GASTRIC SECRETION

AND

THE ADRENAL CORTEX

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I N T R O D U C T I O N

The role of secretions from the adrenal cortex and other endocrine glands in the regulation of gastric secretory activity is a field of enquiry which has attracted increasing attention in recent years both from clinicians and physiologists.

These studies have been greatly stimulated by the recognition that digestive disturbances - in particular peptic ulceration - not infrequently attend the therapeutic administration to patients of corticotrophin and adrenocortical steroids (Hall, 1953; Henderson, 1955; Ridley, 1962).

A permissive action for adrenocortical hormones on acid and pepsin secretion by the stomach has been suggested by Gray and his colleagues in Boston (Gray et al., 1951). Their further hypothesis that stress

mediated through the hypothalamic-pituitary-adrenal axis increases gastric secretion and results in subsequent peptic ulceration has not, however, been generally accepted (Kirsner, 1953; Meltzer et al., 1958; Bachrach, 1963; Zukoski et al., 1963). The pharmacological actions of steroids on the stomach also have been the subject of many reports but the findings are confusing (Clarke et al., 1960; Wiederanders et al., 1960; Cooper et al., 1961; Kirsner, 1964).

In this thesis the evidence which suggests links between the adrenal cortex, the physiological regulation of gastric secretion and the pathogenesis of peptic ulceration is evaluated. Thereafter, the results of animal experiments designed to elucidate the nature of the inter-relationship between the glucocorticoids of the adrenal cortex and gastric secretion are presented. These experiments involved the study of the effect of a pharmacological inhibitor of adrenocortical activity on the secretion of gastric juice in dogs.

A C K N O W L E D G E M E N T S

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REVIEW

OF THE

LITERATURE

NORMAL MECHANISM OF GASTRIC SECRETION

The gastric glands secrete constantly, at a low rate, fluid poor in acid and pepsin content but rich in mucus (James, 1957). The sight, smell or taste of food stimulates the vagus through conditioned and unconditioned reflexes and produces a flow of gastric juice rich in both acid and pepsin. Following this nervous phase the entry of food into the stomach initiates a gastric phase of secretion in which there is released from the antral mucosa the hormone gastrin which causes the secretion of a highly acid juice but little pepsin. Vagal impulses facilitate the release of gastrin (Woodward and Nyhus, 1960), and distention of the body of the stomach has been shown by Grossman (1962) also to cause gastrin release via local vagal reflex pathways. As far as acid secretion is concerned, the nervous and gastrin systems appear to act synergistically on the parietal cells (Nyhus et al., 1963). The hypothesis, first advanced by Babkin (1938), that histamine forms the final common pathways for both avenues of excitation is not now generally accepted (Lin et al., 1962).

The third or intestinal phase of gastric secretion which occurs when food reaching the duodenum provokes the release of a gastrin-like agent as well as the inhibitor enterogastrone is relatively of much less importance than the preceding two phases (Gregory, 1962).

Although it is 140 years since William Prout discovered that the acidity of the stomach was due to hydrochloric acid, the method of formation and secretion of gastric acid is still undecided. Acid is formed in the mucosa of the body of the stomach. Studies on the rabbit foetus, which showed that the secretion of HCl began on the twenty-third day with the first appearance of parietal cells (Menzies, 1958), indicated that it originated from these cells. Experiments on isolated cat mucosa, utilizing the ability of parietal cells to secrete dyes which are also indicators of pH (Bradford and Davies, 1960), have revealed that the contents of the intracellular canaliculi of actively secreting parietal cells are more acid than pH 1.4. The maximum acidity of gastric secretion found by numerous observers is 154 mN per litre in man and 160 mN per litre in the dog (Table I) which represents a hydrogen ion concentration 10^6 times

that of the blood. The energy required - 9,650 calories per gramme of acid formed (Gray, 1948) - is probably derived from the breakdown of high energy phosphate bonds during the aerobic metabolism of glucose. Intracellular cytochrome systems are involved in the transfer of this energy and probably play a part also in the transport of hydrogen ions, formed from dissociation of water, to the gastric lumen (Davies and Ogston, 1950). It seems likely that minimum concentrations of potassium, and possibly other cations, are essential for the efficient running of this "hydrogen ion pump" (Hogben, 1960).

More detailed consideration of the actual intracellular mechanisms involved in gastric acid production form the subject of reviews by Davies (1951), Conway (1953), Heinz and Öbrink (1954), Rehm (1959) and Hogben (1960).

Theoretically, gastric acid secretion must depend on the number of secreting units multiplied by the rate of secretion per unit (Card, 1952). When the gastric glands are stimulated to secrete at the maximum possible rate by injection of the appropriate body-weight dose of histamine, a highly significant correlation is found to

exist between the output of acid and the total number of parietal cells in the stomach mucosa (Card and Marks, 1960; Marks, Komarov and Shay, 1960).

TABLE I : MAXIMUM CONCENTRATION OF GASTRIC ACID

1. MAN

Author	Concentration (mN)
Hanson, Grossman, Ivy (1948)	140
Nordgren (1958)	143
Hirschowitz (1961)	150
Ihre (1939)	152
Wolin and Frisk (1936)	154
2. DOG	
Hanson, Grossman, Ivy (1948)	155
Marks, Komarov, Shay (1958)	158
Thull and Rehm (1956)	160
Marks, Komarov, Shay (1960)	160
Obrink (1948)	161

In addition to hydrochloric acid, gastric juice also contains pepsin, mucin, the intrinsic haemopoetic factor and various salts. Pepsin is secreted as its inactive precursor pepsinogen by the chief cells in response to vagal stimulation. Its optimal digestive activity occurs at a pH of 2.3 and it is inactive in an environment more alkaline than pH 5 (Hollander, 1949). However, gastric juice retains significant digestive capacity at pH values too high to be the result of pepsin activity alone. There is now evidence that gastric juice contains several acid proteases which are optimally active at different pHs (Hollander, 1962).

The secretions of all gastric cells other than the parietal cells are either neutral or alkaline so that the wide range of pH occurring in different specimens of gastric juice has to be explained. The most reasonable explanation, first advanced by Pavlov (1910), is that hydrochloric acid is secreted by the stomach at a constant concentration but widely varying rate and is subsequently partially neutralised by the other constituents of gastric juice. This hypothesis was developed by Hollander (1931) into his two-component theory of gastric secretion which states:

1. Under normal osmotic and acid-base conditions, the concentration of acid in the acid component is 155 ± 10 m.Eq./litre. This value is independent of the mode of stimulation and of the secretory rate but is precisely dependent on the osmotic activity of the mucosal interstitial fluid.

2. The alkaline component, formed of all non-parietal secretions, is presumed to be a dispersion of mucin and other organic substances in a menstrum with an inorganic composition similar to that of extracellular fluid, i.e. its pH is above 7 and it contains the buffer ions bicarbonate, phosphate and proteinate, as well as chloride and all common cations.

3. The reaction of mixed acid juice is then the resultant of the dilution and neutralisation which occurs in admixture of the acid and alkaline components and all concentration values must fall between the limit set by these two extremes (Hollander, 1958).

An alternative hypothesis proposed by Teorell (1947) that, after acid has been secreted, hydrogen ions can diffuse back into the blood and be replaced by metallic cations, predominantly sodium, has some experimental support (Linde et al., 1947).

More recently Hirschowitz (1961) has suggested that the chief cells in the base of the gastric tubule secrete sodium and potassium ions while the parietal cells in the neck of the tubule modify this primary secretion only to the extent of adding hydrogen ions in exchange for sodium.

An inverse relationship between sodium and acid concentrations at very high rates of acid production are well documented (Werther et al., 1960), but studies on the chloride and potassium content of gastric juice under different types of stimulation and at different rates of secretion show conflicting results.

The early view by Roseman (1907) that the chloride content of gastric juice remained constant at all rates of secretion has been disproved by the experiments of Hollander and Covgill (1931) and numerous other investigators. Since chloride is for practical purposes the only anion present in gastric juice (Hirschowitz,

1961), it is probable that it is secreted in both parietal and non-parietal components in order to maintain ionic equilibrium. Due to the difficulties in separating the different glandular elements of the stomach, even *in vitro*, it is impossible to measure directly the electrolyte concentrations of secretions coming from the different types of cells.

Data published by Ihre (1939) on the volume and composition of gastric secretion stimulated by histamine and insulin in normal subjects and patients with peptic ulcer were analysed by Fisher and Hunt (1950). The results of this mathematical approach to the problem are combined in Table II with later work by Hunt (1950, 1950) to indicate the chief constituents and their concentrations in parietal and non-parietal components of human gastric juice.

TABLE II : COMPOSITION OF HUMAN GASTRIC JUICE

	Parietal	Non-Parietal
Chloride	170 mN	125 mN
Hydrochloric Acid	160 mN	-
Bicarbonate	-	-
Potassium	10 mN	10 mN
Other Alkaline Metals	-	160 mN

The most reliable data on gastric secretion have been obtained from pouches of the gastric mucosa formed in dogs or occasionally in cats. Hunt (1950) considers that data for dogs, cats and man can quite legitimately be synthesised into a single hypothesis, provided some allowance is made for the slightly higher total concentration of ions in the gastric secretion of cats (Gudiksen, 1950), but no simple quantitative hypothesis accounts for all the observed variations in the concentrations of ions encountered in gastric juice, even where experimental error has been reduced to the minimum.

The constancy of potassium concentration in gastric juice (Austin and Common, 1931; Gray and Bucher, 1941; Lindo and Obrink, 1950), in spite of variations in secretory rate, has been questioned by Martin (1950) and Bernstein (1952). The latter authors have shown that the potassium concentration in the gastric contents increases with a rise in acidity but Werther et al.,(1960), could not elicit any satisfactory temporal relationship between potassium and hydrogen ion concentration following histamine stimulation in man. The concentration of potassium in gastric juice appears to bear far more

relation to the type of stimulant or inhibitor used rather than to the concentration or rate of secretion of acid (Colcher and Hollander, 1950; Hirschowitz, 1961; Milton, et al., 1963). Since a reduction in the concentration of potassium in the blood leads to reduced secretion of acid in dogs (De Muro et al., 1961), it appears that a minimum blood level of potassium is required for gastric parietal cells to function at optimum efficiency. This however in no way explains the presence of potassium within the gastric lumen. As the concentration of potassium in the gastric juice is always higher than the plasma level, it must be actively secreted into the stomach. In fact, studies with ^{42}K confirm that potassium in gastric juice does originate partly from the intracellular space in the gastric mucosa (Nordgren, 1963) but does not, of course, indicate the particular type of cell of origin.

The consensus of opinion is that potassium forms part of both the parietal and non-parietal components of gastric juice.

Mucus, by virtue of its physical properties, its viscosity, adhesiveness and cohesiveness, is responsible for most of the stomach's resistance to injurious agents (Hollander, 1954). It is formed by three types of cell:

1. Columnar
2. Neck chief or mucoid
3. Pyloric gland cell

and contains a large number of different biologically active fractions (Glass, 1963), consideration of which is beyond the scope of this review. However, the various dializable substances present in gastric mucus influence the chemical composition of the contents of the stomach by means of their acid-binding properties (Mitchell, 1931; James, 1957) and electrolyte content.

The actual buffering capacity of samples of mucus varies within wide limits (Hollander, 1962) depending on its purity and the stimulus used to provoke secretion, but its electrolyte content appears to be more constant. Gastric mucus in dogs contains the same electrolytes as are present in plasma and, as far as sodium, potassium and chloride are concerned, in essentially the same concentrations (Hollander, 1963).

THE RELATIONSHIP OF ADRENAL CORTEX TO GASTRIC SECRETION AND PEPTIC ULCER

(a) Adrenocortical Insufficiency

In the original description of the syndrome which bears his name, Thomas Addison (1855) noted " ... sickness, vomiting and pain in the stomach - symptoms which have constituted a more or less prominent feature in every case that has fallen under my care".

Such symptoms occurred in 88 per cent of the 160 cases of chronic suprarenal insufficiency reviewed by Maranon et al. (1934) while of 94 patients with Addison's disease reported by Thorn (1951), no fewer than 84 complained of vomiting and 32 of abdominal pain. Gastrointestinal symptoms were stated to be common in the 108 cases considered by Rowntree and Snell (1931) in their monograph, but varied a good deal in severity.

Further reviews on the subject include papers by Sorkin (1949) and Kirsner (1953) but no satisfactory explanation for the digestive disturbances in Addison's disease emerges.

Originally the abdominal symptoms were ascribed by Addison, on evidence at autopsy, to acute ulceration of

the stomach. Although it is true that patients in states of adrenal crisis may develop acute gastric ulcers (which heal promptly when adrenal function is restored to normal by appropriate treatment), these are not present in all patients with Addison's disease (Soffer, 1956). Thus Turner (1951) found gastroduodenal ulceration post mortem in only 4 out of 29 patients who had died from adrenal insufficiency. These ulcers are of the acute type seen in the terminal phases of many wasting diseases. It is unlikely that they are responsible for the protracted digestive disturbances which often are among the earlier symptoms of adrenocortical insufficiency in man.

In experimental animals too, adrenocortical insufficiency resulting from bilateral adrenalectomy has been followed in some series by the appearance of acute gastric ulceration in nearly 100 per cent of cases (Elliott, 1915; Mann, 1916a; Banting and Gairns, 1926). Normally, spontaneous peptic ulceration is very uncommon in animals and Mann (1916b) did not discover one peptic ulcer in autopsies on more than 200 practically normal dogs and cats. While superficial erosions of the gastric mucosa were found by Ivy (1920) in 31 out of 900 dogs

anaesthetised with ether for between two and three hours and subjected to a variety of surgical procedures, actual acute ulceration of the stomach occurred in only one case - an old debilitated animal. Even in diseased dogs, peptic ulceration is very rare (Ivy, 1920).

While the shock and trauma of operation may have been factors in the development of peptic ulceration in animals succumbing within a few hours of bilateral adrenalectomy, prolongation of survival for longer periods of up to 15 days by the administration of extracts of fresh bovine adrenal cortex did not prevent this complication. Thus Rogoff and Stewart (1928-29) found that 48 out of 118 dogs so treated developed gastric or duodenal ulcers which occasionally perforated.

Absence of the adrenal glands therefore appears to predispose to acute ulceration of the stomach and duodenum, but chronic peptic ulceration is uncommon in adrenocortical insufficiency. In a series of 363 cases of Addison's disease collected from the literature, Gray et al. (1956) found clinical evidence of chronic peptic ulceration in only 3 patients. This is certainly less than the generally accepted incidence of peptic ulcer in the adult population

of between 5 per cent and 10 per cent (Ivy et al., 1950; Doll and Jones, 1951).

Three of the 160 cases of Addison's disease revieved by Marenon et al. (1934) had peptic ulcer diagnosed during life on clinical grounds. None were found post mortem in 25 patients. Four of the 180 cases reported from the Mayo Clinic by Rowntree and Snell (1931) had ulceration of the stomach or duodenum shown radiologically and there were strong grounds clinically for suspecting an ulcer in a fifth case who had a negative series of radiological investigations. While such figures as are available do not permit a proper statistical appreciation of the exact incidence of chronic peptic ulceration in adrenocortical insufficiency, most revievers consider it to be fairly rare (Sorkin, 1949; Kirsner, 1953; Soffer, 1956).

