidity and the lower the pH.

The composition of gastric juice changes, depending on whether the stomach is at rest, or whether it secretes in response to a meal or other stimuli.

Ivy et al. (1950) divided the secretion of HCl into the following periods and phases:

Period I. Interdigestive secretion.

Period II. Digestive secretion.

Phase 1. The cephalic phase

a) unconditional reflexes

b) conditional reflexes

Phase 2. The gastric phase

a) mechanical distention

b) secretagogues

Phase 3. The intestinal phase

a) secretagogues

This subdivision, however, proved to be a rather rigid classification, which did not take into consideration the interplay between phases.

On the basis of experimental evidence, which was discussed by several investigators, and which will be presented in detail, pure vagal (cephalic phase) or pure antral (gastric phase) mechanisms do not exist in the intact stomach.

In recent years a new scheme was presented by Perey (1963) in which the "parietal cell mass" is the central factor (Fig. 1) upon which the vagus, gastrin, histamine and local nerve reflexes act.

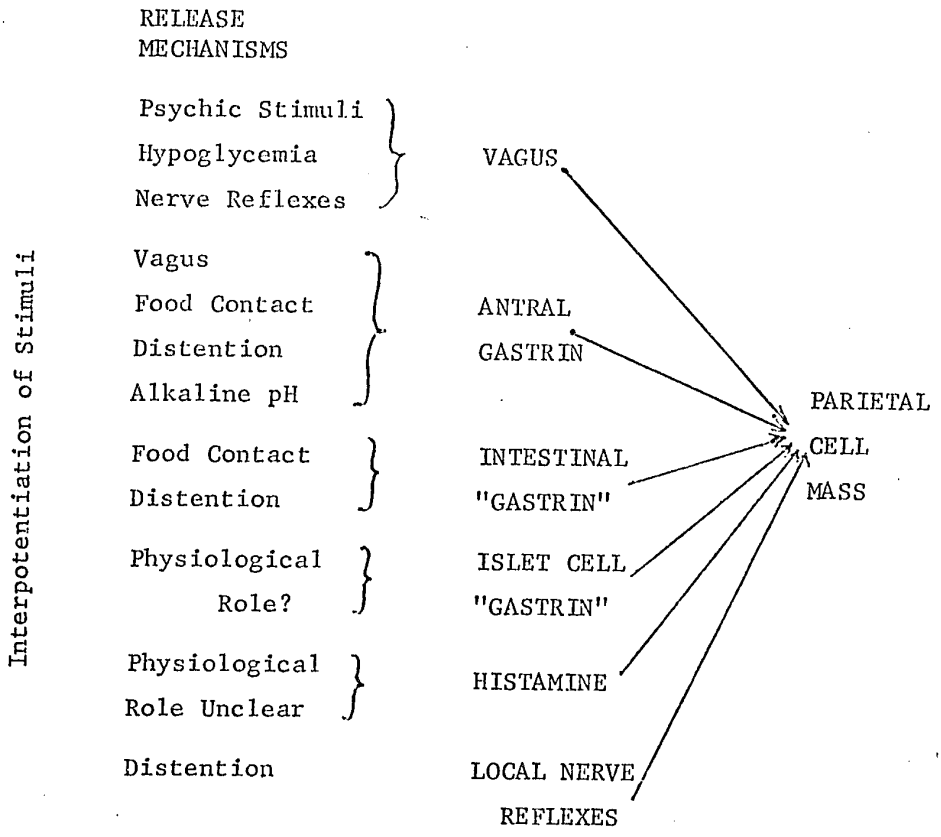


Fig. 1.

The scheme offered by Perey involves the action of three agents on the parietal cell mass. They are all present in the mucosa of the stomach

and are all very potent in stimulating gastric acid secretion. The substances are: acetylcholine, histamine and gastrin.

Vagal activity is still the most important single mechanism in the secretory response to meals. The vagus nerves constitute the entire cranial outflow of the parasympathetic nervous system. Its anatomic relationship to the stomach is a dual neuron system, with a central and a peripheral ganglion, the latter located in the wall of the stomach (Woodward and Nyhus, 1960). The parasympathetic system is cholinergic, with acetylcholine as the chemo-effector. Both the secretory and motor effect of the vagus on the stomach can be duplicated by exogenously given acetylcholine.

The role of the vagus was established by Pavlov in 1889. He prepared a dog with cervical esophagostomy and gastric fistula. When the animal was fed (sham feeding) the food escaped through the opening in the neck. Sham feeding was followed by a copious flow of highly acidic juice from the gastric fistula. The mere sight, smell, thought or taste of appetizing food will elicit secretion of gastric juice. After section of the vagi, sham feeding will not be followed by a gastric secretory response.

Gastric secretion can also be activated through stimulation of the vagus, by insulin-induced hypoglycemia. The response is abolished by vagotomy.

Nerve reflexes also stimulate gastric secretion through the vagus. The afferent stimuli originate from mechano- and chemoreceptors in the stomach.

Vagal stimulation activates both the acid and the enzyme-forming

.cells; in fact a high enzyme activity generally serves to identify a specimen as vagal or vagomimetic in origin. Electrical and pharmacological stimulation of the vagus trunks evokes the output of viscous mucous also, but only if the intensity of stimulus is low. Otherwise the secretory product is typically fluid with high acidity and pepsin activity (Hollander, 1952).

The vagus also releases much of antral gastrin and potentiates markedly the effect of all types of stimuli on the parietal cells.

B. STIMULATION OF GASTRIC ACID SECRETION

In many of our experiments either histamine or gastrin served as stimulants to induce gastric acid hypersecretion and accordingly a more detailed review of their history -in connection to acid secretion- will be given.

Whether histamine is the final, local, common, chemostimulator of parietal cell is a much debated question that has still not been fully answered. In the present review we will try to give an account of experiments, which in most cases are for, but in some cases against this theory.

I. Histamine

1. Effect of Histamine on Gastric Acid Secretion

It is well known that the parenteral administration of histamine causes a marked stimulation of gastric acid secretion in most species.

This effect of histamine was first observed by Popielski (1920)

and by Keeton et al. (1920). Popielski described the effect of β-imidazolylethylamine or " β-i", and stated that a strongly acidic gastric juice was obtained in dogs, after a s.c. injection of histamine, with no observable side effects. However, he did not observe any augmentation of gastric secretion, when histamine was injected intravenously. From the evidence that increased gastric secretion in response to histamine was still present after cutting the vagi or administering atropine, Popielski concluded that histamine acts directly on secretory glands and not through nervous stimulation. Keeton et al. (1920) established the gastric secretory effect of histamine in several species, including that of the dog, cat, rabbit, duck and frog. In most species peak secretion occurred within the first hour.

Ivy et al. (1923) were the first to show that histamine is also effective after oral administration. 50 mg histamine was sufficient to provoke gastric secretion in a Pavlov pouch dog, while 150 mg histamine produced a gastric secretion that was qualitatively and quantitatively equivalent to secretion provoked by a meal of meat. Although these were large doses, no toxic side effects were noted.

Though Popielski (1920) failed to obtain gastric acid hypersecretion in response to i.v. histamine, Ivy and Javois (1925) had obtained positive results. They showed that low concentrations of histamine infused at a slow rate definitely stimulated acid secretion in dogs, provided with Pavlov pouches.

The fact, that histamine acts directly upon secretory cells in the gastric mucosa was shown by Ivy and Farrell (1925). They transplanted

. fundic pouches into s.c. mammary glands of female dogs, and obtained an increase in both free HCl and total acid in response to histamine, although the latent period was longer than that observed in Heidenhain pouch dogs.

There are only few species which are relatively insensitive to histamine. One of them is the rat (Friedman, 1953) in which doses as high as 3.5 - 5.5 mg/kg fail to stimulate an increase in gastric acid secretion. Kaplan (1947) made similar observations in mice using the pylorus ligation method. However, Davenport and Chavré (1950) were able to demonstrate in vitro an increase in acid secretion by the isolated mouse stomach, if histamine was placed into the incubating medium in sufficiently large concentrations.

The amounts of histamine which can induce gastric acid secretion are exceedingly small, according to Hanson et al. (1948) and Öbrink (1948). Hanson et al. (1948) established that the minimal effective s.c. dose of histamine is 0.03 µg/kg/min, both in intact dogs and dogs with denervated pouches, while the minimal effective i.v. dose is 0.045 µg/kg/min. Man is highly sensitive to histamine; the continuous i.v. administration of 0.004 µg/kg/min produces an increase in gastric acid secretion. According to Öbrink (1948), there is no threshold value for the effect of histamine on gastric acid secretion, i.e. the slightest amount of histamine will initiate secretion. This applies, however, only to exogenous histamine, because histamine can be found in the blood of most species (Rocha e Silva, 1955a) which has no secretory effect. On the other hand extremely large amounts of histamine (100 mg) are required, to induce acid secretion when the drug is topically applied (Lim et al. 1925).

The characteristics of the secretory response to histamine are the following: after the s.c. administration of an average dose (0.5 - 1.0 mg in the dog or cat, also in humans), the volume and acidity of the gastric juice begins to rise above the basal level after a latent period of up to 15 minutes. There is a rapid climb in the volume, the rise in acid secretion is somewhat more gradual; similarly the volume will first decline to basal values, while the curve of total acid output shows a slower down-slope (Ivy and Bachrach, 1966).

2. Effect of Histamine on Pepsin Secretion

The effect of histamine on the body chief cells, which elaborate pepsin, has been the subject of much controversy. Babkin (1950) stated that the initial, relatively high pepsin content of gastric juice following the administration of histamine is due to the "washing-out" of the enzyme from the glandular tubules, where presumably it accumulates while the glandular cell is at rest. In the absence of a stimulation the parietal cells practically cease to secrete, whereas the peptic cells continue to elaborate zymogen granules. Babkin (1950) cited, among others, the experiments of Bowie and Vineberg (1935). These workers stained pepsinogen granules in the cells of the gastric mucosa of dog, prior to and following stimulation with histamine and stimulation through the vagi. When sections of control and stimulated mucosa were compared it could be seen that the quantity of granular pepsinogen in the cells did not diminish during prolonged stimulation with histamine, while during vagal stimulation they have disappeared.

Code (1956) also concluded that "the predominant, if not the entire

·secretory effect of histamine on the gastric mucosa is one of stimulation of the production of acid".

Long and Brooks (1965) compared gastric acid and pepsin secretion in response to histamine, insulin hypoglycemia and feeding in dogs, having vagally innervated gastric pouches. They concluded that while the greatest acid output occurred after a maximal dose of histamine, the greatest pepsin output was induced by stimulation with insulin. A submaximal dose of histamine elicited a persisting pepsin secretion, while the maximal dose resulted in lower pepsin output, being therefore a relative inhibitor of pepsin secretion. However, the pepsin output following even the submaximal dose of histamine was less than pepsin values observed after stimulation either through insulin hypoglycemia or feeding.

3. Presence of Histamine in Gastric Mucosa

Studies by Gavin et al. (1933) have revealed that histamine is present in large amounts in the gastric mucosa of normal dogs. They showed that 80% of the total amount of histamine found in the stomach is in the fundic mucosa.

Hollander (1952) established that the parietal cells are concentrated in the neck of the gastric glands. This is also one of the main areas where the mucosal histamine concentration is the largest, as shown by Feldberg and Harris (1953). They prepared horizontal plane serial sections of both the mucosa and submucosa, which were assayed for their histamine content, and examined microscopically. Their work revealed that the body of the stomach contains two histamine peaks, one is near the lumen

.in the region of the maximal concentration of parietal cells, while the other peak occurs in the muscularis mucosae. Feldberg and Harris (1953) performed similar experiments along the G.I. tract and found relatively high histamine content throughout it, although the overall histamine content decreased from stomach towards the colon.

Quantitative differences in histamine distribution between fundic mucosa and antral mucosa were documented by Stubrin et al. (1965) in the dog.

Murray and Wyllie (1964) found however, no differences in histamine distribution in human stomachs. They also noted that in man, as distinct from the dog, the muscularis mucosae is not particularly rich in histamine.

Histamine is present in large concentrations in the mast cells in most organs, but there is a large fraction of histamine in the stomach that does not originate from this source. Riley and West (1956) determined the mast cell content and histamine content of both mucosal and submucosal tissue, and found that while there is a rapid fall in mast cell content in the mucosa compared to the submucosa, no corresponding fall occurs in tissue histamine concentration. They found an almost four-fold difference between histamine and mast cell ratio in submucosa and mucosa.

4. Relationship between Gastric Histamine Content and Gastric Acid Secretion

Recent work by Shore (1965) and Haverback et al. (1965) have revealed, that there is a close relationship in the rat, between histamine levels in the stomach and acid secretion.

Shore (1965) demonstrated, that reserpine administration or insulin induced hypoglycemia, both acting through central vagal stimulation, lowered the histamine levels in the stomach in association with gastric acid secretion. Vagotomy blocked both histamine release and acid secretion.

Similar observation were described by Haverback et al. (1965) inducing acid hypersecretion by gastrin, Urecholine and betazole hydrochloride (Histalog). Kahlson and Rosengren (1965) showed, that when fasting rats are fed, or when gastrin is injected into rats, histamine is liberated from preformed mucosal stores, and the mucosal histamine forming capacity is greatly increased.

5. Maximal Acid Output and its Relation to Parietal Cell Population

Popielski (1920), was the first who observed that larger doses of histamine, administered subcutaneously, gave a response which could be characterized by: (a) higher volume of secreted gastric juice, (b) higher acidity, and (c) longer duration, compared to a response that was elicited by a smaller dose of histamine.

However, to see the secretory capacity of the stomach, with regard to "maximal", "submaximal" and "supramaximal" secretory responses the secretory maximum has to be determined.

Öbrink (1946) found that about 1.7 µg/kg/min of histamine, administered intravenously produced maximal acid secretion in a Heidenhain pouch.

Minimal and maximal histamine doses were also determined by Hanson et al. (1948) in intact dogs and in dogs provided with a vagotomized pouch.

Kay (1953) was the first who established a method in human subjects, by which the maximal secretory capacity of the stomach could be determined. He administered subcutaneously single and multiple body weight doses of histamine (one body weight dose refers to 0.1 mg histamine acid phosphate/10 kg body weight) and observed the response, which was dose-dependent. The administration of four body weight doses of histamine resulted in maximal HCl output. If the dose of histamine was further increased no appreciable change in acid output occurred. Kay suggested, that with increasing doses of histamine, increasing number of parietal cells are stimulated, until the entire parietal cell population is functioning.

The dose-response curves were S-shaped and the usual interpretation of such curves is a relationship of the "all or none" nature, i.e. there is no secretion by the parietal cell until an effective stimulus is applied, then the cell secretes maximally. This observation is not in agreement with that of Öbrink (1948), who stated that there is no threshold value for the effect of histamine on gastric acid secretion.

Adam et al. (1954) also described the secretory maximum as the most important parameter for defining a secretory response. Although the secretory maximum implies that all acid secreting cells have been stimulated, the maximum could vary under conditions which enhance or depress the rate of acid formation, but which do not alter the number of secreting cells.

The maximal acid secretory response to a given dose of histamine is related to the number of parietal cells both in man and dog, as was shown by several workers.

Human subjects with a proven gastro-intestinal disease were

observed by Card and Marks (1960). They found a highly significant correlation between the maximal acid output and the number of parietal cells of the resected stomach.

Ragins et al. (1957) studied the secretory characteristics of total innervated fundic pouches of the stomach in dogs by three methods: (a) the 24-hour collection, (b) the response to a standard insulin test, and (c) the response to a standard histamine test.

The pouches were resected in three stages proceeding towards the cardiac end. The nerve supply was kept intact. Secretion was studied after each operation. An almost direct relationship was found to exist between the amount of fundic mucosa present and the 24-hour daily gastric secretion.

Marks et al. (1958) determined maximal histamine response and parietal cell mass in acute experiments on anesthetized dogs, and in a later study in non-anesthetized, trained dogs (Marks et al. 1960). They concluded that maximal histamine response is a linear function of the total number of parietal cells.

II. Gastrin

The existence of a gastric secretory hormone in the antral mucosa was first postulated by Edkins (1905, 1906). He prepared extracts from pyloric and fundic mucosa and observed, that the i.v. injection of the pyloric extract led to an increased secretion of gastric juice, while the fundic extract did not possess this property. Edkins suggested that the substance, which occurs in the cells of the pyloric mucosa, passes into the blood and later stimulates the secretory cells of the stomach.

The gastrin supposition was subject to controversy during the next 40 years. Edkins' work was criticized on the grounds that the response observed was due to the presence of histamine.

New interest in gastrin started by the work of Komarov (1938, 1942), who recognized that the antral hormone might be of protein nature. According to his method, gastrin was precipitated by trichloracetic acid from an acid aqueous extract of antral mucosa and it was free from histamine. Upon i.v. injection it elicited an increased secretion of gastric juice, which was rich in acid and had low peptic activity. The effect was elicited only by extracts of the pyloric mucosa, and to a much smaller extent by extracts of the proximal duodenal mucosa. Uvnäs (1943) confirmed Komarov's results, but could not observe any activity in extracts from the duodenal mucosa.

In the last decade, Gregory and Tracy (1961) have devoted much effort to the extraction and identification of gastrin. Recently they reported the isolation of two gastrins from hog antral mucosa, and evaluated their physiological properties (Gregory and Tracy, 1964, 1966). Porcine gastrins, namely gastrin I and gastrin II, are heptadecapeptides, with identical amino acid constitution and sequence. The only difference between the 2 gastrins is that gastrin II contains a sulphate group attached in an ester linkage to the tyrosine residue.

Out of the seventeen amino acid residues of the total molecule, only the C-terminal tetrapeptide: Try. Met. Asp. Phe. NH_2 is required for the full range of physiological activity, but the amide group is of vital significance.

The inactivation of gastrin is not yet known, but possible routes of metabolism are (a) removal of the C-terminal amide group, or (b) oxidation of the S in the methionine residue. The action of gastrin on gastric secretion is as follows: a single s.c. injection, in the range of 0.75 - 2.5 µg/kg, given to a conscious dog provided with a denervated fundic pouch, will stimulate the secretion of a highly acidic gastric juice containing little pepsin. The magnitude of the response is proportional to the dose. However, high doses of gastrin inhibit acid secretion, but stimulate pepsin secretion.

Gastrin I and gastrin II are many times more potent than histamine (Gregory and Tracy, 1964) in stimulating gastric acid secretion both in man and dog. For example, a single s.c. injection of 20 µg of gastrin I in a conscious dog, will give a total acid output of 2.3 mEq HCl, compared to 1.2 mEq HCl obtained in the same preparation after the injection of 360 µg of histamine.

Makhlouf et al. (1964) found that the s.c. administration of gastrin II in a dose of 0.5 µg/kg, and of histamine in a dose of 40 µg/kg to normal subjects and ulcer patients, elicited acid secretion which resulted in essentially equal peak values. They also noted a slower rise of secretion with gastrin as compared with histamine. In an attempt to establish the dose of s.c. gastrin that produces a maximal response, increasing graded doses of gastrin up to 4 µg/kg were given to three patients. They found that responses to 3 and 4 µg/kg subcutaneously were similar to the responses obtained from 2 µg/kg subcutaneously. Thus, 2 µg/kg appears to represent the s.c. dose of gastrin II that produces a maximal secretory response.

If the effectiveness, in terms of equal response of gastrin to histamine base, are compared weight for weight, it seems that gastrin is some 30 times more effective. If the comparison of gastrin to histamine is made on a molar basis, then it seems that gastrin is some 500 times more effective than histamine.

Semb and Myren (1965) also found that 2 µg/kg of gastrin LEO (which is a mixture of gastrin I and II by Gregory) elicited maximal acid secretion in human subjects.

III. Influence of Cholinergic Stimuli on Gastric Secretory Responses to Histamine and Gastrin

As mentioned earlier, acetylcholine, histamine and gastrin are three potent stimulants of gastric acid secretion.

While gastrin and histamine potentiate each others effect (Passaro et al., 1963), both will be potentiated by cholinergic stimuli although to different degrees.

If parasympathomimetic drugs are given in stimulating doses during a secretory response to histamine, or if histamine is superimposed on parasympathomimetic stimulation, an augmentation of secretion is observed.

Necheles et al. (1938) reported that both histamine and acetylcholine or histamine and acetyl-β-methyl-choline act synergistically in Heidenhain-pouch dogs. However, a significant depression of secretion occurs when the administration of either drug is discontinued.

Robertson and Grossman (1948) observed a marked increase in acid production when subthreshold doses of urecholine or prostigmine were super-

imposed on a steady secretory rate established by histamine. The experiments were performed in dogs provided with a vagotomized pouch of the entire stomach.

Grossman (1961) determined the maximal rate of acid secretion in response to both gastrin and histamine. The stimulants were administered subcutaneously every 15 minutes to dogs with totally denervated fundic pouches. When mechothane was also administered every 15 minutes for 3 hours prior to and during stimulation with either gastrin or histamine, an augmented response was measured.

The maximal rate of secretion in response to the combined administration of gastrin and mechothane was 2-8 times higher than that achieved without mechothane. However, the maximal rate of acid secretion in response to histamine and mechothane was only 1.2 - 1.4 times higher than observed without mechothane. The author concluded, that cholinergic stimulation potentiates the action of gastrin more than the action of histamine.

Gillespie and Grossman (1964) noted that the pattern of potentiation differed between urecholine and gastrin, and between urecholine and histamine in two respects: (a) the maximal potentiation was greater between urecholine and gastrin, and (b) the dose of urecholine to produce maximal potentiation was more critical in the case of gastrin than of histamine. While the authors observed equal maximal acid secretory response to both gastrin and histamine in vagally innervated pouches, in Heidenhain-pouch dogs the maximal response to gastrin was lower, indicating a decrease in cholinergic tone, to which gastrin is more sensitive than histamine.

Marks et al. (1966) investigated the influence of cholinergic stimuli on gastric secretory responses to histamine in dogs, which were

provided with a vagally innervated gastric fistula. The authors first determined the dose of histamine which gave a maximal acid response and then combined histamine with pharmacological vagal stimulation (mecholyl) or physiological vagal stimulation (sham feeding). They noted that the maximal acid output, in response to combined histamine and cholinergic stimulation was greater than to histamine alone.

This finding, however, was not considered as a direct evidence against the view, that histamine is the final, common, local chemostimulator of the parietal cells in the gastric mucosa. The authors suggest two possible mechanisms to explain their observations: (a) cholinergic activity may increase sentivity of the parietal cells to histamine, or (b) vagal stimulation may release sufficiently large amounts of histamine in the vicinity of the parietal cell membrane which will augment the response to maximal histamine stimulation.

C. INHIBITION OF GASTRIC ACID SECRETION

Gastric secretory inhibition may be classified as physiological, pharmacological and pathological (Code, 1951). The pathological gastric secretory inhibition is not within the scope of this review. However, some aspects of physiological and pharmacological gastric secretory inhibition will be discussed.

I. Physiological Gastric Secretory Inhibition

1. Effect of Vagal Innervation on Histamine or Gastrin-induced Gastric Acid Hypersecretion

(a) Histamine: It is generally known that vagotomy decreases

the gastric secretory response to histamine. However, the extent of the reduction depends upon the extent to which the vagi are sectioned, i.e. whether the entire stomach or only the pouch is vagotomized.

The early observations of Popielski (1920) and Keeton et al. (1920) showed, that histamine was able to increase acid secretion after sectioning the vagi, however, no quantitative comparisons were made.

Oberhelman and Dragstedt (1948) observed a significant decrease in both volume and acid output in response to histamine after vagotomizing innervated total gastric fistulas. The authors noted similar effects in peptic ulcer patients, in whom vagotomy markedly reduced the gastric secretory response to a standard dose of histamine.

Vagotomy produced a similar decrease in acid secretion in response to the augmented histamine test in ulcer patients, as observed by Gillespie et al. (1960).

Muren (1959) however, found no change or only small reductions in gastric acid secretion in dogs, in response to histamine, when the Pavlov pouch was transformed into a Heidenhain pouch.

Similar results were described by Anderson and Olbe (1964), who investigated acid output in response to increasing doses of histamine. The authors noted no difference in acid output between Pavlov and Heidenhain pouches in response to a given dose of histamine.

In the experiments described by Muren (1959), and also by Andersson and Olbe (1964), vagal denervation was limited to the pouch, while the rest of the stomach, including the antrum was innervated. This meant that (a) sub-

threshold vagal impulses were continuously reaching the parietal cells and (b) continuous vagal impulses released antral gastrin (Thein and Schofield, 1959; Nyhus et al., 1962; Olbe, 1963) which is known to potentiate the effect of histamine (Passaro et al., 1963).

However, if the vagotomy included not only the pouch, but the main stomach as well (Oberhelman and Dragstedt, 1948; Gillespie et al. 1960), a marked reduction of the histamine-induced secretion was observed. Gillespie's work also showed, that the same reduction in acid secretion was obtained when the pyloric gland area was surgically removed.

Similar results were claimed by Andersson and Grossman (1966). Increasing acid responses were obtained by the administration of increasing doses of histamine in dogs, which were provided both with Heidenhain and innervated antral pouches. When the antral pouch was either denervated or resected, the acid response to all doses of histamine was markedly reduced. However, no differences were noted between the denervated and resected responses.

(b) Gastrin: As it was indicated in the foregoing references gastric acid responses to endogenous gastrin are greatly dependent upon intact vagal innervation; although to a lesser extent gastrin can be released in response to local mechanical or chemical stimuli, which are independent of innervation.

The physiological significance of vagal release of gastrin was not accepted for a long period of time, because of the observation that in dogs, provided with innervated antrum and denervated fundic pouch, neither sham feeding nor insulin could induce acid secretion from the pouch. If

gastrin were released from the innervated antrum into the blood stream, it should elicit acid secretion from a denervated pouch.

With more sophisticated techniques Thein and Schofield (1959) showed that central vagal activation (sham feeding) produces a slight but definite acid secretion in Heidenhain pouches. Their preparation consisted of a Heidenhain pouch and an innervated antral pouch, the latter was separated by a mucosal septum from the main stomach. The mucosal septum ensured the avoidance of acidification of the antrum, which is known to block gastrin release. Under the conditions described, sham feeding increased acid secretion. If however, they acidified or denervated the antral pouch, the response was completely abolished. Their work established: (a) the antrum as the principal source of vagally-released gastrin, and (b) the role of the vagus in liberating gastrin.

Nyhus et al. (1962) demonstrated, that gastrin can be released from the antrum through (a) vagal stimulation (insulin hypoglycemia), (b) mechanical stimulation (pressure via inflated balloon), and (c) chemical stimulation (10% alcohol). These investigators also showed that antro-neurolysis, i.e. the separation of antral mucosa from submucosa, which disrupts intramural nerve connections caused a 20 to 80% drop in Heidenhain-pouch secretion, indicating that gastrin released by vagal stimulation represents the most significant portion of the total released gastrin. The chemical and mechanical release of gastrin was largely undisturbed by this procedure. Olbe (1963) showed that gastrin released by mechanical means from a denervated antrum and physiological vagal excitation (sham feeding) act in a synergistic manner in Pavlov pouch dogs.

Uvnäs et al. (1966) reported that electrical stimulation of the vagus for 4 hours (20 impulses/sec) resulted in a significant reduction of the gastrin content of the antrum in cats.

Several workers showed that the gastric secretory response to either exogenous or endogenous gastrin was drastically reduced after vagotomy.

Andersson and Olbe (1964) determined acid output in response to graded doses of gastrin in Pavlov-pouch dogs. Each dose of gastrin elicited a much smaller response, when the Pavlov pouch was transformed into a Heidenhain pouch. In the presence of cholinergic stimulus (carbachol) the response to gastrin could be increased and the acid output from the Heidenhain pouch became almost equal to the acid output from the Pavlov pouch.

Passaro and Grossman (1964) obtained almost equal maximal acid responses to histamine and gastrin, in vagally innervated gastric fistulas, while in a denervated pouch the maximal secretion was always significantly lower in response to gastrin. They assumed that the effects of vagotomy are attributable to a decrease in cholinergic activity, which causes little or no interference with histamine, but markedly impairs the responsiveness of the parietal cell to gastrin.

Baron et al. (1965) observed a "paradoxical" increase in acid output, in response to food, alcohol or histamine in a Heidenhain pouch, following transthoracic vagotomy of the main stomach. This was due, according to their postulation, to the following effects: - the parietal cells of the main stomach are less sensitive to histamine in the absence of a "background" cholinergic stimuli. Accordingly, less acid is produced, raising antral pH,

that will increase gastrin release, which in turn acts on the parietal cells of the Heidenhain pouch.

De La Rosa et al. (1966) induced acid secretion in response to endogenous gastrin. The dogs were provided with a Pavlov pouch and an innervated antrum, separated from the corpus. Infusion of a liver solution into the antrum, liberated endogenous gastrin, that stimulated acid secretion from the Pavlov pouch. After vagotomy of the pouch, the response to the same stimulus decreased by 50%.

The abolished or strongly reduced secretory response to vagal impulses after curtailment of antral function, as well as the low sensitivity of the vagus denervated fundic mucose to exogenous or endogenous gastrin illustrate the inability of the nervous and humoral mechanisms to operate effectively and independently of each other.

2. Enterogastrone

Enterogastrone is a hormone, which is released mainly by the mucosa of the upper, small intestine, when an adequate concentration of fat is in contact with the mucosa.

The fact that the ingestion of fat inhibits gastric secretion, was demonstrated as early as 1886 by Ewald and Boas (Code 1951).

However, Farrell and Ivy (1926) were the first to show that the effects induced by fat ingestion were humoral in nature. They fed olive oil to a dog with a subcutaneously autotransplanted pouch of the fundic portion of the stomach, which inhibited the motor activity of the pouch. Thus they established that nervous connections between the duodenum

and stomach were not necessary for this effect.

The inhibitory effect of enterogastrone on gastric acid secretion was first demonstrated by Kosaka and Lim (1930), who also suggested the name of the hormone. These investigators ligated the small intestine of an anesthetized dog, in 10 cm segments, and introduced oil into alternate segments. Extracts were prepared both from exposed and unexposed mucosal segments and injected into Heidenhain-pouch dogs, which were secreting in response to a meat meal.

Extract prepared from those segments, which were exposed to oil inhibited acid secretion, while the extract prepared from the unexposed segments was inactive. They also demonstrated that the inhibitory effect of the extract was removed when it was heated for 10 minutes at 80-100°C.

Recently, Kaulbersz and Konturek (1962) compared the gastric inhibitory potency of various sections of dog and hog intestine. Constant gastric secretion was maintained by subcutaneous doses of histamine. Enterogastrone derived from the initial parts of the small intestine elicited the strongest inhibition during the first hour of stimulation. Enterogastrone extracted from the terminal ileum and of the large intestine was a more potent inhibitor in the second hour. Extracts from the middle section of the small intestine did not inhibit gastric secretion and in some cases stimulated it. Enterogastrone derived from dog intestine was more potent, than enterogastrone from hogs.

Data in the literature dealing with the chemical properties of enterogastrone are insufficient and rather contradictory. Therefore it is hard to make conclusions about the possible chemical nature of the hormone.

Gray et al. (1937) showed that enterogastrone is an odorless, water-soluble, orally totally ineffective substance, which decreased gastric acid secretion in dogs, provided with a vagotomized pouch of the entire stomach.

Millen et al. (1952) identified 13 amino acids in the enterogastrone molecule, glutamic acid, lysine, arginine and leucine being the most prominent. The ether-soluble component of the substance was very small. If the aqueous solution of enterogastrone was repeatedly shaken by chloroform, more than 92% was recovered from the aqueous solution, with no change in activity, and less than 5% of the starting material occurred at the interfacial boundary. Enterogastrone was easily dialysable. It did not manifest pyrogenicity.

The hormone is digested by pepsin (Spector, 1956).

Enterogastrone is a potent inhibitor of gastric acid secretion in several species. It inhibits acid secretion in the dog (Kosaka and Lim, 1930; Mao Chih Li, 1934; Gray et al. 1937; Ferayorni et al. 1948), in the pylorus ligated rat preparation (Katz et al. 1948), and in cats (Öbrink, 1950; Howat and Schofield, 1948, 1954).

In contrast to results obtained from animal experiments, enterogastrone seems to be much less potent in human subjects. Kirsner et al. (1948, 1949) investigated the effect of intramuscularly administered enterogastrone upon nocturnal and 24-hour gastric secretion in patients with duodenal ulcer. Enterogastrone given intramuscularly in doses up to 2000 mg left the HCl output unchanged, while doses of 5000 mg caused a slight reduction. Inhibition consisted chiefly of a marked reduction in HCl con-

centration, the volume was only slightly or moderately reduced. Levin et al. (1948) showed that enterogastrone in doses up to 2000 mg did not influence histamine-induced gastric acid hypersecretion.

Ferayorni et al. (1948) also observed the lack of inhibition of acid secretion against the double histamine test following the i.m. administration of 200 mg of enterogastrone.

Linde et al. (1952) showed that the administration of enterogastrone, to Heidenhain-pouch dogs caused an inhibition of the volume output of gastric juice. If HCl output was plotted against the rate of secretion a linear relationship existed, where the points of observation were the same whether the experiment was performed in the presence or absence of enterogastrone. The authors concluded from this work that the relationship which normally exists between acidity and secretion rate was undisturbed by enterogastrone, and the decrease of acidity was a consequence of the decreased volume output.

The work of Kaulbersz et al. (1954, 1962) suggested that enterogastrone and urogastrone are separate entities. Urogastrone prepared from the urine of hypophysectomized dogs failed to inhibit histamine-induced gastric acid hypersecretion (Kaulbersz, et al. 1954); while enterogastrone prepared from the duodenum of hypophysectomized dogs gave the same inhibition as enterogastrone obtained from normal dogs (Kaulbersz et al. 1962).

On the basis of paper electrophoretic studies Queisnerova and Svatos (1961) also suggested that enterogastrone and urogastrone have different characteristics. The enterogastrone and urogastrone activity was present at different spots.

In summary, enterogastrone (1) is a naturally-occurring, water-soluble substance, formed mainly in the mucosa of the upper small intestine, (2) potently inhibits gastric acid secretion in experimental animals when given i.v., i.m., or s.c., but is ineffective on oral administration, (3) seems to be far less effective in man, (4) is digested by pepsin, and (5) does not cause changes in body temperature.

3. Urogastrone

Three groups of investigators reported in 1939, that extracts of human urine were able to inhibit gastric acid hypersecretion.

The first group, Gray et al. (1939), who described a method for the extraction of enterogastrone from hog duodenal mucosa (Gray et al. 1937) thought of the possibility that enterogastrone was excreted in the urine, and could be extracted from this source using the same (tannic acid precipitation) method. They noted that the material extracted from the urine was nearly 16 times as potent as the material obtained from hog duodenal mucosa. The extract was very potent in inhibiting gastric acid secretion, even in those dogs, which have become refractory to the duodenal mucosal preparation. The dose which blocked gastric acid secretion had no effect on gastric motility. The extract did not exhibit gonadotrophic activity and was unaffected by boiling for 5 minutes.

The work of the second group (Friedman et al. 1939) was initiated by reports of Sandweiss et al. (1938) who found that extracts prepared from urine of pregnant women as well as from urine of normal, non-pregnant women, exerted a strong protection against Mann-Williamson ulcers. The latter workers also observed that extracts of urine from ulcer patients did not have

the protecting action on this experimentally induced ulcer.

Friedman et al. (1939) used urine obtained from both normal female subjects and duodenal ulcer patients. Vagotomized dogs with gastric fistulas of the whole stomach were used. Acid secretion was induced by hourly s.c. injections of histamine phosphate, while the extract was given intravenously. All extracts effectively inhibited acid secretion, regardless of whether they were administered prior to or following histamine. The extract remained active after boiling for 30 or 60 minutes.

The third group (Necheles et al. 1939) used extracts, which were obtained from normal, human urine. The antisecretory activity was assayed on dogs, provided with a Pavlov pouch, following a standard meat meal. Doses capable of inhibiting gastric acid secretion were not large enough to block gastric motility. Similar observation was made by Gray et al. (1939). Necheles et al. (1939) also noted the lack of inhibition upon oral administration of the extract.

The preparation and properties of urogastrone were later described, among others by Gray et al. (1942), Gregory (1955) and Mongar and Rosenoer (1962).

Gray et al. (1942) described urogastrone chemically as a water soluble substance, which is completely insoluble in organic solvents, such as ether, chloroform, benzene, etc. These investigators observed an effective inhibition of gastric acid hypersecretion in response to histamine, when urogastrone was given intravenously, or in larger doses subcutaneously to dogs, provided with a vagotomized, entire stomach pouch or a Heidenhain pouch. However, urogastrone was ineffective, even in large doses, when it was placed directly into the duodenum by a tube.

A further purification of urogastrone was achieved by Gregory (1955). His method was based upon the following principles and steps: granular charcoal removed the activity from untreated urine. The active principle could be eluted, together with protein and pigment, by acid 80% acetone. When the pH of the eluate was adjusted to 5 - 5.5, a heavy, tan-coloured protein precipitate fell. The wet precipitate was almost completely soluble in strongly acid methanol and could be precipitated from this solution by ether. This ether precipitate contained all of the activity which was originally present in the urine. The product was a tan-coloured powder, freely soluble in water.

Its activity was preserved for days at 0 - 5°C in a neutral or faintly acid solution, and no appreciable loss of activity occurred in 24 hours at room temperature, if the pH of the solution was not above 7 - 7.5.

0.10 - 0.15 mg urogastrone which was equivalent to 1 liter of urine, promptly inhibited secretion in dogs, in response to histamine, when given as a single i.v. dose. Gregory also noted, that the inhibition depended on the rate of secretion, and that a greater degree of inhibition was observed following submaximal secretory rates. Urogastrone appeared to have no specific inhibitory effect on the secretion of gastric HCl as distinct from water, the depression of acidity was no greater than was expected from the effect on the volume-rate of secretion. Gregory's extract was pyrogen-free, causing no changes in body temperature of conscious rabbits at a urogastrone dose that corresponded to 15 - 20 liters of urine.

Another method for the purification of urogastrone was recently

described by Mongar and Rosenoer (1962). They percolated clear, acidified urine through a Cabunite column. The active material was eluted with 10% ammonium acetate buffered with ammonium hydroxyde to pH 9.5 in 40% ethanol. Urogastrone was then precipitated by raising the ethanol concentration of the eluate to 85%. The precipitate was dried, ground up and dissolved in distilled water. It was effective in inhibiting carbachol-stimulated gastric acid hypersecretion in anesthetized rats. This urogastrone preparation was also pyrogen-free.

Rosenoer (1962) also established that urogastrone is a polypeptide, which is destroyed by pepsin and chymotrypsin, while trypsin only slightly inactivates it. The fact that reducing agents, such as sodium sulphite and ∝-cysteine inactivate urogastrone is a strong evidence for the existence of one or more disulphide bonds in the molecule.

In summary, urogastrone (1) is a naturally-occurring, water-soluble substance, found in urine, (2) can be obtained from aqueous solutions, by precipitation with ether or concentrated ethanol, (3) potently inhibits gastric acid secretion when given intravenously, intramuscularly or subcutaneously, but is ineffective on oral or duodenal administration, and (4) does not cause changes in body temperature.

II. Pharmacological Gastric Secretory Inhibition

1. Effect of Atropine on Histamine or Gastrin-induced Gastric Acid Hypersecretion

(a) Histamine: In general atropine is not an effective inhibitor of histamine-induced gastric acid hypersecretion. However, a re-

lationship exists between the dose of atropine and the degree of inhibition. Larger amounts of atropine produce a greater reduction of secretion, but atropine never seems to erase completely the effects of histamine (Code, 1951).

(i) Effects observed in humans. Polland (1930) reported a marked decrease in the secreted volume of gastric juice when atropine and histamine were concomitantly given, compared to values obtained after histamine alone. However, the changes in acid output were small due to an actual increase in the titratable acidity.

Atkinson and Ivy (1938) reported that 2 mg atropine reduced the volume of gastric juice, secreted in response to 0.5 mg histamine acid phosphate, and consequently the acid output was also lowered, although the titratable acidity did not change. The authors noted, however, that in order to obtain a depression of gastric secretion, atropine had to be given in amounts, which caused systemic, toxic effects.

Kirsner and Palmer (1940) observed a decreased volume and unchanged pH in patients with duodenal ulcer when they tried to suppress histamine-induced hypersecretion with orally administered atropine.

(ii) Effects observed in dogs. Gray (1937) found in dogs, which were provided with a vagotomized entire stomach pouch, that at a steady rate of HCl production, maintained by repeated s.c. injections of histamine, increasing doses of atropine resulted in increased inhibition. When the rate of secretion was increased in response to histamine the inhibitory potency of atropine decreased. The maximum inhibition of acid secretion, however, was never more than 60%.

Atkinson and Ivy (1938) reported a 50% decrease of the secreted

volume and a 33% decrease of total acid output in Pavlov-pouch dogs after atropine administration, compared to controls receiving only histamine.

Ivy and Bachrach (1940) also noted a definite, but limited inhibition of acid secretion in Pavlov-pouch dogs after the administration of atropine. The inhibition caused by a given dose of atropine was less with increasing doses of histamine.

Gregory and Tracy (1961) observed in Heidenhain-pouch dogs, that a single i.v. dose of atropine, which was able to completely abolish the acid secretory response to gastrin, had little or no effect on histamine-induced acid hypersecretion.

(b) Gastrin: It was shown by Bennett (1965), that the administration of 0.1 - 4 µg/ml gastrin to the isolated guinea-pig ileum preparation, elicited a rapid contraction. The antihistamine Mepyramine (0.4 - 100 ng/ml), the 5-HT antagonist methysergide (10 - 100 ng/ml) and the ganglionic blocking agent hexamethonium (10 µg/ml) had no effect on the response to gastrin. On the other hand, hyoscine (2 - 200 ng/ml) either completely abolished or greatly reduced the contractions induced by gastrin.

Grossman (1961) observed in dogs which were provided with denervated fundic pouches, that submaximal acid secretion in response to gastrin fell almost to basal levels after the i.v. administration of 1.2 mg atropine. Submaximal acid secretion in response to histamine, was only slightly inhibited by the same dose of atropine. Gregory and Tracy (1961), found that in dogs which were provided either with a gastric fistula or with a denervated fundic pouch, i.v. atropine considerably reduced both volume and acid output

in response to repeated s.c. injections of gastrin. In the same animals a similar acid secretory response to histamine was hardly affected.

Gregory and Tracy (1961) showed in man that atropine, administered intramuscularly greatly reduced the acid secretory response to gastrin, in a dose which caused no toxic side effects except for suppression of salivation.

2. Antihistamines

It is well-known that the synthetic antihistamines are able to antagonize the effects of histamine by competitive inhibition. However, histamine-induced gastric acid hypersecretion or experimental ulceration could not be prevented by the administration of synthetic antihistamines, although many trials are recorded in the literature.

Loew and Chickering (1941) tried out Fourneau's compound (929F) in dogs which were provided with a Heidenhain pouch. The antihistamine did not reduce histamine-induced hypersecretion, on the contrary, it augmented the response.

Loew et al. (1946) investigated the effect of another antihistamine preparation, Benadryl, in Heidenhain-pouch dogs. This antihistamine decreased both volume and total acid output in large doses (10 mg/kg) in 3 out of 4 dogs. However, evidence of inconsistency in the results and lack of a pronounced inhibitory effect, reduced the probability that a direct antagonism of the secretagogue action of histamine was involved.

Friesen et al. (1946) examined the effect of Benadryl in dogs provided with Heidenhain and Pavlov pouches, and Sangster et al. (1946) in

dogs provided with entire stomach pouches. Neither group of investigators observed any inhibitory effect of this antihistamine against histamine-induced hypersecretion.

McGavack et al. (1946) reported a significant decrease both in free and total acidity, following chronic (2-7 weeks) administration of Benadryl. However, in these experiments acid secretion was stimulated by a 7% alcohol meal.

Hartman and Moore (1948) found no inhibition of acid secretion in human subjects with peptic ulcer, following chronic administration (up to 30 days) of Pyribenzamine.

Wood (1948) and Paton and Schachter (1951) reported an augmentation of gastric acid secretion following treatment with mepyramine, compared to control studies, where only histamine was given.

Rocha e Silva (1955b) and Ash and Schild (1966) put forward the hypothesis, that while one type of histamine receptor can be specifically antagonized by low concentrations of antihistamine drugs, gastric acid secretion, induced by histamine, cannot be antagonized by antihistamines suggesting that these actions are mediated by other receptors.

3. Effect of Enzymes - Concerned with the Production or Breakdown of Histamine - on Gastric Acid Secretion

(a) Effect of histidine decarboxylase inhibitors: The enzymes which are responsible for the formation of histamine, from its amino acid precursor, L-histidine, are the specific and non-specific histidine decarboxylases. The specific histidine decarboxylase was found to be present in rat and rabbit gastric mucosa. Potent inhibitors of the enzyme are known;

such as a hydrazine analogue of histidine, and 4-bromo-3-hydroxy-benzyloxamine. Levine (1965) observed that pretreatment with these inhibitors greatly reduced acid secretion in the Shay rat preparation. He investigated the effect of the following secretagogues: reserpine, insulin, gastrin and bethanechol. The finding, that the inhibition of histamine formation significantly reduced gastric acid secretion in response to all stimulants, would indicate that histamine is involved in the acid-secreting mechanism of these agents.

(b) Effect of histamine catabolic enzymes: The inactivations of histamine occurs along several metabolic pathways.

Histaminase, first shown by Best and McHenry (1930) blocks oxidative deamination into imidazole acetic acid.

The effect of a highly purified histaminase preparation of low toxicity upon acid secretion, was investigated by Grossman and Robertson (1948). They showed that the i.v. administration of histaminase greatly depressed acid secretion in response to histamine, mecholyl and urecholine. The experiments were performed in dogs, with vagotomized, entire stomach pouches. I.v. histaminase similarly depressed acid secretion in response to a meal in Pavlov-pouch dogs.

On the other hand, the inhibition of the enzyme can cause an accumulation of histamine. Indeed, Lin et al. (1956) were able to demonstrate that the i.v. administration of aminoguanidine (a histaminase inhibitor) led to acid secretion in an untreated Heidenhain-pouch dog, and augmented the effect of exogenous histamine.

Ivy et al. (1956) reported, that the gastric acid secretory response induced by a histamine-free, cooked oat test meal could be potentiated

by aminoguanidine pretreatment.

Furthermore, Ghosh and Schild (1958) observed a strong potentiation of histamine-induced acid secretion in the rat, after the i.v. administration of aminoguanidine or semicarbazide.

Another major catabolic pathway of histamine is ring methylation, by the enzyme N-methyl-transferase, which can be inhibited by chlorpromazine.

Amure and Ginsburg (1964) demonstrated an enhanced acid secretory response in the rat, to exogenous histamine, following pretreatment with chlorpromazine.

These observations supported the hypothesis that histamine has an important role in the chain of events of gastric acid secretion. However, the assumption that histamine is the final, local, common, chemostimulator of the parietal cell could be proven only, if histaminase were chemically known, and both the histaminase activity and the gastric secretory depressant activity were due to the same substance.

D. GENERAL INTRODUCTION TO TEMPERATURE REGULATION AND ANESTHESIA

In homeothermic species temperature varies considerably from one part of the body to another. The temperature of the skin of the extremities varies with the environment, while the temperature of deeper structures in the trunk and head varies much less.

Central body temperature is controlled by balancing heat production and heat loss.

Heat is produced by metabolic activity, and to the basal rate of heat production is added that due to activity, the metabolism of food and perhaps emotion. For purposes of thermal regulation heat production may be increased rapidly by increased muscular activity or shivering.

Heat loss may be increased most powerfully by sweating, which is largely controlled by sympathetic efferents and also by evaporation of water from the respiratory tract in panting.

Radient heat loss is largely a function of skin blood flow and one may consider the regulation of skin blood flow as a fine adjustment mechanism for controlling temperature with shivering and sweating as relatively coarse adjustments (Cranston, 1966).

The regulation of body temperature occurs in the hypothalamus. It is believed that there is a "heat loss center" in the rostral hypothalamus and a "heat production and conservation center" in the caudal hypothalamus (Patton, 1965).

Lesions in the rostral hypothalamus render the animal incapable of lowering its body temperature in warm environment, that is, neither cutaneous vasodilation nor panting will occur. Lesions in the caudal hypo-

thalamus render the animal poikilothermic, which means that its ability to maintain normal body temperature is seriously impaired in both warm and cold environment.

Two receptive mechanisms are available for detecting changes in temperature, the skin and the central receptors.

Skin receptors respond to both heat and cold. It is a reflex pathway, the afferents arise from the skin and extend above the cervical cord, but it is uncertain whether they extend to the hypothalamus (Cranston 1966). The efferent pathway is sympathetic.

There is good evidence from several studies that central receptors exist in the hypothalamus which are sensitive to very small changes in their temperature. Von Euler (1963) described these hypothalamic thermoceptive structures as true receptors, which show specific thermosensitivity, generator potential and impulse discharge directly related to the stimulus. He demonstrated in unanesthetized rabbits that when the thermoceptive region of the hypothalamus was heated with diathermy the temperature increased a few tenths of ^{o}C above control which induced a decrease in body temperature.

Johnson and Spalding (1966) demonstrated the existence of central temperature receptors in man. The subjects of these experiments had spinal cord transections. During the experiment their feet and lower legs were placed in a mixture of ice and water which induced an artificial lowering of core temperature. Areas of the skin which had nervous connections to the brain were well covered with clothes. Shivering and increased metabolism was evoked when the central temperature fell and ceased when the central temperature rose again.

These observations are compatible with the view, that there are central receptors which can cause shivering when stimulated by a fall in core temperature.

Changes in environmental temperature induce a number of responses to compensate this change. For example, normal body temperature will be maintained in cold exposure by vasoconstriction, piloerection and metabolic responses which arise from shivering and non-shivering thermogenesis (Hemingway 1963). During initial exposure to cold heat production is increased by shivering which is an emergency mechanism.

Keller (1959) demonstrated the absolute dependence of shivering not only on the central nervous system, but on the hypothalamus in particular. He showed that an intact dog exposed to cold shivered violently but maintained its normal body temperature. However, lesions in the caudal hypothalamus, depending on their location, greatly impaired or abolished shivering and the body temperature fell dramatically.

When the brain stem was transected through the lower pons sparing however, the pyramidal bundles, the dog exhibited shivering when its core temperature fell to 35°C. This indicated, that at the level of the pons the efferent nerve fibers descend in the pyramidal bundles. The fibers must join these bundles somewhere caudal to the hypothalamus.

Davis (1959) demonstrated in mice that shivering is regulated mainly by the skin temperature but is also influenced by the thermal state of the core.

(a) If the core temperature of mice was between 34° and 40°C, shivering could be demonstrated providing skin temperatures were low. However, raising the skin temperature inhibited shivering.

(b) If the core temperature was more than 40°C shivering was inhibited and there was a reflex peripheral vasodilation.

(c) If the core temperature was less than 34°C shivering could not be inhibited even if the skin temperature was above 40°C.

On the basis of these experiments, Davis suggested that in normothermic animals shivering is stimulated fundamentally by a fall in skin temperature but its quantitative regulation may be dependent upon changes in core temperature. In the truly hypothermic homeotherm, however, shivering is stimulated entirely by the low central temperature.

During shivering due to an increase in muscular contraction the rate of ATP hydrolysis is increased. The greatly elevated ADP concentration stimulates the electron transport system and oxygen consumption. Thus, substrate is utilized at a much higher rate. Heat is also released from the increased hydrolysis of the terminal pyrophosphate bond of ATP.

Apart from shivering-induced heat production, thermogenesis can also be initiated by an increased release of biologically active substances, mainly norepinephrine.

Rats, exposed to cold, excrete large amounts of norepinephrine and slightly lesser quantities of epinephrine (Leduc, 1961). In cold exposure norepinephrine is also released at nerve endings (Hsieh et al. 1957). The release of norepinephrine and epinephrine sets off a series of thermogenic activities, such as activation of glycogenolysis, activation of lipase in fat depots and release of free fatty acids into the general circulation (Smith, 1963).

In the unacclimatized rat non-shivering thermogenesis contributes

about 45% of the cold-induced metabolism. In the acclimatized rat all of the metabolism is arising from non-shivering sources (Davis, 1959).

Maickel et al. (1963) showed the absolute necessity of the presence of epinephrine and norepinephrine in the rat to survive cold exposure. Adrenal demedullated rats did not show an elevation of blood glucose concentration and their survival time was greatly decreased. Administration of epinephrine restored the ability to mobilize glucose and the rats survived their stay in the cold. Similarly, following repeated administration of reserpine neither blood glucose, nor plasma free fatty acid levels were increased. Vasoconstriction, piloerection and shivering were also abolished. These experiments showed that in the absence of sympathetic function the animal cannot mobilize energy substrates and is incapable of conserving body heat.

The hypothalamus contains a relatively high concentration of norepinephrine and 5-hydroxytryptamine. The possibility that these substances might be concerned in temperature regulation was raised by Brodie and Shore (1957) and by von Euler (1963).

The hypothesis received support from the work of Feldberg and coworkers. Feldberg (1965) showed that the injection of norepinephrine and epinephrine into the cerebral ventricle lowered rectal temperature for several hours, while the injection of 5-hydroxytryptamine caused a rise in rectal temperature lasting for many hours. The hypo- and hyper-thermia, which was observed, resulted from the action of the amines on the anterior hypothalamus because the same changes occurred when the amines were applied by microinjections to this part of the hypothalamus. However, when the microinjections

were made on the posterior or ventromedial hypothalamus, the amines did not affect body temperature.

It is now apparent that wakefulness results from enhanced activity of the different parts of the brain, while sleep represents lack of adequate brain excitation.

Wakefulness, under normal circumstances, is associated with consciousness which according to Cobb (1948) is "awareness of environment and of self." If one is not fully aware either of environment or of oneself, some degree of unconsciousness is present. Unconsciousness occurs when one sleeps, faints or is anesthetized. Sleep and anesthesia are two closely related phenomena, the main difference between the two is that a sleeping person can be woken up. Until the late 1940's, prior to the description of the ascending reticular activating system, it was thought that consciousness was exclusively a function of the cerebral cortex. However as early as 1892, Goltz succeeded in removing both cerebral hemispheres in a dog and observed that the animal was able to walk in a perfectly normal fashion. Removal of the cerebral cortex produced a loss of all understanding, intelligence and memory but the dog had periods of sleep and wakefulness, of unconsciousness and consciousness, although the content of its consciousness was enormously different from that of a normal dog.

Throughout the years also more and more clinical evidence accumulated, which showed that disturbances of consciousness are not necessarily associated with lesions in the cerebral cortex but rather other parts of the central nervous sytem. Tumors of the cervical cord as high up as C_1 do not cause disturbances of consciousness as long as they do not invade the medulla oblongata (Feldberg, 1959).

Haemorrhages around the pons and medulla oblongata, in the substance of the pons or in the fourth ventricle produce deep coma and unconsciousness. Furthermore, unconsciousness is characteristic of lesions in the upper brain stem and thalamus (Feldberg, 1959). In the central core of the brain stem is a system, which begins at the upper end of the spinal cord and extends into the posterior part of the hypothalamus, to the sides of the thalamus and upward through the central portion of the thalamus. This system is the reticular formation.

Those portions of the reticular formation and thalamus that enter into the wakefulness response are called the ascending reticular activating system. Although this system has little intrinsic activity it is stimulated by sensory signals from collaterals of all ascending fibers, and the cerebral cortex too. Direct fiber pathways enter the reticular activating system from almost all parts of the cerebral cortex. Large number of nerve fibers from the motor regions of the cerebral cortex enter the reticular formation and therefore motor activity is particularly associated with a high degree of wakefulness. During anesthesia afferent stimulation loses its normal capacity to evoke EEG, behavioral and emotional arousal, and there is an equivalent failure of EEG arousal upon the direct excitation of the ascending reticular system in the brain stem (Magoun, 1958).

The loss of wakefulness and the incapacity of afferent stimuli to induce EEG on behavioral arousal in anesthesia have been attributed to a depression of activity within the ascending reticular system (Magoun, 1958).

Anesthetics are apparently able to block transmission across a synapse much more readily than conduction along a nerve fiber and the reticular formation is characterized by its multisynaptic organization so that impulses have to pass one synapse after another.

This does not mean that the reticular activating system is exclusively susceptible to anesthetic agents, although they have a predilection for the system (Feldberg, 1959).

Newer concepts of anesthetic activity suggest that interference with transmission of impulses in the reticular activating system is the primary event and if cerebral oxygen consumption is decreased it is only a manifestation of decreased neural activity. The reticular activating system modifies central transmission of pain and other sensations, controls the state of consciousness, and regulates reflex activity in the spinal cord, thus it is not unreasonable to locate the site of anesthetic action here (Vandam, 1965).

E. EFFECT OF DRUGS ON BODY TEMPERATURE AND ANESTHESIA

I. Picrotoxin

Picrotoxin is a powerful central nervous system stimulant, which exerts its main effect in the midbrain and medullary region.

1. Effect on Body Temperature

Picrotoxin injected in convulsive doses into experimental animals, increases body temperature (Trendelenburg, 1920).

On the other hand, subconvulsive doses of the drug lowered body

temperature in normal animals as was observed as early as 1894 (Trendelenburg, 1920).

The hypothermic action of picrotoxin is more pronounced than that of pentylenetetrazol (Hahn, 1943), and is mainly due to an increased loss of heat (Hahn, 1943), but there is also a reduction of heat production.

The hypothermic action of picrotoxin is of central origin, as was first demonstrated by Rosenthal et al. (1925). They found that picrotoxin did not lower body temperature in animals in which the cervical cord had been transected. Rosenthal (1941) also showed that the injection of subconvulsive doses of picrotoxin into the para-infundibular region of rabbits, lowered body temperature by 4°C.

In spite of its cooling action in normal animals, picrotoxin prevents or antagonizes the fall in body temperature normally caused by anesthetics, especially by barbiturates.

This was shown by Maloney and Tatum (1932) in rabbits, by Koppanyi et al. (1936) in dogs and by Hahn et al. (1963) in mice and rabbits. Rosenthal (1941) was also able to demonstrate in rabbits that the simultaneous administration of phenobarbital and picrotoxin caused a significantly smaller drop in body temperature, than the hypothermia observed after either of the drugs was given alone. He concluded that because the cooling effect of picrotoxin could be suppressed by the barbiturate, it could not be due to paralysis of the temperature center, but acted probably through the stimulation of a cooling center in the hypothalamus. When he injected simultaneously picrotoxin and aconitine, he observed a much greater hypothermia than the algebraic sum of the action of each drug. This potentiated effect suggested that both drugs stimulated the same (cooling) centers.

2. Antianesthetic Action

Although the mutual antagonism between picrotoxin and various hypnotics was known in the last century, only the discovery of its very pronounced antagonism to the barbiturates (Maloney et al. 1931) led to its widespread clinical use.

Maloney et al. (1931) found that picrotoxin was effective against acute poisoning induced by the shorter acting barbiturates, Nembutal and Amytal, both in rabbits and dogs. They also found that the process of treatment was reversible, that is, picrotoxin was effective against barbiturate depression, and the barbiturates were effective in controlling picrotoxin convulsion.

Animal experiments by several investigators demonstrated the life-saving effect of picrotoxin after pretreatment with lethal amounts of barbiturates.

Barlow (1935) investigated the analeptic effect of pentylenetetrazol, picrotoxin and nikethamide against both a maximal hypnotic dose and a 100% lethal dose of several hypnotics (pentobarbital, chloral hydrate and tribromethanol (Avertin)) in adult, albino rabbits. He found that the degree and duration of effect of each analeptic was inverse to the depth of anesthesia, and the order of antidotal efficiency of the series of agents was the same for each of the hypnotics. Picrotoxin was the most potent analeptic, with pentylenetetrazol and nikethamide following in this order.

Chakravarti (1939) compared in mice amphetamine, pentylenetetrazol, picrotoxin and nikethamide as "awakening" agents against a narcotic dose of Nembutal and as antidotes in barbiturate poisoning. Amphetamine was the most

potent in arousing animals from Nembutal narcosis, pentylenetetrazol and picrotoxin were almost equipotent, while nikethamide proved to be much less effective. However, only picrotoxin and pentylenetetrazol were able to antagonize barbiturate poisoning; both amphetamine and nikethamide even increased mortality. These observations indicated that true pharmacological antagonism existed only between picrotoxin and pentylenetetrazol on the one hand and Nembutal on the other.

Werner and Tatum (1939) found that picrotoxin and pentylenetetrazol were able to arouse depressed rabbits injected with sublethal doses of Nembutal. Picrotoxin was the more effective analeptic in their studies.

Järvinen and Vartiainen (1949) demonstrated in rabbits that 0.5 g/kg barbital sodium, administered subcutaneously, caused 100% mortality. In the presence of picrotoxin only 1 g/kg barbital sodium proved fatal to all animals. Doses of barbital sodium greater than about double the lethal dose could not be overcome by any amount of picrotoxin.

Lavenson et al. (1958) were able to reinstate spontaneous respiration in cats with picrotoxin after the animals received twice the "respiratory arrest dose" of pentobarbital sodium.

Hahn and Oberdorf (1963) determined in mice the LD 50 of phenobarbital and hydroxydione alone and in the presence of picrotoxin. Pretreatment with picrotoxin considerably raised the LD 50 dose of both drugs.

In summary, picrotoxin (a) causes hypothermia in normal animals in subconvulsive doses; (b) reverses hypothermia in barbiturate pretreated animals, and (c) potently antagonizes sublethal and lethal doses of anesthetic agents.

II. Pentylenetetrazol

Pentylenetetrazol was introduced into therapy as an analeptic agent by German workers in the mid 1920's.

1. Effect on Body Temperature

Schnepel (1928) was the first to show that pentylenetetrazol decreased body temperature in subconvulsive doses and potentiated the effect of antipyretic agents (Pyramidon) in experimental animals.

Hahn (1943) showed that the hypothermia induced by subconvulsive doses of pentylenetetrazol was dose dependent, lasted for several hours and became less pronounced with increasing the environmental temperature.

In subsequent years this hypothermic action of pentylenetetrazol was demonstrated in several species (Hahn, 1960).

Shemano and Nickerson (1959) observed in dogs, which were completely paralysed with a neuromuscular blocking agent, that the effect of repeated, convulsive doses of pentylenetetrazol produced either hypothermia or hyperthermia, depending on the ambient temperature at which they were administered. If the ambient temperature was below 23°C, hypothermia was observed, which was probably the result of an increased cutaneous blood flow resulting in loss of body heat. The hyperthermic response was noted above 25°C and appeared to be due to an increased heat production, independent of skeletal muscle activity. Neither skin temperature, nor rectal temperature was affected by pentylenetetrazol in C_4 to C_6 spinal cord sectioned dogs, which indicated that the thermal responses of pentylenetetrazol were of central origin.

Bartsokas (1940) described that the administration of pentylenetetrazol into rabbits resulted in panting; the phenomenon resembled that seen after increasing the room temperature. Similarly, pentylenetetrazol pretreated dogs showed tachypnea at lower rectal temperatures than did control animals. These observations suggested that the central regulatory mechanism was adjusted to a lower temperature by pentylenetetrazol.

Hahn et al. (1963) showed in rabbits, which were anesthetized with barbital sodium, that analeptic agents (pentylenetetrazol and picrotoxin) raised body temperature in connection with their arousal action. In the case of pentylenetetrazol, this even led to hyperthermia, while after picrotoxin, rectal temperature of rabbits returned to normal but did not exceed pretreatment levels. This hyperthermic effect of pentylenetetrazol was considered to be an expression of a temporary lability of the heat regulatory system during the arousal of the narcotized animal.

2. Antianesthetic Action

The arousing and life-saving effect of pentylenetetrazol against the anesthetic and lethal doses of several anesthetic drugs has been investigated, but much less extensively than its antagonism to barbiturates. In general, the antagonism offered by pentylenetetrazol against paraldehyde, chloral hydrate, urethane, chloralose and ethyl ether appears to be weaker than that against barbiturates (Hahn, 1960).

The life saving effect of pentylenetetrazol against lethal doses of the barbiturates was described by several investigators (Barlow, 1935; Chakravarti, 1939; Werner and Tatum, 1939; Loewe, 1955; Kimura and Richards, 1957 and Hahn and Oberdorf, 1963).

Picrotoxin and pentylenetetrazol manifest many similarities in antagonizing either the hypnotic or the lethal effects of barbiturates, and accordingly several of the foregoing references were discussed in detail in the previous chapter.

The findings of Carlsson and Theander (1946) were not in agreement with the previous references. These workers investigated the effect of pentylenetetrazol in guinea-pigs, which were depressed or anesthetized with barbital sodium. The analeptic potency of pentylenetetrazol was increased by increasing the dose but only to a certain limit and a further increase induced a depression rather than an arousal. Furthermore Carlsson and Theander (1946) noted that the dose of the barbiturate was also very important in evaluating the analeptic potency of pentylenetetrazol.

Kimura and Richards (1957) observed in mice that the stimulant action of pentylenetetrazol resulted both in the reduction of the depth and in the shortening of the duration of pentobarbital sodium induced anesthesia. A transitory peak within one minute of analeptic administration could be noted with pentylenetetrazol prior to its real awakening effect in contrast to picrotoxin, which did not show such transitory peak.

In summary, pentylenetetrazol (a) causes hypothermia in subconvulsive doses in normal animals; (b) reverses hypothermia in barbiturate pretreated animals often causing even hyperthermia, and (c) is an effective antagonist to barbiturates.

III. Nikethamide

Nikethamide was synthetised in the mid 1920's in Germany and in-

troduced as a brainstem stimulant. However, its analeptic potency is generally considered to be much less effective when compared to picrotoxin or pentylenetetrazol.

1. Effect on Body Temperature

Subconvulsive doses of nikethamide, in contrast to picrotoxin and pentylenetetrazol, were found to raise the body temperature of several species. Hahn (1943) described the hyperthermic effect of nikethamide in rabbits, Flacke et al. (1953) in mice, and Frommel et al. (1963) in guinea-pigs.

The hyperthermic effect of nikethamide was not strong enough however, to counteract hypothermia induced by barbiturate anesthesia. Instead, the administration of nikethamide to animals anesthetized by barbiturate accentuated and prolonged the fall in body temperature (Hahn, 1943).

Hahn (1943) observed an increased motor activity which accompanied nikethamide-induced hyperthermia, and suggested that the hyperthermic effect was due to stimulation of lower motor centers.

On the other hand, nikethamide augmented hyperthermia in guinea-pigs, which were pretreated with fever causing vaccines. This augmentation of hyperthermia occurred in the absence of any muscular exercise (Frommel et al., 1963).