Investigations in cases of adrenocortical insufficiency have not always included analysis of the gastric contents although as far back as 1907 von Gravitz noted a weak acid response to a test meal in an adult male with Addison's disease. The patient later developed achlorhydria. The marked symptomatic and objective improvement in this and in another similar case, which

followed gastric lavage with saline and the addition of hydrochloric acid to their diet, was taken by von Gravitz as confirmation of the role of reduced gastric acid secretion in causing the gastrointestinal disturbances of Addison's disease. It is of course more likely that the saline corrected the patients' electrolyte deficiency. We must however consider further the changes which occur in gastric secretion in adrenocortical insufficiency.

Fractional test meals were carried out in 4 only of 29 cases of Addison's disease described by Conybeare and Millis (1924) but all 4 showed hypochlorhydria. Reduced secretion of acid in response to a test meal of gruel was described by Rowntree and Snell (1931), who found that 36 of 38 patients tested had hypochlorhydria and of these 20 produced no free acid. Thirteen in the series of 160 cases reported by Maranon et al. (1934) had their gastric contents tested after an Ewald-Boas breakfast. Three of the 13 had complete achlorhydria, while a further 4 had a reduced secretion of acid. Soffer (1936) reports the finding of achlorhydria in 50 per cent of his cases of Addison's disease and a similar figure is given by Sorkin (1949), although it is not clear how many patients were tested.

By modern standards the type of test meal used in these earlier studies was not a satisfactory index of gastric acid secretion. However, using the augmented histamine test of Kay (1953), Smith et al. (1961) confirmed these impressions by the demonstration that 5 of 14 patients with adrenocortical insufficiency did not secrete any free acid after the subcutaneous injection of 0.04 mg. histamine acid phosphate per kg. body weight. In the remainder, the response was only 25 per cent of normal.

The atrophy of the adrenals which follows reduced pituitary activity has been held responsible for the high incidence of hypochlorhydria in Simmond's disease (Kyle, 1956; Gray, 1964). In a review of the 595 cases of Simmond's disease reported in the literature up to that time, Escamilla and Lisser (1942) found hypochlorhydria recorded

1. In 17 of 20 patients tested in a group of 101 typical cases in which verification of the diagnosis was available at autopsy;
2. In 21 of 38 patients tested out of 158 typical cases without pathological confirmation;

3. In 19 of 33 patients tested in a group of 180 cases with features suggestive, but not quite typical, of the disease.

Examining gastric acid output in the hour following a maximal dose of histamine in 10 female patients with hypopituitarism, Smith et al. (1961) found the mean acid output to be 0.6 m.Eq. compared with 17.2 m.Eq. in a control series of 28 normal women. This difference is highly significant. It is probable that the reduced gastric secretory activity in deficiency of anterior pituitary secretion is mediated, at least in part, through impairment of adrenocortical function.

Restoration of acid secretion occurs in patients with Addison's disease treated with replacement doses of cortisone (Stempien and Dogradi, 1954; Engel, 1955). This suggests that the reduced acid output is due to deficiency of the glucocorticoid fraction of adrenocortical secretion. It is believed by Gray (1964) that cortisol produced from the adrenal cortex plays an essential role in the physiological regulation of gastric secretion. The mechanism by which such an action is achieved is not known.

The only reference to gastric secretion in patients with disorders of adrenocortical steroid synthesis presenting as the adrenogenital syndrome appears to be by Broster (1934) who reviewed 60 cases of virilism, and of these 14 were possibly the adrenogenital syndrome; he states that the gastric juice was within normal limits in every case. With improved biochemical techniques, cases of congenital adrenal hyperplasia, in which the absence of cortisol in the plasma and urine is due to an inborn lack of 11 β-hydroxylase in the adrenal cortex, are now being detected (Eberlein and Bongiovanni, 1956). Information on gastric acid and pepsin secretion in such patients would be of considerable interest.

(b) Adrenocortical Hyperfunction

Whereas adrenocortical insufficiency would appear to lead to a reduction in gastric secretory activity, or at least to reduced hydrochloric acid output, over-production of glucocorticoids by a hyperfunctioning adrenal cortex has opposite effects. Gastric secretion was studied, using gruel fractional test meals, by Kyle et al. (1956) in 11 patients with Cushing's syndrome. Above-average concentrations of free HCl were obtained in 7 out of 8

patients tested preoperatively, in 2 of whom values in excess of 100 clinical units were found. In the 4 cases in which preoperative and postoperative tests were performed, there was a marked reduction in the concentration of acid after adrenalectomy. The acidity of the resting juice was not affected significantly by operation in these 4 cases.

It has been stated that the increased secretion of cortisol in Cushing's syndrome does not carry with it an increased liability to peptic ulceration (Kirsner and Palmer, 1952; Kyle, 1956) but Hurxthal and O'Sullivan (1959) found 3 proved cases of duodenal ulcer among 34 patients who had attended the Lahey Clinic for treatment of Cushing's syndrome. Two of these presented with melaena and the third had a perforation. Figures derived from retrospective surveys may tend to underestimate the incidence of this complication.

(c) Adrenal Steroids

Iatrogenic Cushing's syndrome caused by the therapeutic administration of corticotrophin or cortisone may be accompanied by increased secretion of hydrochloric acid and pepsin by the stomach (Gray et al., 1956).

Since the synthesis of cortisone by Kendall and his co-workers in 1945, a variety of adrenocortical hormones and their synthetic analogues have become available as therapeutic agents in a wide range of conditions. Not surprisingly, their widespread use has been followed by side-effects, of which a large proportion is referable to the gastrointestinal tract. Initially, these were considered to be infrequent. Only 1 of 66 patients treated with A.C.T.H. or cortisone (Thayer, 1952) complained of symptoms suggestive of peptic ulceration which, however, was not confirmed radiologically. The patient's post-prandial epigastric distress disappeared on stopping the A.C.T.H. The prominent side-effects in Thayer's series were neuropsychiatric disturbances, hypertension, electrolyte imbalance and fluid retention. In an editorial on the untoward effects on the stomach of corticotrophin and cortisone, Bloomfield (1952) stated that gastrointestinal complications were rare but warned that peptic ulcers could be reactivated during steroid treatment.

In a review of the literature, Sandweiss (1954) found that peptic ulceration had been reported in 50 patients

following A.C.T.H. or cortisone. He reported 25 ulcers in 520 patients (5.2 per cent) on long-term therapy and a total of 34 ulcers in 980 patients (3.5 per cent) given steroids for short or long periods. An incidence of 5.3 per cent in 1440 patients was recorded by Henderson (1955) and Bollet et al. (1955), after reporting 3 cases of peptic ulceration among 18 patients with rheumatoid arthritis treated for up to 7 months with prednisolone or prednisone, tabulate the findings from 6 other reports in the literature which yield a total of 36 ulcers in 477 patients (7.5 per cent).

It has been pointed out by Segal (1960) that ulcers attributable to steroids usually appear within a few weeks or months of starting therapy. Their incidence should be compared with the rate of occurrence of spontaneous ulceration in a similar population during a comparable period of time. While it is true that approximately 10 per cent of the adult population develop peptic ulceration at some time in their life, Ivy et al. (1950) state that the incidence in any survey over a period of 12 months is likely to lie between 1 per cent and 3 per cent. In certain communities, such as

York (Pulvertaft, 1959), the incidence of proved peptic ulcer is much less -- 0.258 per cent per annum. Other reasons for variation in reported figures are given by Doll and Jones (1951). While it has been estimated that 0.15 per cent to 0.38 per cent of the general adult population over the age of 20 years are admitted to hospital annually due to peptic ulcer (Ivy et al., 1950), many more are treated at home.

Since many of the side-effects from steroids have been in arthritic patients, the investigation by Boyen et al. (1960) into the incidence of peptic ulcer in this condition is of special importance. Of 2114 patients with rheumatoid arthritis attending the Mayo Clinic in the years 1954 and 1957, peptic ulcer was proved in 8.1 per cent of 877 patients not treated with systemic steroids and in 7.5 per cent of 1237 who had received steroids. Of 331 cases who showed signs of hyper-cortisonism, 8.8 per cent only developed ulcers. Gastric ulcers represented 7 per cent of all ulcers in the group not receiving steroids but accounted for 18.8 per cent of those in the group receiving steroid therapy. The overall annual incidence of peptic ulcer among all

patients attending the Mayo Clinic is stated to lie between 1 per cent and 2 per cent.

The dose of steroid used has considerable bearing on the occurrence of side-effects. Nine of 116 patients treated by Kern et al. (1957) with 50 mg. cortisone or 15 mg. prednisone per day complained of dyspepsia, and 2 others were shown to have peptic ulcers. Of 22 given up to 100 mg. cortisone or 40 mg. prednisone per day, 5 developed ulcers, 2 had exacerbations of old ulcers and 4 had non-ulcer dyspepsia. Seventeen of 63 patients (27 per cent) treated with steroids in the series reported by Badgin et al. (1960) were found on barium meal examination to have peptic ulcers which were predominantly gastric in site. The incidence varied from 13 per cent with doses of steroids equivalent to less than 20 mg. prednisone daily to 8 cases in 17 receiving over 60 mg. per day. Indisputable evidence of peptic ulceration was found by Hilbish and Black (1958) in 12 of 49 patients (24 per cent) undergoing corticosteroid therapy for rheumatoid arthritis on doses of prednisone ranging from 7.5 mg. to 30 mg. daily.

Reviewing experience in treating 215 dermatological cases with A.C.T.H. (47), cortisone (90), prednisone (125) and triamcinolone (37), Ridley (1962) reported that indigestion was the most frequently encountered complication, occurring in 34 patients. These were all on oral therapy and it is conceivable that local gastric irritation may have been partly responsible in some instances but cannot account for haematemesis in one patient while on A.C.T.H.

The clinical importance of steroid ulceration lies not so much in its frequency as in the atypical presentation in many cases and its lethal potentialities. Silent ulcers may occur, presenting as rapidly developing anaemia (Hilbish and Black, 1958) or the first indication may be haematemesis or perforation (Nordin, 1960). In a short review of the 1958-1959 literature on side-effects from adrenocorticosteroid therapy, Nordin (1960) found that 13 of 68 deaths during treatment could be quite properly attributed to the steroids. Seven of these 13 deaths were the result of haemorrhage from the stomach or small intestine.

An unduly high proportion of ulcers developing during steroid therapy is situated in the stomach as opposed to the duodenum. In a study of 114 rheumatoid patients who had been on steroid therapy for more than 6 months, Freiberger et al. (1958) found 35 (31 per cent) with peptic ulcers, of which 30 were gastric and only 5 duodenal. In the series of Bowen et al. (1960), gastric ulceration was two-and-a-half times more common than duodenal and, of 85 patients, treated with steroids and followed up with periodic barium meal examinations, Dubois et al. (1960) found 10 with gastric and 6 with duodenal ulcers. All 3 cases of peptic ulcer discovered by Bollet et al. (1955) in radiological studies on 18 patients suffering from rheumatoid arthritis and treated with steroids were gastric and asymptomatic.

In seeking the mechanism by which steroid ulcers are produced, most studies have been directed to discovering alterations in gastric secretion but the pharmacological actions on the stomach which have been ascribed to the adrenocortical steroids are conflicting.

Replacement cortisone therapy restores the concentration of acid in the gastric secretion of patients

with Addison's disease tested with histamine (Stempien and Dagradi, 1954; Engel, 1955). In longstanding cases, this is not complete and Smith et al. (1961) ascribe this to irreversible atrophic changes in the gastric mucosa which they observed in biopsies of the stomach in 9 out of 20 in a series of 24 patients suffering from adrenocortical insufficiency of varying duration. The restoration of hydrochloric acid secretion with steroid therapy in Addison's disease is not without its complications. Seven patients of Gray et al. (1956) developed chronic peptic ulcers while on 12.5 mg. - 25 mg. cortisone daily for 2-3 years. Gastric ulcer in Addison's disease during treatment with 12.5 mg. cortisone b.d. and 2.5 mg. D.C.A. daily has been reported by Engel (1955). These doses are considerably less than have generally been responsible for producing peptic ulceration in patients with intact adrenal glands.

In short-term studies, steroids have been ineffective in stimulating gastric secretion in normal subjects. Single intravenous injections of 20 units corticotrophin and 50 mg. hydrocortisone to each of 5 patients was without significant effect on basal gastric acid output, measured

for two hours before and 6 hours after administration (Kirsner and Ford, 1957). Gastric secretion was measured by Hirschovits et al. (1957) in 13 normal subjects during a control period of 3 hours followed by intravenous infusions lasting 3 hours and consisting of 25 units A.C.T.H. in 13 men, 100 mg. hydrocortisone in 11 men, 100 mg. corticosterone in 9 men and 25 mg. prednisolone in 2 men. No consistent effect was noted in the volume or acidity of the half-hourly collections but the viscosity of the gastric juice was reduced by A.C.T.H. and corticosterone. This effect was not observed with hydrocortisone or prednisolone. In another acute study by Dreiling et al. (1958) on 57 patients with and without ulcer diathesis, the intravenous administration of 40 units A.C.T.H. to 22 patients, 100 mg. hydrocortisone to 10, and 50 mg. metacortelone to 16 failed to produce any increase during a period of 6 hours in the rate of flow of gastric juice, in its free and total acidity, or in the rate of pepsin secretion.

The effect on the stomach of administration of adrenocortical steroids in pharmacological doses for longer periods is less certain. The results vary widely in

different reports, despite comparable levels of dosage and duration of treatment. Differences appear to exist between species and between individuals. Only studies on human subjects will be considered in this section.

Corticotrophin, in an intramuscular dose of 100-160 mg. once per day, was administered to 6 normal adults for 7-21 days by Gray et al. (1951). The output of free acid and pepsin in 12-hour night secretion and morning fasting juice was measured before starting the course of corticotrophin and at weekly intervals thereafter. Nocturnal acid output increased by a mean of 162 per cent, while the morning fasting hourly output increased by 175-510 per cent, with a mean increase of 270 per cent over control values. The output of pepsin in the morning collections in 5 subjects showed a mean increase of 209 per cent while on corticotrophin. A slight rise in gastric acid and pepsin concentrations was noted by Hirschowitz et al. (1955) in fasting specimens of gastric juice obtained by stomach tube from 5 subjects given 25 units corticotrophin gel intramuscularly twice daily for 6 days, but a coincident reduction in the volume of secretions meant that the output of acid and pepsin did not rise appreciably.