2. Antianesthetic Action

The antagonistic action of nikethamide to barbiturates had been studied in several species but most authors agreed that nikethamide is a much weaker antagonist than either picrotoxin or pentylenetetrazol.

Werner and Tatum (1939) performed experiments in rabbits, and stated that nikethamide was neither able to cause complete arousal when animals were anesthetized with hypnotic doses of Nembutal, nor did it have life saving or life prolonging effect against lethal doses of Nembutal.

Chakravarti (1939) described nikethamide as a weak "awakening" agent compared to pentylenetetrazol or picrotoxin, and observed that nikethamide increased mortality in rabbits, which were pretreated with sub-lethal doses of Nembutal. Airaksinen and Mattila (1962) found in mice, that nikethamide was able to decrease the sedative and lethal effects of reserpine both at room temperature and in cold (9°C). They suggested that nikethamide probably acted as a non-specific stimulant by increasing motor activity, thus increasing heat production and antagonizing hypothermia.

Brazda et al. (1965) observed that nikethamide decreased the pentobarbital sleeping time in rats and rabbits. This effect was due to an increase in the activity of liver microsomal enzymes, which are responsible for the oxydation of pentobarbital. However, in poikilothermic animals, furthermore in chickens and guinea-pigs, nikethamide did not influence liver microsomal enzyme activity, consequently the drug did not reduce pentobarbital sleeping time in these species.

In summary, nikethamide (a) is a weak hyperthermic agent, which in experimentally-induced hypnosis rather potentiates than reverses hypothermia; (b) its arousal activity is weak, and because of its depressant effects, the initial excitation is mostly followed by a relapse into a prolonged depression.

IV. Amphetamine

Although the synthesis of amphetamine was described as early as 1912 and 1913, systemic studies on its pharmacological effects did not take place until the late 1920's.

The central stimulant action of amphetamine was first observed by Alles (1933).

1. Effect on Body Temperature

Several investigators observed and described the hyperthermic effect of amphetamine in experimental animals (Jacobsen, 1939; Werner, 1941; Kiessig, 1941, and Simonyi and Szentgyörgyi, 1949).

According to Jacobsen (1939), hyperthermia induced by amphetamine is probably due to increase in motor activity in combination with vasoconstriction.

Feitelberg and Pick (1940) noted that amphetamine increased heat production in the brain. The effect was due to central stimulation but was not specific for the amine, since analeptics which cause a fall in body temperature, have the same effect.

The fall in body temperature following the administration of ethanol, was effectively antagonized in rabbits by amphetamine, more effectively than by pentylenetetrazol or picrotoxin (Werner, 1941).

In contrast to pentylenetetrazol and picrotoxin, however, amphetamine did not antagonize the fall in body temperature induced by phenobarbital. On the contrary, Simonyi and Szentgyörgyi (1949) described a collapse-like drop in temperature accompanied by deep narcosis, when phenobarbital and

amphetamine were simultaneously administered. In recent experiments Weiss and Laties (1963) investigated the effect of amphetamine on behavioral thermoregulation. Rats were exposed to cold but they had access to a lever that turned on a heat lamp. Amphetamine greatly increased the frequency with which the rats turned the lamp on, compared to controls, even though their skin temperature was driven above normal values. The authors suggested that amphetamine apart from its effect upon heat production, impaired behavioral thermoregulation.

2. Antianesthetic Action

Several investigators concluded on the basis of their experiments that amphetamine antagonized sublethal amounts of nearly all anesthetic agents.

Hjort et al. (1938) investigated the effect of a number of analeptic drugs against hypnotic doses of a urea derivative (unsymmetrical n-propyl-o-tolyl-urea) and pentobarbital in mice. They found that amphetamine closely followed the effectiveness of pentylenetetrazol and picrotoxin. However, they also noted that the toxicity of these drugs was differently affected by anesthesia. While the minimal lethal dose of picrotoxin decreased more than 2 fold, the toxicity of amphetamine increased 5 fold in anesthetized animals.

Trevan (1938/39) described the analeptic potency of amphetamine in mice which were anesthetized with paraldehyde, and found that amphetamine greatly reduced the sleeping time.

Chakravarti (1939) found amphetamine to be the most potent drug to arouse mice from a pentobarbital anesthesia when compared to pentylene-

tetrazol, picrotoxin and nikethamide.

Tainter et al. (1939) noted that low doses of amphetamine reduced the time interval between the loss and the recovery of the righting reflex in rats following pretreatment with chloral hydrate, Avertin or pentobarbital sodium. Higher doses of amphetamine rather prolonged the depression reduced by chloral hydrate or Avertin than shortened it, and only against pentobarbital sodium was there a clear analeptic effect.

The ability of amphetamine to antagonize alcohol-induced narcosis in rabbits was described by Reifenstein (1941). Hess (1941) made similar observation using methamphetamine (Pervitin).

Lumière and Meyer (1938); Chakravarti (1939) and Reifenstein (1941) noted that the effectiveness of amphetamine, as an analeptic agent, declined rapidly with increasing depth of anesthesia, and not only did it lack a protective effect against lethal doses of narcotic drugs, but it even increased their toxicity.

The analeptic potency of amphetamine could not be restored by increasing the dose for synergism with the anesthetic agent was then observed (Lumière and Meyer, 1938; Trevan, 1938/39; Chakravarti, 1939, and Tainter et al. 1939).

The possible mechanism of action, by which amphetamine brings about its central effect, have been recently investigated by several workers.

Grana and Lilla (1959) compared the stereoisomers of amphetamine and 1-phenylethylamine as central stimulants and as inhibitors of monoamine oxidase (MAO). They found no correlation between these two effects. While the two agents were almost equally potent in inhibiting MAO, (+)-1-phenyl-

ethylamine was 5-6 times less potent in antagonizing chloral hydrate-induced sleep in rats than dexamphetamine. The authors concluded on the basis of their findings that it is unlikely that the central stimulating action of amphetamine is due to inhibition of MAO.

Work of McLean and McCartney (1961) in rats and of Sanan and Vogt (1962) in rabbits, revealed that amphetamine lowered the norepinephrine content of the brain, although large doses (20 mg/kg or more) were required for pronounced effect. Recent work by Stein (1964) and Hanson (1966) claimed that the central action of amphetamine was mediated via catecholamine liberation.

Stein (1964) described experiments in male rats which were based upon the self stimulation method. Amphetamine induced a high rate of self stimulation which declined as the drug effect wore off. The effect of amphetamine was diminished or shortened when the rats were pretreated with reserpine. The time course of the diminished response and the time course of depletion of catecholamines by reserpine roughly followed the same pattern. The facilitating effect of amphetamine on self stimulation was strongly potentiated by MAO inhibitors. Furthermore, amphetamine-like facilitation of self stimulation was observed after the administration of α-methyl-meta-tyrosine (α-MMT), which is known to liberate catecholamines rapidly from brain stores.

Hanson (1966) showed that untreated, trained cats responded to a conditioned stimulus in 100% of the trials. Reserpine greatly decreased this performance, but amphetamine was able to restore it by liberating the small amounts of catecholamines that were available. If α-methyl-tyrosine

(∝-MT), an agent which acts by inhibiting the biosynthesis of DOPA from tyrosine, was simultaneously administered with reserpine, the cats did not respond to the conditioned stimulus at all and amphetamine was unable to restore their performance. However, amphetamine was active again if small amounts of catecholamine precursor (∝-DOPA) was given prior to amphetamine.

These experiments supported the idea that the central stimulating action of amphetamine was mediated by the release of catecholamines.

In summary, amphetamine (a) causes hyperthermia in normal animals; (b) is unable to restore barbiturate-induced hypothermia, but reverses alcohol-induced hypothermia; (c) antagonizes hypnotic doses of anesthetic drugs; (d) potentiates sublethal doses of anesthetic drugs, and (e) according to recent experimental evidence, mediates its central action by the release of catecholamines.

V. Iproniazid

Iproniazid, together with Isoniazid, was originally introduced into therapy in the early 1950's as an antituberculotic agent.

It was noted, that in tuberculotic patients, iproniazid produced euphoria and sometimes conditions resembling maniac psychoses.

The possible mechanism of this action was first investigated by Zeller *et al.* (1952) and Zeller and Barsky (1952). These authors found that iproniazid was able to inhibit monoamine oxidase (MAO) both *in vitro* and *in vivo*.

Borowitz and North (1959) observed that pretreatment with iproniazid potentiated the toxicity and increased the mortality of intravenously adminis-

tered epinephrine and norepinephrine.

Spector et al. (1958, 1960) measured norepinephrine concentration in rabbit brain. Iproniazid, at doses of 25 or 50 mg/kg, increased brain norepinephrine concentration which reached a plateau by the 3rd day.

According to Green and Erickson (1960) in the rat brain, maximal increase in norepinephrine concentration was obtained 8 hours after oral administration of 100 mg/kg of iproniazid but norepinephrine levels returned to normal only after about 7 days.

Similar observations were made in human subjects by Ganrot et al. (1962). Determining, postmortem, the norepinephrine concentration in the hypothalamus, they found that in patients who were under iproniazid treatment, the norepinephrine concentration was about 2 times as high as in untreated subjects.

Spector et al. (1960) observed that the repeated administration of iproniazid increased spontaneous motor activity and intensified motor responses to tactile stimuli in rabbits. The time of increased spontaneous motor activity coincided with the increase in brain norepinephrine concentration.

Eltherington and Horita (1960) found that iproniazid was able to reverse reserpine induced sedation.

On the other hand, Furgiuele et al. (1962) noted that the administration of iproniazid induced an immediate decrease in spontaneous activity in mice. The decrease in motor activity was inversely proportional to the dosage used. 24 hours later however, no appreciable difference in activity was observed between control and treated groups.

1. Effect on Body Temperature

Chodera (1963) investigated the effect of morphine alone and that of iproniazid and morphine on the rectal temperature of female rats. He found that iproniazid pretreatment enhanced and prolonged morphine-induced hypothermia.

2. Antianesthetic Action

Iproniazid potentiates barbiturate anesthesia as was observed by several investigators.

Goldin et al. (1955) measured sleeping time in mice in response to pentobarbital alone and in response to the combined effect of pentobarbital and iproniazid. They noted that iproniazid prolonged pentobarbital-induced anesthesia. The drug was also able to induce anesthesia in animals which were pretreated with subanesthetic doses of pentobarbital. However, iproniazid alone did not cause anesthesia even in such high doses as 1500 mg/kg s.c.

Fouts and Brodie (1956) found in mice that iproniazid pretreatment caused a 3 fold increase in hexobarbital sleeping time. When they determined the concentration of hexobarbital in the whole animal, they found that it was 2 times as high in the iproniazid pretreated group than in mice receiving only hexobarbital. This showed that iproniazid inhibited the metabolism of the barbiturate. If they injected iproniazid into mice which just recovered from hexobarbital hypnosis, none of the animals returned to sleep. Thus, they concluded that iproniazid increased the duration of action of hexobarbital only by inhibiting its metabolic transformation.

Buchel and Lévy (1960) described the hexobarbital potentiating effect

of iproniazid in rats, which effect was more pronounced in female animals. They concluded, similarly to Fouts and Brodie (1956), that the mechanism of this potentiation was due to inhibition of the enzymes responsible for detoxifying the barbiturates. The same authors noted, however, that iproniazid potentiated chloral hydrate anesthesia. Although the exact mechanism is not known, iproniazid did not interfere with cloral hydrate metabolism.

In summary, iproniazid (a) increases spontaneous motor activity by inhibiting MAO; (b) enhances and prolongs morphine induced hypothermia, and (c) potentiates hexobarbital anesthesia which effect is due to inhibition of detoxifying enzymes and not to a direct central effect.

VI. α-Methyl-Meta-Tyrosine

The finding of Smith (1959), that α-methyl-Dopa lowered tissue levels of serotonin, prompted Hess et al. (1961) to investigate the action of α-methyl-meta-tyrosine (referred to as α-MMT), which is a closely related analogue of α-methyl-Dopa, on tissue levels of serotonin and norepinephrine. They found that α-MMT depleted endogenous norepinephrine in the brain and heart of rats and guinea-pigs. The results were confirmed by Costa et al. (1961), Costa et al. (1962) and Carlsson and Lindqvist (1962), who all showed that α-MMT caused a long lasting depletion of brain norepinephrine while serotonin was only mildly and transiently affected.

Udenfriend et al. (1961) showed that in the presence of a decarboxylase inhibitor, which blocks the conversion of α-MMT to α-methyl-meta-tyramine, norepinephrine depletion still takes place, suggesting that α-MMT itself is the effective norepinephrine releasing agent.

Later work however, indicated that ∝-MMT, which is decarboxylated *in vivo* to ∝-methyl-meta-tyramine and subsequently β-hydroxylated to metaraminol, does not act *per se* but through its decarboxylated derivatives (Gessa *et al.* 1962; Carlsson and Lindqvist, 1962; and Carlsson, 1964).

Carlsson and Lindqvist (1962) reported that when they injected 400 mg/kg ∝-MMT into mice, within 24 hours the norepinephrine content had almost completely disappeared from the brain. Metaraminol was found however, in the brain and its amount corresponded approximately to the missing norepinephrine.

The effect of ∝-MMT on motor activity and behavior was investigated by Costa *et al.* (1961), Carlton (1963), Van Rossum (1963), Scheckel and Boff (1964) and Moore (1966).

Costa *et al.* (1961) performed their experiments in mice. After the administration of a MAO inhibitor (MO- 911) ∝-MMT was injected. The behavior of the animals was similar to that observed after amphetamine treatment. Their movements were well coordinated and they were hyperactive, running up and down in their cages.

The animals also showed other effects associated with stimulation of adrenergic centers, such as an extreme exophtalmos and hyperthermia. The authors concluded that the remarkable behavioral effects resulted from released norepinephrine. Van Rossum (1963) also reported that the i.p. administration of ∝-MMT induced greatly increased motor activity comparable to the action of amphetamine. Both the behavioral studies and the biochemical investigations reported by Sourkes *et al.* (1961) suggested that the increase in motor activity occurred when brain norepinephrine was selectively released.

According to Scheckel and Boff (1964), behavioral stimulation should not occur in animals which are nearly devoid of endogenous norepinephrine, if the assumption is true that this stimulation is due to the selective release of norepinephrine. Indeed, they observed that while a marked increase in the rate of avoidance responding occurred 45 minutes after the administration of α-MMT, in α-MMT pretreated animals a second injection 16 hours later did not cause stimulation. In summary, α-MMT releases brain norepinephrine acting probably through its decarboxylated derivatives. The drug causes increased motor activity, stimulated behavioral effects and hyperthermia. All these effects are probably brought about through the release of norepinephrine.

F. STEROIDS

The effect of steroids on body temperature and their anesthetic action was reviewed briefly, because our experiments were performed with extracts obtained from pregnant mare urine and it is known that steroids and/or their metabolites are present in human and animal urine.

The most extensively studied steroids are those possessing hormonal properties. The fact that hormonal steroids exert neuropharmacological effects as well was first observed and reported by Selye.

1. Effect on Body Temperature

(a) Hyperthermia: Progesterone appears to be the first steroid shown to have temperature elevating properties. Its thermogenic activity has been thoroughly confirmed. Intramuscular administration of this steroid

consistently induced small but significant increase in temperature both in humans and in several species of experimental animals. The increase in temperature was usually 1 - 1.5°F, which developed several hours after the injection of the hormone, and lasted for 48 hours or more (Palmer and Kappas, 1963).

The in vivo metabolites of many steroids were looked upon for a long time as "waste products", devoid of significant physiological activity. Recent studies by Kappas et al. (1961) and Palmer and Kappas (1963) showed however, that a number of steroid metabolites possess fever inducing activity.

The following steroid derivatives are the prototypes of this new class of pyrogens: etiocholanolone (C_{19} steroid), pregnanolone (C_{21} steroid) and litocholic acid (C_{24} steroid). The first two metabolites are derived from gonadal and adrenal hormones, litocholic acid is a degradation product of cholesterol.

(b) Hypothermia: Selye (1941) reported first that several steroids, which were capable of inducing general anesthesia, caused peripheral vasodilation, resulting in hyperemia of the paws and ears, and a slight decrease in body temperature at the height of anesthesia.

Overbeek and Bonta (1964) investigated several steroids, which act on the central nervous system, but are devoid of hormonal activity. They found that androstane derivatives, that contained a N function on C_{16}, and pregnane and pregnene compounds, which possessed and $-NH_2$ group on C_3, were able to lower body temperature in mice after s.c. administration. The first group was synthetically prepared, while the second group was obtained from plant leaves.

2. Anesthetic Effect

Selye (1941) reported that the i.p. administration of deoxycorticosterone, progesterone, and testosterone induced general anesthesia in rats, while α-estradiol was inactive. In a later work Selye (1942) studied and described the anesthetic property of 75 steroids in partially hepatectomized rats. Pregnanedione, which is found in urine and is hormonally inactive, manifested the most pronounced anesthetic action. Selye concluded that the highest anesthetic effect was exhibited by steroids, oxygenated at the two extreme ends of the molecule, and the activity depended on the rate of absorption. Esterification could render any compound more potent or less potent, depending on whether it increased or delayed its absorption.

P'An et al. (1955) described the anesthetic action of a water soluble steroid, hydroxydione (21-hydroxypregnanedione sodium succinate). This compound possessed a pronounced central nervous sytem depressant property in a number of species, but was devoid of all hormonal activity.

Overbeek and Bonta (1964) and Bonta and Overbeek (1964) described several hormonally inactive steroids with central depressant activity. Some compounds - which had a pregnane nucleus - caused sedation and augmented barbiturate anesthesia.

Atkinson et al. (1964) investigated the possible central nervous system activity of 142 synthetic pregnane derivatives. They found that 67 of these possessed hypnotic activity.

In summary: A number of naturally occurring steroids, steroid metabolites or synthetic steroid derivatives, can induce hyperthermia, hypothermia, hypnosis and anesthesia.

MATERIALS AND METHODS

1. Extraction of Pregnant Mare Urine

All experiments described in this work, were carried out with extracts obtained from pregnant mare urine. Our starting material was a hexane-hexanol fraction of the pregnant mare urine which was obtained as by-product in the course of the isolation of estrons. This partially purified fraction is a dark brown, viscous substance and each gram of the preparation corresponds to approximately 5 liters of horse urine. The fraction is insoluble in water but is readily soluble in organic solvents, such as chloroform, ether, petroleum, etc.

In order to eliminate simple phenols (phenol, cresol, etc.) 1 g residue was dissolved in 50 ml chloroform and extracted 4 times with 0.1 N NaOH, then 4 times with distilled water. The volume of the extracting fluid was 100 ml in each extraction.

The watery layer was discarded and the chloroform layer which contained the biologically active substance, retained. The chloroform was evaporated under vacuum using a flash-evaporator, and the substance dried to constant weight. 1 g of the original hexane-hexanol fraction yielded 420-460 mg of chloroform soluble extract.

The material was stored in a 250 ml quickfit flask under Argon at $-10^{\circ}C$ until further use.

When the antisecretory effect of the urine extract was investigated in Heidenhain-pouch dogs, large quantities were needed. The steps of the extraction were the same as described above but instead of 1 g, 4 g starting

material was extracted at one time. This amount was dissolved in 200 ml chloroform and extracted 4 times with 0.1 N NaOH, then 4 times with distilled water. The volume of the extracting fluid was 400 ml in each extraction. The evaporation and storage procedures were unchanged.

2. The Antihistamine Assay Using the Isolated Guinea-Pig Ileum Preparation

A male guinea-pig, weighing between 200-300 g, was sacrificed by a blow on the back of the neck. The abdomen was opened and a piece of terminal ileum, 20-30 cm long, was removed by stripping from the attached mesentery. It was gently washed through with Tyrode solution then subdivided into segments of approximately 6 cm in length.

A segment was suspended in a 20 ml organ bath. A platinum hook on the oxygen inlet, which was suspended above the bath, provided a means for attaching the gut within the bath. The other end was passed through a small hook attached to a thread which fixed this upper end of the gut to the writing lever located directly above it. This lever recorded the isotonic contractions of the strip of ileum on a smoked drum using the same lever and the same magnification of 1:2. The bath contained Tyrode solution at 34°C through which a mixture of 95% oxygen and 5% CO_2 was bubbled.

The Tyrode solution contained the following constituents per liter: NaCl, 8.0 g; KCl, 0.2 g; $NaHCO_3$, 1.0 g; dextrose, 1.0 g; $CaCl_2$, 0.2 g; $MgCl_2$, 0.2 g; NaH_2PO_4, 0.05 g. The 20 ml organ bath was situated in a plastic water bath of 30 x 20 x 23 cm (Palmer Co.), and its solution was replaced by fresh Tyrode after each addition of histamine. The Tyrode solution was pre-circulated through a series of coils placed into the water bath; the

latter was heated to 34°C and kept at this temperature.

The ileum was allowed to stabilize for one-half hour in the organ bath. The response of the ileum was standardized by injections of doses of standard histamine dihydrochloride, ranging from 0.1 - 0.4 µg depending on the sensitivity of the preparation.

The standard dose of histamine was repeatedly administered into the organ bath at 3 minutes intervals. Histamine was left in contact with the ileum for 20 seconds, during which time the segment contracted, and then the drug was washed out for 10 seconds. Two minutes and 30 seconds were allowed to elapse before the next injection. The extract was injected into the bath after three closely identical responses were obtained with the standard dose of histamine. The extract was dissolved in ethanol (10 mg/0.5 ml) immediately prior to its introduction into the bath. The ethanol solution was diluted with Tyrode in two steps: the "stock solution" contained 10 mg/ml, the "test solution" 1 mg/ml of the extract. The effect of "test solutions" which did not contain the extract were also investigated using identical volumes.

Graded doses of the extract were introduced into the bath and left in contact with the tissue for 2 minutes and 30 seconds prior to histamine administration. The standard histamine injections were repeated until contractions were the same as those before the extract, that is the same as the control contractions.

3. Gastric Secretion

A. Animals

(a) Guinea-pigs: multicolored, short haired variety of both

sexes. The males weighed 320 - 420 g, the females weighed 280 - 380 g at the time of the experiment.

(b) Rats: hooded, male weighing between 180 - 200 g at the time of the experiment.

Both the guinea-pigs and the rats were purchased one week prior to experimentation from the Quebec Breeding Farm Inc. or Robidoux Laboratory Animal Farm. They were kept on a normal diet (Purina guinea-pig chow, Purina Labena) and water ad libitum.

(c) Dogs: one male dog weighing 60 lbs (27 kg) and one female dog weighing 34 lbs (15.3 kg) at the time of the operation (preparation of Heidenhain pouch) were used. The dogs received normal dog food (Purina Dog Chow and Ken.L Ration dog food) and water ad libitum. After each experiment they also received half a quart of milk. The dogs were purchased from J.B. Gauthier's Kennel, Ste. Anne de Plain.

B. Operative Procedure and Analysis of Gastric Juice

(a) Guinea-pigs and rats: The animals were fasted for 24 hours before the experiment; water was allowed at all time.

To induce anesthesia, a mixture of 10% urethane and 5% sodium pentobarbital (Nembutal) solution (ratio 19:1) was used. The mixture was injected intraperitoneally in a dose of 4 ml/kg 20 minutes prior to the operation. When necessary this dosage was supplemented with additional quantities.

The method used for collecting and analysing the gastric juice was similar to that described by Herr and Porszasz (1951). Through a midline

incision the stomach was gently pulled out, following which the duodenum was ligated about 2 cm distal from the pylorus, carefully avoiding all nearby blood vessels. Through a small incision, 1 cm proximal to the pylorus a polyethylene tube (10 cm long, 3 mm diameter) was inserted into the stomach. The position of the tube was carefully fixed by a thread in such a way that its tip penetrated no deeper than 2 to 3 mm into the cavity of the stomach. A soft rubber catheter (Ingram-Bell, No.8) was inserted orally and the stomach was washed with physiological saline kept at 37°C, until all food particles were removed and the washing fluid seemed clear. The abdominal cavity was then closed.

The animals were placed on an elevated holder, face down, and remained in this position throughout the experiment. Heating lamps were used to maintain normal body temperature.

The stomach was washed with 3 ml saline (37°C) at 15 min intervals. To assure a complete recovery of the fluid, 2 ml air was gently pushed through the catheter after the fluid, taking care not to cause any mechanical distention. The gastric samples were collected directly from the polyethylene cannulae into a graduated cylinder; and the actual volume of gastric juice was then obtained by subtracting the 3 ml washing fluid from the total volume measured.

The amount of free and total acid was determined by titrating against 0.01 N NaOH, with Töpfer's reagent and phenolphthalein as indicators. The amount of acid secreted was expressed in mEq per 15 minutes. Total acidity, considered a truer reflection of parietal cell secretion than free acidity (Shay et al. 1950), was chosen to illustrate the acid output in all experiments.

Basal acid output was determined for at least 30 minutes. In the experiments in which dose-response curves were determined, the stimulant (histamine or gastrin) was injected as a single s.c. dose, and collections were made every 15 minutes until the secretory rate returned toward basal levels. In the experiments in which the gastric acid inhibitory effect of urine extract was determined, the extract (or vehicle) was injected as a single i.p. dose and 60 minutes later the secretagogue was injected as a single s.c. dose. Collections were made every 15 minutes until the secretory rate returned toward basal levels.

Both male and female guinea-pigs received histamine dihydrochloride (calculated as a base) in doses ranging from 0.1 to 0.8 mg/kg. Histamine was dissolved in distilled water and the concentration of the solution was such, that all animals received 0.5 ml/100 g body weight.

Gastrin (Leo Pharmaceutical Products) which was prepared according to the method of Gregory, was used in all experiments. We used batch number 65031 which contained 160 Leo units per vial. One Leo unit was equivalent to 1 µg of the highly purified gastrin, and was standardized by the manufacturers according to IAI's method.

The male guinea-pigs received gastrin in doses ranging from 0.5 µg/kg to 400 µg/kg. The female guinea-pigs received gastrin in doses ranging from 2.0 µg/kg to 400 µg/kg. Gastrin was kept in sealed vials at -4°C and dissolved immediately prior to use in distilled water; the concentration of the solution was such that the guinea-pigs received between 0.25 and 0.5 ml/100 g body weight. Hooded rats received gastrin in a dose of 2.0 µg/kg. The concentration of the solution was such that the rats

received 0.2 ml/100 g body weight. The extract was administered intraperitoneally in a dose of 1.5 g/kg. It was dissolved in isopropyl myristate in a concentration of 500 mg/ml.

(b) Dogs: The dogs were fasted for 24 hours preoperatively. I.v. sodium pentobarbital (Nembutal) provided satisfactory anesthesia. It was given in the following dose: 1 ml/5 lbs. body weight from a 6% solution. Nembutal was easily supplemented as required during operation via the large sublingual veins.

The abdomen was shaved first with an electric clipper, then with a razor, and the skin was disinfected with Metaphen (Nitromersol N.F.) tincture. Sterile drapes were applied to cover the entire animal except for the operative field.

As guide-line in the operative technic we relied upon the methods described by Friedman (1951) and de Vito (1959).

A midline incision from xiphoid to umbilicus was made, first cutting the skin, then the muscle layer and finally the peritoneum. A self-retaining retractor was placed into the abdominal cavity to provide exposure of the stomach. The right gastroepiploic artery was tied off on the greater curvature, several cm proximal to the antrum. The small vessels were divided between ligatures for a distance of 1 cm. Two curved Mayo Robson stainless steel gastrointestinal clamps (10 inches long) were used; the "arms" of the forceps were covered with soft rubber tubing.

The clamps were introduced through the devascularized area and directed cephalid to divide the stomach lengthwise. The stomach was kept stretched and flattened while the clamps were applied so that optimal placement

was possible. The placement of the forceps resulted in a gastric segment composed of the greater curvature and anterior and posterior walls of part of the corpus. Next, all layers of the anterior and posterior walls of the stomach were cut by passing scissors between the clamps. The clamp on the main stomach was moved back from the edge, taking care to avoid spilling of any gastric content. The mucosal layer was closed by a continuous suture, the muscular and serosal layer by single stitches. The clamp was removed and the incision line covered with omentum. The clamp on the segment was moved back from the edge and the segment formed into a pouch by inverting continuous sutures of the mucosal layer, starting at the cardiac end. The stainless steel cannula (3 inches long, with a diameter of 9 mm) was placed in the central portion of the pouch through a small incision in the anterior wall. A single, rather loose purse-string held it in place. The muscle and serosal layers were closed by single stitches.

A stab wound was made on the left side of the abdominal wall near the midline and the cannula was drawn through it. The pouch was covered with omentum. The abdomen was closed in layers. The peritoneum was closed with continuous sutures, both the muscle and skin layers by single stitches.

Postoperative care: the dogs were given 500 ml of a 5% glucose solution in normal saline, by i.v. drop infusion, lasting for about 2 hours. They also received an i.m. injection of Fortimycin-½ suspension (vials of 2 ml). The 2 ml dose contained 400.000 I.U. of Penicillin G Procaine in aqueous suspension with ½ g of Streptomycin sulfate (as base). This dose of antibiotic was repeated 24 and 48 hours postoperatively. Following the operation, the dogs received only water for 24 hours. They were kept on milk and water

for the next three days. On the fifth postoperative day the administration of semisolid food was started, slowly increasing the solid content. From the tenth day on, the dogs received normal dog food and water ad lib.

Not less than four weeks after the operation the dogs were trained to stand quietly in an up-right position, loosely strapped in a Pavlov frame. Before each test the dogs were fasted for 18 hours. On the morning of each test, the pouch was gently washed with luke-warm distilled water. Histamine dihydrochloride (calculated as base) was injected subcutaneously into the hind leg. The dose of the histamine was 0.175 mg/kg, using a solution with a concentration of 5 mg/ml. The gastric juice was collected from the cannula at 15 minutes intervals throughout the experiment, directly into a 50 ml Erlenmeyer flask which was kept in place by a rubber tube loosely tied around the animal.

The volume of each specimen was measured to the nearest 0.1 ml and the acidity determined by titration with 0.1 N NaOH, using Töpfer's reagent and phenolphthalein as indicators. The amount of acid secreted was expressed in mEq per 15 minutes.

Experiments were performed at weekly intervals. The response to a single s.c. dose of histamine was determined in five experiments. The extract or vehicle was administered orally two hours before stimulation with histamine. Four experiments were performed with the vehicle+histamine and four experiments with the extract+histamine in each dog. The vehicle or extract were administered at alternative weeks.

The dose of extract administered to the dogs was 1.3 g/kg. It was dissolved in isopropyl myristate, in a concentration of 1 g/ml.

4. Ulcer Production in Experimental Animals

A. Shay Method

Animals: Male, hooded rats weighing between 160 and 180 g at the start of the experiment, were used.

Procedure: The method of Shay et al. (1945) was used with slight modification. For 48 hours before each experiment the animals were fasted in cages provided with wire mesh bottoms to prevent coprophagy, however, water was allowed during this period. The water was removed from the cage at the start of the experiment, at which time the animals were weighed. This weight was used in all subsequent calculations. Under light ether anesthesia the abdomen was opened, through a mid-line incision, and the pylorus exposed without handling the stomach. A fine thread was then tied around the pylorus, care being taken to avoid inclusion of adjacent blood vessels. The wound was then closed and the animal returned to its cage where it regained consciousness. 17 hours later the animal was again anesthetized, the abdomen opened and the stomach removed after tying the esophagus, following which the animal was killed without regaining consciousness. By opening the stomach along its greater curvature gastric juice was collected individually in graduated centrifuge tubes. The stomach was microscopically examined. Ulcers appeared in the ruminal part. The severity of ulceration was graded and expressed according to Pauls et al. (1947). With increasing severity the ulcer was graded from +1 to +4; +1 described a few small ulcers; +2, several small ulcers and 1 or 2 large ulcers; +3, several large ulcers, while +4 a perforated ulcer. By multiplying the average + ulceration in a given group by the percentage of animals showing

ulceration in that group, the "index of ulceration" is obtained.

The gastric juice was centrifuged and its volume measured in milliliters. All rats were excluded from the experiment, when the gastric juice collected from their stomach, contained 0.5 ml or more of solid particle after centrifuging the sample. The acidity was determined by titrating the sample with 0.1 N NaOH, using Töpfer's reagent and phenolphthalein as indicators. Acid output of the whole sample was determined and expressed in milliequivalents.

Urine extract was administered either orally, 6 or 24 hours prior to pylorus ligation, or intraduodenally immediately after ligation.

Oral administration was performed with the help of a soft rubber catheter (Ingram-Bell, No.8); intraduodenal injection was given with a 23-gauge needle distal to ligation. Control animals received the corresponding volumes of the vehicle both orally and intraduodenally. The extract was dissolved in isopropyl myristate and was administered in the following doses: 2.0, 2.5 and 3.0 g/kg orally, and 0.5, 1.0 and 1.5 g/kg intraduodenally.

B. Restraint (Stress) Method

Animals. Female albino rats weighing between 170 and 190 g at the start of each experiment were used. They were purchased from the Quebec Breeding Farm Inc.

Procedure. The method of Bonfils and Lambling (1963) was used with slight modification. For 24 hours before each experiment, the animals were fasted in cages provided with a wire mesh bottom to prevent coprophagy.