Gastric intubation for 24 hours before and after giving 14 volunteers hydrocortisone 20-60 mg., prednisone 7.5-15 mg. or prednisolone 7.5-15 mg. per day for 1 week allowed Kammerer and Rinaldi (1960) to obtain hourly specimens of juice both in the fasting state and while on semi-solid diet. An average increase in concentration of hydrochloric acid of 10 clinical units over a 24-hour period was recorded in the group of 6 subjects on hydrocortisone and prednisone but no increase occurred in the group of 8 receiving prednisolone. Prednisone 20 mg. daily for 24 days administered to 12 healthy young adults did not alter the output of gastric acid in basal juice, or that secreted in response to histamine 0.01 mg. per kg. body weight or mecholyl chloride 0.1 mg. per kg. (Beck et al., 1960). Also, doses of 90 mg. prednisone per day for 7 days did not affect the gastric secretory response to mecholyl.

Maximal histamine stimulation was used by Green (1960) in testing 18 patients during prolonged treatment with A.C.T.H. and steroids. Gastric secretion of acid was depressed by therapy in 4, unchanged in 8 and increased in 5 by 33 per cent to 100 per cent over control figures.

Further inconsistent findings were reported by Carbone and Liebowitz (1958) who investigated the effect of 40 mg. prednisone daily on basal and histamine-stimulated acid output in 14 medical students. Eight of the 14 showed a significant rise in gastric acidity while receiving prednisone, while the remaining 6 showed an equally significant fall. No significant change occurred in mean acid values for the whole group.

In a number of reports, the estimation of the output of pepsinogen in the urine over the 24 hours has been used as a convenient method of assessing variations in secretion of pepsin by the stomach without resorting to uncomfortable intubation. For a time it was thought that 1 per cent of that amount of pepsin secreted into the stomach is to be found in the plasma as pepsinogen, which is subsequently excreted by the kidneys (Grey and Ramsay, 1957). However, the amount of pepsin excreted in the urine has been shown to bear an inconstant relationship to both blood pepsinogen levels and to gastric pepsin production (Hirschowitz, 1957). Corticotrophin and the glucocorticoids greatly increase the renal clearance of pepsinogen (Spiro et al., 1959; Hirschowitz et al., 1957) and urinary pepsinogen output

probably reflects alterations in adrenocortical activity more faithfully than changes in gastric secretion (Hirschowitz, 1957).

The peptic ulcers which may complicate treatment with steroids are generally believed to be due to a steroid-induced hypersecretion of hydrochloric acid, in spite of the evidence, which is far from convincing, that such a factor is consistently involved. The anatomical situation of steroid ulcers in the pyloric canal and duodenum, where hyperchlorhydria is generally accepted as the most important aetiological factor, provides support for this view (Jones and Gummer, 1960). This view is contested by Monguy (1964) who considers that the increases in secretion produced by steroids are on the whole too modest to explain the ulcerogenic action of these compounds and directs attention to the question of possible alterations in the protection offered by mucus in preventing digestion of the gastric and duodenal mucosa by acid pepsin. The administration of steroids has been found to decrease the viscosity of gastric juice in the human (Hirschowitz et al., 1957) and in experimental animals (Kyle, 1956; Beck et al., 1960; Clarke, 1959; Monguy and Masters, 1963). This is

due to two factors: a marked reduction in mucus secretion and changes in the composition of the mucus. As a result, Menguy believes that the peptic ulcers which follow steroids are due to interference with the mucus protective barrier. The difficulties associated with measuring and analysing the composition of gastric mucus, which are apparent from the work of Glass and his colleagues (1953, 1958 and 1963), have deterred most investigators from this field of investigation. It is likely, however, that both hypersecretion and decreased mucosal resistance are operative factors in the genesis of steroid ulcers.

Stress, for example from myocardial infarction, burns, surgical operation, trauma, shock and pain, may all induce an increase in gastric secretory activity. According to Gray (1961), this hypersecretion coincides in time with an increased output of cortisol from the adrenals. "Stress ulcers" which may occur under these circumstances are of the acute variety.

Evidence of increased secretion of adrenocortical steroids in the more common chronic duodenal and gastric ulcers is lacking. In patients with peptic ulcers, the 24-hour urinary excretion of 17-hydroxycorticoids does not

differ from that in control subjects (Cummins and Comperèz, 1957; Sleisenger et al., 1958; Grey, 1958). Some have found it less (Sandweiss et al., 1950; Green and Pulvertaft, 1962). Thus Green and Pulvertaft (1962) reported that the 24-hour excretion of 17-hydroxycorticosteroids in 107 male patients with active ulcers was only 78 per cent that in 56 normal male controls and 17-ketosteroid excretion was 93 per cent of normal; in the quiescent phase (50 men) the differences were 71 per cent and 86 per cent of normal respectively. These differences are small. The relationship of adrenocortical function to peptic ulceration cannot be further clarified until the physiological actions of steroids on the stomach and duodenum are defined.

The conflicting results noted above concerning the effect of steroids on gastric secretion in men may partly be due to individual susceptibility. On the other hand, accurate assessment of gastric function in man under various conditions is difficult. Spontaneous fluctuations in basal secretion can occur in the same subject on different days, and gastric juice may be lost through the pylorus. Regurgitation of bile may affect acidity

measurements (Hunt, 1958). The more rigorous control of experimental conditions possible in animals has afforded a more satisfactory assessment of the effects of the adrenocortical steroids on gastric function.

EFFECT OF ADRENOCORTICAL STEROIDS ON GASTRIC SECRETION IN EXPERIMENTAL ANIMALS

Before examining the evidence from published reports on animal experiments, it is well to emphasise a few of the differences which exist between species and may in part account for some of the confusing and contradictory findings of different authors.

1. Variation in sensitivity to drugs in different species is widely recognised. For instance, the dose of histamine per kg. of body weight required to stimulate a maximum output of acid from the stomach of a dog is some 20 times greater than that which exerts a similar effect in man (Marks et al., 1960). In rats, even more massive doses of histamine are necessary to stimulate secretion. Also, while histamine may stimulate the secretion of both acid and pepsin in man (Friedman et al., 1957), its action on pepsin secretion in the dog is minimal.

2. The existence of accessory adrenal tissue is of great importance when the effects of adrenalectomy are being studied. Functioning adrenal tissue rarely remains after bilateral adrenalectomy in the dog (Rogoff and Stewart, 1926), yet it is not uncommon in cats (7 of 21) (Rogoff and Stewart, 1926), relatively common in rabbits (20 per cent) and frequent in rats (50 per cent) (Biedl, 1912). Where small numbers of animals are used, these considerations will obviously gain increased importance.

3. Possibly related to the foregoing, the requirement for maintenance steroids after adrenalectomy varies in different species. Following this operation, the rat can be maintained in good health by simply substituting 1 per cent saline for its normal drinking water (Kyle, 1956), whereas dogs resemble men in their need for replacement of cortisol.

4. The adrenocortical steroids responsible for the physiological actions of the adrenals vary in different species. In rats, the main

glucocorticoid produced in the adrenal cortex is corticosterone (Hechter and Pincus, 1954), while in both man and the dog the chief glucocorticoid is cortisol.

5. When assessing the effect of the administration of steroids, it should be borne in mind that any dose of steroid which lies within the normal range of endogenous production by the the adrenal cortex mainly inhibits pituitary release of corticotrophin, resulting in decreased stimulation of the subject's adrenal cortex and consequently of cortisol secretion (Sayers and Sayers, 1949; Ingle, 1954).

Rodents

In order to minimise the effects on gastric secretion of laparotomy, anaesthesia, fall in blood pressure and cardiac output, acidosis and alterations in blood glucose concentration and plasma electrolytes, which may occur in animals as a result of bilateral adrenalectomy, Davenport and Chevre (1950) conducted experiments on the stomachs of mice *in vitro*. They found the rate of acid production in this preparation in adrenalectomised mice was the same as in controls. The addition of desoxycorticosterone or

cortisone acetate to the incubation fluid in a variety of concentrations did not influence secretion. The injection of DOCA (2, 4, 8 mg. per 15-20 G. mouse) or cortisone acetate (2 mg./mouse) 14-16 hours before, and again 2 hours before removal of the stomach in mice with intact adrenals was also without demonstrable effect.

Following ligation of the pylorus in the rat, the secretion of acid by the stomach continues at a constant rate close to the maximal, even in the fasting state (Shay et al., 1945). Since the rat is unable to vomit, gastric juice is not lost and can be collected conveniently by excising the intact stomach after 4-6 hours. Using this type of preparation, commonly referred to as "the Shay rat", Tuerkischer and Wertheimer (1945) found the mean volume of secretion in the stomachs of 28 controls after the injection of the parasympathomimetic drug Doryl (8 µg./kg. s.c.) was 8.6 ml. with a pH of 1 - 2 compared to a mean of 1.1 ml. and a pH of 6 - 8 in 26 rats after bilateral adrenalectomy. Less striking changes were observed in Shay rats by Madden and Ramsburg (1951), who noted mean volumes of 6.8 ± 0.18 ml. (standard error of the mean) in controls and 3.2 - 0.14 ml. in rats subjected

to adrenalectomy. Although the mean pH showed no significant difference, the total output of acid (volume x concentration) was reduced by over 50 per cent. Similar results of adrenalectomy in the Shay rat have been reported by Welbourn and Code (1953) and by Kyle and Welbourn (1956). Restoration of normal volumes of juice is achieved by administering DOCA, cortisone, prednisone or extracts of whole adrenal cortex (Turkischer and Wertheimer, 1945; Madden and Ramsburg, 1951; Welbourn and Code, 1953; Kyle and Welbourn, 1956) but DOCA differs from the other drugs mentioned in not correcting the acidity (Turkischer and Wertheimer, 1945; Kyle and Welbourn, 1956).

In Shay rats with intact adrenals, the effect of DOCA and cortisone has been unimpressive. In Madden and Ramsburg's (1951) study, DOCA (1.9 mg. subcutaneously 21 and 5 hours before pyloric ligation) increased the mean volume of gastric juice by only 15 per cent and the acidity was reduced by 0.2 of a pH, while Kyle and Welbourn (1956) reported that DOCA (2.5 mg. on the day before and 1.25 mg. at the time of pyloric ligation) caused a small, but probably significant, decrease in volume and acidity.

In this latter study, cortisone acetate (2 mg. daily for 3 days) was without effect on secretion in a separate group of 9 rats, confirming earlier work by Welbourn and Code (1953).

Ligation of the pylorus produces ulcers of the stomach in 60 per cent of rats sacrificed 6 hours after operation (Bonta, 1961). The incidence of such ulceration in control animals was compared by Bonta with acid output and with the occurrence of ulcers in groups of rats (all weighing 90 G. - 110 G.) given various steroids by subcutaneous injection daily for 10 days. These included cortisone 5 mg., hydrocortisone 4 mg., prednisone 1 mg., dexamethasone 0.02 mg., corticosterone 5 mg. and A.C.T.H. 2 units per day. No correlation was found between the ulcerogenic effect of the compounds and their effect on gastric juice. With the exception of cortisone and hydrocortisone, all corticoids and A.C.T.H. considerably enhanced the ulcer response.

The effect on the secretion of mucus by the stomach of 5 mg. prednisolone administered subcutaneously to rats for 1-5 days was investigated by Robert and Nezamis (1963). They adopted the hexosamine content of the gastric juice 5

hours after pyloric ligation as a measure of mucus secreted. Treated animals showed a higher incidence of ulceration, while the acidity of the gastric juice and its content of mucus was markedly reduced.

It appears reasonable to conclude that, except after adrenalectomy, steroids do not enhance gastric secretion in the rat and the ulcerogenic effect of steroids in this species, in the artificial conditions of the experiments quoted, must be mediated through other imperfectly understood mechanisms.

Dogs

As is well-known, repeated collections of pure uncontaminated gastric juice from unanaesthetised dogs can be made from pouches of the stomach. Collections of juice from such pouches may be accurately measured and permit precise appraisal of the temporal relations of any secretagogue or inhibitor, as well as an accurate estimate of the intensity and duration of effect produced (Code et al., 1949).

The effect of adrenalectomy on acid gastric secretion in dogs was first studied by Sigel et al. (1957). In their experiments, the secretory response of separated

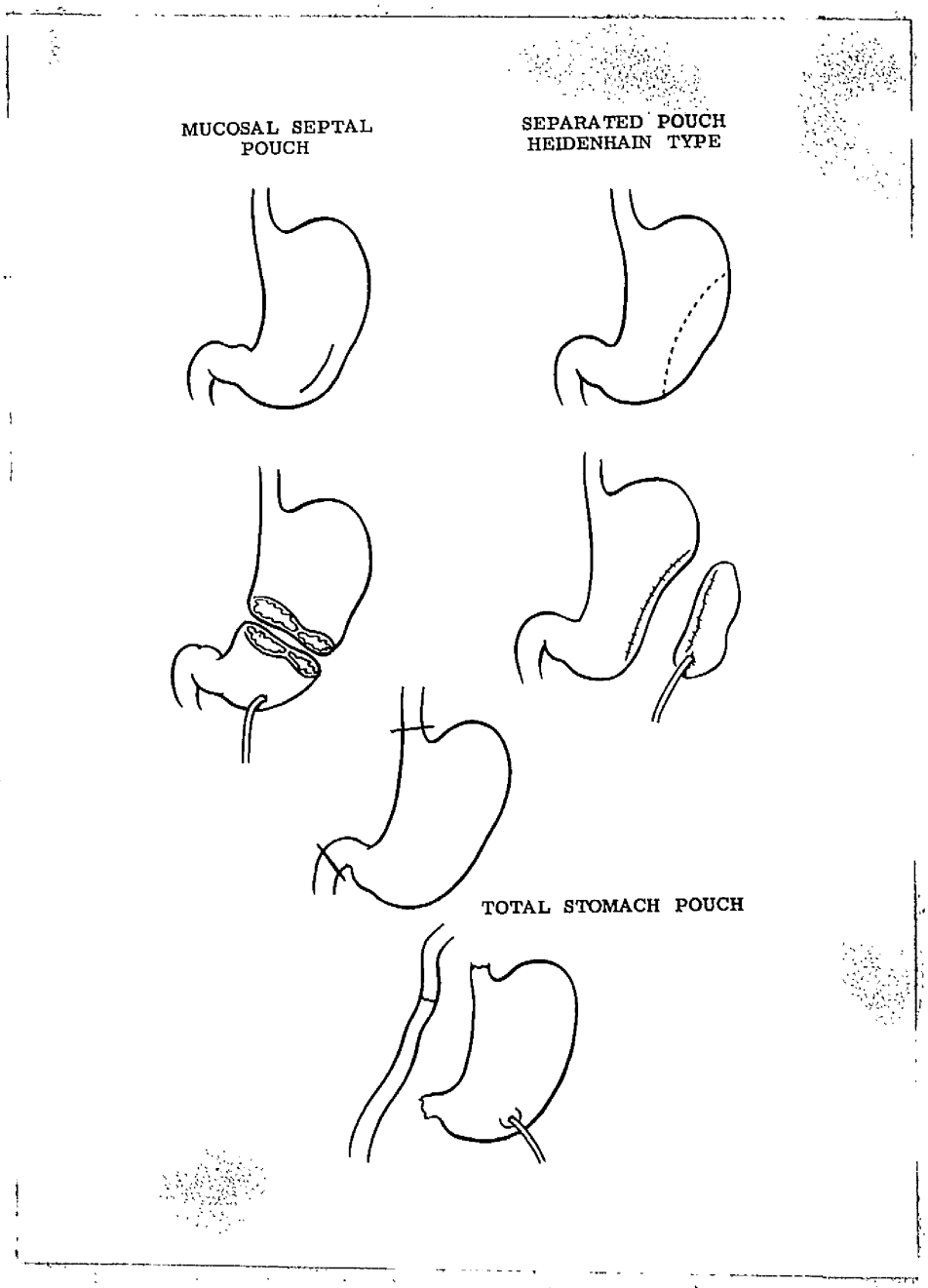


Figure 1 : Three types of pouch

gastric pouches to a subcutaneous injection of histamine was measured before and after bilateral adrenalectomy. The operation led to a marked reduction in both the volume and concentration of acid. Even when frank signs of adrenocortical insufficiency were present, however, some acid was still secreted when histamine was given. Replacement doses of cortisone restored the acid response to histamine to control levels, in spite of continuing low serum concentrations of sodium and chloride. Thus, the effect of adrenal steroids in restoring gastric secretion was not secondary to alterations in electrolyte concentrations. The dose of cortisone required to restore gastric secretion to preoperative levels in adrenalectomised dogs varies markedly. In a study of 8 dogs with Heidenhain pouches, Nicoloff et al. (1961) assessed gastric secretory activity by analysing 24-hour collections of juice from the pouches while the animals received a carefully controlled diet. Following a control period of 20 days, the dogs were submitted to bilateral adrenalectomy by a one-stage procedure and were thereafter given varying doses of cortisone acetate (12.5 - 500 mg.) daily by intramuscular injection. At least ten 24-hour