Only water was allowed during this period, which was discontinued at the start of the test at which time the animals were weighed. This weight was used in all subsequent calculations. Under light ether anesthesia the animal was restrained by placing around its body a wire mesh screen so that it could not move. The shape of the screen was formed to be identical with the shape of the animal's body. Thus the restriction volume closely corresponded to the rat's body volume. After 24 hours restraint the wire mesh was removed and the animal was anesthetized. Its stomach was removed, opened along the greater curvature gently washed in saline and examined macroscopically for lesions of the gastric mucosa. The lesions appeared in the glandular part of the rat stomach. They were evaluated according to the "all or nothing" criterion of Bonfils and Lambling (1963). According to this classification all lesions present, regardless of their severity and number, are considered to be "positive", while all stomach samples without lesion, are considered to be "negative". Ulceration is expressed as percentage. The extract was dissolved in isopropyl myristate and administered either intraperitoneally or intramuscularly, immediately prior to immobilization, in the following doses: 0.75 and 1.0 g/kg intraperitoneally and 1.0 and 1.5 g/kg intramuscularly; 2.0 ml physiological saline was injected subcutaneously immediately prior to immobilization, to avoid dehydration.

5. Recording of Blood Pressure and Electrocardiogram

The cardiovascular effects of urine extract were tested in both rats and guinea-pigs. Male hooded rats, weighing 300 - 370 g, and male guinea-pigs, weighing 380 - 440 g were used.

All animals were anesthetized with urethane 30 minutes prior to

operation. Urethane was given in a dose of 1 g/kg intraperitoneally, as a 20% solution.

Heparin was given in a dose of 1 mg/kg intravenously as a 0.1% solution, to prevent clotting. Carotid arterial pressure was measured in all experiments with a Statham P 23 AA blood pressure transducer. Recordings were made on a Gilson polygraph. Electrocardiogram (LEAD II) was taken on a Sanborn Visocardiette.

Artificial respiration was maintained with a Palmer respiratory pump, the rate of respiration being kept at about 88/minute, and the tidal volume between 4.5 - 5 ml. The mean arterial blood pressure was calculated according to the following formula:

$$\frac{\text{systolic pressure} + 2 \text{ times the diastolic pressure}}{3}$$

Blood pressure and electrocardiographic readings were taken every 15 minutes throughout the experiment.

Control readings were taken for 30 minutes prior to the i.p. administration of the urine extract. The rats received the extract at two dose levels, 1.0 and 1.5 g/kg; the guinea-pigs received the extract at a dose of 1.5 g/kg. Following treatment with the extract, blood pressure and electrocardiogram were measured for two hours.

The blood pressure and heart rate, recorded at 15 minutes intervals, was statistically compared to values obtained prior to the administration of the extract.

6. Investigation of Central Nervous System Activity

A. Animals

Male, hooded rats were employed as test animals in all experiments.

Their weights ranged from 120 - 160 g. They were kept on a normal diet (Purina Labena) and water ad libitum for one week prior to the experiment.

All food and water was taken away from the animals on the morning of the experiment. No animal was used more than once. The great majority of experiments were conducted in an environment of 20 - 22°C, and a 40 - 60% relative humidity.

B. Measurement of Body Temperature

Rectal temperature was measured in all experiments by using a Telethermometer apparatus (Yellow Springs Instrument Co.).

The rat was held in the left hand and the probe was inserted rectally, 1½ inch deep, using vaseline. The reading was taken when the temperature became stable, i.e. after 30 seconds.

The temperature was measured before starting an experiment and 1, 2, 3, 4, 5 and 24 (sometimes 48) hours after the administration of the urine extract.

C. Elevated Environmental Temperature

Some of the experiments were performed at elevated environmental temperature. A wooden box was build in our laboratory for this purpose, 50 x 30 x 32 cm. A wire-mesh was build in, one inch from the bottom, to prevent coprophagy. The top was made of glass, which enabled the experimenter to observe the rats without opening the box.

A 100 W bulb served as heat source, while a built-in thermostat maintained the temperature between 30.7 - 31.6°C.

D. Estimation of the Hypnotic ED 50

The procedure was based upon the method of Miller and Tainter (1944). Groups of 5 rats each were injected intraperitoneally with graded, logarithmic doses of the urine extract. An equal volume of the vehicle (isopropyl myristate) was administered to the control group.

The animal was considered asleep when it had lost its righting reflex, that is the ability to right itself from the dorsal to the ventral position for at least one minute. The percentage of sleeping animals was plotted against the dose administered. The doses employed were rather close to each other and it was impossible to plot them on a logarithmic scale. Therefore we plotted the dose on an ordinary mm scale against the percentage probability.

In drawing the best fitting line, one has to bear in mind that the points farthest from 50% have the least weight per animal in fixing the true position of the line. Accordingly the line best fitting the points of observation was drawn. The estimated ED 50 was the dosage value at 50% and was read directly from the graph paper in original dosage units.

The exact position of points representing the 0 and 100% effect, was corrected on the basis of a correction table taken from Litchfield and Wilcoxon (1949). In the course of this experiment we also determined acute mortality, that is death occurring within 24 hours, and mortality occurring after 48 hours and after one week. Body weight changes were also recorded for the same length of time.

E. Potentiation of Subhypnotic Doses of Hexobarbital

The method used was similar to the one described by Toman and

Everett (1958). The rats were divided into 3 groups, with group 1 receiving the vehicle, group 2 the hypnotic ED 5 dose of the extract, and group 3 the hypnotic ED 95 dose of the extract. All these injections were given intraperitoneally.

30 minutes later all groups received a subhypnotic dose of hexobarbital sodium. The sleeping time was recorded until spontaneous waking occurred. The same experiment was performed both at room temperature (20 - 22°C) and at elevated ambient temperature (30.7 - 31.6°C). Rectal temperature was measured in all animals throughout the experiment.

F. Antagonism of Urine Extract-Induced Hypnosis and Hypothermia

A number of drugs were investigated with respect to their effect upon hypnosis and hypothermia induced by the i.p. injection of the hypnotic ED 50 dose of the extract.

(a) Drugs: All solutions were freshly prepared on the day of the experiment and were administered intraperitoneally or subcutaneously, by means of a tuberculine syringe and a 23 gauge needle, and orally, by means of a tuberculine syringe and a rubber catheter (Ingram and Bell, No.8).

The concentration of all solutions was so prepared that between 0.3 and 0.8 ml/100 g body weight represented the doses intended, with the exception of α-methyl-meta-tyrosine where the dose/100 g body weight was in 1.6 ml. All drugs were dissolved in distilled water.

The antagonist drugs were investigated at the following dose levels:

picrotoxin: 0.25, 0.5 and 1.0 mg/kg;

pentylenetetrazol: 5.0, 10.0, 20.0 and 40.0 mg/kg;

nikethamide: 15.0, 30.0 and 60.0 mg/kg and

amphetamine: 3.75, 7.5, 15.0 and 30.0 mg/kg.

The dose of the extract was 1110 mg/kg, in a concentration of 200 mg/ml. The extract was dissolved in isopropyl myristate.

(b) Experimental arrangement I.: The experiment consisted of the following steps: oral administration of graded doses of the antagonist drug; 30 minutes later i.p. administration of the extract and observation of the occurrance of hypnosis and hypothermia.

(c) Experimental arrangement II.: Rats were divided into 3 groups: group 1 received the extract; group 2 iproniazid, 100 mg/kg subcutaneously for 3 consecutive days and the extract on the third day, 4 hours after the last injection of iproniazid. Group 3 received iproniazid, 100 mg/kg subcutaneously for 3 consecutive days, α-methyl-meta-tyrosine, 500 mg/kg intraperitoneally in the morning of the third day and 4 hours later the extract.

G. Spontaneous Motility

Spontaneous motility was determined only on a qualitative basis. Two rats were placed into a cage prepared from a half inch wire mesh and measuring 13 x 13 x 13 cm. Each cage was suspended on a flexible iron blade. The bottom of the cage was attached with the help of a hook and thread to a Gilson Force Transducer. Recordings were made on a Gilson polygraph. Each recording channel was set to the same sensitivity. Recordings were started at the end of the first hour following administration of the extract, and were carried on throughout the subsequent 4 hours. Motility of normal

control and antagonist-pretreated "control" rats was also observed.

In the extract treated groups, the 2 rats were selected according to observations made during the first hour following administration of the extract. Animals who lost their righting reflex were paired with those in whom hypnosis was not induced.

7. Statistical Analysis

In the statistical evaluation of the results, Student's "t" was used as a significant test, except in the "restraint" method, where the results were evaluated according to the Chi Square test.

8. Agents and Drugs Used

Ethyl Carbamate (Ethyl Urethane), Fisher Scientific Co., Montreal, Que.

Pentobarbital Sodium (Nembutal), May and Baker Ltd., Dagenham, England.

Histamine Dihydrochloride, Matheson Coleman & Bell, Norwood, Ohio, East Rutherford, N.J.

Gastrin, Leo Pharmaceutical Products, Denmark.

Picrotoxin, The British Drug Houses Ltd., Poole, England.

Pentylenetetrazol (Metrazol), Bell- Craig Pharmaceuticals, Toronto, Ont.

Nikethamide (Coramine) liquid, Ciba Co. Ltd., Dorval, Que.

d-Amphetamine (Dexedrine), Smith, Kline & French, Montreal, Que.

Iproniazid (Marsilid), Hoffman - La Roche Ltd., Montreal, Que.

DL-alpha-methyl-meta-tyrosine, Regis Chem Co., Chicago, Ill.

Pentobarbital Sodium, 6%; Siegfried S.A., Zofingue, Switzerland.

Metaphen (Nitromersol N.F.), Abbott Laboratories Ltd., Montreal, Que.

Fortimycin-1/2 suspension, Ayerst Laboratories, Montreal, Que.

RESULTS

I. Antihistamine Activity of Urine Extract on the Isolated Guinea-Pig Ileum

As a routine procedure, every extract was tested on an isolated guinea-pig ileum preparation in order to establish its antihistamine activity. The potency of the extracts was standardized by this method. Only those extracts were used in in vivo experiments, which were able, in a dose of 1 mg or less, to exert a complete inhibition against the histamine-induced contractions when introduced into the 20 ml bath.

A typical example of the results obtained by the extracts, is shown in Fig.2.

The administration of the extract in a concentration of 1.25×10^{-5} g/ml elicited about a 50% reduction in the contractions induced by 0.2 µg of histamine. The addition of the extract in a concentration of 2.5×10^{-5} g/ml produced about a 90% inhibition. After repeated washing, however, the gut regained its original sensitivity to the standard dose of histamine.

The administration of equal volumes of the vehicle had no effect upon the histamine-induced contractions.

II. Studies on Gastric Secretion

A. Effect of Graded Doses of Histamine and Gastrin on Gastric Acid Secretion in Male and Female Guinea-Pigs

1. Effect of Histamine

Gastric secretory changes recorded in response to increasing doses of histamine in male and female guinea-pigs, are illustrated in Fig.3 A and B respectively.

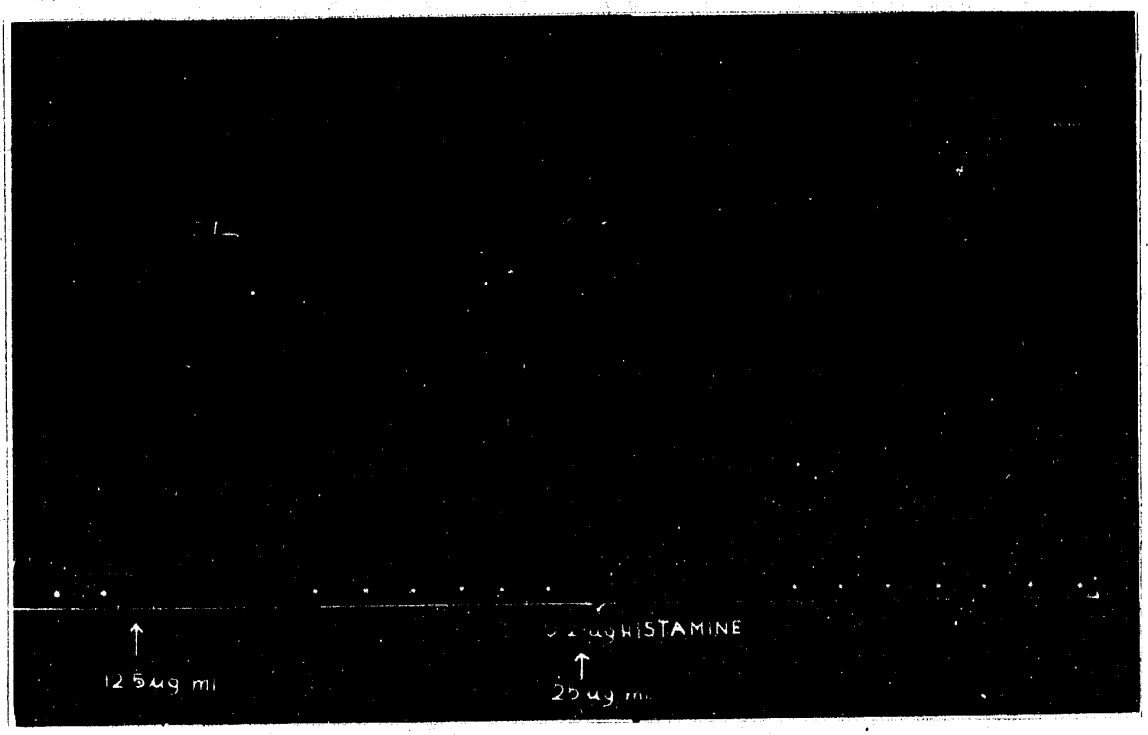
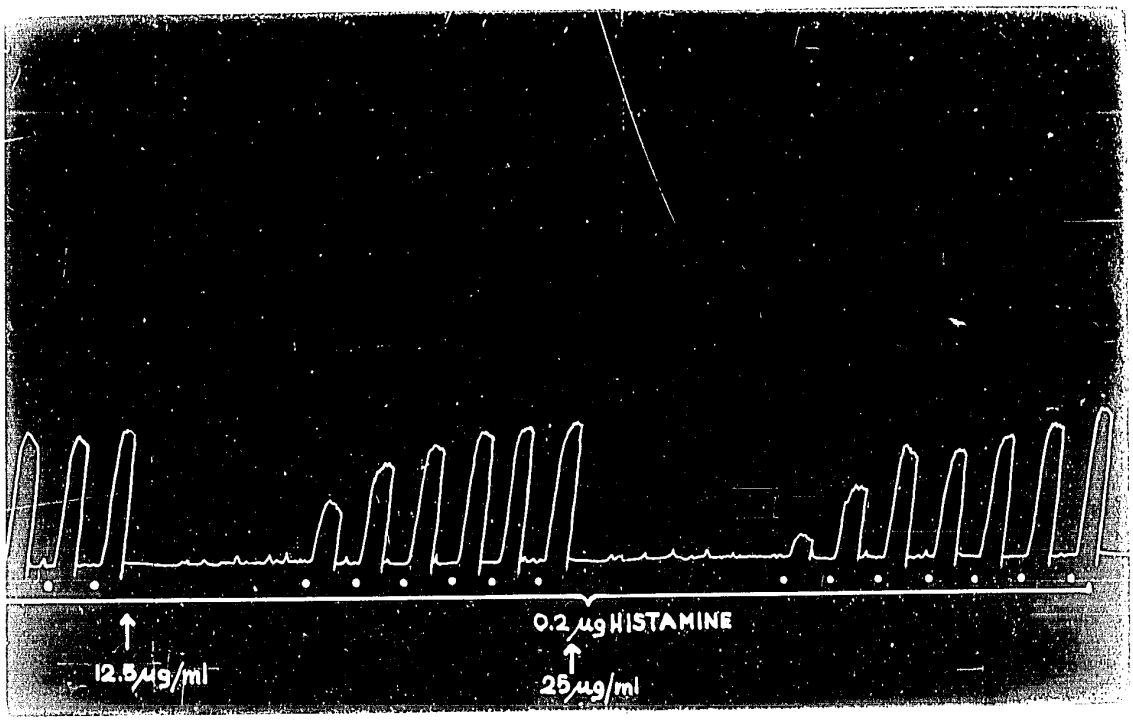


Fig.2: Response of guinea-pig ileum preparation to histamine (white dots) before and after the administration of horse urine extract (as marked at arrows) in increasing concentrations (12.5 ug/ml and 25 ug/ml). In each instance the drum was temporarily stopped after washing and restarted 20 sec before the next test. After each administration of the extract the record was taken continuously for 3 min.


0.2 µg HISTAMINE
12.5 µg/ml
25 µg/ml

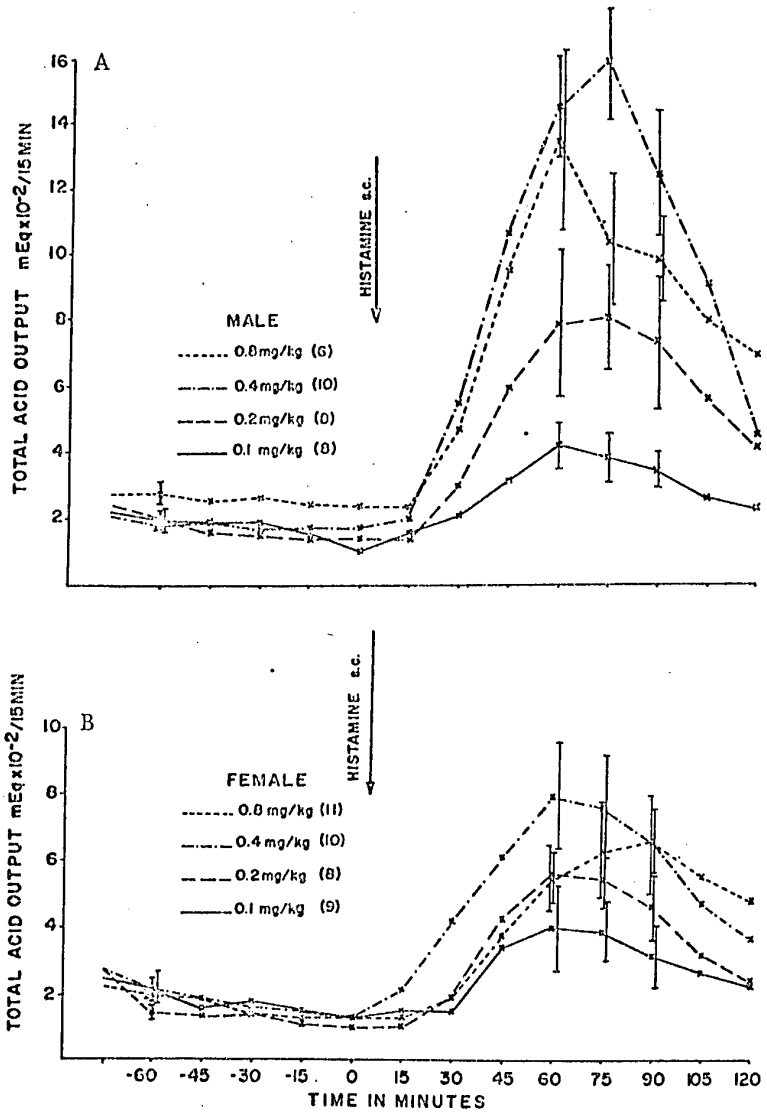


Fig.3: Mean total acid output (mEq x 10^{-2}/15 min) in male (Fig.3/A) and female (Fig.3/B) guinea-pigs in response to graded doses of histamine given in a single s.c. injection. Number in parenthesis refers to number of animals at each dose level. For details see text.

The results of this experiment are discussed in detail in the chapter dealing with the sex difference in gastric acid output in response to histamine.

Identical doses were employed in the 2 sexes. Following a single s.c. injection of histamine, acid secretion increased rapidly at all dose levels, reaching a peak and then declining toward basal levels at the end of the second hour. Even the smallest dose of histamine used in this experiment, 0.1 mg/kg, induced a well-defined increase in acid output in both sexes.

Higher doses of histamine induced correspondingly higher acid output. Maximum histamine response was attained at a dose of 0.4 mg/kg in both male and female guinea-pigs. A higher dose of histamine, 0.8 mg/kg, proved to be a supramaximal dose, inducing a lower acid secretory response when compared to the maximal dose. In the female guinea-pigs, peak acid secretion was reached 60 minutes after the injection of histamine, except with the supramaximal dose with which acid secretion rose slower and reached the peak only 90 minutes after the administration of histamine.

In the male animals peak secretory values were recorded either 60 or 75 minutes after the administration of histamine.

2. Effect of Gastrin

Gastric secretory changes recorded in response to increasing doses of gastrin in male and female guinea-pigs are summarized in Table 1. and illustrated in Fig.4.

i. Response in male guinea-pigs: Table 1/A shows that the in-

Table 1: EFFECT OF GASTRIN ON GASTRIC ACID SECRETION IN GUINEA-PIGS

Section A shows total acid output in male, section B in female guinea-pigs during basal secretion and during peak secretion (30, 45 and 60 min) following the administration of a single s.c. dose of gastrin in graded doses.

	Dose of Gastrin µg/kg	No. of animals	Total acid output mEqx10^{-2}/15 min ± S.E			
			Basal	Peak (min after gastrin)		
				30	45	60
A.	0.5	4	2.89 ± .61	2.61 ± .70	3.32 ± .98	2.46 ± .57
	1.0	4	1.54 ± .22	1.44 ± .024	1.25 ± .10	1.16 ± .11
	2.0	4	2.12 ± .51	2.10 ± .50	2.98 ± 1.2	1.82 ± .68
	10.0	4	2.66 ± 1.44	3.29 ± .95	3.01 ± .95	2.72 ± 1.05
	60.0	4	1.85 ± .39	5.81 ± .77	5.14 ± .99	5.08 ± 1.2
	100.0	10	1.73 ± .24	6.14 ± .69	7.42 ± .76	4.90 ± .69
	200.0	4	2.36 ± .97	9.15 ± 1.85	10.09 ± 2.65	8.01 ± 2.0
	400.0	3	1.02 ± .32	1.45 ± .49	1.97 ± 1.1	3.77 ± 2.2
B.	2.0	6	2.02 ± .29	1.27 ± .14	1.48 ± .23	1.22 ± .16
	100.0	7	1.68 ± .32	4.67 ± .82	5.18 ± 1.06	3.89 ± .58
	200.0	4	1.42 ± .29	8.60 ± 2.55	7.00 ± 2.0	4.09 ± 1.01
	400.0	3	1.42 ± .26	1.50 ± .50	1.68 ± .32	2.27 ± .37

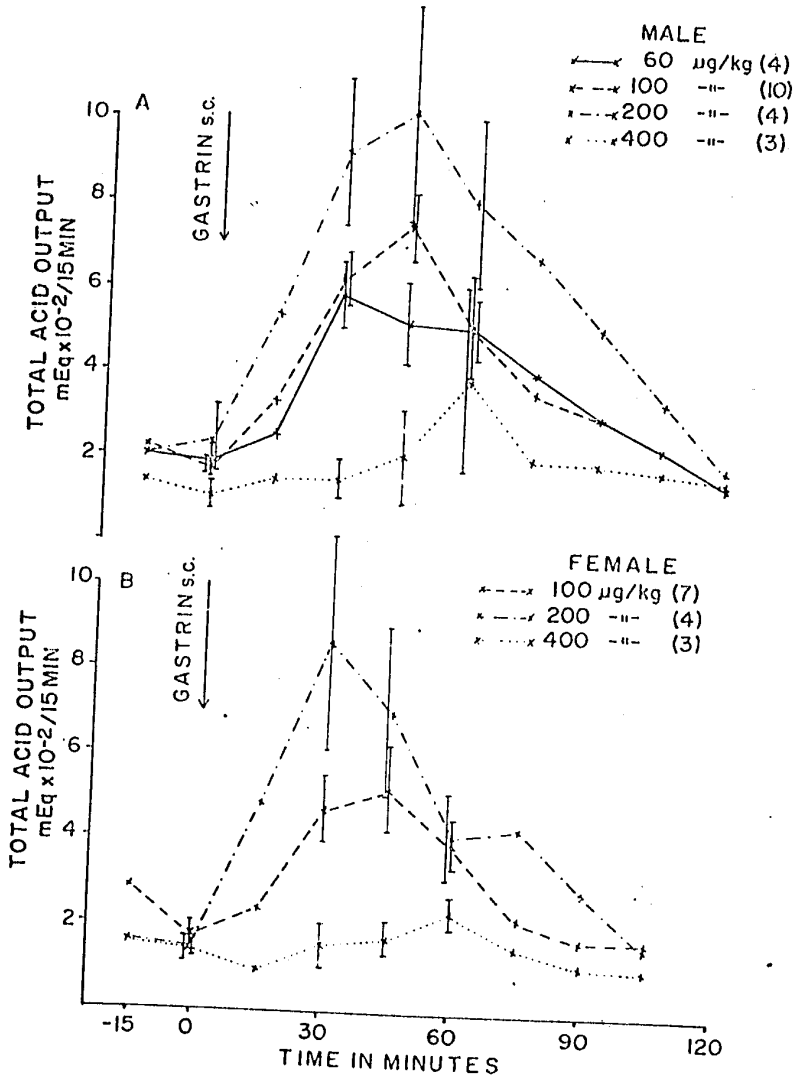


Fig.4: Mean total acid output (mEq x 10^{-2}/15 min) in male (Fig.4/A) and female (Fig.4/B) guinea-pigs in response to graded doses of gastrin given in a single s.c. injection. Number in parenthesis refers to number of animals at each dose level. For details see text.

jection of gastrin in doses of 0.5, 1.0, 2.0 and 10.0 µg/kg did not induce a significant increase in acid secretion. Each dose of gastrin was administered to 4 guinea-pigs, and the results express the mean total acid output. The administration of 60 µg/kg of gastrin to 4 guinea-pigs induced a well-defined acid secretory response. Total acid output reached a peak 30 minutes after the administration of gastrin, then declined slowly reaching basal levels in 2 hours (Fig.4/A).

The administration of 100 µg/kg of gastrin to 10 guinea-pigs and 200 µg/kg of gastrin to 4 guinea-pigs, induced increasing acid secretory responses. Peak values were obtained at 45 minutes with both doses (Fig.4/A).

Maximum response was attained with 200 µg/kg of gastrin in the male guinea-pigs.

400 µg/kg of gastrin injected into 3 guinea-pigs proved to be a supramaximal dose. The acid secretory response was slight and delayed when compared to responses elicited by the lower doses, but a peak could be observed 60 minutes after the administration of gastrin.

ii. Response in female guinea-pigs: In the female animals, among the smaller doses 2.0 µg/kg was the only dose of gastrin tested (Table 1/B). The administration of this dose of gastrin to 6 animals did not result in any gastric acid secretory response.

100 µg/kg of gastrin, administered to 7 animals, induced a well-defined increase in acid secretion, while 200 µg/kg administered to 4 guinea-pigs induced maximal acid secretion in the female animals as well (Fig.8/B).

Acid secretion remained virtually unchanged following the injection

of 400 µg/kg of gastrin and only a slight increase could be observed after 60 minutes. This dose of gastrin was tested in 3 guinea-pigs.

B. Effect of Gastrin on Gastric Acid Secretion in Male, Hooded Rats

Fig.5 illustrates the effect of a single s.c. injection of 2.0 µg/kg of gastrin on acid secretion in 10 male hooded rats. This small dose of gastrin elicited a well-defined increase in acid secretion. Maximal acid output occurred 45 minutes after the administration of gastrin. The effect was relatively short lasting and in 90 minutes acid output declined to basal levels.

C. Effect of Urine Extract on Histamine-Induced Gastric Acid Hypersecretion

1. Experiments in Male Guinea-Pigs

The result of the experiment obtained in 10 treated and 10 control male guinea-pigs is summarized in Fig.6.

Prior to the administration of the extract neither total acid output, nor the volume of the gastric juice secreted was significantly different between the 2 groups.

After establishing basal acid output and the basal rate at which gastric juice was secreted, 10 animals were injected intraperitoneally with the extract, 1.5 g/kg; the control animals received an equal volume of the vehicle.

One hour was allowed for absorption. During this period acid output gradually declined in the treated animals but in the controls it remained virtually unchanged. Thus, 60 minutes after the administration of the extract,

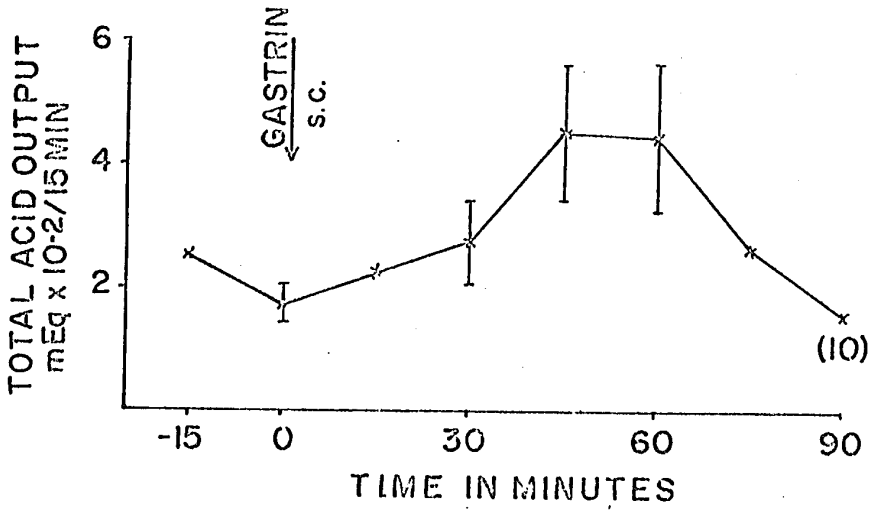


Fig.5: Mean total acid output (mEq x 10^{-2}/15 min) in male hooded rats in response to gastrin given in a dose of 2.0 µg/kg subcutaneously. Number in parenthesis refers to number of animals used in the experiment.

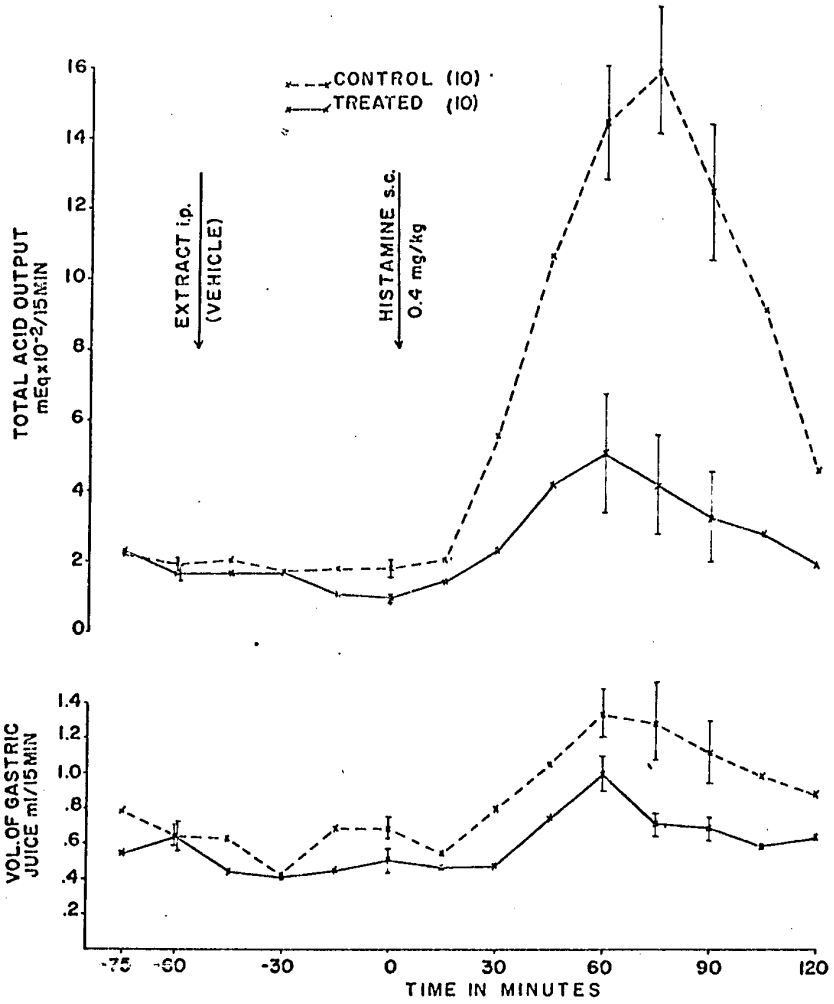


Fig.6: Mean total acid output (mEq x 10^{-2}/15 min) and mean volume of gastric juice secreted (ml/15 min) in control animals and in guinea-pigs treated with horse urine extract before and after the administration of 0.4 mg/kg of histamine. Number in parenthesis refers to number of guinea-pigs in each group.

acid output was already significantly lower ($P < 0.01$) in the treated group prior to the administration of histamine. The volume of gastric juice secreted was also lower at the end of this one hour period, though not significantly. After a single s.c. injection of 0.4 mg/kg of histamine, acid secretion and the rate of volume secretion was stimulated in both groups, although to greatly different degrees. The values of the 2 parameters under investigation increased to a peak, and then decreased toward basal levels.

Peak secretion was observed 60, 75 and 90 minutes after the administration of histamine. These three samples were therefore used for statistical analysis and comparison of the secretory changes occurring in both groups.

The analysis showed that total acid output was significantly different ($P < 0.001$) between treated and control guinea-pigs in all three samples obtained during peak secretion.

Acid concentration did not differ between treated and control guinea-pigs, prior to the administration of either the extract or histamine, but became significantly lower following stimulation with histamine ($P < 0.01$ after 60, $P < 0.001$ after 75 and 90 minutes) as shown in Table 2/B.