collections were made during the time that the dogs were on a particular dose of cortisone. Normal levels of acid secretion were restored or exceeded by 25 mg. cortisone daily in 3 dogs and by 50 mg. daily in a further 3. A dose of 100 mg. was insufficient in one dog and the optimal replacement requirement of cortisone in the remaining animal is not obvious from the data given, but lay between 25 mg. and 100 mg. daily. The difference between dogs was greater than could be explained by the minor expected differences in the requirements for cortisone replacement. When the dose of cortisone given was higher than that required for maintenance alone, the volume of 24-hour collections was increased by up to 50 per cent above control values and the concentration of free-acid by 21 per cent to 614 per cent.

Contradictory results to the foregoing were reported by Weidenanders et al. (1960) in 2 dogs with Heidenhain pouches, that had both adrenals removed and were then placed on a daily dose of cortisone increasing at 10-day intervals from 10 mg. up to 300 mg. or 500 mg. At the 40 mg. level the output of acid had decreased by 30 per

cent in one dog and by 5 per cent in the other. Pepsin had also decreased by 8 per cent and 12 per cent. Higher doses of cortisone failed to restore secretion to normal levels.

As in humans, single injections of corticotrophin and adrenocorticoids do not have much effect on gastric secretion in dogs with intact adrenal glands. Beef broth and histamine were the two stimuli used by Friedman et al. (1951) in testing the effects of A.C.T.H. and cortisone on gastric secretion in 12 normal and 38 Mann-Williamson dogs. Single doses of A.C.T.H. (30 mg. - 68 mg. intravenously) did not affect the concentration of acid and pepsin or duration of secretion. In fasting animals, Ragins et al. (1956) found that A.C.T.H. (40 units - 80 units subcutaneously to 4 dogs), cortisone (200 mg. intramuscularly to 4 dogs) and hydrocortisone (66.8 mg. intravenously to 5 dogs) were without significant effect on secretion from Heidenhain pouches in the 24 hours following injection. Similar negative findings were reported by Zukowski et al. (1961) for A.C.T.H. whether given as an intravenous infusion of 25 units or as a subcutaneous injection of 40 units - 80 units. In Heidenhain-pouch dogs, Zawoiski et al. (1968)

obtained no effect on basal secretion during an observation period of 2½ hours after intravenous injection of prednisolone in the high dose of 1.5 mg. per kilogram of body weight.

Contrasting with these reports is the observation by Shay (1950) that a small but definite decrease in the pH of juice (from neutrality to pH 5) secreted by canine Heidenhain pouches occurs 2½ hours after the intravenous injection of 100 mg. hydrocortisone. He has also reported (in abstract form without data) that single injections of cortisone, hydrocortisone and A.C.T.H. can produce unquestioned increases in acid and pepsin output from Heidenhain pouches even after removal of the antrum (Sun and Shay, 1957). The weight of evidence, however, suggests that corticotrophin and the adrenocorticoids are not gastric secretagogues when given as single injections.

Prolonged administration of A.C.T.H. and adrenocorticoids produces greater and more consistent effects on gastric secretion in the dog than in man, although here again, some dissention exists in respect of the frequency and extent of the changes produced.

A sustained increase in 24-hour secretion from vagally innervated and denervated gastric pouches was noted by Zubiran et al. (1952) to accompany the administration to dogs of corticotrophin (75 mg. per day) for 10 days and cortisone (100 mg. per day) for 14-30 days. Acid output increased by 30 per cent to 75 per cent above preliminary control levels and the increase persisted for 7 to 10 days after the drug was discontinued. In the experiments of McGee et al. (1959), cortisone, in a dose of 300 mg. once daily by intramuscular injection, was given to 6 dogs with Heidenhain pouches for 2 weeks. All 6 animals showed a significant rise in volume of the 24-hour collections of pouch juice compared with a preliminary control period, but there was little change in the pH of the juice. However, in the lower ranges of acidity encountered in this study, estimations of pH give a much less accurate indication of changes in acid concentration than titration against alkali. The average 24-hour output of free acid during the cortisone period rose by 35 per cent to 185 per cent over control values and returned to normal in the second week after stopping the drug. A second group of 5 dogs with denervated pouches

of the stomach had the gastric antrum removed and the experiment repeated. Cortisone did not affect pouch secretion in these animals but, as is the rule in antrectomised dogs, the volume of gastric juice was severely reduced.

It has also been reported by Drye and Schoon (1958) that the administration of cortisone acetate (12.5 mg. twice daily by intramuscular injection) for over a month to 2 antrectomised dogs with gastric pouches was without effect on the 24-hour output of acid. The dose of cortisone used was however small and probably would not exceed the amounts normally produced by the animals' own adrenals. Three of the dogs in the report by Zubiran et al. (1952), which describes the effect of cortisone (100 mg. daily for 14-30 days) on secretion from gastric pouches, were prepared without the gastric antrum. The 24-hour output of acid from all 3 dogs was increased by cortisone, the actual increases being 36 per cent, 50 per cent and 200 per cent over control values.

In the experiments of Clarke et al. (1960) in denervated canine pouches, 3 to 5 days elapsed before an increased secretion occurred in response to corticotrophin

(25 units intramuscularly daily), cortisone (50 mg. intramuscularly twice daily), methyl prednisolone (12 mg. orally daily) or aldosterone (0.2 mg. intramuscularly once daily). The increase in volume and acidity of basal juice and that stimulated by histamine and intragastric meat extract, which occurred with all 4 drugs, was independent of the glucocorticoid or mineralocorticoid activity of the particular hormone used. A.C.T.H. (20 units - 40 units intramuscularly thrice daily) was given by Chaikof et al. (1961) to 3 dogs with Heidenhain pouches for 15-21 days without affecting the concentration of acid, although the volumes of 24-hour collections increased by 20 per cent to 56 per cent. Larger doses of A.C.T.H. increased both the concentration and output of acid from a mean of 24.9 m.Eq. per 24 hours in the preliminary control period to a mean of 55.2 m.Eq. per 24 hours during the administration of A.C.T.H.

The administration of corticotrophin (40 units intramuscularly) daily to 5 dogs with Heidenhain pouches and denervated antral fistulae resulted in an increase in the volume (71 per cent to 168 per cent) and output of free acid (108 per cent to 240 per cent) from the pouches in

response to perfusion of the antrum with 10 per cent peptone for 3 hours (Nicoloff et al., 1963). No alteration in pepsin secretion occurred.

Oral prednisone, in doses ranging from 10 mg. to 60 mg. daily, also increases acid output in dogs (Plainos and Philippu, 1958; Chaikof et al., 1961) when given for several days, and the newer synthetic steroids triamcinolone (50 mg. daily) and dexamethasone (10 mg. daily) have similar effects (Plainos et al., 1962).

In some studies the effects of long-term administration of steroids have been inconsistent. Cortisone, in a dose of 100 mg. daily, was given by Dragstedt et al. (1956) for 43 days to 2 dogs with denervated gastric pouches. The 24-hour output of acid was increased in one dog and decreased in the other. In the study by Weideranders et al. (1960), 300 mg. cortisone was administered daily for 10 days to 4 dogs with Heidenhain pouches. Two dogs showed an increase of 30 per cent and 4 per cent respectively in their 24-hour secretion of free acid while the remaining 2 dogs showed a fall of 60 per cent. An increase in 24-hour acid output from Heidenhain pouches of 20 per cent to 30 per cent was found

by Johnson (1963) in 7 of 8 dogs given 100 mg. cortisone acetate intramuscularly daily for 30 days. The eighth dog showed no increase in secretion yet died from a perforation of a duodenal ulcer.

The digestive capacity of gastric juice depends on the presence of pepsin as well as acid but the effects of steroids on pepsin secretion by the stomach are rather variable. In certain situations, such as fasting juice from denervated pouches, the output of pepsin is normally low and often considered not worth measuring, so that less data is available on this aspect of gastric secretion. Corticotrophin has been reported by Gray and Ramsey (1957) to increase pepsin output from canine gastric pouches and this occurs in denervated (+ 497 per cent) as well as in innervated (+ 261 per cent) pouches (Villareal et al., 1955).

Increases in pepsin output in basal juice following oral prednisone (60 mg. daily for 10 days) averaged 355 per cent in 4 dogs with gastric fistulae (Plainos and Philippu, 1958) while still greater outputs have been reported in basal juice after 50 mg. triamcinolone daily for 10 days (Plainos et al., 1962). However, Clarke et al. (1960) did

not find any significant alteration in the concentration of pepsin in the basal juice of Heidenhain pouch dogs treated with pharmacological doses of corticotrophin, cortisone or aldosterone. A significant reduction occurred in 3 dogs treated with methyl prednisolone in a dose of 12 mg. daily for 7-12 days. No effect on pepsin secretion was noted by Nicoloff et al. (1963) in 5 dogs given 40 units corticotrophin daily for 4 days. The dogs used had Heidenhain pouches and antral fistulae with end-to-side gastrojejunostomy and the secretory stimulus employed was perfusion of the antrum with 10 per cent peptone for 3 hours.

The mechanism by which adrenal steroids influence gastric secretion is still obscure. That their effect is independent of either the pyloric antrum or the vagus is clear from the results on antrectomised dogs with Heidenhain pouches (Zubiran et al., 1952; Sun and Shay, 1957) and it is probable that they act directly on, or at a site close to, the parietal cells. According to Reid et al.,(1961), the numbers of parietal cells in the stomachs of 6 dogs was increased 50 per cent by the administration of cortisone acetate 25 mg. per kilogram

of body weight for 5 days. If this is the explanation for augmentation of secretion, the time taken for the parietal cells to multiply could explain its delay in onset. Support for this concept of an increase in parietal cell mass is given by the increase in the maximal histamine response which follows cortisone administration to dogs with Heidenhain pouches (Clarke et al., 1960). The magnitude of the maximal response is related directly to parietal cell mass (Marks et al., 1960). However, the fact that steroids also increase the hydrogen ion concentration of basal juice (Plainos and Philippu, 1958; Clarke et al., 1960) suggests that the parietal cells may be stimulated to secrete as well as to divide. Possibly the "reactivity" of the parietal cell to other secretory stimuli is increased by steroids (Stavney et al., 1964).

The studies by Reid et al. (1961) have not been confirmed - no doubt on account of the tedious work involved in making parietal cell counts.

In the experiments of Friedman et al. (1951), prolonged treatment with small doses of A.C.T.H. (12 mg. daily) or cortisone (5 mg. - 12 mg. intramuscularly daily) delayed the appearance of peptic ulceration in Mann-

Williamson dogs. Larger doses of steroids have been reported to have deleterious effects on Heidenhain pouches including haemorrhage and partial disruption (Cooper et al., 1961). Of the 5 dogs to whom Nicoloff et al. (1961) administered 200 mg. cortisone daily for 13 days, 2 died from perforation of a stomal ulcer, 2 had multiple stomal ulcers and the remaining one had gastric erosions at autopsy. The type of preparation used involved the formation of an ambral fistula and the restoration of the alimentary tract by end-to-side gastrojejunostomy. Death of animals from perforated peptic ulcer occurred in the series reported by Chaikof et al. (1961) in 2 of 4 dogs on A.C.T.H. 80 units daily, in 2 of 12 dogs on 10 mg. prednisone daily and in 2 of 12 dogs on 25 mg. prednisone daily. Of 8 dogs given 100 mg. cortisone daily for 30 days, 2 died from perforated peptic ulcer shortly after completing the course of treatment (Johnson, 1963).

Not all investigators accept that the ulcerogenic properties of steroids are the result of hypersecretion of acid; steroids also affect the viscosity of gastric juice (Kyle, 1956; Clarke, 1961). This is due to a reduction in the amount of mucus secreted (Mengny and

Masters, 1963) as well as alterations in its physical characteristics, which may impair its ability to protect the gastric mucosa from the digestive properties of acid and pepsin (Monguy, 1964).

The effect of cortisone (2.5 mg. - 20 mg. per kilogram body weight twice daily intramuscularly) and A.C.T.H..(5 mg. - 10 mg. per kilogram body weight intramuscularly daily) on the healing of ulcers following excision of mucosa in explants of the stomach in dogs was studied by Janowitz et al. (1958). Despite a wide range of figures obtained, it is clear that the mean healing time of these lesions was lengthened by both cortisone and by A.C.T.H. in the doses used. However, Rodriguez-Olleros and Galindo (1957) found that the size and rate of healing of gastric ulcers induced in 10 dogs by intramuscular injection of pancreatic juice was not affected by cortisone in a dose of 1.5 mg. per lb. body weight daily. Ulcers produced by atophan also healed satisfactorily while the dogs were on cortisone or corticotrophin.

Consideration of all the available data leads to the conclusion that the mechanism by which the adrenocortical steroids produce peptic ulceration is not the result simply

of a stimulatory action on hydrochloric acid secretion but involves, among other factors, the extent and composition of the mucous protective barrier in the individual subject. Nevertheless, there is ample evidence to suggest that the adrenal steroids are closely involved in the acid secretory process and comparatively slight increases in acid production might be sufficient to cause peptic ulceration in a susceptible case. Of the reports reviewed, only a handful deal with the place of steroids in the normal physiological regulation of gastric acid secretion and impairment of this activity in adrenocortical insufficiency. Research in this direction has been hampered by the difficulties associated with maintaining adrenalectomised animals in good health for the prolonged periods of time required for systematic examination of this aspect of the problem.

DRUG INHIBITION OF ADRENOCORTICAL FUNCTION

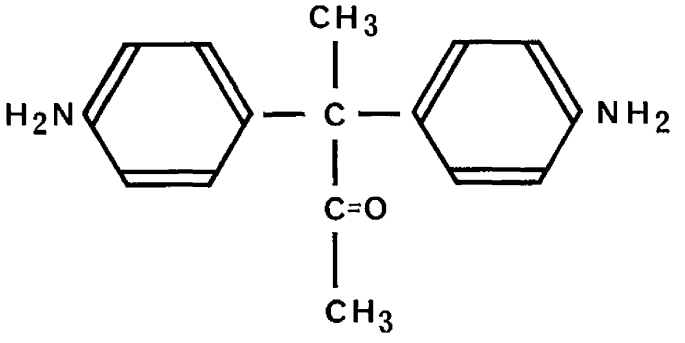
It has been stated in the previous section of this review that the administration of physiological doses of cortisol calls into play an autoregulatory mechanism whereby the anterior pituitary is inhibited and production of corticotrophin reduced. This in turn causes a reduction in the secretory activity of the adrenal cortex which may go on to atrophy (Sayers and Sayers, 1940; Ingle, 1954). Cortisone and a number of other steroids such as dexamethasone may therefore be used as indirect inhibitors of adrenocortical function, but as they themselves are glucocorticoids, they are unsuitable where it is desired to reduce the amount of circulating steroid possessing glucocorticoid activity.

The pharmacology and biochemistry of some adrenocortical inhibitors have been reviewed by Chart and Sheppard (1959) who found that the two most promising substances were the insecticide DDD and its derivative Amphenone B (3,3 - bis (p-aminophenyl) - 2 butanone), which was first synthesised by Allen and Corwin (1950). This latter compound suppresses adrenocorticoid secretion in the dog (Hertz et al., 1955; Nelson and Hume, 1955)

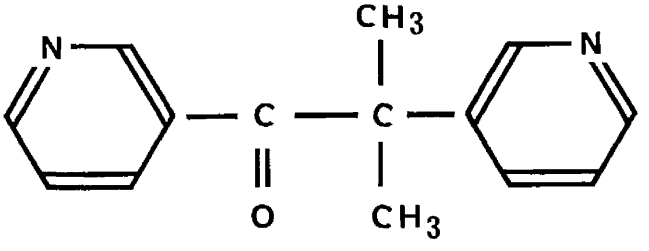
and in man (Thorn et al., 1956) but has many toxic side-effects. Accordingly, Chart and Sheppard (1959) undertook a chemico-biological study of analogues of amphenone B which had the same effect on adrenal function but were less toxic.

The most satisfactory drug tested was 2-methyl-1, 2-di (3'-pyridyl) propan-1-one (Figure 2) which, at a controlled dose level, selectively inhibits hydroxylation at position 11 of the adrenal steroid nucleus (Jenkins et al., 1958) (Figure 3). This drug was introduced by Ciba Laboratories Ltd. under the code numbers SU 4885 and SU 8874; it was later given the proprietary name Metopirone and its official name is now metyrapone.

Metyrapone base has a low melting point (51°-52° C.) and, as it is a basic substance, it is also potentially susceptible to oxidation. Solutions of the base with 7 per cent dimethyl-acetamide or 10 per cent propylene glycol as solvent are extremely light-sensitive and, in biological trials, have shown local irritant effects which have been traced to the base itself and not to the solvent (Müller, 1961). Accordingly, where an injectable preparation of the drug is required, the ditartrate salt



AMPHENONE B



METYRAPONE

Figure 2 : Chemical formulae of amphenone B and metyrapone

is preferred. As metyrapone ditartrate is also light-sensitive, the preparation, sterilisation, storage and filling of ampoules must be carried out in the dark and the simultaneous use of an atmosphere of nitrogen is recommended. When these precautions are taken, metyrapone ditartrate is analytically stable after storage for six months and is also galenically stable at 20° C. Ciba Laboratories Ltd. felt that the free base could probably be autoclaved without significant deleterious effect on its chemical structure, but there is no specific data available on this point and the destruction of any spores present could not be guaranteed (personal communication, 1963).

The minimum effective dose of metyrapone ditartrate is 5 mg. per kilogram body weight and Grant (1962) has confirmed the observation by Liddle et al. (1958) that 15 mg. per kilogram body weight administered intravenously promptly reduces the amount of cortisol in adrenal vein blood to a very low level. The plasma concentration of hydrocortisone also falls, resulting in an increased secretion of corticotrophin and a correspondingly greater production of the "precursors", cortexone (B0C) and

Reichstein's "compound S" - 17 hydroxy - 11-desoxy-corticosterone (Brownie and Sprunt, 1962; Liddle and Island, 1962). Further elaboration of these hormones to cortisol and aldosterone is blocked by metyrapone (Figure 2).

The administration of large doses of metyrapone in the dog causes a generalised suppression of adrenal steroid biosynthesis for a short period only but the formation of 11-hydroxycorticoids is inhibited for approximately 8 hours and the concentration of cortisol in the plasma does not return to pre-injection levels for 20 hours (Jenkins et al., 1958). In dogs, Chart et al. (1953) found that an oral dose of 200 mg. metyrapone per kilogram body weight reduced the output of cortisol from the adrenal cortex to approximately one-third of the average level obtained in 36 control animals.

When metyrapone is used in man, a degree of cortisol suppression persists for at least 12 hours after a single dose and may be detectable for 1-2 days after stopping the drug (Jenkins et al., 1960), but in order to obtain maximum suppression of cortisol secretion throughout the 24 hours, it is recommended that metyrapone be given every 2 hours

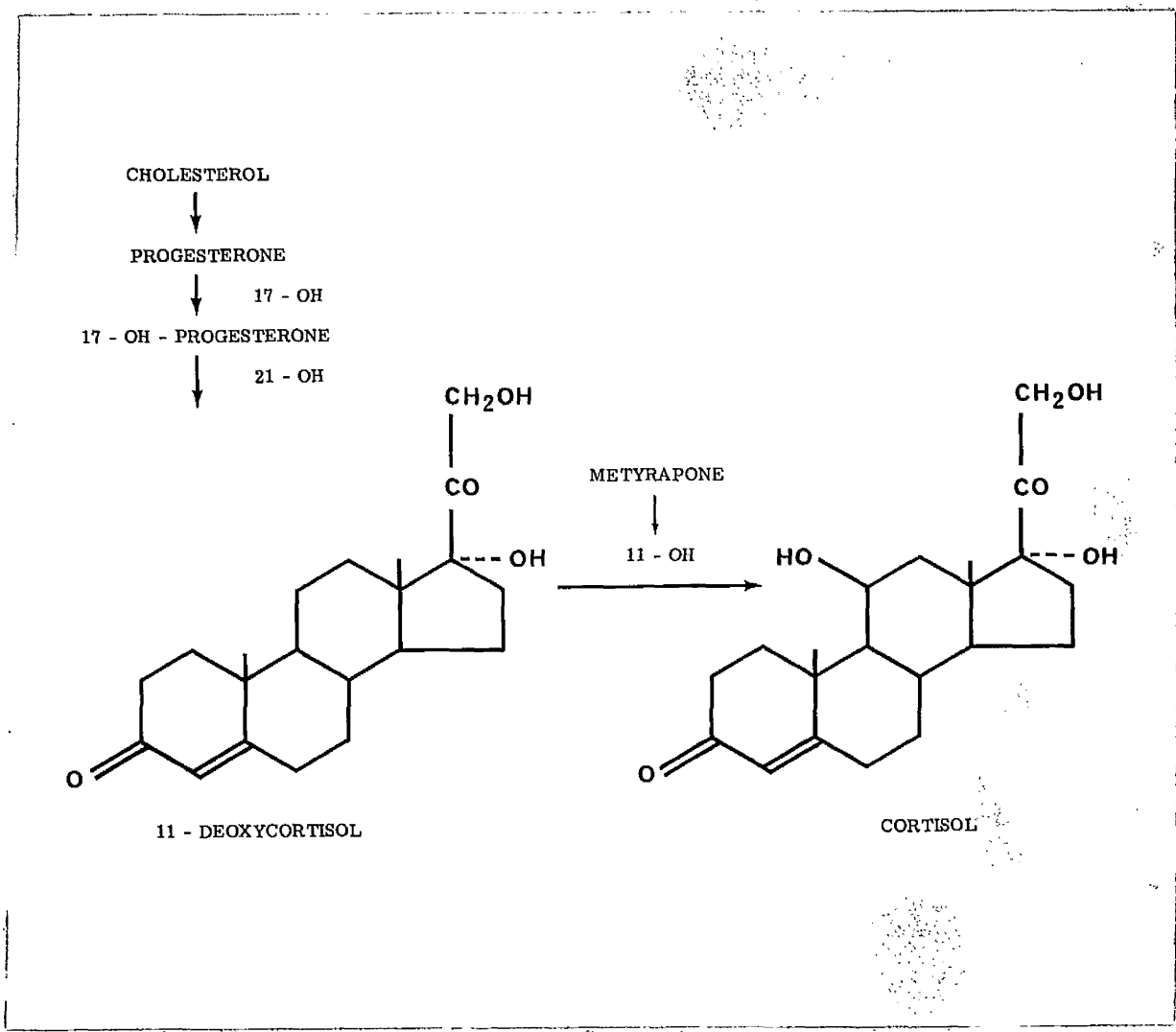


Figure 3 : Site of action of metyrapone on adrenocortical steroid synthesis

(Duus et al., 1962). Even then, the plasma concentration of cortisol cannot be reduced below about 2 µg./100 ml. due to diffusion into the blood from the extravascular cortisol pool which amounts to nearly 2 mg. (Duus et al., 1962).

In chronic toxicity studies in dogs, doses of metyrapone up to 200 mg. per kilogram body weight twice daily for 4 weeks have not been attended by any signs of adrenocortical insufficiency (Chart et al., 1958). Electrolyte balance is maintained by the activity of the intermediate substances involved in adrenocorticoid synthesis, 11-desoxycortisol (compound S) and desoxycorticosterone (DOC).

Very few side-effects have attended the use of metyrapone in pharmacological doses in clinical practice where it is now used in a standard test of corticotrophin reserve (Jenkins et al., 1959). Nausea and some gastric discomfort may be experienced when the oral dose is 6.0 G. or more, but drowsiness, skin rashes and methaemoglobinaemia, which were among the toxic effects of Amphenone B (Hertz et al., 1950) have not been encountered with metyrapone to date.

The specificity of action of metyrapone on adrenal steroid synthesis and its non-toxicity suggests that it should prove a most useful tool in research into the physiological actions of the adrenocortical hormones in the experimental animal as well as in man.

EFFECT OF ADRENOCORTICAL INHIBITOR DRUGS ON GASTRIC SECRETION

In spite of the considerable interest shown by investigators in the effects of adrenocortical steroids on gastric secretion, the pharmacological action of inhibitors of adrenocorticoid secretion on gastric function has attracted little attention. The reason for this omission is that, until recently, no suitable non-toxic drug was available for this purpose.

In an exhaustive search of the literature, the only systematic evaluation of the effect on the stomach of a drug which inhibits adrenocorticosteroid production is the report by Henrique et al. (1958) on the effect of amphenone B (3, 3di (p-aminophenyl) butanone-2 dihydrochloride) on gastric secretion in Shay (pyloric-ligated) rats. In their experiments, a single subcutaneous injection of amphenone (25 mg. per 100 G. body weight) produced a significant decrease in the volume, free acid and potassium content of

gastric secretion compared to control rats injected with a dilute solution of HCl of the same pH. The gastric contents of the amphenone-treated rats showed an increase in pH, sodium and pepsin concentration. Since cortisone (25 mg. intramuscularly daily for 3 days to 10 rats), corticosterone (2.5 mg. intramuscularly daily for 3 days to 8 rats), desoxycorticosterone (2.5 mg. intramuscularly daily for 7 days to 6 rats), aldosterone (10-30 γ daily to 6 rats) or corticotrophin (10 units intramuscularly for 10 days to 10 rats) did not affect their subsequent response to amphenone, these authors suggest that amphenone may have a direct effect on the gastric mucosa which is not mediated through adrenal suppression. On the other hand, amphenone was without effect on gastric secretion in adrenalectomised rats. These findings were taken to indicate a "permissive" rôle for the adrenocortical steroids on gastric secretion.

The limitations of the pyloric-ligated rat preparation in the investigation of adrenocorticosteroid influence on gastric secretion have been mentioned previously. In addition, the various toxic side-effects of amphenone severely restrict the validity of these conclusions.

S T A T E M E N T

O F

P R O J E C T

Evidence from the literature indicates that bilateral adrenalectomy reduces gastric secretion in certain species of experimental animals, including the dog, while states of impaired adrenocortical function in man are also accompanied by hypochlorhydria. That this depression of gastric secretion is the consequence of a deficiency in the glucocorticoid fraction of adrenocortical secretion is shown by the return of acid output to normal levels following the administration of cortisol.

This thesis describes experiments carried out to study the effect on gastric secretion in dogs of selective inhibition of cortisol production for varying periods by the administration of a synthetic adrenocortical-inhibitor drug - metyrapone. The experiments were designed to establish the most effective mode of administration of the drug and to elucidate the mechanism of action of adrenocortical steroids on gastric secretion.

As most authors have found that single doses of adrenal steroids do not affect gastric secretion, it seemed unlikely

that transient reductions in endogenous steroid production would either. However, it must first be demonstrated whether the adrenal-inhibitor drug used has any direct secretagogue or inhibitor effect on gastric secretion. The first part of this thesis deals with the effect of single intravenous injections of metyrapone on the output of acid and pepsin from canine Heidenhain pouches in response to (1) ingestion of a meal of meat, (2) subcutaneous mecothene and (3) subcutaneous histamine.

Repeated administration of adrenocortical steroids, over a number of days, has generally been accompanied, in most reports, by an increase in gastric HCl output and Section II describes the effect on acid, pepsin and electrolyte output from Heidenhain pouches when metyrapone was given orally over a prolonged period, and the reversal of its action obtained by the simultaneous administration of cortisone.

In Section III, histamine dose-response curves are presented before, during and after metyrapone and also during cortisone administration in an attempt to discover a possible site and mode of action of steroids on gastric acid secretion.

The validity and significance of the experimental results is carefully examined in the General Discussion, and, drawing on conclusions reached earlier in this thesis in the critical review of the literature, a reasoned explanation of the action of adrenocortical steroids on gastric secretion is presented.

The experiments described in this thesis were spread over some three-and-a-half years due, in part, to difficulty in obtaining supplies of metyrapone. Accordingly, several different batches of metyrapone were used (Table III) and are distinguished in the description of the experiments by the manufacturer's code number in brackets after the preparation's chemical name. There is no reason to suspect any difference in basic chemical composition or potency in different batches as each was tested by the manufacturer, but such a possibility cannot be excluded completely.