The volumes secreted were not significantly different at 60 minutes, only 75 and 90 minutes ($P < 0.05$) after the administration of histamine. All control guinea-pigs responded to histamine with an increase in acid output.

The extract was able to supress gastric acid secretion in 8 out of 10 treated guinea-pigs. In 2 of the animals acid output reached values similar to those found in control animals.

Table 2: EFFECT OF URINE EXTRACT ON HISTAMINE-INDUCED GASTRIC SECRETION IN MALE GUINEA-PIGS

Section A shows the difference in total acid output, section B the difference in acid concentration, and section C the difference in the volume of gastric juice secreted between control and treated guinea-pigs prior to and 60, 75 and 90 minutes after the administration of histamine.

Group	Total acid output mEqx10^{-2}/15 min ± S.E.				
	Basal	Pre-histamine	min. after histamine		
			60	75	90
A. Control	1.82 ±.20	1.74 ±.25	14.38 ±1.6	15.82 ±1.8	12.40 ±1.9
Treated	1.62 ±.30	0.91[b] ±.10	4.98[c] ±1.7	4.08[c] ±1.4	3.20[c] ±1.3
	Acid concentration (mEq/L) ± S.E.				
B. Control	39.65 ±10.35	26.41 ±3.49	112.72 ±6.14	115.23 ±11.76	125.53 ±17.53
Treated	25.08 ±2.81	18.56 ±4.28	63.32[b] ±13.15	47.82[c] ±3.64	43.04[c] ±8.48
	Volume of gastric juice secreted ml/15 min ±S.E.				
C. Control	.64 ±.08	.68 ±.06	1.32 ±.14	1.27 ±.22	1.10 ±.18
Treated	.64 ±.06	.50 ±.07	.98 ±.10	.70[a] ±.07	.68[a] ±.07

a = $P < 0.05$ (treated versus control)

b = $P < 0.01$ (treated versus control)

c = $P < 0.001$ (treated versus control)

Numerical values of the statistical analysis are summarized in Table 2.

2. Experiments in Dogs, Provided with a Heidenhain pouch

Neither the male nor the female dog, provided with a Heidenhain pouch, secreted gastric juice prior to stimulation with histamine. Several doses of histamine were administered to the male dog in order to establish the dose which induces a well-defined increase in both total acid output and volume secretion, but which is submaximal and does not induce undesirable side effects besides the stimulation of gastric secretion.

1.4 mg/kg histamine given in a single s.c. injection, induced a long lasting gastric secretory response and acid output did not return to normal, even 3 hours after the administration of histamine. The dog manifested salivation, vomited several times and had difficulty in breathing, shortly after the administration of histamine. Peak secretion occurred 120 minutes after the injection of histamine. The total acid output was 17.8×10^{-1} mEq in the 15 minute sample which was collected during this peak secretion. 0.35 mg/kg histamine given in a single s.c. injection, induced a well-defined acid secretory response which declined by the end of the second hour.

However, the dog vomited once, shortly after the injection of histamine. Peak secretion occurred 60 minutes after the administration of histamine. Total acid output was 12.7×10^{-1} mEq in the 15 minute sample collected during this peak secretion.

0.175 mg/kg histamine given in a single s.c. injection did not result in any undesirable side effect, but stimulated gastric secretion

from the Heidenhain pouch. Both total acid output and the volume of gastric juice secreted increased to a peak and then decreased toward basal levels. This dose of histamine was therefore chosen as the secretory stimulant dose in all experiments performed in both dogs.

i) Male dog: The changes in total acid output and volume were investigated under 3 different experimental conditions as shown in Fig.7. (1) The response of the pouch to histamine (5 experiments); (2) the response of the pouch to histamine, 2 hours after the oral administration of the vehicle (4 experiments) and (3) the response of the pouch to histamine, 2 hours after the oral administration of the urine extract (4 experiments).

Peak secretion occurred 30, 45 and 60 minutes after the administration of histamine in all 3 experimental arrangements. Therefore, from samples collected during these periods total acid output and the volume of gastric juice secreted were statistically analyzed and compared.

Total acid output in response to histamine and to "vehicle + histamine", reached the maximum 45 minutes after the administration of histamine. Total acid output was essentially the same under both experimental conditions, except at 30 minutes, when the total acid output in response to the "vehicle + histamine" was significantly higher than in response to histamine alone ($P < 0.05$).

Total acid output in response to "extract + histamine" reached the peak 30 minutes after the administration of histamine and started to decline thereafter. During the peak secretory period acid output in response to "extract + histamine" was significantly less than acid output in response to"vehicle + histamine" ($P < 0.01$ at 30 and 45 minutes, $P < 0.05$ at 60

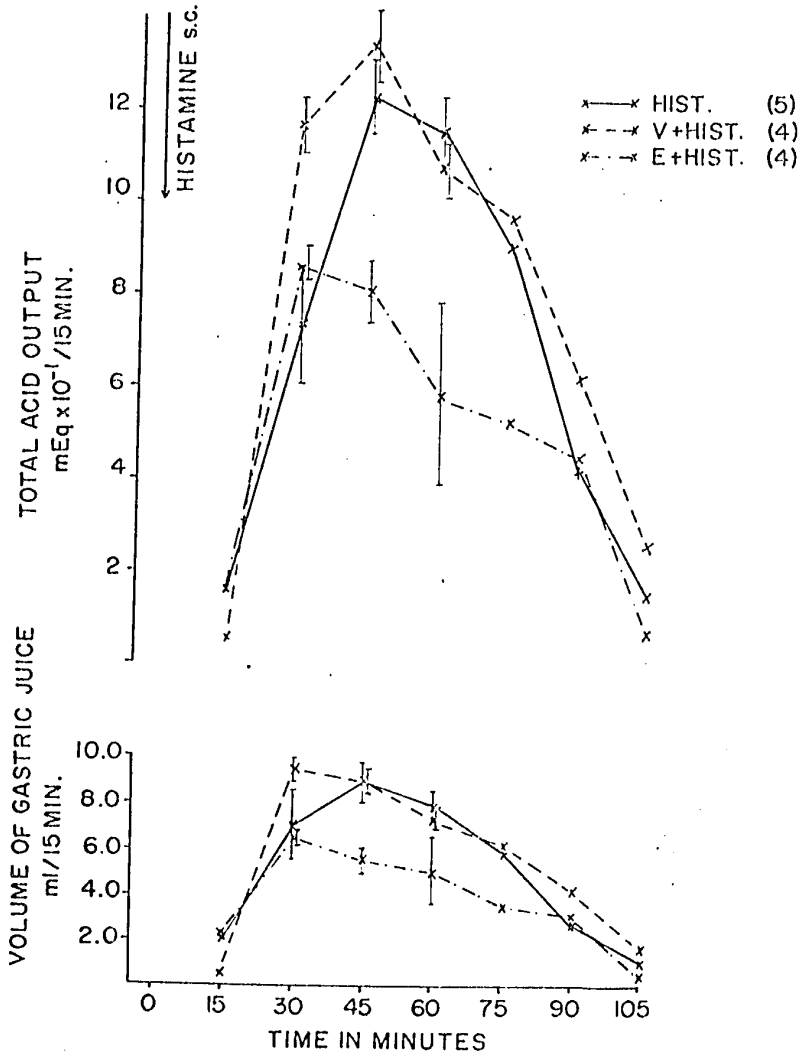


Fig. 7: Mean total acid output (mEq x 10^{-1}/15 min) and mean volume of gastric juice secreted (ml/15 min) in a Heidenhain pouch (male dog) in response to histamine (Hist.), vehicle + histamine (V + Hist.) and urine extract + histamine (E + Hist.). Number in parenthesis refers to number of experiments per treatment.

minutes). However, the concentration of the acid did not change significantly in response to the various treatments (Table 3/B).

The volume of gastric juice secreted was not significantly different at any time in response to histamine or to "vehicle + histamine". However, the volume of gastric juice secreted was significantly less in samples collected 30 and 45 minutes after the administration of histamine when the dog received the extract as compared to the vehicle ($P < 0.01$ at 30 and 45 minutes). At 60 minutes no significant differences were obtained.

Numerical values obtained during peak secretion are summarized in Table 3.

ii) Female dog: Changes in total acid output and the volume of gastric juice secreted by the female dog, provided with the Heidenhain pouch, were investigated under similar experimental conditions as previously described for the male dog. The results are summarized in Fig.8.

Total acid output in response to histamine and to "vehicle + histamine" reached the maximum 45 minutes after the administration of histamine. Both total acid output and the volume of gastric juice secreted in response to only histamine or to "vehicle + histamine" were essentially the same throughout the experiments.

Total acid output in response to the "extract + histamine" also reached the peak at 45 minutes but maintained a plateau during the next 15 minutes and started to decline only after the 60^{th} minute. When the dog was pretreated with the extract as compared to the vehicle, acid output was significantly lower ($P < 0.01$), both 30 and 45 minutes after the injection of histamine. No difference was noted, however, after 60 minutes.

Table 3: EFFECT OF URINE EXTRACT ON HISTAMINE-INDUCED GASTRIC SECRETION IN A MALE HEIDENHAIN-POUCH DOG

Section A shows the differences in total acid output, sectionB the differences in acid concentration and section C the differences in the volume of gastric juice secreted 30, 45 and 60 minutes after the administration of histamine.

Treatment	Total acid output mEq x 10^{-1}/15 min ± S.E. min. after histamine		
	30	45	60
A. H	7.31 ±1.33	12.21 ±.86	11.44 ±.81
V + H	11.61[a'] ±.61	13.27 ±.84	10.65 ±.62
E + H	8.55[b] ±.38	8.00[b] ±.74	5.75[a] ±1.97
	Acid concentration (mEq/L) ± S.E.		
B. H	111.57 ±14.47	139.74 ±6.56	147.49 ±3.78
V + H	124.40 ±2.59	149.08 ±3.46	149.85 ±17.1
E + H	131.47 ±3.20	145.47 ±4.87	126.05 ±17.96
	Volume of gastric juice secreted ml/15 min ±S.E.		
C. H	6.86 ±1.56	8.88 ±.90	7.80 ±.66
V + H	9.40 ±.50	8.90 ±.51	7.10 ±.37
E + H	6.50[b] ±.21	5.55[b] ±.64	4.95 ±1.52

H = histamine
V = vehicle (isopropyl myristate)
E = urine extract

a = $P < 0.05$ (E+H versus V+H)
b = $P < 0.01$ (E+H versus V+H)
a' = $P < 0.05$ (V+H versus H)

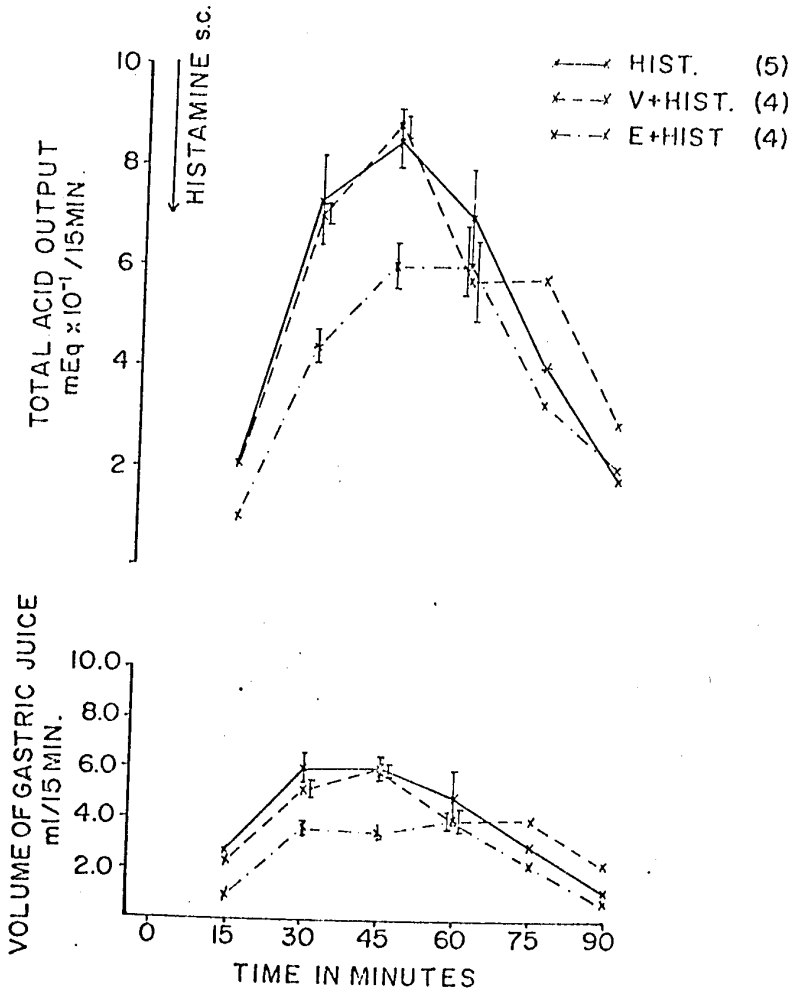


Fig.8: Mean total acid output (mEq x 10^{-1}/15 min) and mean volume of gastric juice secreted (ml/15 min) in a Heidenhain pouch (female dog) in response to histamine (Hist.), vehicle + histamine (V + Hist.) and urine extract + histamine (E + Hist.). Number in parenthesis refers to number of experiments per treatment.

The concentration of the acid did not change significantly in response to the various treatments (Table 4/B). Acid concentration was significantly lower ($P < 0.01$) only in response to "extract + histamine" as compared to "vehicle + histamine" 30 minutes after the administration of histamine.

The volume of gastric juice secreted was also significantly less at 30 and 45 minutes when the dog received the extract as compared to the vehicle ($P < 0.01$ at 30 minutes and $P < 0.001$ at 45 minutes). At 60 minutes no significant differences were observed.

Numerical values obtained during peak secretion are summarized in Table 4.

Both total acid output and the volume of gastric juice secreted returned toward basal levels 105 minutes after the administration of histamine in the male dog and 90 minutes after the administration of histamine in the female dog.

iii) Side effects: No toxic side effects were observed in the dogs throughout the experimentation despite the fairly large doses of urine extract administered.

The only side effect which was observed occurred in the female animal. After the second administration of the extract the dog lost all hair from a round area on its back (diameter about 3 inches, close to the tail). The area seemed strongly inflamed and was constantly licked and scratched. Ten days of treatment with antibiotics and tranquilizers allowed for complete healing. No experiments were performed during this period.

Neither dog lost any weight but rather gained a few lbs. by the end of the experimental period.

Table 4: EFFECT OF URINE EXTRACT ON HISTAMINE-INDUCED GASTRIC SECRETION IN FEMALE HEIDENHAIN-POUCH DOG

Section A shows the differences in total acid output, section B the differences in acid concentration and section C the differences in the volume of gastric juice secreted 30, 45 and 60 minutes after the administration of histamine.

Treatment	Total acid output mEq x 10^{-1}/15 min ± S.E. min. after histamine		
	30	45	60
A.			
H	7.31 ±.89	8.50 ±.62	7.00 ±.95
V + H	6.95 ±.39	8.80 ±.45	5.74 ±.85
E + H	4.38[a] ±.34	6.00[a] ±.45	6.18 ±.67
B.	Acid concentration (mEq/L) ± S.E.		
H	121.44 ±3.91	138.49 ±3.49	142.91 ±3.29
V + H	132.94 ±2.89	147.65 ±2.84	142.69 ±2.24
E + H	119.04[a] ±1.76	139.86 ±3.22	152.01 ±7.35
C.	Volume of gastric juice secreted ml/15 min ± S.E.		
H	5.96 ±.56	6.12 ±.36	4.88 ±.60
V + H	5.25 ±.28	5.95 ±.20	4.00 ±.53
E + H	3.68[a] ±.28	3.49[b] ±.35	4.05 ±.35

H = histamine

V = vehicle (isopropyl myristate)

E = urine extract

a = $P < 0.01$ (E+H versus V+H)

b = $P < 0.001$ (E+H versus V+H)

On each occasion, 25-30 minutes after the oral administration of the extract both dogs manifested tranquility which lasted for about one hour.

D. Effect of Urine Extract on Gastrin-Induced Gastric Acid Hypersecretion in Male Guinea-Pigs

The effect of urine extract upon gastrin-induced gastric secretory changes was investigated in male guinea-pigs. The results obtained are summarized in Fig.9 A and B.

1. Response to a Submaximal Dose of Gastrin

Acid secretion was stimulated by 100 µg/kg of gastrin, which induces a submaximal gastric secretory response in male guinea-pigs.

The results of the experiment obtained in 8 treated and 8 control animals are illustrated in Fig. 9/A.

Prior to the administration of the extract, neither total acid output nor the volume of the gastric juice secreted was significantly different between the two groups.

After establishing basal acid output and the basal rate at which gastric juice was secreted, 8 animals were intraperitoneally injected with the extract, 1.5 g/kg; the control animals received an equal volume of the vehicle. One hour was allowed for absorption. During this period, acid output gradually declined in the treated animals, while in the controls it remained virtually unchanged. Thus, 60 minutes after the administration of the extract acid output was already significantly lower ($P < 0.01$) in the treated group prior to the administration of gastrin.

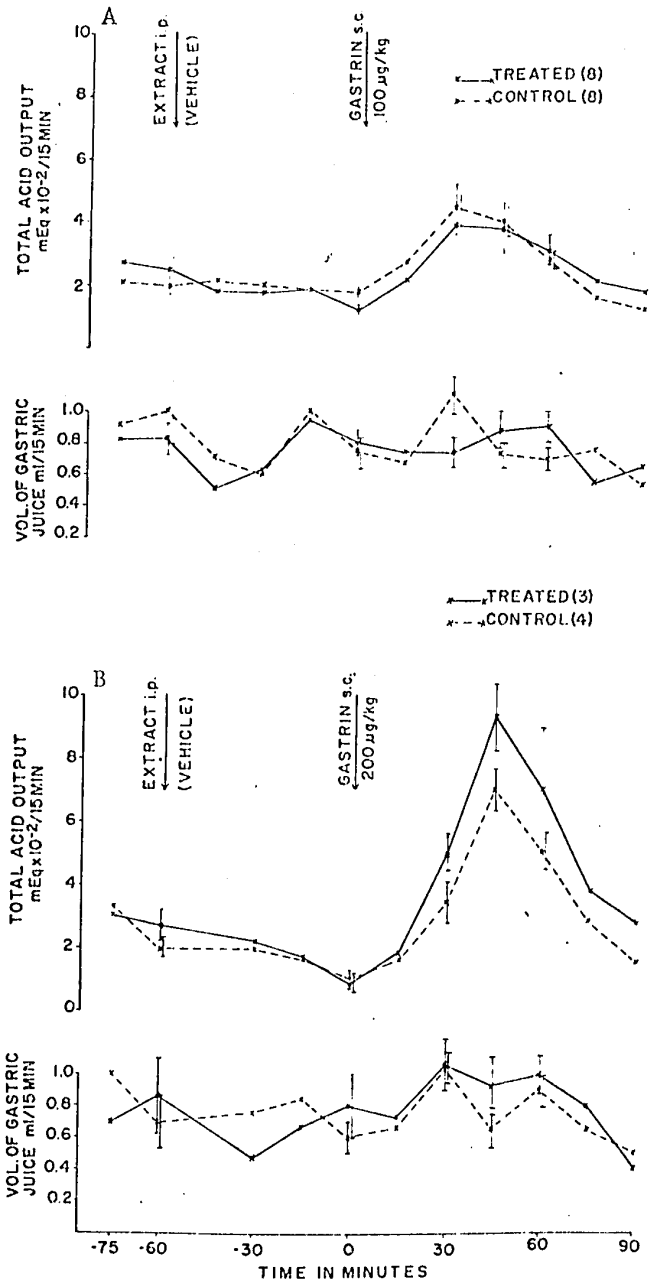


Fig.9: Mean total acid output (mEq x 10^{-2}/15 min) and mean volume of gastric juice secreted (ml/15 min) in control animals and in guinea-pigs treated with horse urine extract before and after the administration of 100 µg/kg of gastrin (Fig.9/A) and 200 µg/kg of gastrin (Fig.9/B). Number in parenthesis refers to number of guinea-pigs in each group.

Following a single s.c. injection of gastrin, acid output increased in both treated and control groups, reaching a peak in 30 minutes. Without maintaining a plateau, acid output declined after the peak and reached basal values within 90 minutes. Although acid output determined from the peak 15-minute sample was slightly lower in the treated group, this difference was not statistically significant.

The volume of gastric juice secreted by the treated and control animals was not significantly different, neither prior to the administration of the extract nor prior to the administration of gastrin. The volume of gastric juice secreted by the treated guinea-pigs was significantly lower ($P < 0.05$) 30 minutes after the administration of gastrin as compared to the volume secreted by the controls. However, 45 and 60 minutes after the administration of gastrin the volume of gastric juice secreted was even somewhat higher in extract-pretreated animals.

2. Response to a Maximal Dose of Gastrin

Acid secretion was stimulated by 200 µg/kg of gastrin which induces a maximal gastric secretory response in male guinea-pigs. The result of the experiment obtained in 3 treated and 4 control guinea-pigs is summarized in Fig. 9/B.

Prior to the administration of the extract, neither total acid output nor the volume of the gastric juice secreted was significantly different between the 2 groups.

After establishing the basal secretory response, the extract was administered intraperitoneally in a dose of 1.5 g/kg; control animals received an equal volume of the vehicle. One hour was allowed for absorption.

During this period acid output declined in both groups. The decline was more pronounced in the treated guinea-pigs but not significantly lower when compared to control values.

Following a single s.c. injection of gastrin, a well-defined acid secretory response was obtained in both groups reaching a peak in 45 minutes. Total acid output returned to basal levels in all guinea-pigs 90 minutes after the administration of gastrin.

In all samples collected during the 90-minute period, total acid output was even higher in the treated group when compared to values obtained in the control group. However, none of the differences were statistically significant.

The volumes secreted did slightly increase in both groups after the administration of gastrin. This increase was also more pronounced in the treated animals but not significantly different.

E. Differences in Gastric Acid Output in Response to Histamine and Gastrin in Male and Female Guinea-Pigs

1. Differences in Response to Histamine

Fig.10 summarizes the differences which were observed after comparing total acid output and volume of gastric juice secreted in male and female guinea-pigs, following the administration of increasing doses of histamine.

Peak secretory values were obtained in samples collected 60, 75 and 90 minutes after histamine administration. These 3 samples were, therefore, used for analysis and comparison of the secretion changes for each dose.

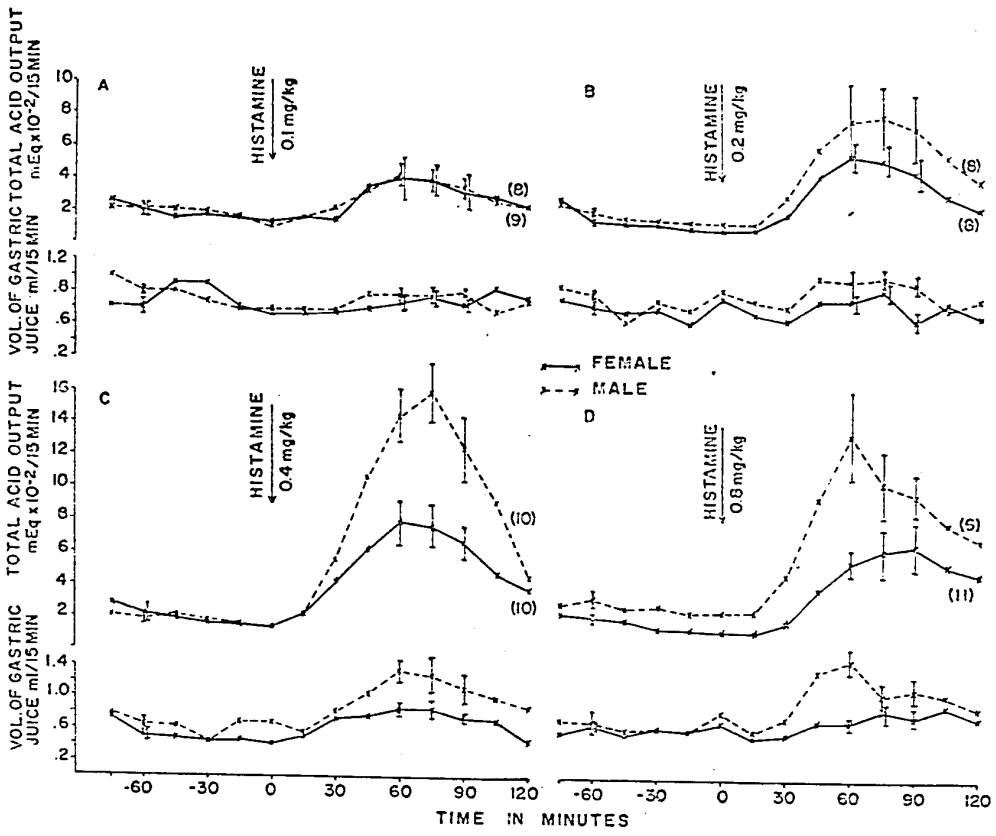


Fig.10: Mean total acid output (mEq x 10^{-2}/15 min) and mean volume of gastric juice secreted (ml/15 min) in male and female guinea-pigs before and after the administration of 0.1 mg/kg histamine (Fig.10/A); 0.2 mg/kg histamine (Fig.10/B); 0.4 mg/kg histamine (Fig.10/C) and 0.8 mg/kg histamine (Fig.10/D). Number in parenthesis refers to number of guinea-pigs in each group.

Results obtained after the administration of 0.1 mg/kg of histamine to 8 male and 9 female guinea-pigs are shown in Fig. 10/A. With respect to acid secretion, histamine at this dose level brought about a well-defined, moderate and identical increase in both sexes. However, the volume of gastric juice secreted remained unchanged throughout.

Fig. 10/B summarizes the results obtained in 8 male and 8 female guinea-pigs after the administration of 0.2 mg/kg histamine. Total acid output, determined from the secreted gastric juice of male guinea-pigs, was higher than that of female guinea-pigs. This difference, however, was not statistically significant. The volume of gastric juice also showed a definite increase in both sexes. In females, however, the volume of gastric juice returned to normal values 90 minutes after the histamine administration, yet during the same period in males, the volume of gastric juice was still well above the control values. This noted difference between the 2 groups was statistically significant ($P < 0.05$).

Fig. 10/C illustrates the effect of 0.4 mg/kg of histamine in 10 male and 10 female guinea-pigs. This dose of histamine elicited a maximal response of acid secretion in both sexes. It is illustrated that the acid output of males and females became significantly different under conditions of maximal histamine stimulation. In three subsequent samples which were collected during the peak of the secretory response, the total acid output values were found to be significantly greater in males than in females ($P < 0.01$ for the 60 and 75-minute samples, $P < 0.05$ for the 90-minute sample).

The difference between the sexes was also clearly manifested in the volume of gastric juice secreted. The males, in general, secreted much

higher quantities of gastric juice, and this difference was highly significant in samples collected 60 minutes after the administration of histamine ($P < 0.01$).

The administration of 0.8 mg/kg of histamine to 6 male and 11 female guinea-pigs proved to be a supramaximal dose in both sexes. The results obtained in this experiment are shown in Fig. 10/D. The acid output and the volume of gastric juice secreted was significantly different between the 2 sexes ($P < 0.05$ for acid output and $P < 0.001$ for volume), in the sample collected 60 minutes after the administration of histamine.

2. Differences in Response to Gastrin

Acid secretory responses obtained in 4 male and 4 female guinea-pigs were compared after the s.c. administration of 200 µg/kg of gastrin, which induced a maximal acid secretory response in both sexes. Fig. 11 shows that, although acid secretion in response to gastrin declined earlier in the female guinea-pigs as compared to males, none of the differences noted were statistically significant. Similarly no significant differences were observed in the volumes of gastric juice secreted.

F. Effect of Urine Extract in the Shay Rat

1. Action on Ulcer Formation

Table 5 summarizes the results which were obtained when the effect of urine extract was investigated in the Shay rat preparation.

In the first series of experiments urine extract was administered in a dose of 2.0 and 3.0 g/kg orally 6 hours prior to pylorus ligation (Table 5/A). Though in the treated animals ulcer formation was strongly

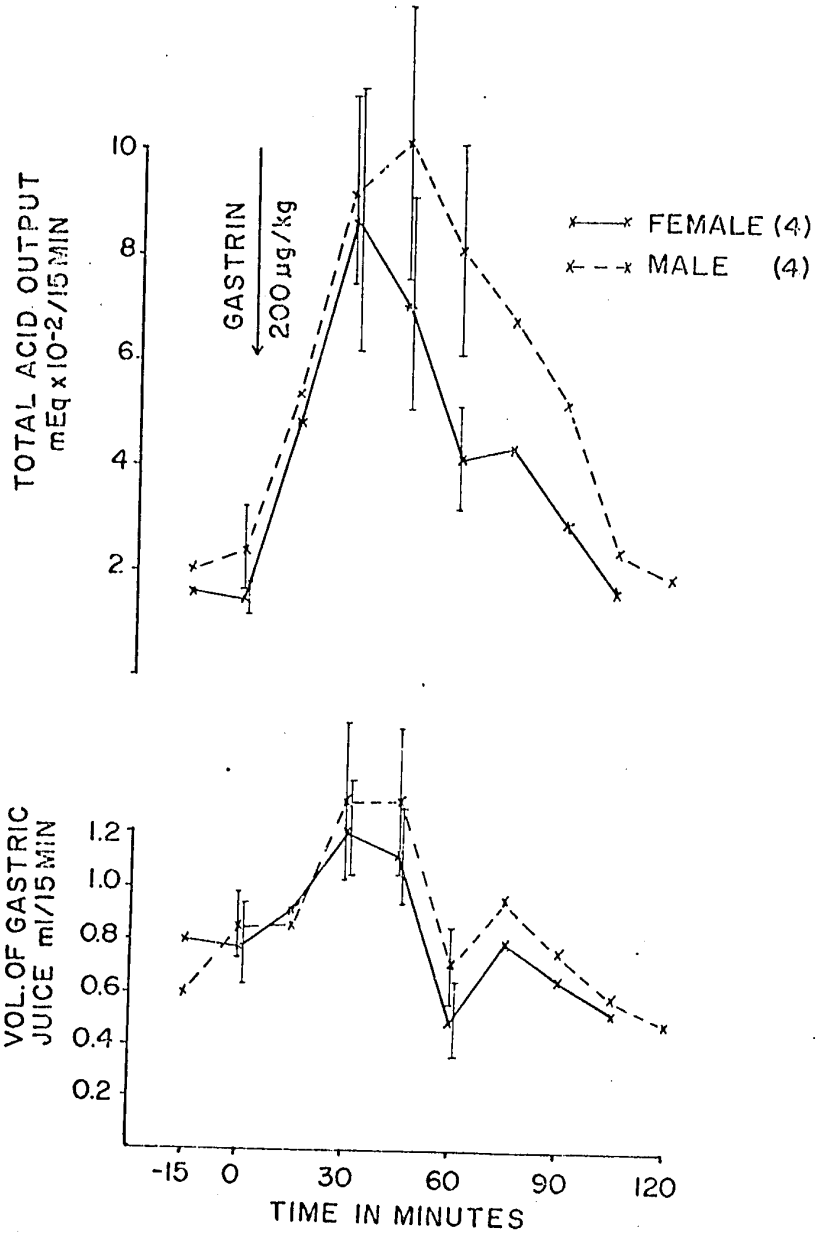


Fig.11: Mean total acid output (mEq x 10^{-2}/15 min) and mean volume of gastric juice secreted (ml/15 min) in male and female guinea-pigs before and after the administration of 200 µg/kg of gastrin. Number in parenthesis refers to number of guinea-pigs in each group.

Table 5: EFFECT OF URINE EXTRACT ON ULCER FORMATION IN SHAY RATS

The extract or solvent was administered orally 6 hours prior to pylorus ligation (Section A); 24 hours prior to pylorus ligation (Section B), or injected intraduodenally immediately after pylorus ligation (Section C). Animals were killed 17 hours after ligation.

	Treatment	No. of animals	Dose g/kg	Ulceration	Ulcer Index
A.	Solvent	6	(1.0 ml)	1.56 ± .67	99.9
	Extract	6	2.0	0.58 ± .30	38.6
	Extract	6	3.0	0.17 ± .10	5.7
B.	Solvent	35	(1.5 ml)	2.54 ± .22	225.0
	Extract	6	2.0	2.08 ± .56	208.0
	Extract	5	2.5	1.00 ± .49[a]	60.0
	Extract	20	3.0	0.85 ± .30[b]	34.0
C.	Solvent	22	(0.6 ml)	3.0 ± .24	286.2
	Extract	6	0.5	2.0 ± .78	133.2
	Extract	20	1.0	0.75 ± .25[b]	45.0

a = $P<0.01$ (extract versus solvent)

b = $P<0.001$ (extract versus solvent)

inhibited, these results could not be fully evaluated, since traces of the substance could be seen in the stomach of some animals during autopsy. Thus the possibility of a local effect could not be excluded.

Table 5/B summarizes the results of those experiments in which the extract was given orally 24 hours prior to pylorus ligation. It is shown that the administration of 2.0 g/kg extract to 6 rats produced a slight protection. However, when the dose was increased to 2.5 g/kg (5 rats), ulceration became significantly lower ($P < 0.01$) in the extract treated rats as compared to untreated animals. The administration of 3.0 g/kg of extract to 20 rats resulted in a highly significant reduction in ulceration ($P < 0.001$).