TABLE III

Preparation	Chemical Description
SU 4885 injection	Metyrapone bitartrate
SU 4885 tablets	Metyrapone base
SU 8874 tablets	Metyrapone base
SU 8874 capsules 'A'	Metyrapone dihydrochloride
Metopirone capsules	Metyrapone base in propylene glycol

GENERAL

METHODS

PREPARATIONS

All the animal experiments described in this thesis were performed on dogs with separated pouches of the stomach of Heidenhain (1878) type. Under general anaesthesia, a full thickness segment of the stomach, constituting about one-third of the greater curvature, was formed into a pouch supplied by a pedicle of short gastric vessels (Figure 4). In the process of dividing the stomach, the vagal nerve supply to the pouch is interrupted and so the pouch is vagally denervated.

The pouches were cannulated with non-irritant nylon or stainless steel cannulae which were brought out through the anterior abdominal wall in the mammary line.

During the shorter experiments, the dogs were loosely tethered in a dog-stand and pouch secretion was collected via the cannula into graduated centrifuge tubes (Figure 5). Complete 24-hour collections were obtained more conveniently by attaching a screw-top polythene bottle to the nylon or metal cannula by means of a water-tight fitting, thus allowing the animal unrestricted movement (Figure 6).

The dogs, which were kept under identical living conditions for the duration of each group of experiments,

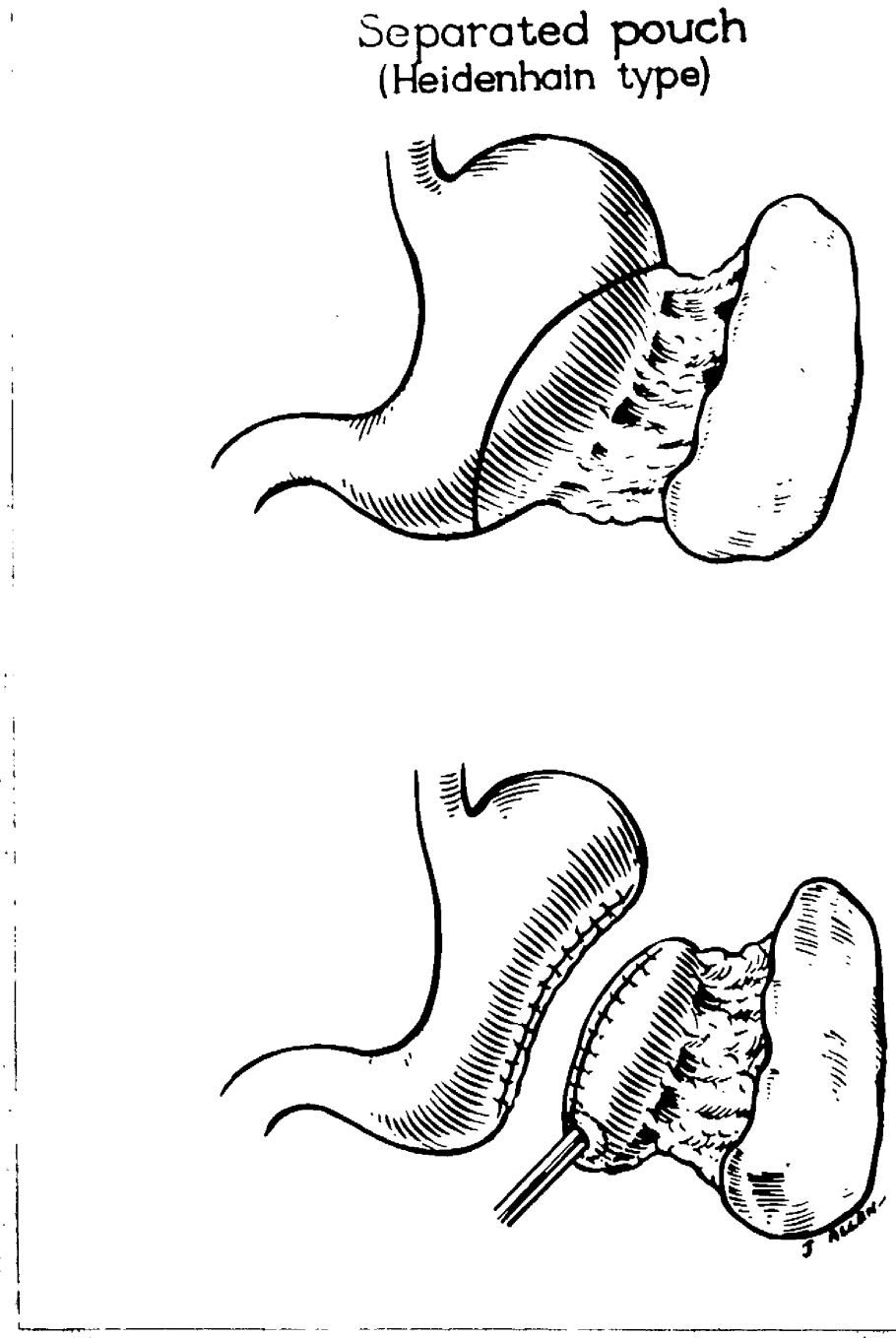


Figure 4 : Heidenhain pouch.

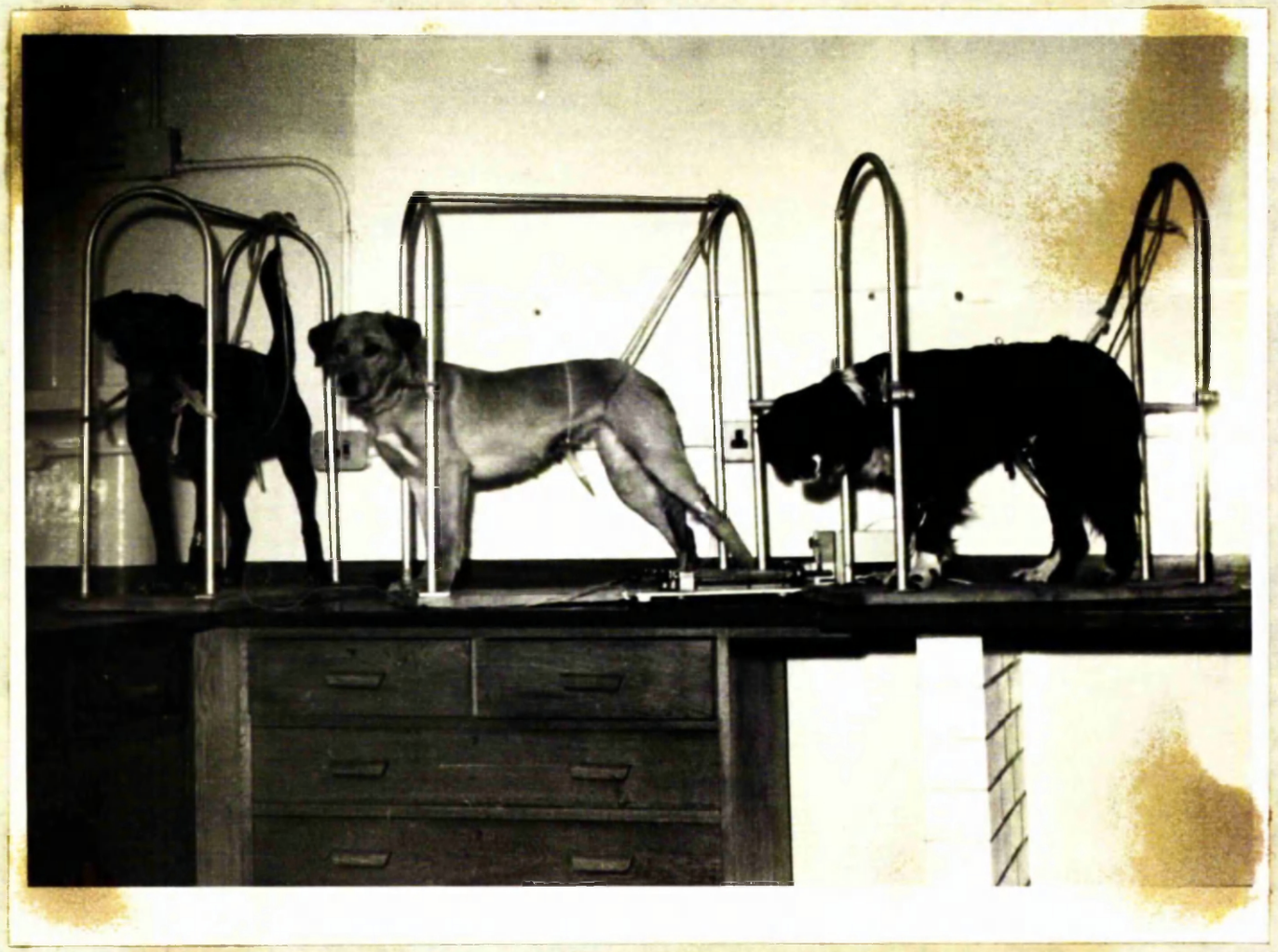


Figure 5 : Three dogs undergoing continuous intravenous histamine infusion by means of a Palmer slow injection apparatus fitted with three syringes. Gastric juice from the Heidenhain pouches is being collected in graduated centrifuge tubes.

Figure 6 : Method of collection of 24-hour output of secretion from Heidenhain pouch into screw-top polythene bottle.

were fed a fixed amount of a well-balanced diet each day. Times and duration of daily exercise were kept as constant as possible. Initially the animals all weighed between 10 and 15 kg. and their weight remained within plus or minus 1 kg. during the complete series of experiments.

METHODS OF ANALYSIS

Acidity: Two methods were used:

(a) In the experiments in Sections I and II, only free acid was estimated. Duplicate aliquots of gastric juice were titrated with decinormal sodium hydroxide, using Topfer's solution (p-dimethyl amino-azo benzene) as indicator. The end-point was when the mixture turned canary yellow, corresponding to a pH of 3.5.

(b) In the experiments in Section III, total acidity was measured. Duplicate samples of 2 ml. gastric juice were titrated with N/10 NaOH to an end-point of pH 7.0, using a glass electrode coupled to an E.I.L. 23a pH meter which has an accuracy of $\pm$ 0.05 of a pH. Magnetic stirring was employed.

In dealing with specimens of strong acidity, both methods give comparable results with an accuracy of ± 1 m.Eq/l but titration to pH 7.0 gives a truer indication of the amount of acid present when the initial pH of gastric juice is greater than pH 2.5 or so. This is illustrated by the three titration curves shown in Figure 7. The gentle slope of the titration curve in the specimen with the highest initial pH is due to the presence of mucus and other buffering substances. A comparison of the pH of a number of specimens with the acid content measured by titration to neutrality (Figure 8) indicates the superiority of titration over simple pH measurements in highly acid juice.

Sodium and Potassium

The sodium and potassium content in gastric juice samples was determined with an EEL flame-photometer (Evans Electro-Selenium Ltd.). Calibration curves were drawn using separate standard dilutions of known strength sodium and potassium chloride solutions. For sodium estimations the sample was diluted 1/1,000 with de-ionised water and standards of 0.1 m.Eq/l. and 0.2 m.Eq/l. sodium chloride were used to set the meter range each day. For potassium chloride, a 1/50 dilution of the sample in de-ionised water was used. Duplicate estimations were carried out

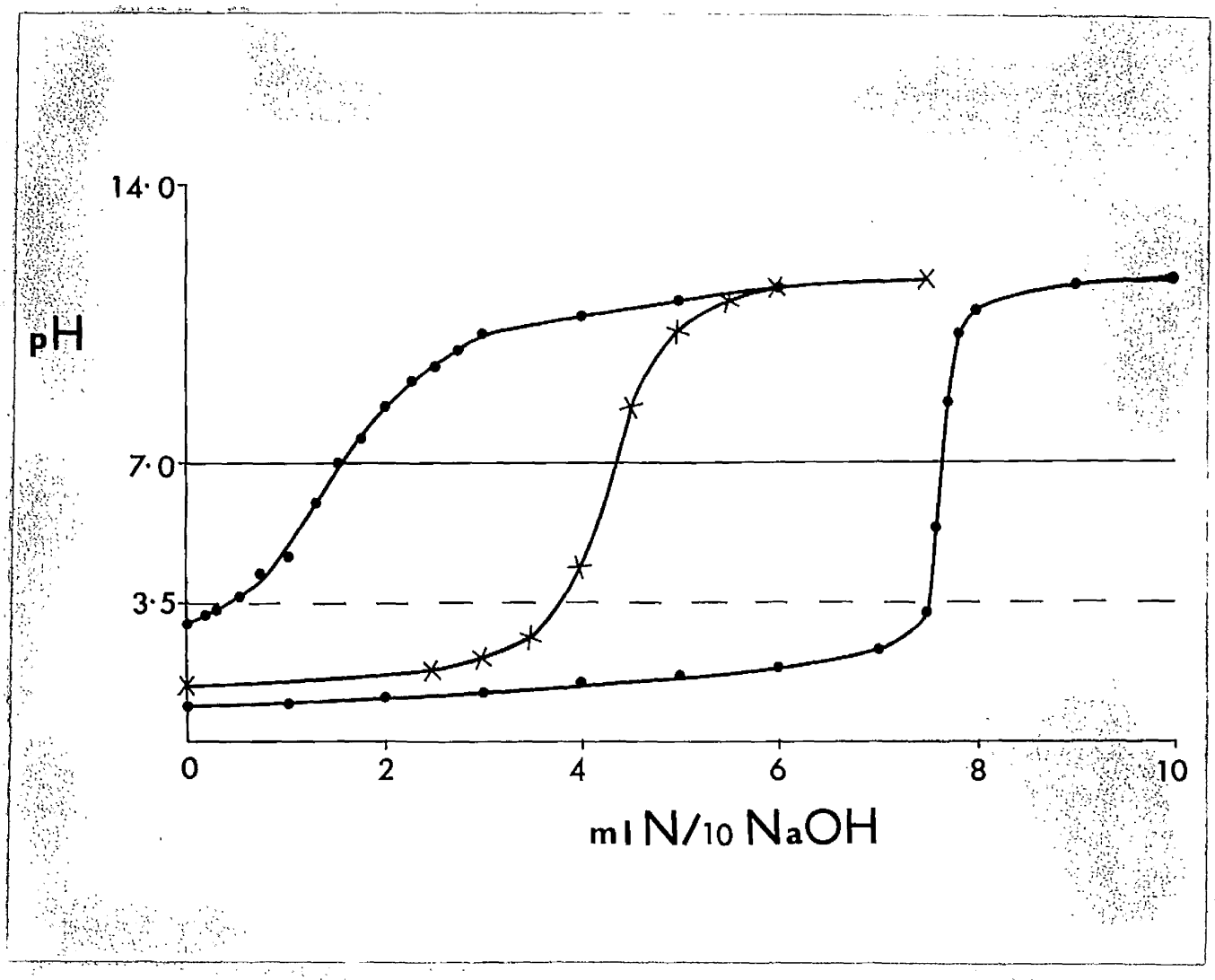


Figure 7 : Titration curves of three specimens of gastric juice of different initial pH.