In order to exclude any possibility of a local effect in a series of experiments, the extract was injected intraduodenally into rats immediately following pylorus ligation. Table 5/C illustrates the results obtained after the intraduodenal administration of 0.5 and 1.0 g/kg urine extract. It is shown that extract administered in a dose of 0.5 g/kg to 6 rats did not produce any significant reduction in ulcer formation, while the injection of 1.0 g/kg of extract to 20 rats resulted in a highly significant protection ($P < 0.001$).

2. Action on Acid Secretion

Gastric juice, which accumulated in the stomach during the entire period of ligation (17 hours), was individually analyzed after centrifuging the samples. Significantly lower volumes of gastric juice were secreted ($P < 0.001$) by rats which received the extract orally 24 hours prior to ligation (Table 6/A) when compared to rats which received the vehicle. Similarly, the total acid output was significantly lower ($P < 0.01$) in these

Table 6: EFFECT OF URINE EXTRACT ON GASTRIC SECRETION IN SHAY RATS

The extract or solvent was administered orally 24 hours prior to pylorus ligation (Section A), or injected intraduodenally immediately after pylorus ligation (Section B). Animals were killed 17 hours later. Determinations were made individually, results express mean values and standard errors.

Treatment	No. of animals	Dose g/kg	Volume of gastric juice ml	Total acid output mEq $.10^{-2}$/17 hrs
A.				
Solvent	15	1.5 ml	7.3 ± .65	68.9 ± 8.5
Extract	8	3.0	4.4 ± .31[b]	39.4 ± 4.0[a]
B.				
Solvent	14	0.6 ml	8.6 ± .9	86.5 ± 3.4
Extract	4	0.5	6.7 ± 1.5	77.4 ± 18.2
Extract	16	1.0	4.8 ± .2[b]	60.4 ± 3.2[b]

a = $P<0.01$ (extract versus solvent)

b = $P<0.001$ (extract versus solvent)

animals. When the extract was injected intraduodenally (Table 6/B) in a dose of 1.0 g/kg, the volume of gastric juice secreted was significantly lower ($P<0.001$) than in response to the vehicle. The total acid output was similarly greatly reduced ($P<0.001$) in treated animals. The lower dose of the extract (0.5 g/kg) caused only insignificant reductions in both volume and total acid output.

G. Effect of Urine Extract on Restraint-Induced Gastric Ulcer in Rats

The action of urine extract against restraint-induced ulceration was investigated in a total of 57 female albino rats, while 30 animals served as controls.

Table 7/A illustrates the protective effect of 0.75 and 1.0 g/kg of extract, administered intraperitoneally immediately prior to immobilization. The 24-hour immobilization period led to ulcer formation in 66% of the control rats. In rats treated with 0.75 g/kg of the extract ulcer formation was found to be reduced and occurred in only 38% of the animals. In rats which received 1.0 g/kg of the extract ulceration was found in 21% of the animals.

Table 7/B shows that the i.m. injection of the vehicle led to ulcer formation in 80% of the rats. In animals treated with 1.0 g/kg of the extract ulceration was reduced to 50%; in animals receiving 1.5 g/kg to 37%. The protective effect of the extract was significantly different ($P<0.05$) from the controls, both after i.p. and i.m. administration at the higher dose levels.

Table 7: EFFECT OF URINE EXTRACT ON ULCER FORMATION IN IMMOBILIZED RATS

The extract or solvent was administered either intraperitoneally (Section A), or intramuscularly (Section B) immediately prior to immobilization. Animals were killed 24 hours later.

	Treatment	No. of animals	Dose g/kg	% Showing Ulceration
A.	Solvent	15	0.3 ml	66.66
	Extract	13	0.75	38.46
	Extract	14	1.00	21.43[a]
B.	Solvent	15	0.3 ml	80.00
	Extract	16	1.0	50.00
	Extract	14	1.5	37.71[a]

a = $P < 0.05$ (extract versus solvent)

III. Effect of Urine Extract on the Cardiovascular System.

The effect of the urine extract was investigated on the cardiovascular system both in male rats and male guinea-pigs.

1. Response in Rats

The extract was given intraperitoneally at a dose of 1.0 g/kg to 6 rats and 1.5 g/kg to 3 rats.

The control mean arterial blood pressure was 95 $\pm$ 4.9 and the heart rate 390 $\pm$ 21.1 in rats treated with the lower dose; 102 $\pm$ 6.0 and 380 $\pm$ 20.0 in rats treated with the higher dose of the extract. Following the administration of the urine extract the blood pressure remained essentially unchanged, while the heart rate decreased slightly but not significantly during the 2 hour observation period at both dose levels.

2. Response in Guinea-Pigs

The extract was administered in a dose of 1.5 g/kg to 3 male guinea-pigs. The control mean arterial blood pressure was 63 $\pm$ 4.4 and the heart rate 290 $\pm$ 10.8. During the 2 hour observation period following the administration of the extract both blood pressure and heart rate remained virtually unchanged.

IV. Studies on the Central Nervous System

A. Hypnotic and Hypothermic Effect of Graded Doses of Urine Extract in Hooded Male Rats

1. Hypnosis

The hypnotic effect of the urine extract was determined in

50 rats, which were divided into groups of five. The rats were injected intraperitoneally with graded doses of the extract (Fig.12).

The occurrence of hypnosis was dose-dependent, and was induced by the extract within 10-20 minutes following its administration. All rats were awake at the end of the first hour.

The extract injected into 10 rats in a dose of 1000 mg/kg proved to be subhypnotic since none of the animals lost its righting reflex for 1 minute, the criterion defining "sleep". (This dose will be referred to as the hypnotic ED 5).

The administration of 1050 mg/kg of extract caused sleep in 2 out of 10 rats (20%); 1102 mg/kg in 4 out of 10 rats (40%); 1157 mg/kg in 8 out of 10 rats (80%), while 1215 mg/kg caused sleep in all 10 rats. (This dose will be referred to as the hypnotic ED 95).

Figure 12 illustrates that in male rats the intraperitoneal hypnotic ED 50 dose of the urine extract is 1110 mg/kg.

2. Hypothermia

Table 8 summarizes the results obtained in rats, when the effect of graded doses of urine extract was investigated on body temperature. A total of 30 rats, divided into 6 groups of 5 rats each, were injected intraperitoneally with the extract, while 5 rats received the vehicle.

The injection of the vehicle itself caused a slight fall in body temperature.

The extract in a dose of 200 mg/kg induced hypothermia, which

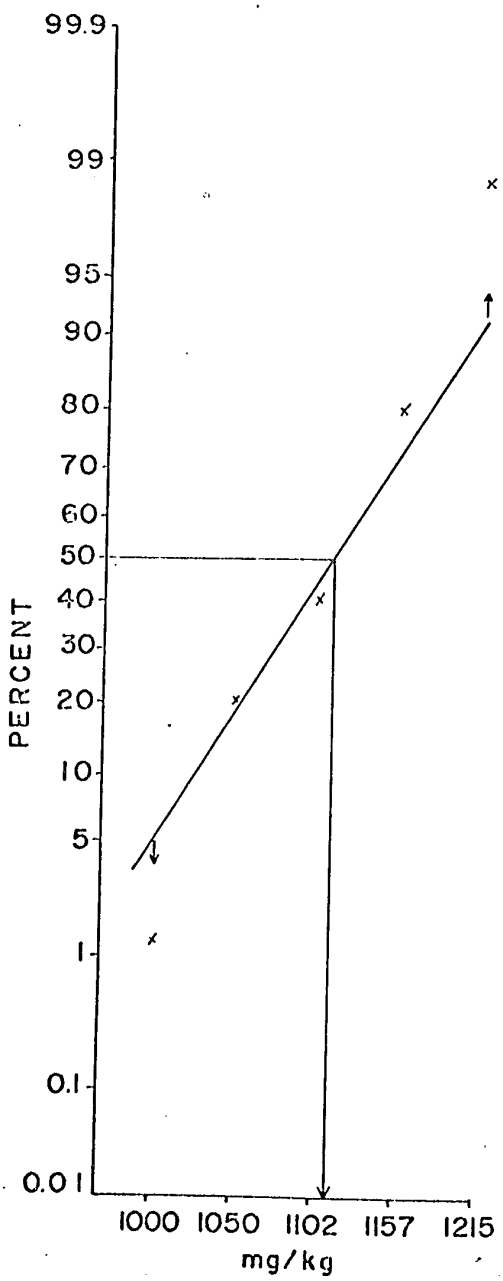


Fig. 12: Determination of hypnotic ED 50 dose of urine extract. Each dose of the extract was administered intraperitoneally to 10 male hooded rats. Dose administered is plotted against the per cent of rats which lost their righting reflex.
For details see text.

Table 8: TEMPERATURE CHANGES FOLLOWING THE ADMINISTRATION OF GRADED DOSES OF URINE EXTRACT

The vehicle or the extract was administered intraperitoneally. Rectal temperature of rats was recorded at hourly intervals for the first 5 hours as well as at 24 and 48 hours. Peak hypothermia occurred either 1 or 2 hours after the administration of the extract.

Extract mg/kg	No. of rats	Rectal temperature ($^{o}C \pm$ S.E.)					
		Control	Hours after i. p. injection of extract				
			1	2	5	24	48
Control (Vehicle)	5	38.2 ±.29	37.6 ±.23	37.6 ±.33	37.4 ±.24	$36.7^{(1)}$ ±.43	37.5 ±.20
200	5	38.4 ±.06	36.6 ±.44	35.7^{b} ±.20	36.6^{a} ±.24	$37.2^{(11)}$ ±.30	38.0 ±.16
1000	5	38.4 ±.15	31.2^{c} ±.20	31.1^{c} ±.41	33.9^{c} ±.63	$36.2^{(11)}$ ±.48	36.7 ±.57
1050	5	38.3 ±.14	31.3^{c} ±.25	31.8^{c} ±.26	34.7^{c} ±.17	$36.3^{(11)}$ ±.39	37.4 ±.29
1102	5	38.3 ±.18	30.5^{c} ±.16	29.7^{c} ±.36	32.0^{c} ±.46	$36.1^{(111)}$ ±.40	36.6 ±.22
1157	5	37.8 ±.14	30.8^{c} ±.75	31.4^{c} ±.70	34.3^{c} ±.48	$37.1^{(11)}$ ±.16	37.4 ±.25
1215	5	38.1 ±.32	31.9^{c} ±.29	30.7^{c} ±.31	32.7^{c} ±.45	$35.8^{(11)}$ ±.54	37.2 ±.33

a = $P < 0.05$ (extract versus vehicle)
b = $P < 0.01$ (extract versus vehicle)
c = $P < 0.001$ (extract versus vehicle)

(1) $P < 0.05$ (24 hrs versus control within group)
(11) $P < 0.01$ (24 hrs versus control within group)
(111) $P < 0.001$ (24 hrs versus control within group)

reached its peak after 2 hours, at which point the rectal temperature of the extract-treated animals was significantly different ($P < 0.01$) from the controls (vehicle). The temperature was still significantly lower ($P < 0.05$) after 5 hours.

Following administration of the extract in doses of 1000, 1050, 1102, 1157 and 1215 mg/kg, the temperature fell rapidly, often by more than $8^{o}C$ within one hour of the injection.

Maximal hypothermia was observed either 1 hour or 2 hours after the administration of the extract. The rectal temperature of all rats was significantly decreased ($P < 0.001$) after the administration of these high doses of the extract, compared to controls, even 5 hours later. Twenty four hours after the injection of the extract the rectal temperature was found to be essentially identical with temperature values recorded in control rats. However, rectal temperatures measured 24 hours after the administration of either the vehicle or the extract, were significantly lower, when compared to values measured during the control period within each group. Rectal temperatures measured 48 hours later were somewhat higher in all groups compared to values recorded after 24 hours, but still did not reach control values in any of the groups.

All animals which received the high doses of the urine extract, were observed for a period of seven days. For the first 24 hours following the administration of the extract no mortality occurred, even with the highest dose of the extract. Only 1 rat died within 48 hours from the group, which received 1157 mg/kg of the extract.

Within 7 days none of the rats died in the control group or in

the group which was treated with 1000 mg/kg of extract.

One animal each was lost from 3 groups and 2 animals from the group which received 1102 mg/kg of extract.

This indicated that with increasing doses the mortality did not increase in the dose range used.

However, the gain in weight was significantly less in all groups after 7 days, compared to control animals and an actual loss in weight of 4.0 g occurred in rats treated with the highest dose.

B. Hypnotic and Hypothermic Effect of Urine Extract at Elevated Environmental Temperature

Ten rats were placed for 24 hours in a box maintained at an ambient temperature of 30.7 - 31.6°C.

Immediately prior to placing the animals into the box, 5 rats were injected intraperitoneally with the vehicle, while 5 rats received the extract (hypnotic ED 50 dose). Table 9 shows that 40% of the extract-treated, but none of the control rats lost their righting reflex. Fig.13 summarizes the changes which occurred in the rectal temperature of both control and treated rats. Rectal temperatures decreased in treated animals 60 minutes after the administration of the extract, and the recorded values were significantly lower ($P < 0.01$) than temperature values measured in control rats.

After this initial decrease, rectal temperatures increased in the treated group and were maintained at slightly, but not significantly, higher levels than those of the controls.

Table 9: ACTIVITY OF URINE EXTRACT (HYPNOTIC ED 50 DOSE) AT ELEVATED AMBIENT TEMPERATURE

The vehicle or the extract was administered intraperitoneally immediately prior to placing the rats into a box maintained at an elevated ambient temperature. Table also shows the mortality for 48 hours observed within the groups.

Group	No. rats	% hypn.	% Mortality	
			24 hrs.	48 hrs.
Control	5	0	0	0
Treated	5	40	60	80

% hypn. = % of rats that lost righting reflex

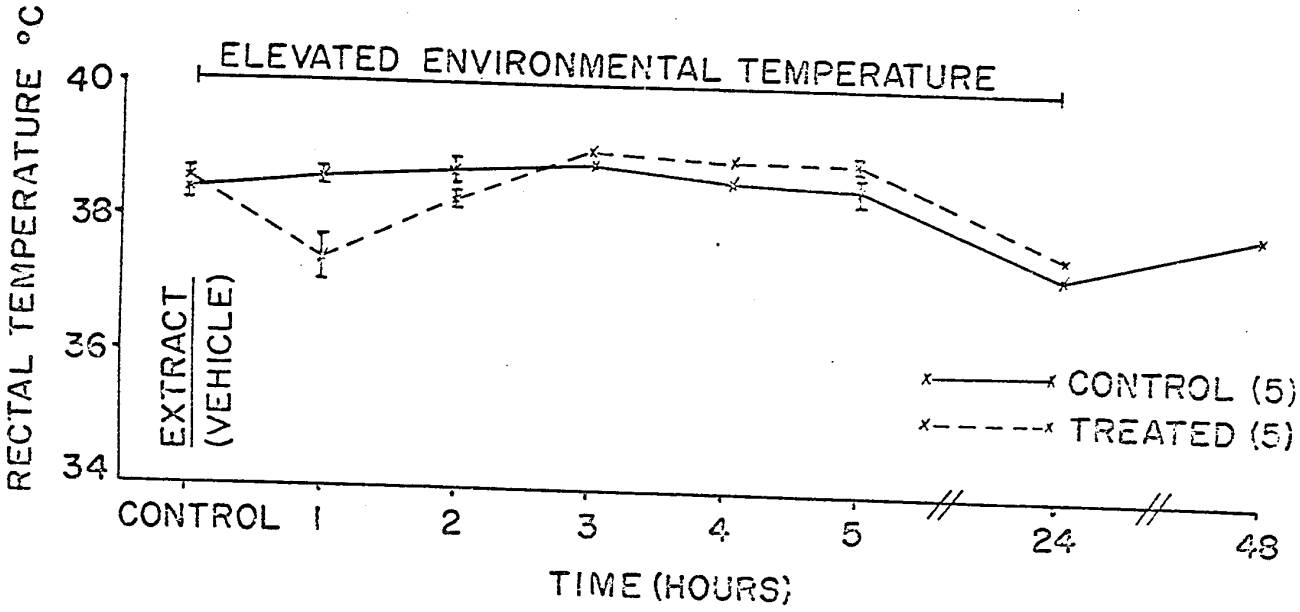


Fig.13: Mean rectal temperature changes in control or urine extract (hypnotic ED 50 dose) treated male hooded rats at an elevated environmental temperature. Number in parenthesis refers to number of rats in each group. For details see text.

In the control group no mortality occurred within 48 hours (Table 9). However, 60% of the treated animals died within 24 hours, and 80% within 48 hours.

C. Potentiation of the Hypnotic Effect of Hexobarbital Sodium by Urine Extract

1. Determination of the Subhypnotic Dose of Hexobarbital Sodium

Hexobarbital sodium was administered subcutaneously in doses of 80, 100 and 125 mg/kg to 3 groups of 5 rats each. From the group of rats treated with 80 mg/kg of hexobarbital sodium, no animal lost its righting reflex. One rat lost its righting reflex after the injection of 100 mg/kg, and 3 rats lost their righting reflex after the injection of 125 mg/kg of hexobarbital sodium. On the basis of this experiment, 80 mg/kg hexobarbital sodium was chosen as the subhypnotic dose in subsequent experiments.

2. Potentiation of the Hypnotic Effect of Hexobarbital Sodium at Room Temperature and at Elevated Environmental Temperature

Table 10/A illustrates that after the administration of hexobarbital sodium at a dose of 80 mg/kg, and urine extract at a dose of 1000 mg/kg, none of the rats lost its righting reflex. Following the administration of 1215 mg/kg of extract all rats lost their righting reflex, and the mean duration of sleep was 9 ± 2.5 minutes.

The potentiating effect of the extract was investigated at room temperature (Table 10/B) and at an elevated environmental temperature (Table 10/C).

Table 10: POTENTIATION OF THE HYPNOTIC EFFECT OF HEXOBARBITAL SODIUM BY URINE EXTRACT

The vehicle or the extract was administered intraperitoneally, followed 30 minutes later (in Sections B and C) by a subcutaneous injection of hexobarbital sodium. For details see text.

	Group	No. rats	Sleeping time min.	Ambient temperature (°C)
A.	Hexob.	5	0	20 - 22
	E ED5	5	0	
	E ED95	5	9 ± 2.5	
B.	V + Hexob.	6	0	20 - 22
	E ED5 + Hexob.	6	64.3 ± 14[b]	
	E ED95 + Hexob.	6	136.7 ± 20.6[c]	
C.	V + Hexob.	5	0	30.7 - 31.6
	E ED5 + Hexob.	5	50.0 ± 17[a]	
	E ED95 + Hexob.	5	89.2 ± 21.3[b']	

a = $P < 0.05$ (E ED5+Hexob. versus V+Hexob. at elevated ambient temperature)

b = $P < 0.01$ (E ED5+Hexob. versus V+Hexob.)

b' = $P < 0.01$ (E ED95+Hexob. versus V+Hexob. at elevated ambient temperature)

c = $P < 0.001$ (E ED95+Hexob. versus V+Hexob.)

Hexob = hexobarbital sodium: 80 mg/kg s.c.

E ED5 = extract ED5 = 1000 mg/kg i.p.

E ED95 = extract ED95 = 1215 mg/kg i.p.

V = vehicle (isopropyl myristate)

Three groups of 6 rats each were used at room temperature, and three groups of 5 rats each were used at the elevated temperature.

None of the rats injected with the vehicle + hexobarbital sodium (controls) slept either at room temperature or at the elevated temperature.

Pretreatment with the hypnotic ED 5 dose of the extract induced sleep in 5 out of 6 rats at room temperature, and in all 5 rats at the elevated temperature. The mean duration of sleep was 64.3 $\pm$ 14 minutes at room temperature and 50.0 $\pm$ 17 minutes at elevated temperature. Both values are significantly different from controls, $P<0.01$ and $P<0.05$ respectively. However, the sleeping time within the 2 treated groups did not differ statistically from each other.

Pretreatment with the hypnotic ED 95 dose of the extract induced sleep in all animals, both at room temperature and at the elevated environmental temperature.

The mean duration of sleep was 136.7 $\pm$ 20.6 minutes at room temperature and 89.2 $\pm$ 21.3 minutes at the elevated environmental temperature. Both values are significantly different from controls, $P<0.001$ and $P<0.01$ respectively. However, the sleeping time in the 2 treated groups did not differ statistically from each other.

3. The Effect of Hexobarbital Sodium and Urine Extract on Rectal Temperatures

Rectal temperatures were also measured in the above-mentioned experiment. The changes observed are summarized in Table 11 and Fig.14/A

.(room temperature), and Table 12 and Fig.14/B (elevated temperature).

At room temperature, rectal temperatures decreased significantly ($P < 0.001$) in both groups pretreated with the extract, when compared to the temperature of rats receiving only vehicle + hexobarbital sodium. This highly significant decrease in body temperature was observed both 2 and 5 hours after the injection of the extract. Furthermore, rectal temperatures of rats pretreated with the hypnotic ED 95 dose of the extract, were significantly lower when compared to rectal temperatures of rats pretreated with the hypnotic ED 5 dose, both after 2 hours ($P < 0.05$) and after 5 hours ($P < 0.001$).

Twenty four hours after the administration of the hypnotic ED 95 dose of the extract, rectal temperatures of rats were still significantly lower than those of vehicle + hexobarbital, and extract ED 5 + hexobarbital treated rats at $P < 0.01$ and $P < 0.05$ levels respectively.

At the elevated ambient temperature, a slight but significant ($P < 0.05$) decrease was noted in rectal temperatures in the extract ED 5 + hexobarbital sodium group, 1 hour after the administration of the extract. Twenty four hours later rectal temperatures of this group were slightly but significantly higher ($P < 0.05$) when compared to vehicle + hexobarbital controls.

No significant differences were observed at any time in the rectal temperatures of control animals and animals treated with extract ED 95 + hexobarbital sodium.

Table 11: THE EFFECT OF HEXOBARBITAL SODIUM AND URINE EXTRACT ON RECTAL TEMPERATURES AT ROOM TEMPERATURE.

The vehicle or the extract was administered intraperitoneally followed by a s.c. injection of hexobarbital sodium 30 minutes later.

Group	No. rats	Rectal temperature (°C ± S.E.)			
		Control	Hrs after i.p. injection of extract (vehicle)		
			2	5	24
V + Hexob.	6	37.3 ± .10	36.8 ± .37	36.9 ± .19	35.8 ± .12
E ED5 + Hexob.	6	37.2 ± .11	32.1 ± .53^{c}	34.9 ± .15^{c}	35.9 ± .19
E ED95 + Hexob.	6	37.4 ± .14	30.4 ± .24$^{ca'}$	31.8 ± .57$^{cc'}$	35.0 ± .22$^{ba'}$

b = $P < 0.01$ (extract versus vehicle)

c = $P < 0.001$ (extract versus vehicle)

a' = $P < 0.05$ (E ED95 versus E ED5)

c' = $P < 0.001$ (E ED95 versus E ED5)

Hexob. = hexobarbital sodium: 80 mg/kg s.c.

E ED5 = extract ED5 = 1000 mg/kg i.p.

E ED95 = extract ED95 = 1215 mg/kg i.p.

V = vehicle (isopropyl myristate)

Table 12: THE EFFECT OF HEXOBARBITAL SODIUM AND URINE EXTRACT ON RECTAL TEMPERATURES AT ELEVATED ENVIRONMENTAL TEMPERATURE

The vehicle or the extract was administered intraperitoneally followed by a s.c. injection of hexobarbital sodium 30 minutes later.

Group	No. rats	Rectal temperature ($^{o}C \pm S.E.$)		
		Control	Hrs after i.p. injection of extract (vehicle)	
			1	24
V + Hexob.	5	$38.3 \pm .09$	$38.6 \pm .10$	$37.1 \pm .19$
E ED5 + Hexob.	5	$38.5 \pm .34$	$38.0 \pm .15^{a}$	$38.0 \pm .11^{a}$
E ED95 + Hexob.	5	$38.4 \pm .08$	$38.4 \pm .09$	$37.4 \pm .24$

a = $P < 0.05$ (extract versus vehicle)

Hexob. = hexobarbital sodium: 80 mg/kg s.c.
E ED5 = extract ED5 = 1000 mg/kg i.p.
E ED95 = extract ED95 = 1215 mg/kg i.p.
V = vehicle (isopropyl myristate)

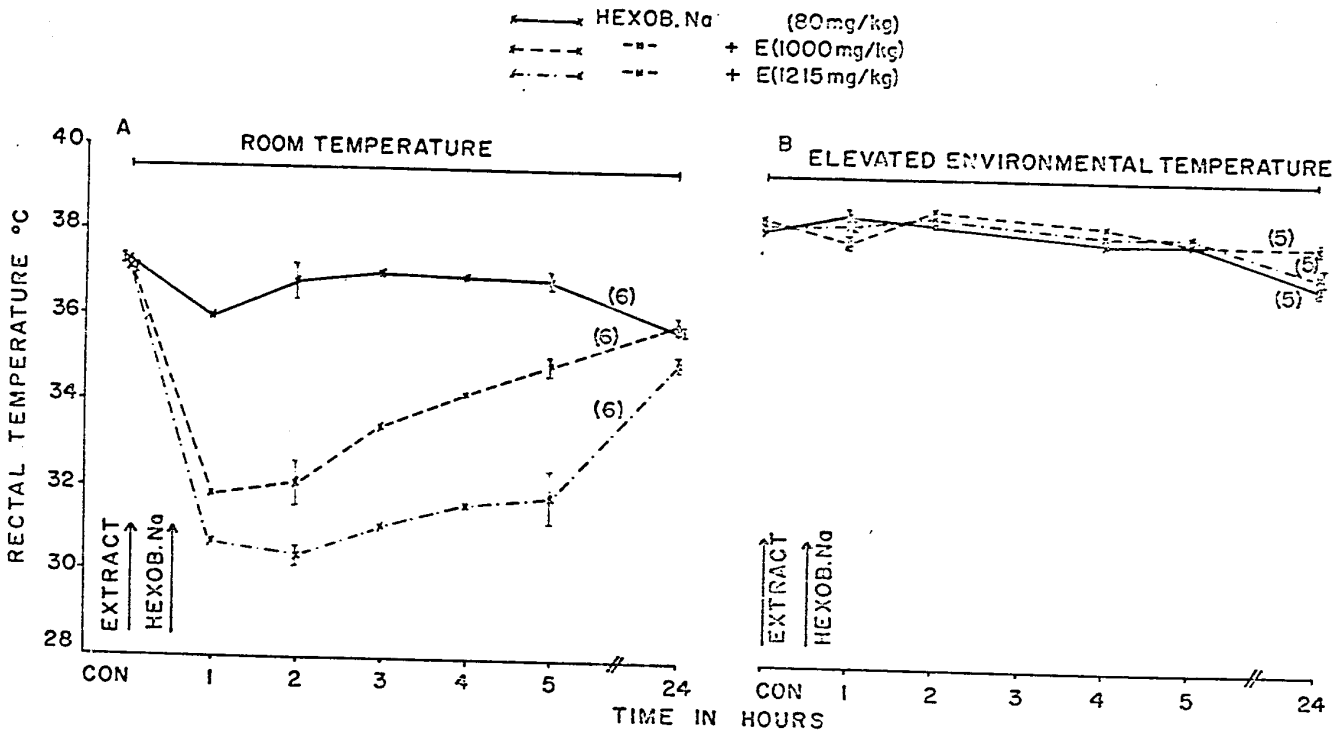


Fig.14: Mean rectal temperature changes in hexobarbital sodium, hexobarbital sodium + urine extract (1000 mg/kg) and hexobarbital sodium + urine extract (1215 mg/kg) treated male hooded rats.

Fig.14/A illustrates the changes occuring at room temperature and Fig.14/B illustrates the changes occuring at elevated environmental temperature.

Number in parenthesis refers to number of rats in each group.

For details see text.

D. Effect of Picrotoxin on Hypnosis and Hypothermia Induced by Urine Extract

1. Hypnosis

Table 13 summarizes the results obtained after the oral administration of graded doses of picrotoxin 30 minutes prior to the i.p. administration of the extract in the hypnotic ED 50 dose.

The administration of the extract to 9 vehicle pretreated rats caused a loss of righting reflex in 55.5% of the animals. Picrotoxin given to 5 rats in a dose of 0.25 mg/kg did not antagonize extract-induced hypnosis and gave essentially the same results as was observed in the vehicle pretreated group. However, 0.5 mg/kg of picrotoxin given to 5 rats caused loss of righting reflex only in 20% of the animals while 1.0 mg/kg of picrotoxin given to 10 rats caused a loss of righting reflex in 10% of the animals. This indicated that increasing doses of picrotoxin effectively antagonized extract-induced hypnosis.

2. Temperature

Table 14 shows that picrotoxin was unable to influence the extract-induced hypothermia. All rats, whether pretreated with picrotoxin or the vehicle, manifested the same dramatic drop in rectal temperatures. Peak hypothermia was recorded 2 hours after the administration of the extract. No statistical differences were noted between control and treated groups. Five hours after the administration of the extract rectal temperatures of all groups were still low with no differences between groups, although slightly higher when compared to values recorded during peak hypothermia.

Table 13: EFFECT OF PICROTOXIN ON URINE EXTRACT-INDUCED HYPNOSIS

The vehicle or graded doses of picrotoxin were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Picrotoxin mg/kg	No. rats	% Hypn.
Vehicle	9	55.5
0.25	5	60.0
0.50	5	20.0
1.00	10	10.0

% Hypn. = % of rats, that lost righting reflex

Table 14: EFFECT OF PICROTOXIN ON URINE EXTRACT-INDUCED HYPOTHERMIA

The vehicle or graded doses of picrotoxin were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Picrotoxin mg/kg	No. rats	Rectal temperature ($^{o}C \pm S.E.$)			
		Control	Hours after i.p. injection of extract		
			2	5	24
Vehicle	4	37.5 ± .12	29.8 ± .28	31.8 ± .52	36.2 ± .40
0.25	5	37.5 ± .16	29.3 ± .47	31.2 ± .54	36.6 ± .34
0.50	5	37.4 ± .11	30.4 ± .53	32.6 ± .47	36.3 ± .20
1.00	5	37.5 ± .25	29.7 ± .79	31.7 ± .90	36.2 ± .18

Within 24 hours rectal temperatures of all groups returned toward normal but were still lower when compared to control values within groups. No mortality occurred during this period in any of the groups. Identical, graded oral doses of picrotoxin did not elicit significant hypothermia in normal rats (Table 24) when compared to controls.

E. Effect of Pentylenetetrazol on Hypnosis and Hypothermia Induced by Urine Extract

1. Hypnosis

Five groups of 5 rats each were used. Four groups were pre-treated with increasing, oral doses of pentylenetetrazol (5.0, 10.0, 20.0 and 40.0 mg/kg) 30 minutes prior to the administration of the extract in the hypnotic ED 50 dose. The results are summarized in Table 15. The administration of the extract to 5 vehicle pretreated rats caused a loss of righting reflex in 40% of the rats. One out of a group of 5 animals (20%) lost its righting reflex when pretreated with the lowest dose of pentylenetetrazol (5.0 mg/kg). However, the pretreatment of rats with higher doses of pentylenetetrazol entirely prevented the loss of righting reflex following the administration of the extract.

2. Temperature

Table 16 illustrates that there was no difference between control and treated groups in the degree of decrease of rectal temperatures following the administration of the extract. Peak hypothermia was recorded 2 hours after the administration of the extract. Rectal temperatures in pentylene-tetrazol pretreated animals did not differ from those of vehicle pretreated

Table 15: EFFECT OF PENTYLENETETRAZOL ON URINE EXTRACT-INDUCED HYPNOSIS

The vehicle or graded doses of pentylenetetrazol were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Pentylenetetrazol mg/kg	No. rats	% Hypn.
Vehicle	5	40
5.0	5	20
10.0	5	0
20.0	5	0
40.0	5	0

% hypn. = % of rats that lost righting reflex

Table 16: EFFECT OF PENTYLENETETRAZOL ON URINE EXTRACT-INDUCED HYPOTHERMIA

The vehicle or graded doses of pentylenetetrazol were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Pentylene-tetrazol mg/kg	No. rats	Rectal temperature (°C ± S.E.)			
		Control	Hours after i.p. injection of extract		
			2	5	24
Vehicle	5	37.2 ± .16	29.7 ± .40	32.2 ± .48	36.3 ± .40
5.0	5	37.8 ± .71	30.1 ± .37	33.1 ± .86	36.6 ± .24
10.0	5	37.8 ± .19	30.3 ± .18	32.9 ± .13	36.3 ± .15
20.0	5	37.5 ± .17	30.0 ± .28	32.0 ± .66	36.5 ± .20
40.0	5	37.4 ± .18	30.1 ± .48	31.9 ± .89	36.1 ± .10

rats, neither during peak hypothermia nor 5 hours after the administration of the extract. Rectal temperatures returned toward normal in all groups within 24 hours, but were still lower when compared to control values within groups. No mortality occurred during this period in any of the groups. Identical, graded oral doses of pentylenetetrazol did not elicit significant hypothermia in normal rats (Table 24) when compared to controls.