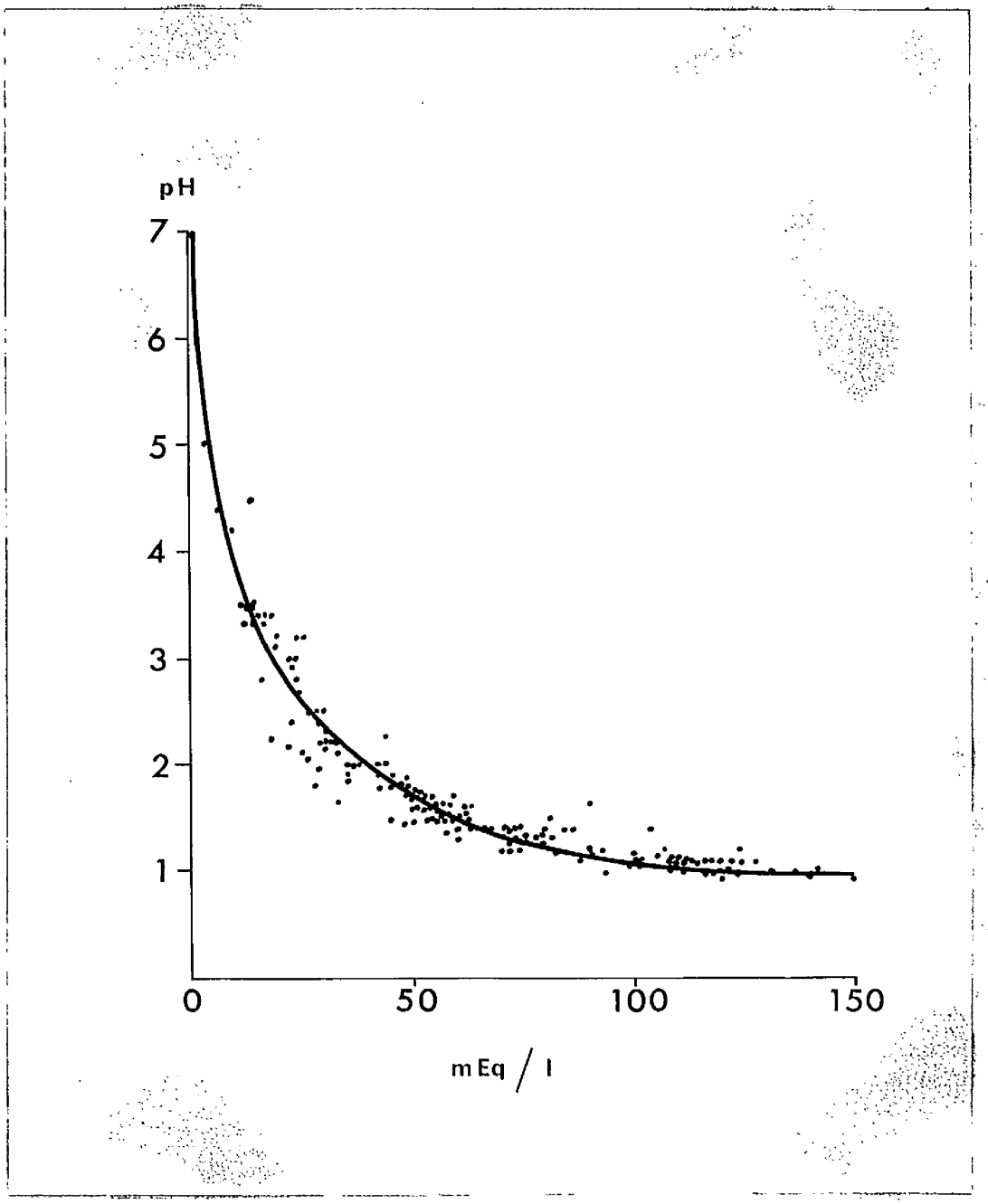


Figure 8 : Graph of pH against titrated acidity.

on each sample and the coefficient of variation of the method was 1.4 per cent for sodium and 2.6 per cent in the case of potassium (Table IV).

Chloride

Two methods were used at different times during this investigation:

(a) 1.0 ml. of sample was diluted to 10 ml. with distilled water and duplicate 2.0 ml. aliquots were titrated with mercuric nitrate solution using diphenylcarbazone as indicator (Schales and Schales, 1941).

(b) In the experiments in Section III, total chlorides were determined by coulometric titration using an EEL chloride meter (Evans Electro-Selenium Ltd.). 0.2 ml. gastric juice was mixed with 13.8 ml. citrate buffer and 6 drops of gelatin indicator solution in a glass beaker which was placed on the platform of the EEL chloride meter. The chloride concentration was read off directly in mEq./litre. The meter was checked daily with a standard solution of sodium chloride which was made up fresh at weekly intervals. The coefficient of variation using this particular method was 1.1 per cent (Table IV).

In order to obtain some estimate of the errors involved in the electrolyte estimations, the sodium, potassium and chloride content of one standard samples of gastric juice was tested 30 times. The estimations were all done by the same person on the same day and the results are shown in Table IV.

Pepsin

The concentration of pepsin in a 1 in 2 dilution of gastric juice was determined by Hunt's (1948) method in which the ability of juice to digest human plasma is estimated. The released amino-acids are estimated colorimetrically by the Folin and Ciocalteau reaction and the results expressed in milligrams tyrosine equivalents per millilitre of juice. From these estimations the output of pepsin in milligram tyrosine equivalents per 24 hours was calculated.

TABLE IV : ACCURACY OF SODIUM, POTASSIUM AND CHLORIDE ESTIMATIONS

	Sodium mEq./l.	Potassium mEq./l.	Chloride mEq./l.
Standard	135	5.0	135
Test 1	138	5.0	138
2	138	5.2	135
3	134	5.1	133
4	138	5.2	136
5	138	5.0	135
6	134	5.0	136
7	136	5.2	134
8	136	5.2	137
9	138	5.0	135
10	138	5.1	135
11	136	5.1	138
12	136	5.1	134
13	134	5.0	137
14	136	5.0	133
15	136	4.9	136
16	136	4.9	135
17	134	4.8	133
18	132	5.0	137
19	134	5.0	136
20	134	5.0	135
21	136	4.9	136
22	134	4.8	137
23	138	5.0	135
24	134	4.9	134
25	138	4.9	138
26	136	5.0	133
27	132	5.2	135
28	132	5.1	132
29	134	5.0	136
30	134	4.9	133
Mean	135.33	5.02	135.2
Standard Deviation	1.9	0.12	1.5
Coefficient of Variation	1.4	2.3	1.1

S E C T I O N I

THE EFFECT OF SINGLE INJECTIONS OF METYRAPONE (SU 4885 TH ON GASTRIC SECRETION IN DOGS

ON GASTRIC SECRETION IN DOGS

PROCEDURE

The effect of the administration of metyrapone (SU 4885) by single intravenous injection and continuous intravenous infusion on the secretion of separated (vagally denervated) gastric pouches under stimulation by a meal of meat, a parasympathomimetric drug and histamine was determined in 3 dogs.

Three tests and 3 controls were done on each dog and a statistical analysis of the differences of the results obtained with and without metyrapone was carried out.

METHOD

These tests were carried out on 3 mongrel bitches weighing between 10 and 12 kg., on which Heidenhain pouches had been constructed at least 6 weeks previously. The dogs were kept under identical living conditions and fed on standard kennel rations. Tests were carried out after a 16-hour fast and only when there was no free acid in the basal juice. Each dog acted as its own control and each test was repeated 3 times with an interval of at least one day between tests.

The effect of intravenous injection of metyrapone bitartrate (SU 4885) (15 mg. per kg. body weight) on the secretory response of the pouches to meat, to a para-sympathomimetric drug and to histamine was determined.

Meal of Meat

After the ingestion of half-a-pound of proprietary tinned meat, juice was collected at 15-minute intervals for 4 hours. In 3 tests on each dog an intravenous injection of metyrapone bitartrate in 0.9 per cent saline (15 mg. per kg. body weight) was given 15 minutes after the meat had been eaten, while in 3 further control tests a similar volume of 0.9 per cent saline alone was given.

Mecothane

In these tests, pouch secretion was stimulated by the continuous subcutaneous infusion of 6 mg. Mecothane (carbaminoyl-β-methyl choline chloride) per hour at a constant rate, using a Palmer injection apparatus. The injection was continued for 4 hours during which juice was collected from the pouch at 15-minute intervals. In 3 tests on each dog, metyrapone bitartrate in saline was injected intravenously immediately the infusion started and in 3 control tests saline alone was injected.

Histamine

The secretion of juice from the pouch was stimulated by a continuous subcutaneous infusion of histamine acid phosphate 25 µg. base per kg. body weight per hour, by a Palmer injection apparatus. When a steady rate of acid secretion had been reached, usually after 1½ hours to 2 hours, the juice from the pouch was collected for six 15-minute periods. An intravenous injection of metyrapone bitartrate, 15 mg. per kg. body weight, was then given and collections continued for nine 15-minute periods. Cortisol hemisuccinate, 100 mg., was then injected intravenously and a final six 15-minute collections made. Tests were carried out on 3

occasions on each dog, while in 3 further control experiments 0.9 per cent saline was injected in place of the metyrapone and cortisol was omitted.

In all the tests, juice was collected at 15-minute intervals, the volume measured in millilitres and the concentration of free acid determined by titration with N/10 sodium hydroxide, using Topfer's solution (p-dimethyl amino-azo benzene) as indicator. The concentration of pepsin in a 1 in 2 dilution of juice in distilled water was estimated by Hunt's method (1948). From these estimations the outputs of acid in milliequivalents and of pepsin in milligrams tyrosine equivalents per 15-minute period were calculated.

Logarithmic transformation of the total output of acid and of pepsin for the 4 hours of the meat and Mecothane responses allowed statistical comparison between metyrapone and control tests by an analysis of variance.

CONTINUOUS INTRAVENOUS INFUSION OF METYRAPONE (SU 4885)

In 3 fasting dogs with Heidenhain pouches, histamine acid phosphate in a dose of 25 µg. of base per kg. body weight per hour was infused subcutaneously by a Palmer constant rate injection apparatus over a 5-hour period. Juice was collected every 15 minutes and its volume and free acidity measured as before. After 2½ hours, when a steady rate of acid secretion had been obtained, metyrapone bitartrate was administered by continuous intravenous injection while the histamine infusion continued; 15-minute collections of juice were continued for another 2½ hours. The dose of metyrapone infused during this period was varied in different experiments from 10 mg. to 120 mg. per kg. body weight per hour.

RESULTS

SINGLE INTRAVENOUS INJECTION OF METYRAPONE (SU 4885)

The results are for three dogs.

Response to Meat

The intravenous injection of 15 mg. metyrapone bitartrate per kg. body weight was without significant effect on the volume of juice or the outputs of acid or pepsin from the separated pouches in response to ingestion of a meal of meat. The mean outputs of acid and pepsin for each dog are shown in Figures 9 and 10 and the mean total outputs during the 4 hours of the tests in Table V.

The absence of significant effect was confirmed by an analysis of variance of the mean logarithms of the outputs of juice, acid and pepsin in the control tests in all three dogs and in those carried out after metyrapone.

Response to Mecothane

The intravenous injection of metyrapone bitartrate (15 mg. per kg. body weight) was similarly without significant effect on the output of juice, acid and pepsin secreted in response to a continuous subcutaneous infusion of Mecothane (Figures 11, 12 and Table VI). This also was confirmed by an analysis of variance of the logarithms of the outputs.

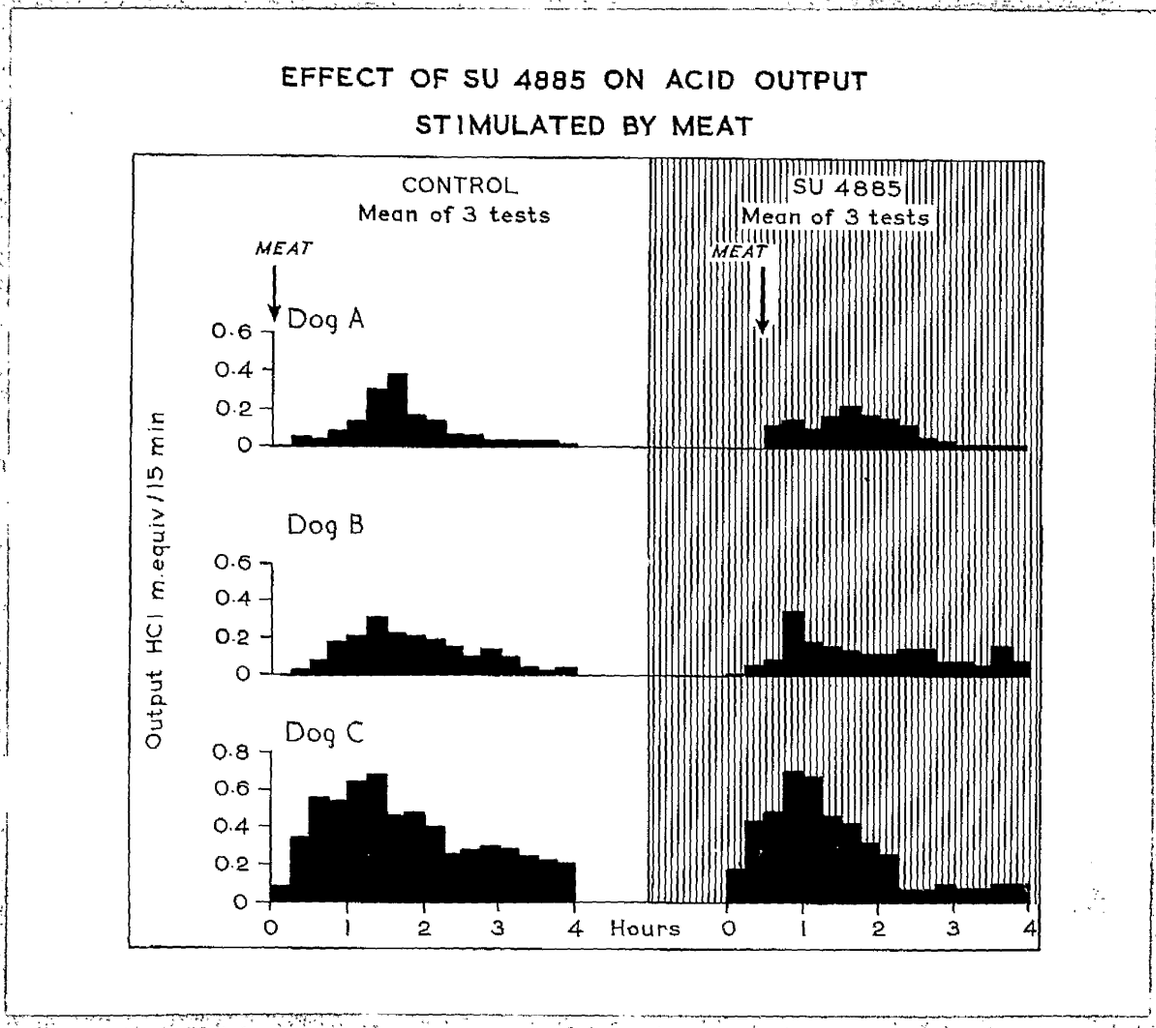


Figure 9 : Effect of intravenous injection of motyrepone bitartrate (15 mg. per kg. body weight) on the output of acid from Heidenhain pouches following a meal of meat.