F. Effect of Nikethamide on Hypnosis and Hypothermia Induced by Urine Extract

1. Hypnosis

Four groups of 5 rats each were used. Three groups were pretreated with increasing oral doses of nikethamide (15.0, 30.0 and 60.0 mg/kg) 30 minutes prior to the administration of the urine extract in the hypnotic ED 50 dose. The results are summarized in Table 17. In the control group, 4 out of 5 rats (80%) lost their righting reflex following the administration of the extract. Nikethamide pretreatment reduced, but could not entirely antagonize the occurrance of hypnosis. Two out of 5 rats (40%) lost their righting reflex following pretreatment with 15.0 mg/kg of nikethamide and 1 out of 5 rats each (20%) lost its righting reflex following pretreatment with 30.0 and 60.0 mg/kg of nikethamide.

2. Temperature

The effect of nikethamide on urine extract-induced hypothermia is summarized in Table 18 and Fig.15. Peak hypothermia occurred 2 hours after the administration of the extract in all groups. Nikethamide potentiated hypothermia. Rats pretreated with the low (15.0 mg/kg) and middle

Table 17: EFFECT OF NIKETHAMIDE ON URINE EXTRACT-INDUCED HYPNOSIS

The vehicle or graded doses of nikethamide were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Nikethamide mg/kg	No. rats	% Hypn.
Vehicle	5	80
15.0	5	40
30.0	5	20
60.0	5	20

% Hypn. = % of rats that lost righting reflex

Table 18: EFFECT OF NIKETHAMIDE ON URINE EXTRACT-INDUCED HYPOTHERMIA

The vehicle or graded doses of nikethamide were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Nikethamide mg/kg	No. rats	Rectal temperature (°C ± S.E.)			
		Control	Hours after i.p. injection of extract		
			2	5	24
Vehicle	5	37.6 ± .13	31.0 ± .30	33.9 ± .53	36.2 ± .15
15.0	5	37.9 ± .18	30.0 ± .08[a]	32.1 ± .57[a]	36.1 ± .20
30.0	5	37.4 ± .15	30.1 ± .25[a]	31.5 ± .28[b]	35.6 ± .18[a]
60.0	5	37.3 ± .12	29.6 ± .24[b]	30.9 ± .36[b]	36.0 ± .14

a = $P < 0.05$ (Nikethamide versus vehicle)

b = $P < 0.01$ (Nikethamide versus vehicle)

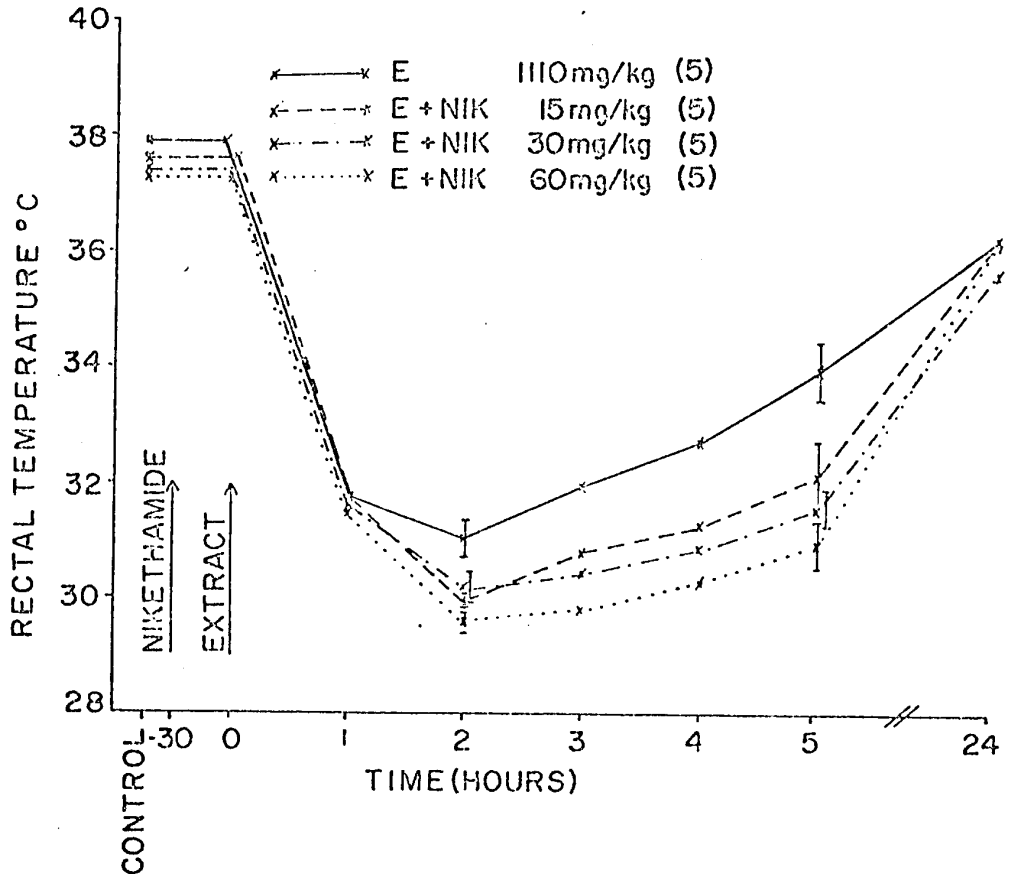


Fig.15: Mean rectal temperature changes in response to urine extract (hypnotic ED 50 dose) in rats pretreated with graded doses of nikethamide (NIK) or vehicle. Number in parenthesis refers to number of rats in each group.
For detail see text.

dose (30.0 mg/kg) of nikethamide showed significantly lower ($P<0.05$) rectal temperatures than did controls; the high dose (60.0 mg/kg) had an even more pronounced potentiating effect ($P<0.01$). Even 5 hours after the injection of the extract rectal temperatures of the nikethamide pretreated animals were significantly lower than those of the control rats ($P<0.05$ at 15.0 mg/kg dose; $P<0.01$ at 30.0 and 60.0 mg/kg dose). Twenty four hours after the administration of the extract only one group (30.0 mg/kg) showed slightly but significantly lower ($P<0.05$) rectal temperatures when compared to the control group. No mortality occurred in any of the groups within 24 hours.

It was shown in control studies (Table 24) that identical, graded oral doses of nikethamide induced slight but significant hypothermia in normal rats. Two hours after the administration of nikethamide the recorded rectal temperature were significantly lower ($P<0.05$ at 15.0 and 30.0 mg/kg and $P<0.01$ at 60.0 mg/kg) from that of vehicle (water) pretreated animals.

G. Effect of Amphetamine on Hypnosis and Hypothermia Induced by Urine Extract

1. Hypnosis

Five groups of rats were used. One group, consisting of 11 rats, served as control. Increasing doses of orally administered amphetamine (3.75, 7.5, 15.0 and 30.0 mg/kg) were given to the other groups. Amphetamine had a varying effect on urine extract-induced hypnosis depending on the dose administered. The results are summarized in Table 19. Of the control animals

Table 19: EFFECT OF AMPHETAMINE ON URINE EXTRACT-INDUCED HYPNOSIS

The vehicle or graded doses of amphetamine were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Amphetamine mg/kg	No. rats	% Hypn.
Vehicle	11	54.5
3.75	5	60.0
7.50	11	18.2
15.00	5	80.0
30.00	6	83.3

% Hypn. = % of rats that lost righting reflex

54.5% lost their righting reflex after the administration of the extract. The low dose of amphetamine, 3.75 mg/kg given to 5 rats, caused loss of righting reflex in 60% of the animals. Amphetamine given in a dose of 7.5 mg/kg to 11 rats decreased hypnosis, as the loss of righting reflex occurred only in 18.2% of the rats. Amphetamine, in a dose of 15.0 and 30.0 mg/kg, given to 5 and 6 rats respectively potentiated urine extract induced hypnosis causing loss of righting reflex in 80% of the animals at the 15.0 mg/kg dose and in 83.3% of the animals at the 30.0 mg/kg dose.

2. Temperature

Amphetamine was able to prevent and to reverse the extract-induced hypothermia. These results are summarized in Table 20 and 21 and Fig.16/A and B.

Table 20 and Fig.16/A summarizes the results obtained when the rats were pretreated with amphetamine 30 minutes prior to the administration of the extract. Table 20/A shows in 4 groups of 5 rats each that a progressive decrease in the degree of hypothermia was observed as the dose of amphetamine was raised from 3.75 to 15.0 mg/kg. Peak hypothermia occurred 2 hours after the administration of the extract in all 4 groups. Rectal temperatures of rats which received the vehicle were much lower than those of amphetamine pretreated rats the difference being highly significant ($P<0.001$). The difference in rectal temperatures between rats receiving the vehicle or amphetamine was still significantly different 5 hours after the administration of the extract.

Table 20/B summarizes the results of another experiment in 3 groups of 6 rats each. The administration of amphetamine in a dose of

Table 20: EFFECT OF AMPHETAMINE ON URINE EXTRACT-INDUCED HYPOTHERMIA

The vehicle or graded doses of amphetamine were administered orally followed 30 minutes later by the i.p. injection to all rats of the hypnotic ED 50 dose of the urine extract.

Amphetamine mg/kg	No. rats	Rectal temperature (°C ± S.E.)			
		Control	Hours after i.p. injection of extract		
			2	5	24
A.					
Vehicle	5	37.8 ± .11	28.9 ± .39	32.1 ± .71	36.0 ± .33
3.75	5	37.7 ± .17	32.9 ± .27[b]	34.8 ± .37[a]	35.9 ± .47
7.50	5	37.9 ± .17	33.7 ± .43[b]	35.8 ± .17[b]	35.7 ± .10
15.00	5	38.0 ± .13	36.6 ± .87[b]	37.4 ± .31[b]	36.2 ± .37
B.					
Vehicle	6	37.5 ± .22	28.4 ± .26	29.6 ± .56	35.4 ± .16
7.5	6	37.5 ± .14	34.0 ± .56[b]	34.6 ± .20[b]	35.2 ± .16
30.0	6	37.3 ± .11	37.9 ± .68[b]	36.2 ± .29[b]	35.8 ± .55

a = $P < 0.01$ (Amphetamine versus vehicle)

b = $P < 0.001$ (Amphetamine versus vehicle)

Table 21: EFFECT OF AMPHETAMINE ON URINE EXTRACT-INDUCED HYPOTHERMIA

The hypnotic ED 50 dose of urine extract was injected intraperitoneally to all rats followed 60 minutes later by the vehicle or graded doses of orally administered amphetamine.

Amphetamine mg/kg	No. rats	Rectal temperature (°C ± S.E.)					
		Extract induced hypothermia	Hours after oral injection of amphetamine				
			1	2	3	4	24
Vehicle	5	31.0 ± .34	29.6 ± .29	29.5 ± .34	30.5 ± .38	31.2 ± .34	37.1 ± .38
3.75	5	31.3 ± .28	30.2 ± .12	31.2 ± .07[b]	32.5 ± .18[b]	33.5 ± .21[c]	36.2 ± .27
7.50	5	31.2 ± .36	30.7 ± .60	31.8 ± .82[a]	33.0 ± .74[a]	34.6 ± .49[c]	35.6 ± .38[a]
15.00	4	31.0 ± .29	31.4 ± .60[a]	34.3 ± 1.29[b]	36.2 ± 1.64[b]	36.9 ± 1.32[b]	36.8[o]

a = $P < 0.05$ (Amphetamine versus vehicle)

b = $P < 0.01$ (Amphetamine versus vehicle)

c = $P < 0.001$ (Amphetamine versus vehicle)

o = single animal

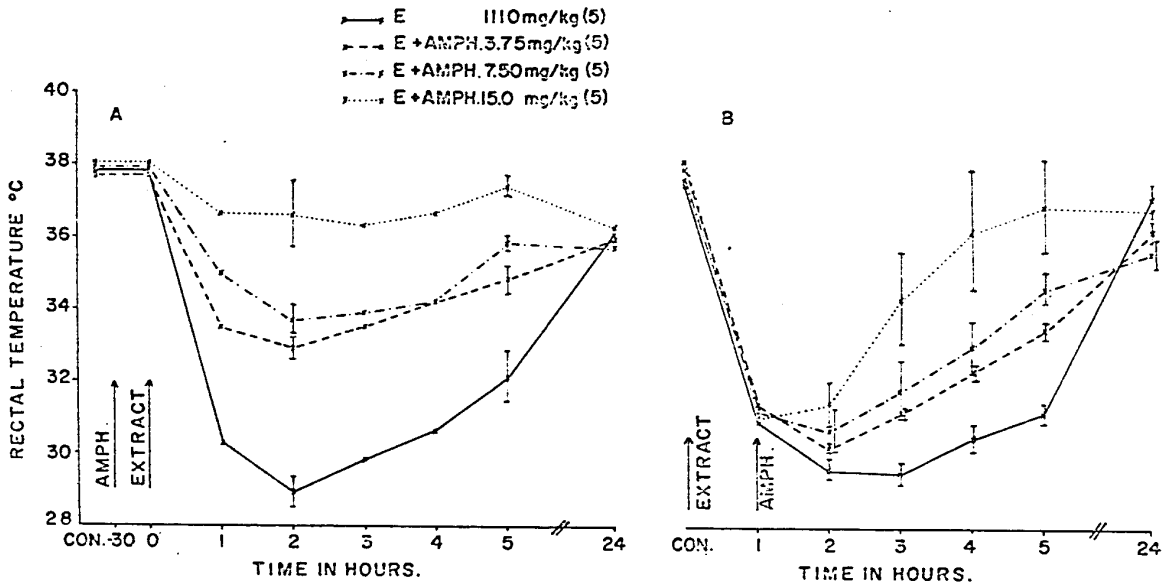


Fig.16/A: Mean rectal temperature changes in response to urine extract (hypnotic ED 50 dose) in rats pretreated with graded doses of amphetamine (AMPH) or vehicle. Number in parenthesis refers to number of rats in each group. For detail see text.

Fig.16/B: Mean rectal temperature changes in hypothermic rats in response to the administration of graded doses of amphetamine (AMPH) or vehicle. Hypothermia was induced by the urine extract (hypnotic ED 50 dose). Number in parenthesis refers to number of rats in each group. For detail see text.

7.5 mg/kg significantly reduced ($P<0.001$) the fall in body temperature induced by the administration of the extract compared to the vehicle pretreated group both at 2 and 5 hours. The results obtained by this dose are well comparable to the results described in Table 20/A. Those rats which received 30.0 mg/kg of amphetamine did not manifest any hypothermia, on the contrary, their rectal temperatures increased slightly 2 hours after the injection of the extract and were close to normal after 5 hours. Temperatures returned toward normal in all groups investigated (Table 20/A and B) 24 hours after the injection of the extract and no differences were observed among amphetamine treated and control groups. Mortality occurred in all groups within 24 hours. Two rats died from both the control group and the group pretreated with 7.5 mg/kg of amphetamine, each group consisting of 11 animals. Two out of 5 rats died in 2 groups, which were pretreated with 3.75 and 15.0 mg/kg of amphetamine respectively. Half of the group died which received the highest dose of amphetamine.

Table 21 and Fig.16/B summarizes the results obtained when the effect of different doses of amphetamine was investigated on established hypothermia. Four groups of 5 rats each were injected intraperitoneally with the extract in the hypnotic ED 50 dose. An equal drop in body temperature occurred in all groups. Sixty minutes after the injection of the extract, the rats were treated orally with amphetamine in doses of 3.75, 7.5 and 15.0 mg/kg; the controls received water. Rectal temperatures were recorded in all groups at hourly intervals for the next 4 hours and at 24 hours. The hypothermic effect of the extract was rapidly reversed by amphetamine. One hour after its administration the mean rectal temperature

of the group receiving the highest dose of amphetamine (15.0 mg/kg) was significantly different ($P<0.05$) from that of controls while 2 hours later all treated groups showed significantly higher temperatures than did controls. This significant difference was maintained during the subsequent 2 hours as well. Twenty four hours after the injection of the extract the treated groups had slightly lower rectal temperatures than did the control group. In one of the groups (7.5 mg/kg) this difference was significant ($P<0.05$).

Mortality increased with increasing doses of amphetamine. Only 1 rat survived in the group which received the highest dose of amphetamine (15.0 mg/kg). Identical, graded oral doses of amphetamine did not induce significant changes in the rectal temperature of normal rats (Table 24).

3. Spontaneous Motility

Due to the well known fact that amphetamine greatly increases the spontaneous motility, which might influence body temperature, it seemed of interest to investigate the effect of amphetamine on spontaneous motility in extract treated rats. Fig.17 illustrates the spontaneous motility of normal, amphetamine + extract, and extract treated rats.

A slight hypermotility was noted when the 2 normal rats were first introduced into the test cages, however, this hypermotility soon decreased. The 2 rats moved and sniffed around the cage in the next 3 hours and no great differences could be observed in their spontaneous motility between the subsequent hourly intervals. Rats which were pretreated with 30.0 mg/kg of amphetamine, 30 minutes prior to the administration of the

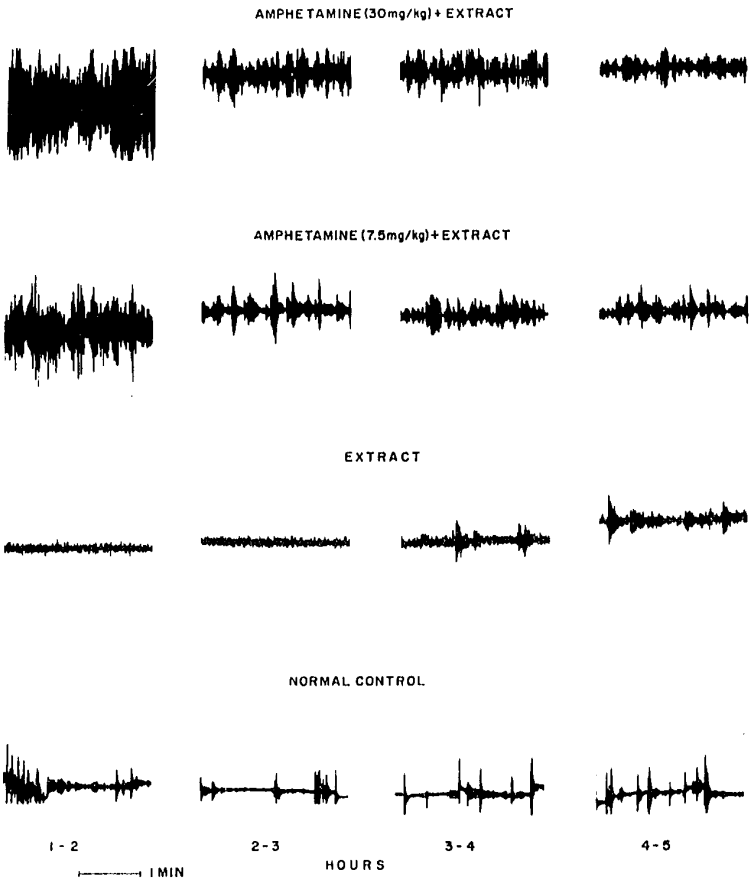


Fig.17: Spontaneous motility observed in amphetamine (30 mg/kg) + extract, amphetamine (7.5 mg/kg) + extract, only extract treated and normal rats.
The record represents the spontaneous motility of 2 rats in each group. Continuous recordings were taken throughout the experiment for 4 hours. The samples shown represent the typical motility observed for each hour. Recording speed: 25 mm/min.

extract, showed a tremendous hypermotility in the course of the second hour following the administration of the extract. This hypermotility gradually decreased during the next 3 hours but was still considerably greater than the motility shown by normal rats.

Rats which received amphetamine in a dose of 7.5 mg/kg 30 minutes prior to the administration of the extract manifested essentially the same pattern of spontaneous motility as observed in rats treated with 30.0 mg/kg of amphetamine + extract, although to a lesser degree.

Rats receiving only the extract showed complete tranquility in the 2nd and 3rd hour following the administration of the extract. During the 4th and 5th hour the rats started to move around in their cage. The administration of amphetamine alone at both dose levels gave essentially the same response as observed following treatment with amphetamine + extract and is therefore not shown separately.

H. Effect of Iproniazid and ∝-Methyl-Meta-Tyrosine on Hypnosis and Hypothermia Induced by Urine Extract

1. Hypnosis

Three groups of 6 rats each were used. Group 1 was injected intraperitoneally with the extract in the hypnotic ED 50 dose. Group 2 was pretreated for 3 consecutive days with iproniazid, given subcutaneously, and the extract was administered in the hypnotic ED 50 dose 4 hours after the last injection of iproniazid. Group 3 was pretreated for 3 consecutive days with iproniazid. On the morning of the 3rd day ∝-MMT was injected intraperitoneally, followed 4 hours later by the injection of the extract.

The results are summarized in Table 22. After the injection of the extract 50% of the animals lost their righting reflex. Pretreatment both with iproniazid and iproniazid + ∝ -MMT potentiated the effect of the extract, since 66.6% of the animals in the iproniazid pretreated group (4 out of 6 rats) and 83.3% of the animals in the iproniazid + ∝ -MMT pretreated group (5 out of 6 rats) lost their righting reflex.

2. Temperature

The effect of iproniazid and iproniazid + ∝ -MMT on urine extract-induced hypothermia is summarized in Table 23 and Fig.18.

Iproniazid pretreatment was unable to prevent extract-induced hypothermia. Rectal temperatures of rats pretreated with iproniazid were even lower, although not significantly, than rectal temperatures of extract treated animals both 2 and 5 hours after the injection of the extract. Iproniazid + ∝ -MMT pretreatment was able to antagonize hypothermia induced by the extract. The maximal decrease of rectal temperature in this group occurred 1 hour after the injection of the extract (see Fig.18). Rectal temperatures were significantly higher ($P < 0.01$) when compared to control animals both 2 and 5 hours after the administration of the extract. The temperature of this group was significantly lower ($P < 0.05$) 24 hours after the injection of the extract when compared to both extract and iproniazid + extract treated animals.

3. Spontaneous Motility

The spontaneous motility of rats treated with iproniazid + ∝-MMT + extract, iproniazid + extract, extract, iproniazid + ∝-MMT, and iproniazid

Table 22: THE EFFECT OF IPRONIAZID AND IPRONIAZID + α-METHYL-META-TYROSINE ON URINE EXTRACT-INDUCED HYPNOSIS

For details see text

Groups	No. rats	% Hypn.
E^*	6	50.0
$Ipr.^y + E^*$	6	66.6
$Ipr.^y + \alpha\text{-}MMT^z + E^*$	6	83.3

% Hypn. = % of rats that lost righting reflex

E^* = urine extract, hypnotic ED 50 intraperitoneally

Ipr^y = Iproniazid, 100 mg/kg subcutaneously

α - MMT^z = α-methyl-meta tyrosine, 500 mg/kg intraperitoneally

Table 23: THE EFFECT OF IPRONIAZID AND IPRONIAZID + α-METHYL-META-TYROSINE ON URINE EXTRACT-INDUCED HYPOTHERMIA

For details see text.

Group	No. rats	Rectal temperature ($^{o}C \pm$ S.E.)			
		Control	Hours after i.p. injection of extract		
			2	5	24
E^{*}	6	$36.7 \pm .12$	$30.1 \pm .21$	$31.8 \pm .46$	$35.9 \pm .22$
$Ipr^{y} + E^{*}$	6	$36.7 \pm .32$	$29.5 \pm .31$	$30.6 \pm .35$	$35.6 \pm .27$
$Ipr^{y} + \alpha$-$MMT^{z} + E^{*}$	6	$37.8 \pm .26$	$35.6 \pm .31^{b}$	36.3 ± 1.03^{b}	$33.8 \pm .68^{a\ a'}$

a = $P < 0.05$ (treated versus extract)

b = $P < 0.01$ (treated versus extract)

a' = $P < 0.05$ (Ipr. + α-MMT versus Ipr.)

E^{*} = urine extract, hypnotic ED 50 intraperitoneally

Ipr^{y} = Iproniazid, 100 mg/kg subcutaneously

α-MMT^{z} = α-methyl-meta-tyrosine, 500 mg/kg intraperitoneally

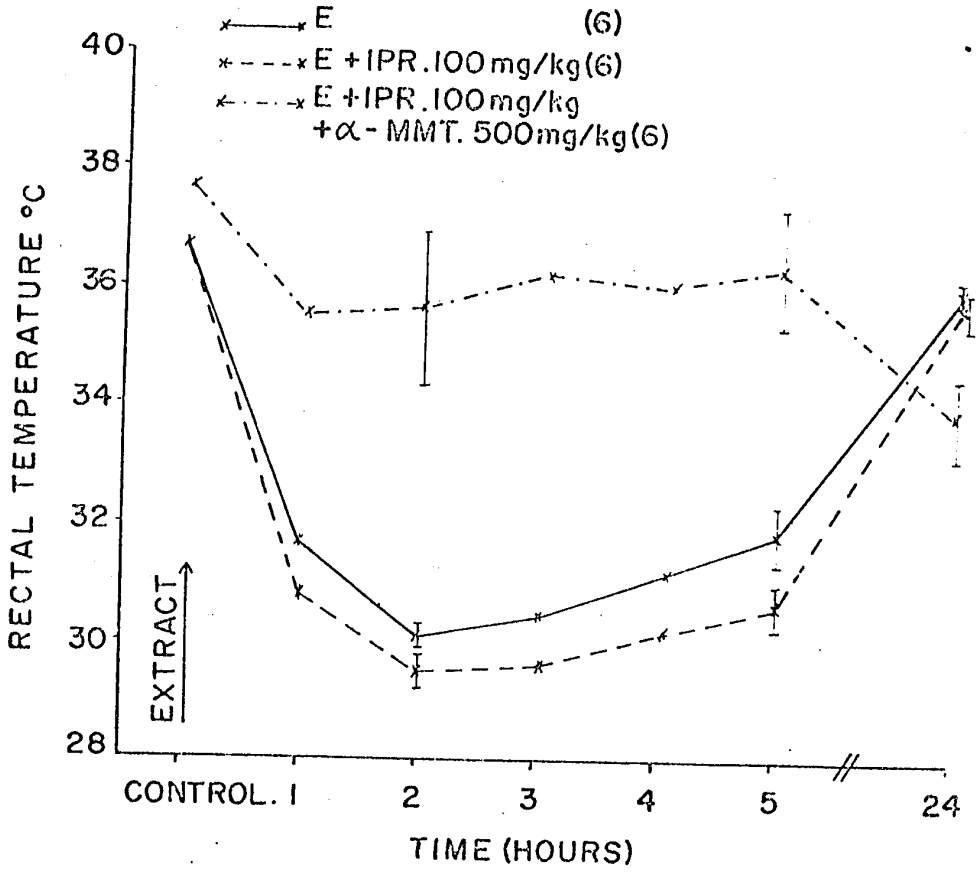


Fig. 18: Mean rectal temperature changes in response to urine extract (hypnotic ED 50 dose) in rats pretreated with iproniazid (IPR), iproniazid + ∝-methyl-meta-tyrosine (IPR + ∝-MMT) or vehicle.
Number in parenthesis refers to number of rats in each group. For detail see text.

is shown in Fig.19.

It is illustrated that 2 and 3 hours following the administration of the extract in the iproniazid + α-MMT treated group the behavior of the rats was characterized by a hypermotility which decreased slightly during the course of the 4th and 5th hour.

The spontaneous motility of rats treated with iproniazid + extract was characterized by the same pattern, although to a lesser degree when compared to the previous group.

Rats treated with extract alone were completely quiet for 2 hours while during the 4th and 5th hour they started to move around. Control studies showed that while the administration of iproniazid + α-MMT gave essentially a similar response in the presence or absence of the extract, the spontaneous motility of rats pretreated with iproniazid alone, was characterized by a depression of activity if compared to the spontaneous motility of normal animals, shown in Table 17.

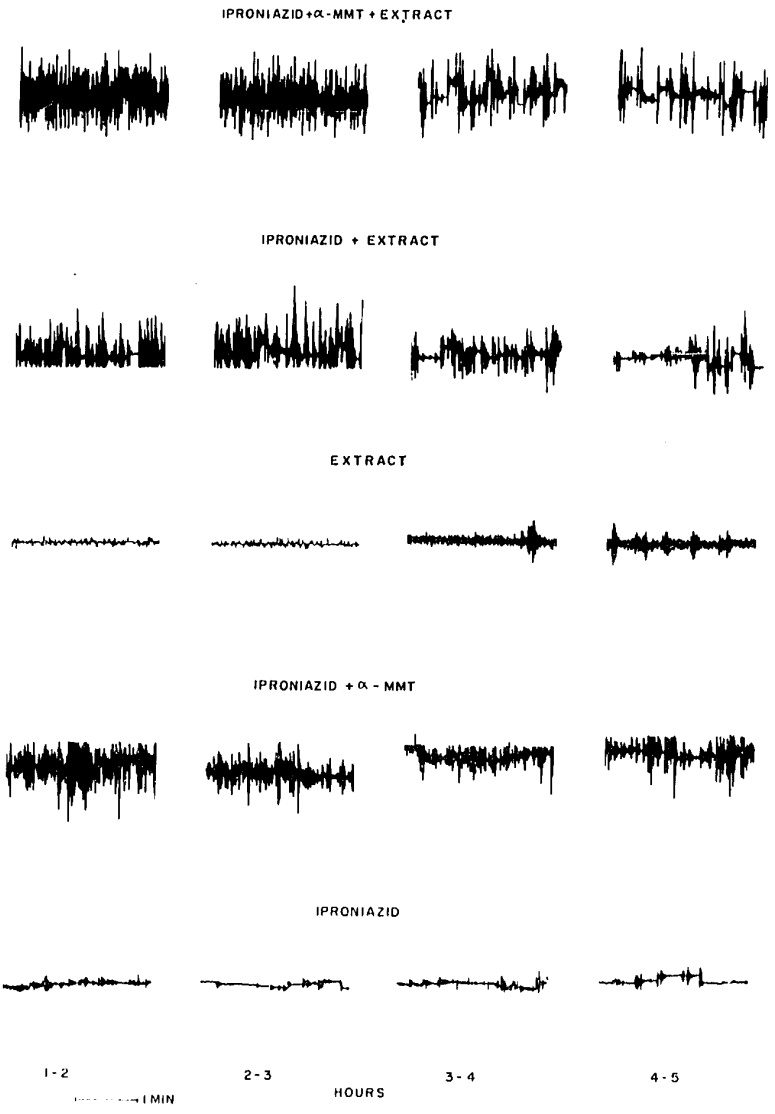


Fig. 19: Spontaneous motility observed in iproniazid + ∝-methyl-meta-tyrosine (∝-MMT) + extract, iproniazid + extract, and only extract treated rats.
Record also shows the spontaneous motility of rats treated with iproniazid + ∝-methyl-meta-tyrosine (∝-MMT) or iproniazid.
The record represents the spontaneous motility of 2 rats in each group. Continuous recordings were taken throughout the experiment for 4 hours. The samples shown represent the typical motility observed for each hour. Recording speed: 25 mm/min.

Table 24: TEMPERATURE CHANGES IN RATS, FOLLOWING THE ADMINISTRATION OF VARIOUS DRUGS

Graded doses of various drugs were administered orally to rats. Rectal temperature was measured prior to and 2 hours following drug administration. The values in the table express the difference obtained by subtracting 2-hour values from controls.

Drug mg/kg	No. rats	$^{o}C \pm$ S.E.
Vehicle	4	$-.32 \pm .15$
Picrotoxin 0.25	4	$-.28 \pm .14$
" 0.50	4	$-.78 \pm .35$
" 1.00	4	$-.58 \pm .19$
Pentylenetetrazol 5.0	4	$-.45 \pm .21$
" 10.0	4	$-.28 \pm .12$
" 20.0	4	$-.30 \pm .07$
" 40.0	3	$-.20 \pm .62$
Nikethamide 15.0	3	$-.76 \pm .04^{a}$
" 30.0	4	$-.90 \pm .16^{a}$
" 60.0	4	$-1.22 \pm .16^{b}$
Amphetamine 3.75	5	Ø
" 7.50	5	$-.10 \pm .08$
" 15.00	5	$-.10 \pm .07$

a = $P < 0.05$ (treated versus vehicle)

b = $P < 0.01$ (treated versus vehicle)

DISCUSSION

A. General Discussion

The results presented in this work showed that urine extract prepared from a fraction of pregnant mare urine exerted strong biological activity.

It was shown that the extract possessed a strong antisecretory activity antagonizing histamine-induced acid hypersecretion in guinea-pigs and dogs, but interestingly it rather potentiated than inhibited gastrin-induced acid hypersecretion in guinea-pigs. The extract also exerted a strong anti-ulcerogenic effect both in the pylorus-ligated and immobilized (restraint) rat.

The extract elicited a definite hypnotic action both at room and elevated temperature. Since the strong hypothermic effect of the extract was almost completely abolished at elevated temperature it seems reasonable to assume that both the hypnotic and hypothermic action are primary and independent effects of the extract.

Investigations using centrally acting agents seemed to confirm this observation, since a certain class of drugs (brain stem stimulants) antagonized hypnosis but were without any effect on the hypothermia. On the other hand, prevention of hypothermia did not inhibit hypnosis, the number of rats that lost their righting reflex was even increased.

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The results presented in this thesis, however, were obtained with a purified but not a chemically pure substance. Thus it could not be stated with certainty that the effects described are the property of a single

substance. The possibility exists that it may be due to the combined effect of various constituents of the extract. This possibility is remote however, if one considers that (a) the extract was highly active in antagonizing histamine-induced acid hypersecretion, while it had no effect on gastrin-induced acid hypersecretion; (b) hypnosis (ED 5 - ED 95) was obtained within a relatively narrow dose range (1000 - 1215 mg/kg of extract), and (c) small doses of extract induced a significant drop in body temperature. It is questionable furthermore, even if one assumes that the effects are due to a single substance, whether this is of known or unknown chemical nature.

It is well known that certain steroids or steroid metabolites have anesthetic property. It is also known that both human and animal urine contain large numbers of steroids and/or steroid metabolites and the possibility existed that hypnotic activity was brought about by pregnane and pregnene derivatives. However, the analysis of the extract using gas chromatography (kindly performed by Dr. D.J. Marshall of Ayerst Laboratories) revealed that "the sample which was chromatographed under several conditions contained traces of estrogens (estrone, equilin, 17α-dihydroequilin) together with possible traces of pregnanes, terpenes and many unknowns". This determination excluded the possibility that loss of righting reflex was due to known steroids present in the urine.

B. Detailed Discussion

1. Effect of Graded Doses of Histamine and Gastrin on Gastric Acid Secretion in Male and Female Guinea-pigs

Prior to the investigation of the effect of urine extract on his-

.tamine or gastrin-induced gastric acid hypersecretion experiments were carried out in male and female guinea-pigs to establish the dose range for both histamine and gastrin which induces a submaximal, maximal and supramaximal acid secretory response. The experiments demonstrated that a single s.c. injection of histamine, at the dose levels used, uniformly induced a well-defined acid secretory response. The results obtained were in agreement with the established effect of histamine in most mammalian species when similar doses were used. Progressive increases in histamine dosage resulted in correspondingly higher acid output in both sexes. Maximal acid secretory response was obtained following the administration of 0.4 mg/kg of histamine in both male and female animals; 0.8 mg/kg of histamine proved to be a supramaximal dose because acid secretion declined in both sexes when compared with the results obtained after the administration of 0.4 mg/kg histamine. This finding however, is in accord with the observation of Lin et al. (1962) who found that when histamine was intravenously infused in Heidenhain-pouch dogs dose-response curves were obtained which rose to a maximum and then fell off with further increase in dosage.

The experiments performed with a highly purified gastrin preparation (Gastrin Leo) clearly showed that guinea-pigs are much less sensitive to gastrin than some other species.

Gregory and Tracy (1964) have reported that gastrin I and II stimulate the flow of a highly acidic gastric juice in a conscious dog when given subcutaneously in a dose range of 0.25 - 2.5 µg/kg. Doses of 2.0 µg/kg of gastrin were described by Makhlouf et al. (1964) and Semb and Myren (1965) as potent stimulants of acid secretion in man. (The latter investi-

gators used the same purified gastrin preparation (Gastrin Leo) in humans as we did in the guinea-pigs.) However, gastrin in doses reported to be able to stimulate acid secretion in other species had no effect on gastric acid secretion in male or female guinea-pigs. In guinea-pigs gastrin was able to induce maximal acid secretion only in the dose range of 100-200 µg/kg. This dose is about 100 times larger than the doses which produced maximal effect in humans and dogs. The extreme sensitivity of the guinea-pigs to histamine is well known. Therefore it was unexpected to find that the guinea-pig was so insensitive to gastrin.

When dose-response curves obtained in guinea-pigs, were compared in response to either histamine or gastrin the following striking differences were noted:

(i) the secretory response became evident earlier in response to gastrin,

(ii) peak secretion was recorded earlier in response to gastrin,

(iii) the secretory response declined earlier in response to gastrin,

(iv) maximal total acid output was higher (although not significantly) in response to histamine in the male guinea-pigs,

(v) the dose which induced a maximal secretory response following the administration of gastrin was only half the dose of histamine on a weight basis,

(vi) a supramaximal dose of gastrin hardly stimulated acid secretion; a supramaximal dose of histamine induced a smaller response than the maximal dose but only slightly so.

In human subjects Kay (1953) observed the maximal gastric secretory response between 20-40 minutes after the s.c. injection of large doses of histamine; Semb and Myren (1965) found that on gastrin stimulation maximal values were not reached until 40-60 minutes after the administration of the hormone.

Differences in maximal responses to gastrin and histamine are described in the literature but in most cases the maximal response to gastrin is greater than to histamine (Makhlouf, 1964 and Konturek and Grossman, 1966).

The results obtained in the experiments do not explain the striking lack of sensitivity of guinea-pigs in response to gastrin. However, from data available from the literature certain comparisons can be made which may enable one to draw some conclusions.

The mechanism by which gastrin stimulates gastric acid secretion is not known. Most experiments in which the possible mechanism of action was investigated were performed in rats. Haverback et al. (1965) observed a decrease in gastric histamine content in association with acid secretion following injection of gastrin. Kahlson and Rosengren (1965) reported a greatly increased histamine forming capacity in the stomach after the administration of gastrin. On the basis of these observations, it was suggested that the effect of gastrin on the acid-producing cells might be mediated through histamine, thus rendering histamine the final common pathway in the stimulation of acid secretion.

It is also known that histamine metabolism is dynamic in the acid-secreting gastric mucosa of the rat and the stomach has the highest histamine

forming capacity of any organ. Histamine forming capacity is a measure of histidine decarboxylase activity and specific histidine decarboxylase has not been identified in the gastric mucosa of species other than the rat and rabbit.

All these observations indicate that histamine formation is different in the rat stomach from that of other species. Thus, the suggestion that the effect of gastrin is mediated via histamine might be true in the rat, but it seems very unlikely that the mechanism of action of gastrin would be the same in other species particularly in the guinea-pig.

The existence of highly purified and synthetic gastrin is fairly new and most studies recorded in the literature describe the effect of the hormone in humans, dog and rat. We did not find data with reference to the effect of gastrin on acid secretion in the guinea-pig.

The administration of gastrin (same batch as used in guinea-pigs) to hooded male rats in a dose of 2.0 µg/kg was able to induce acid secretion and peak secretory responses were recorded 45-60 minutes after injection. No other doses of gastrin were investigated in the rat: the only aim of the experiment was to establish whether 2.0 µg/kg of gastrin (which is a secretory stimulant in other species) is able to stimulate acid secretion in the rat. The experiment provided an affirmative answer.

In summary: gastrin had to be administered in large doses to guinea-pigs in order to stimulate acid secretion. The earlier secretory response, and earlier peak secretion, the lower maximal secretory response, and finally the earlier return to basal secretory levels (as compared to responses following histamine) suggested that: (1) in the guinea-pig gastrin-induced acid

secretion was not mediated via histamine and (2) the guinea-pig is a species highly insensitive to the antral hormone.

2. The Effect of Urine Extract on Histamine and Gastrin-induced Gastric Acid Hypersecretion

On the basis of the results presented, it is evident that urine extract obtained from fractions of pregnant mare urine, antagonized histamine-induced gastric acid hypersecretion both in anesthetized male guinea-pigs and in conscious dogs provided with a Heidenhain pouch. The extract however, did not antagonize gastrin-induced gastric acid hypersecretion in anesthetized male guinea-pigs.

The dose of histamine used induced maximal acid secretion in the guinea-pigs and submaximal acid secretion in the dogs. Gastrin-induced gastric acid hypersecretion was investigated in response to both a maximal and a submaximal stimulatory dose.

It is evident from the results presented that although urine extract inhibited histamine-induced acid hypersecretion in both guinea-pigs and dogs, this inhibition was both quantitatively and qualitatively different. In the guinea-pigs the extract decreased total acid output during peak secretion by an average of 71%. Furthermore, in extract-treated animals the concentration of the acid was also significantly depressed by an average of 56%, calculated similarly for the entire peak secretory period.

In contrast to this total acid output was decreased by 38 and 34% respectively from the pouch of the male and female animal.

Thus % inhibition of total acid output, following treatment with

the urine extract, was less in dogs than in guinea-pigs, although the decrease was highly significant in both species.

However, acid concentration was not decreased following treatment with the extract as compared to treatment with the vehicle. The reason for this difference is not known although similar discrepancies were recorded for example in response to enterogastrone. Kirsner (1948, 1949) noted in human subjects that enterogastrone markedly decreased HCl concentration and only slightly the volume; Linde (1952) showed in Heidenhain-pouch dogs that enterogastrone in response to histamine inhibited the volume output and consequently acid output, while acid concentration remained unchanged.

According to several workers, however, inhibition of histamine-induced acid hypersecretion by urogastrone in dogs (Gregory, 1955), by atropine in either humans (Polland, 1930; Atkinson and Ivy, 1938; Kirsner and Palmer, 1940) or dogs (Atkinson and Ivy, 1938) was usually confined to a decrease of volume and acid output, while acid concentration remained unchanged.

It should be taken into consideration that several differences existed in the experimental conditions, under which investigations were carried out in guinea-pigs and dogs, besides the species difference:

(i) the dose of extract/kg was lower in dogs;

(ii) guinea-pigs were kept under light anesthesia, the dogs were conscious;

(iii) the guinea-pigs received the extract intraperitoneally, the dogs orally, and

(iv) the dose of histamine induced a maximal secretory response in guinea-pigs and a submaximal secretory response in dogs.

This latter difference, however, would mean a disadvantage for the guinea-pigs. Gregory (1955) noted that in general a greater degree of inhibition is observed following submaximal secretory rates.

Due to the fact that there is a considerable lack of knowledge concerning the intimate details of the secretory process in the cells of the gastric mucosa it is not possible to draw conclusions concerning the mechanism of action of the extract.

It was shown by Gray et al. (1939); Friedman et al. (1939) and Necheles et al. (1939) that human and animal urines contain a substance, called urogastrone, which inhibits gastric acid secretion. However, the active principle present in our extracts and urogastrone are most probably two different substances based upon comparisons as follows: urogastrone is a water soluble substance (Gray et al. 1942) which can be precipitated by ether (Gregory, 1955) or by 85% ethanol (Mongar and Rosenoer, 1962). Urogastrone is a polypeptide, digested by pepsin and chymotrypsin (Rosenoer, 1962) and is consequently not active on oral administration. It causes no changes in body temperature of rabbits. In contrast to these properties the active principle present in the extract is soluble in organic solvents, insoluble in water; is active after oral administration (dogs, rats) and causes a profound hypothermia as shown in conscious rats.

When acid secretion was stimulated by gastrin, 1.5 g/kg of extract given intraperitoneally was unable to inhibit hypersecretion in lightly anesthetized male guinea-pigs.

Treated animals secreted slightly, but insignificantly less acid in response to a dose of gastrin which induced submaximal secretion. In

response to a dose, which induced a maximal secretory response, total acid output was even somewhat greater in treated animals as compared to controls. There are several data in the literature which indicate that responses induced by gastrin or histamine, both *in vitro* or *in vivo*, are differently affected by the same pharmacological agent.

Bennett (1965) showed that gastrin-induced contractions of the guinea-pig ileum were unaffected by the antihistamine Mepyramine, while hyoscine either completely abolished or greatly reduced the contractions induced by gastrin.

Gregory and Tracy (1961) showed both in man and dog, and Grossman (1961) in dogs, that while histamine-induced acid secretion was only slightly affected by atropine, gastrin-induced acid secretion was almost completely abolished.

In the experiments presented it was shown that the extract effectively inhibits acid secretion in response to histamine, while it has no effect upon acid secretion in response to gastrin under identical experimental conditions in guinea-pigs.

In summary: it was shown that the urine extract is a potent inhibitor of histamine-induced gastric acid hypersecretion in both guinea-pigs and dogs, provided with a Heidenhain pouch. It is more potent in guinea-pigs in which it significantly decreases the volume, total acid output and acid concentration. The volume and acid output are significantly decreased from the pouches as well, but acid concentration remains unaffected.

Under identical experimental conditions the extract has no effect on gastrin-induced gastric acid hypersecretion in male guinea-pigs.

3. Differences in Gastric Acid Output in Response to Histamine and Gastrin Between Male and Female Guinea-Pigs

Gastric acid secretion in response to graded doses of histamine has been the subject of many investigations in experimental animals, yet the possible significance of the influence of sex has not been studied though it has been reported in man.

The results of our experiments clearly indicate that there is a basic difference in acid secretory capability between male and female guinea-pigs under conditions of maximal histamine stimulation.

The results show a remarkable similarity to the pattern of acid secretion observed in man.

Clinical studies performed by Vanzant et al. (1932, 1933) showed that after a test meal the values obtained for both volume and free and total acidity were higher in men than in women. After the introduction of the Kay test, similar experiments were carried out under conditions of maximal histamine stimulation (Baron, 1963; Vakil and Mulekar, 1965) confirming the original observation.

Investigations carried out in man and dog showed a direct correlation between the parietal cell mass and maximal secretory response (Card and Marks, 1960; Marks et al. 1958; Marks et al. 1960). It was also shown that the number of parietal cells was greater in males than in females (Cox, 1952).

It seems likely, therefore, that a similar difference might also be found between male and female guinea-pigs. There is only one objection

to this possible explanation namely, the finding that the administration of 0.2 mg/kg of histamine brought about a definitely greater (though statistically not significant) response of acid secretion in the male than in the female. Since this dose of histamine was below that which induced a maximal secretory response, it is possible that (a) the same stimulus elicits a lesser response in females, or (b) female guinea-pigs are less sensitive to the same dose of histamine.

Differences in acid secretion between male and female guinea-pigs in response to gastrin were investigated only under conditions of maximal stimulation. Although the differences were not statistically significant it was shown that total acid output was less in females and declined earlier towards basal levels. The dose of gastrin which induced a maximal acid secretory response was investigated only in 4 male and 4 female guinea-pigs, and the large variation in the response obtained is probably responsible for the fact that the differences were not statistically significant. However, there was a definite tendency in female guinea-pigs to secrete less acid in response to gastrin, as was seen in response to histamine.

4. The Antiulcerogenic Effect of Urine Extract

In the past 40 years several experimental procedures were devised to produce gastric ulcer in animals. Two of the established methods were used in our experiments: the pylorus-ligation and the restraint-induced gastric ulcer methods, both of which use the rat as experimental subject.

However, the pathogenesis of the ulceration, produced by these methods differ and the protection afforded by the urine extract in both

the restraint and Shay rat was probably due (at least in part) to different pharmacological actions of the substance.

Shay et al. (1945) demonstrated first that an acute ulcerative process takes place in the forestomach of the pylorus-ligated rat. The lesions appear when unbuffered juice is present in the stomach in sufficient amounts and for a sufficient period of time. It is suggested that the rumenal ulcers in the pylorus-ligated rat are merely the result of corrosion of those parts of the stomach which normally are not exposed to strong acid. Sun and Chen (1963) described that certain surgical procedures and a number of drugs afforded protection against ulceration in the Shay rat. These were as follows: bilateral vagotomy; adrenalectomy; hypophysectomy; anticholinergic drugs (atropine sulfate, scopolamine methylbromide, propantheline); urogastrone and enterogastrone, and chlorpromazine. The decreased incidence of ulceration was always accompanied by a decrease in both acid output and volume of gastric juice secreted. These observations suggested that the depression of gastric secretion is the major mechanism by which ulceration is reduced in the pylorus-ligated rat.

The results of our experiments clearly showed that the volume of gastric juice collected over the entire period of ligation and total acid output were greatly and significantly reduced in rats treated with the urine extract either 24 hours prior to ligation or in correspondingly lower doses if given intraduodenally immediately following pylorus ligation. Parallel to the decrease of volume and acid output, ulceration was always significantly reduced.

The long duration of action was a striking feature of the anti-

ulcerogenic action of urine extract. Administered in sufficient amounts the effect of a single oral dose could exert an inhibitory effect for at least 30-40 hours.

Thus the results of the experiments indicate that the protection against ulcer formation afforded by the urine extract in the Shay rat was most probably due to the suppression of acid secretion.

Bonfils and Lambling (1963) have extensively studied the factors which contribute to the induction of restraint-induced experimental ulcers.

According to Bonfils and Lambling (1963), acute vascular lesions of the gastric mucosa observed half an hour after the application of restraint, are of primary importance.

Furthermore the presence of excess acid in the stomach and a "central nervous system component" are also involved in restraint-induced gastric ulceration (Brodie et al. 1963).

Brodie et al. (1963) remarked that because the lesions do not penetrate into the serosa and do not produce scarring on healing they are not true gastric "ulcers" as the term is applied in clinical gastrointestinal pathology. "Erosion" would be a better term but the word "ulcer" is firmly established in stress literature.

The finding that anticholinergic drugs significantly reduced the incidence of ulceration (Hanson and Brodie, 1960; Brodie et al. 1963) showed the importance of acid in the stomach and pointed to the fact that it played a role in the pathogenesis of the ulcer. Central nervous system depressants also significantly decreased the incidence of ulceration.

Pentobarbital, benactyzine and chlorpromazine were investigated by Hanson and Brodie (1960) and chlorpromazine was the most potent among these drugs in inhibiting ulceration. The results of our experiments performed in female albino rats showed that the incidence of ulceration following a 24 hour immobilization period led to 66 and 80% ulcer formation respectively in the 2 control groups. This was in good agreement with the ulcer incidence of 86% as reported by Bonfils and his group. The urine extract, as was shown in the course of other experiments, significantly decreased gastric acid secretion and exerted a strong depressant effect on the central nervous system.

Based on these observations, it is suggested that the protection afforded by the urine extract against restraint-induced gastric ulceration was most probably due both to its ability to decrease gastric acid secretion and to its depressant effect upon the central nervous system.

5. Effect of Urine Extract Upon the Central Nervous System

The experiments performed with the urine extract can be divided into 2 groups:

(1) determination of the action of urine extract upon the central nervous system and the intensity of these actions;

(2) possible antagonism of these actions by centrally acting drugs.

In general, all the actions of the extract were "depressant". It induced hypnosis, potentiated the hypnotic effect of hexobarbital sodium, decreased the body temperature and greatly suppressed spontaneous motility.

Due to the observation that the extract induces hypnosis within

a relatively small dose range it seems reasonable to assume that this is a specific pharmacological action.

The effects of the urine extract on the central nervous system are well comparable to those of chlorpromazine but are unlike those of reserpine. This comparison is based upon data from Lessin and Parkes (1957) who described and compared the effects of chlorpromazine and reserpine upon sedation and body temperature in the mouse.

They found that chlorpromazine caused a fall in body temperature and peak hypothermia was reached between 1½ - 3 hours after the administration of the drug. It greatly decreased spontaneous activity and potentiated pentobarbital sleeping time both at room temperature and at an elevated ambient temperature. However, chlorpromazine had no sedative or hypothermic actions at 32°C.

Like chlorpromazine, the extract potentiated hexobarbital sleeping time at both room temperature and elevated environmental temperature. The maximal fall in body temperature was also observed between 1 and 3 hours after the administration of the extract.

Unlike chlorpromazine the extract caused a loss of righting reflex in 40% of the rats given at the hypnotic ED 50 dose at an elevated ambient temperature.

The hypothermic effect of the extract was extremely marked. Even a small dose caused a significant drop in rectal temperatures while larger doses caused a dramatic fall of often more than 8°C.

At elevated environmental temperature the hypothermic effect of the hypnotic ED 50 dose was significant 1 hour following its administration,

while during the subsequent hours the temperature rose to even higher levels than observed in the vehicle-pretreated group. Although the drop in body temperature, 1 hour after the administration of the extract, was statistically significant it should be emphasized that the hypnotic ED 50 dose caused a drop in body temperature of about $8^{o}C$ at room temperature and $1.2^{o}C$ at the elevated ambient t^{o}.

Although separate extracts were used for each experiment throughout this study, the repeatability of experimental data was extremely good. For example the fall in rectal temperature, following administration of the hypnotic ED 50 dose was always around $8^{o}C$ and the peak of the temperature decreasing action occurred at about 2 hours.

The hypothermic effect of the extract was more pronounced than its hypnotic effect. Less than $1/5^{th}$ of the hypnotic ED 50 dose induced a significant drop in body temperature.

In none of the experiments performed did the rats exhibit shivering with rectal temperatures as low as $29^{o}C$. It is known that anesthesia abolishes shivering, however shivering was absent in all animals, regardless whether they lost their righting reflex or were conscious.

Davis (1959) suggested on the basis of experiments performed in mice that in the truly hypothermic homeotherm shivering is stimulated only on the basis of core temperature. (Mice with rectal temperatures below $34^{o}C$ were designated as "truly hypothermic").

Keller (1959) demonstrated the absolute dependence of shivering upon the integrity of the caudal hypothalamus.

It is possible therefore that the extract induced the profound hypothermia through its depressant effect on the caudal hypothalamus where the heat conserving mechanism is located. Efferent nerve fibers, responsible for shivering are also known to enter this area.

It is more probable that the heat conserving mechanism of the caudal hypothalamus was inhibited than to suggest stimulation of the rostral hypothalamus, which promotes heat loss.

This suggestion is based upon the observation that at elevated ambient temperature extract-treated rats manifested higher rectal temperatures than did controls and at the end of the 24 hour period 60% of the rats died, while none of the control animals were dead. This showed that extract-treated animals were unable to handle a heat-load. However, if the extract stimulated the heat loss center (rostral hypothalamus) the foregoing observation could probably not have occurred.

In further experiments it was investigated if centrally acting drugs were able to antagonize extract-induced hypnosis and hypothermia. The experiments established that pretreatment with graded doses of drugs, classified as brain stem stimulants, either entirely prevented hypnosis or reduced the frequency of loss of righting reflex, which normally followed the administration of the extract.

Picrotoxin, pentylenetetrazol and nikethamide were used in these experiments.

Most data in the literature revealed that nikethamide is a much weaker antagonist than either picrotoxin or pentylenetetrazol. The latter two drugs were described by some workers as almost equipotent (Chakravarti,

.1939); other investigators found picrotoxin more potent (Barlow, 1935; Werner and Tatum, 1939).

In our experiments pentylenetetrazol was the most potent in antagonizing extract-induced hypnosis. Pentylenetetrazol was administered at 4 different dose levels and, except for the smallest dose, it entirely prevented the loss of righting reflex in rats following administration of the extract.

Increasing doses of picrotoxin and nikethamide could partially antagonize hypnosis but in the dose range used neither drug entirely prevented it.

Pentylenetetrazol and picrotoxin had no effect on extract-induced hypothermia.

These drugs are called "cooling drugs" and in subconvulsive doses they lower body temperature of normal animals. However, if they are concomittantly administered with barbiturates they prevent a fall in body temperature (Hahn, 1943).

Rosenthal (1941) suggested that this effect is due to a true pharmacological antagonism; picrotoxin on the one hand and barbiturates on the other prevent each others action. On the other hand the hypothermic action of other drugs than barbiturates will be potentiated by picrotoxin (Rosenthal, 1941), because probably both stimulate the same (cooling) center.

Due to the fact that picrotoxin and pentylenetetrazol neither potentiated nor antagonized extract-induced hypothermia, no conclusion could be reached from these experiments for the site of hypothermic action of the extract.

Hahn (1943) observed that hypothermia induced by several drugs

could be potentiated by nikethamide. Nikethamide also potentiated significantly the extract-induced hypothermia. However, nikethamide itself caused a significant drop in body temperature in normal rats 2 hours after its administration. This finding was in contrast to the general observation that nikethamide causes hyperthermia in subconvulsive doses.

Amphetamine was the only drug which was tested under 2 different experimental conditions. It was investigated (a) whether amphetamine was able to prevent loss of righting reflex and hypothermia, (b) whether it antagonized established hypothermia.

Although according to the literature amphetamine antagonizes the hypnotic effect of nearly all anesthetic agents (Hjort, 1938; Trevan, 1938/39; Chakravarti, 1939, and Reifenstein, 1941) its antagonism against the urine extract was not marked. The number of rats which lost their righting reflex, was decreased by the administration of 7.5 mg/kg of amphetamine however, increasing doses of amphetamine even potentiated the effect of the extract.

Amphetamine potently antagonized extract-induced hypothermia under both experimental conditions. Higher doses were more potent and the highest dose investigated even caused hyperthermia.

The effect of iproniazid and iproniazid +α-MMT pretreatment was also investigated on extract-induced hypnosis and hypothermia. The time of injection of iproniazid and α-MMT were based upon observations in the literature. Spector et al. (1958, 1960) found that brain norepinephrine concentration reached a plateau by the 3rd day following treatment with iproniazid. Plummer and Furness (1963) noted that maximal changes in activity

and behavior of an animal occurred following pretreatment of iproniazid for 3 days. Costa et al. (1961) noted that 4 hours after the injection of α-MMT more than 80% of brain norepinephrine was released. Such animals manifested hyperactivity and hyperthermia.

The behavior of the rats following pretreatment with both iproniazid + α-MMT, and also with amphetamine was very similar to that described by Costa et al. (1961). However, neither iproniazid alone, nor iproniazid + α-MMT could prevent the loss of righting reflex in rats, but rather potentiated the effect of the extract.

It is known that MAO inhibitors potentiate anesthetic agents (Giarman, 1965) by an inhibition of the oxidative enzyme system in liver microsomes and the potentiating effect observed in our experiments was possibly due to this action of iproniazid. While iproniazid + α-MMT pretreatment prevented hypothermia, iproniazid pretreatment alone potentiated it, although not significantly.

When animals were placed into motility cages from groups which received (i) amphetamine, (ii) iproniazid + α-MMT and (iii) iproniazid prior to the extract, a tremendous hypermotility was noted in all 3 groups. However, while the hypothermia was significantly antagonized by (i) amphetamine and (ii) iproniazid + α-MMT it was potentiated by iproniazid pretreatment alone. This observation largely excluded the possibility that the increase in body temperature was due to heat production arising from an increase in muscular activity. Work of Stein (1964) and Hanson (1966) suggested that the central actions of amphetamine were mediated via catecholamine release; α-MMT is known to cause a long-lasting depletion of brain norepinephrine.

Catecholamines, particularly norepinephrine, are known to induce an increased mobilization of metabolic substrates, this is manifested in both an increase of blood glucose and an increase of plasma levels of free fatty acids (Maickel et al. 1963; Smith, 1963).

It is suggested that amphetamine and iproniazid + α-MMT both antagonized extract-induced hypothermia by releasing catecholamines which induced an increased metabolism with consequent heat production. Unfortunately no biochemical experiments were performed which could support this suggestion.

In summary, it is suggested that urine extract-induced hypnosis and hypothermia are of central origin, and are 2 separate effects not produced as a result of one another. No direct experiments were performed to ascertain the central origin of the effects, however, the observation that both actions were antagonized by centrally acting drugs is a good indirect support of this hypothesis.

SUMMARY

Experiments were performed with partially purified extracts, prepared from pregnant mare urine to determine (1) whether the extract is capable of antagonizing histamine and gastrin-induced gastric acid hypersecretion, (2) whether the extract is capable of antagonizing experimentally-induced gastric ulceration, (3) whether the extract has any effect upon the cardiovascular system and (4) after establishing that the extract exerts definite actions upon the central nervous system, whether these actions can be antagonized.

The results may be summarized as follows:

1. The efficacy of the extracts, which are soluble in organic solvents, was routinely tested and standardized on the isolated guinea-pig ileum preparation by determining their antihistaminic activity.

2. The extract given intraperitoneally 1 hour prior to histamine potently inhibited histamine-induced gastric acid hypersecretion in anesthetized male guinea-pigs. Total acid output, the volume of gastric juice secreted and acid concentration were significantly decreased.

3. The extract, given orally 2 hours prior to histamine, potently inhibited histamine-induced gastric acid hypersecretion in conscious dogs, provided with a Heidenhain pouch. Total acid output and the volume of gastric juice secreted were significantly decreased, however, the acid concentration remained essentially unchanged.

4. The extract administered intraperitoneally 1 hour prior to gastrin did not antagonize gastrin-induced gastric acid hypersecretion in anesthetized male guinea-pigs.

5. The extract prevented or strongly reduced ulcer formation in the Shay rat when given either orally 6 or 24 hours prior to, or intraduodenally immediately following pylorus ligation.

6. The extract potently antagonized gastric secretion in the Shay rat when given either orally 24 hours prior to, or intraduodenally immediately following pylorus ligation. Both volume and total acid output were significantly decreased.

7. The i.p. or i.m. administration of the extract into rats reduced ulcer development induced by the restraint (stress) method.

8. The extract given intraperitoneally did not induce significant changes in the mean arterial blood pressure and heart rate of anesthetized male rats and guinea-pigs.

9. The extract administered intraperitoneally into rats induced hypnosis. The hypnotic ED 50 dose was determined by a graphical method.

10. The hypnotic ED 50 dose of the extract induced hypnosis at an elevated ambient temperature as well.

11. The extract induced hypothermia. Doses which induced hypnosis caused a profound fall of rectal temperatures. Even a small dose of the extract, about $1/5^{th}$ of the hypnotic ED 50 dose, significantly decreased rectal temperatures.

12. Peak hypothermia was recorded either 1 or 2 hours following the administration of the extract. Rectal temperatures returned towards normal 24 hours after the administration of the extract but did not reach pretreatment values.

13. The hypnotic effect of hexobarbital sodium was significantly potentiated by the extract.

14. The urine extract significantly potentiated the hypnotic effect of hexobarbital sodium at an elevated ambient temperature as well.

15. Graded doses of pentylenetetrazol, administered orally 30 minutes prior to the extract (given intraperitoneally in the hypnotic ED 50 dose), decreased or prevented hypnosis.

16. Graded doses of picrotoxin, administered orally 30 minutes prior to the extract (given intraperitoneally in the hypnotic ED 50 dose), decreased hypnosis but did not prevent it.

17. Graded doses of nikethamide, administered orally 30 minutes prior to the extract (given intraperitoneally in the hypnotic ED 50 dose), decreased hypnosis but did not prevent it.

18. Pentylenetetrazol and picrotoxin, in the dose range used, neither prevented nor potentiated extract-induced hypothermia.

19. Nikethamide, in the dose range used, significiantly potentiated extract-induced hypothermia.

20. Graded doses of amphetamine, administered orally 30 minutes prior to the extract (given intraperitoneally in the hypnotic ED 50 dose), exerted a variable effect on hypnosis. A medium dose decreased, whereas higher doses rather potentiated the effect of the extract.

21. Amphetamine pretreatment significantly decreased the degree of extract-induced hypothermia in the dose range used. Amphetamine given orally 1 hour after the administration of the extract potently reversed established hypothermia.

22. Iproniazid, given subcutaneously for 3 consecutive days, potentiated both the hypnotic and the hypothermic effects of the extract.

23. Iproniazid, given subcutaneously for 3 consecutive days, + α-methyl-meta-tyrosine, given intraperitoneally 4 hours prior to the extract (given intraperitoneally in the hypnotic ED 50 dose), potentiated hypnosis but antagonized extract-induced hypothermia.

24. Rats pretreated with amphetamine, iproniazid and iproniazid + α-methyl-meta-tyrosine exhibited hyperactivity 2 and 3 hours after the administration of the urine extract. Rats treated with the extract alone exhibited, during the same period of time, a complete lack of spontaneous motility.

Results are also presented on experiments in which the secretory responses of guinea-pigs were investigated to histamine and gastrin. It was found that:

25. Under conditions of maximal histamine stimulation the secretory response (volume, total acid output) of male guinea-pigs was significantly higher than that of females.

26. Under conditions of maximal gastrin stimulation the secretory response (volume, total acid output) of male guinea-pigs was slightly but not significantly higher than that of females.

27. Gastrin was not found to be a potent stimulant of gastric acid secretion in male and female guinea-pigs. The doses of gastrin which stimulated acid secretion in guinea-pigs were about 100 times larger than doses which were found to be effective in humans and dogs.

On the basis of these observations it is suggested that

(a) urine extracts contain a highly effective substance(s) which is capable to exert a wide range of pharmacological actions.

(b) The substance(s) is soluble in organic solvents and insoluble in water. It is effective when orally administered which seems to indicate that the active principle is not a polypeptide.

(c) The observation that the extract effectively antagonizes histamine-induced gastric acid hypersecretion in several species, while it is ineffective against gastrin-induced gastric acid hypersecretion supports the assumption that the antagonism of hypersecretion is due to one substance and not to the combined effect of various constituents of the urine extract.

(d) Based on the observation that centrally-acting drugs effectively antagonized either the hypnosis or the hypothermia, which the administration of the hypnotic ED 50 dose of the urine extract induced, it is suggested that (i) both hypnosis and hypothermia are of central origin and (ii) the induction of both hypnosis and hypothermia are primary actions of the urine extract and are not brought about as a consequence of each other.

(e) It was established that under conditions of maximal histamine stimulation the secretory response of male guinea-pigs was significantly higher than that of females.

(f) It was established that the guinea-pigs are extremely insensitive to the action of gastrin, the antral hormone, and that the doses which do induce an increase in acid secretion are about 100 times larger,

than doses reported in the literature for other species.

(g) It was suggested that gastrin does not stimulate gastric acid secretion in the guinea-pigs via histamine.

CLAIMS OF ORIGINAL WORK

An antihistamine-like activity of urine extracts, obtained from mammalian sources, has been previously established.

The work presented in this thesis on the pharmacological effects of purified urine extract, extended the research into areas which had not been investigated previously. It was established that the urine extract:

(1) exerts a strong antisecretory activity in several animal species (dog, guinea-pig, rat);

(2) exerts a strong antiulcerogenic activity in rats;

(3) does not significantly alter cardiovascular functions, and

(4) exerts a depressant effect upon the central nervous system.

It was established that the "maximum" response to histamine of gastric secretion is significantly greater in the male than in the female guinea-pig.

It was established that the guinea-pig is highly insensitive to the gastric acid stimulant action of gastrin. The doses capable of inducing increased acid secretion were found to be about 100 times larger than those reported in the literature to induce similar increase in other species.

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