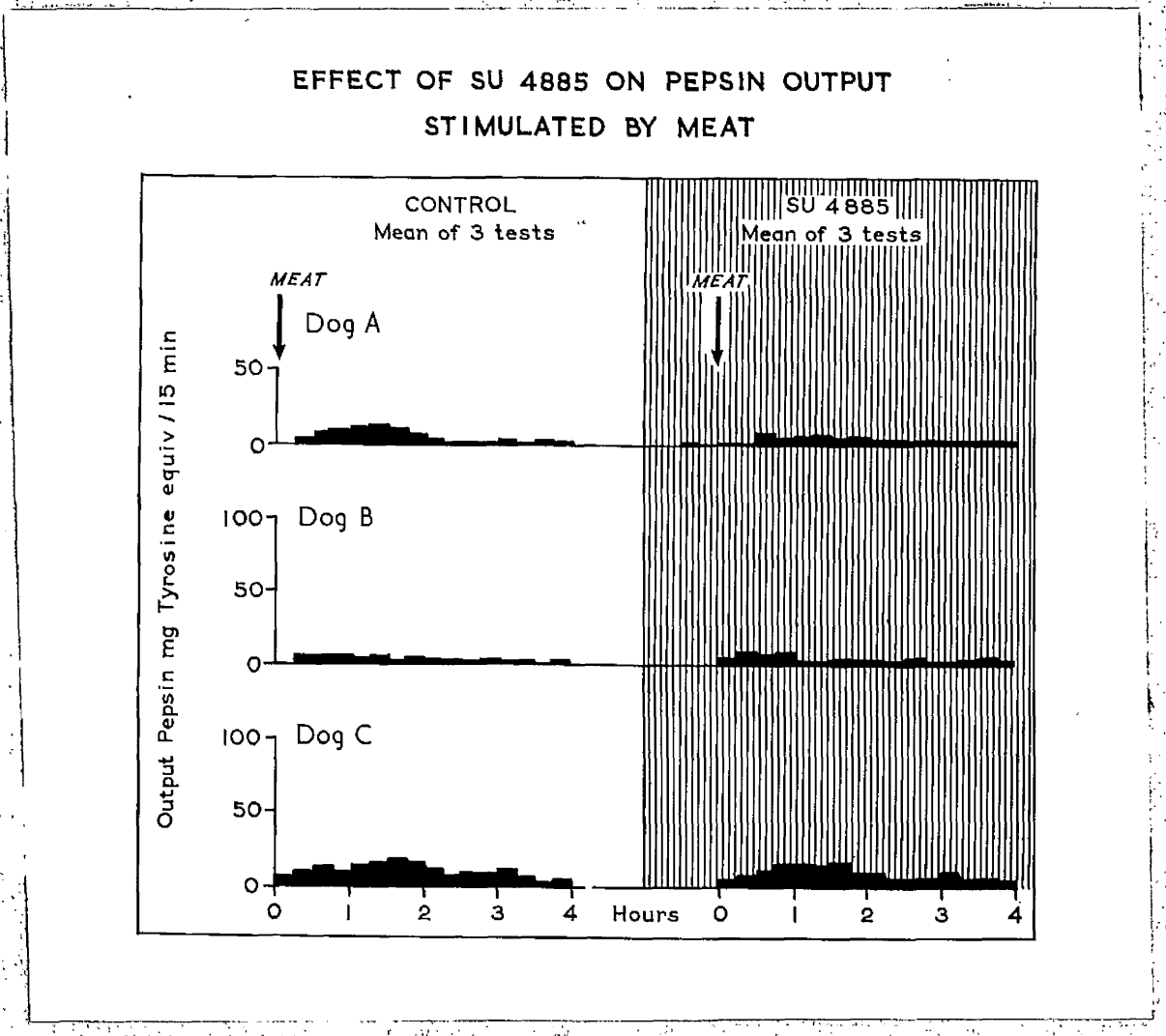


Figure 10 : Effect of intravenous injection of metyrapone bitartrate (15 mg. per kg. body weight) on the output of pepsin from Heidenhain pouches following a meal of meat.

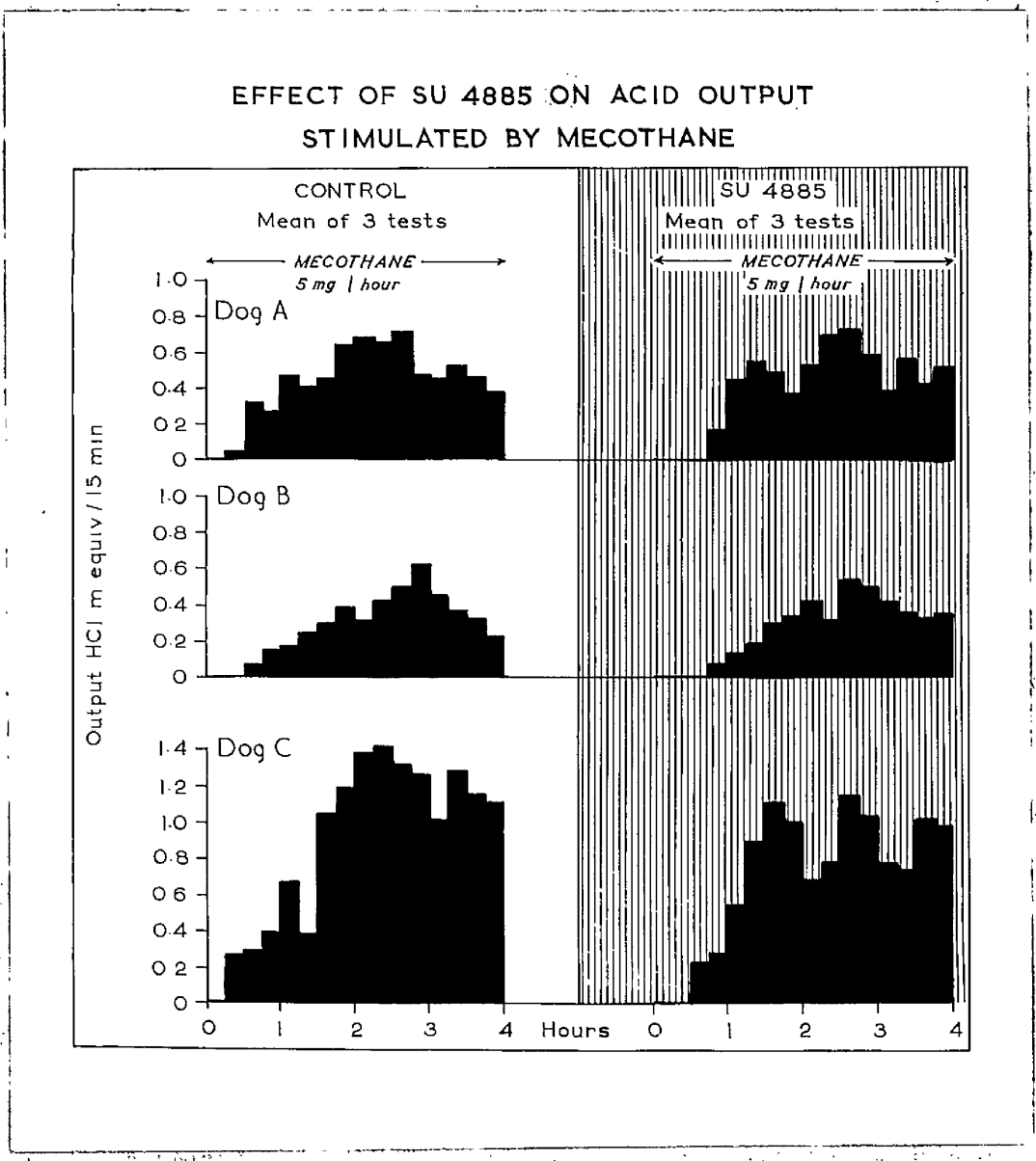


Figure 11 : Effect of intravenous injection of betazopone bitartrate (15 mg. per kg. body weight) on the output of acid from Heidenhain pouches stimulated to secrete by a continuous subcutaneous infusion of 5 mg. Mecothane per hour.

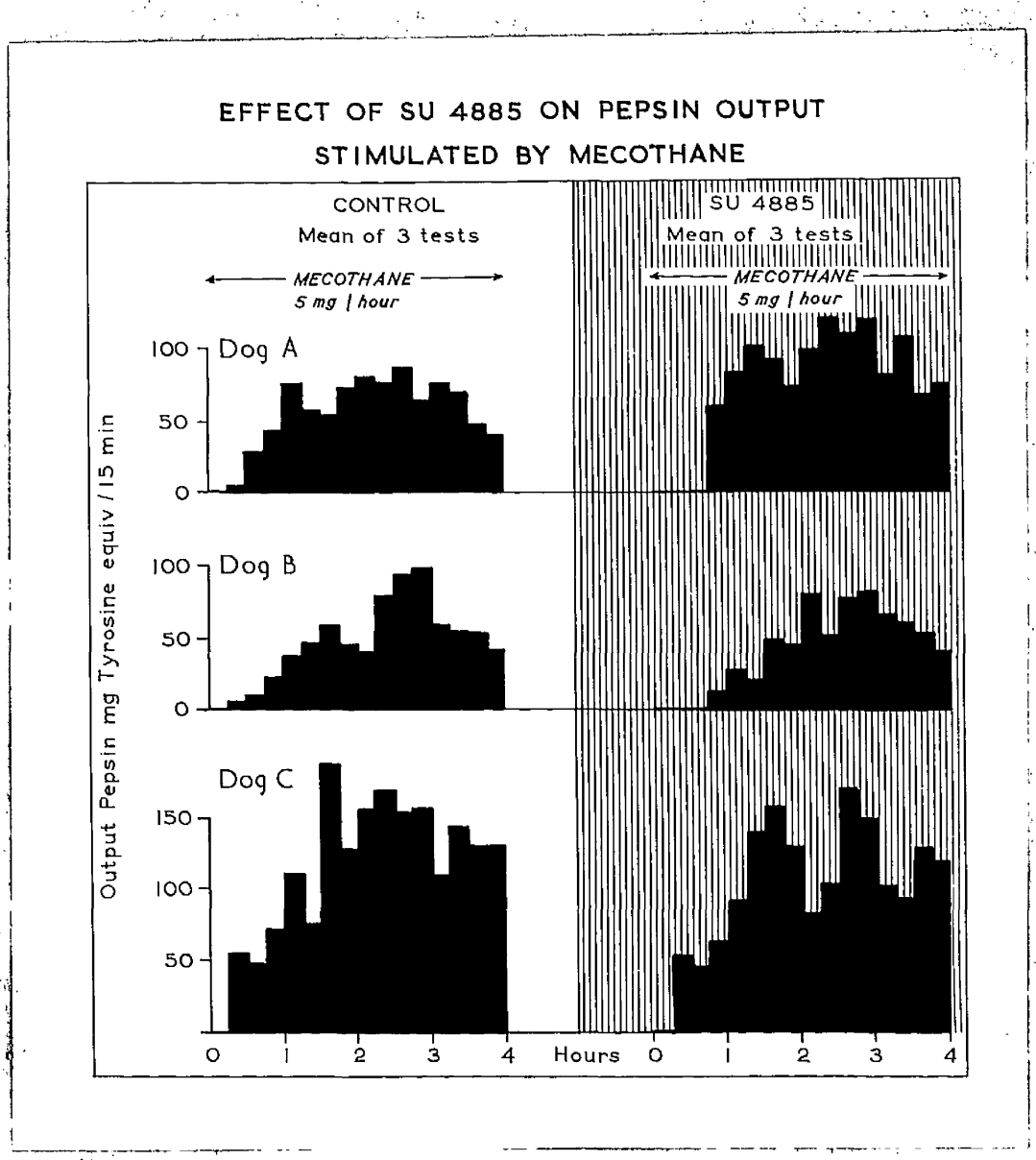


Figure 12 : Effect of intravenous injection of butyrophenone bituroxate (35 mg. per kg. body weight) on the output of pepsin from Heidenhain pouches stimulated to secrete by a continuous subcutaneous infusion of 5 mg. Mecothane per hour.

TABLE V : EFFECT OF METYRAPONE (SU 4885) ON OUTPUTS OF JUICE, ACID AND PEPSIN FROM SEPARATED POUCHES IN THREE DOGS AFTER STANDARD MEAL

Dog	Volume Juice (ml./4 hrs.)		Output Acid (mEq/4 hrs.)		Output Pepsin (mg.tyrosine/4hrs.)	
	Control	Metyrapone	Control	Metyrapone	Control	Metyrapone
A	15.0	13.4	1.490	1.392	66.7	89.5
B	22.7	28.9	1.003	1.983	44.1	66.7
C	42.2	34.5	5.472	4.497	168.6	132.8

TABLE VI : EFFECT OF METYRAPONE (SU 4885) ON OUTPUTS OF JUICE, ACID AND PEPSIN FROM SEPARATED POUCHES IN THREE DOGS STIMULATED BY CONTINUOUS SUBCUTANEOUS INFUSION OF MECOTHANE

Dog	Volume Juice (ml./4 hrs.)		Output Acid (mEq/4 hrs.)		Output Pepsin (mg.tyrosine/4hrs.)	
	Control	Metyrapone	Control	Metyrapone	Control	Metyrapone
A	57.5	54.5	6.703	6.286	860.8	1104.2
B	49.8	46.6	4.468	4.212	707.2	638.9
C	103.3	88.1	14.403	11.285	1859.6	1585.5

Histamine

Neither the injection of metyrapone bitartrate (15 mg. per kg. body weight) nor that of cortisol (100 mg.) had any effect on the secretory response to a continuous subcutaneous infusion of histamine acid phosphate (25 µg. base per kg. body weight per hour). The results of these tests are shown graphically in Figures 13 and 14. The mean outputs of juice, acid and pepsin for six 15-minute collections before metyrapone (Table VII, columns I), nine 15-minute collections after metyrapone (Table VII, columns II) and six 15-minute collections after cortisol (Table VII, columns III) were calculated for each dog and compared with those of the corresponding periods of the control tests. No obvious difference was noted (Table VII).

Response to Histamine Plus Continuous Metyrapone (SU 4885)

In all 3 dogs, the continuous subcutaneous infusion of histamine acid phosphate in a dose of 25 µg. of base per kg. body weight per hour produced a step-wise increase in the 15-minute output of acid from the Heidenhain pouches until a plateau was reached after 1½ hours to 2 hours. Secretion of acid continued thereafter at a fairly constant rate for the duration of the histamine infusion except during one

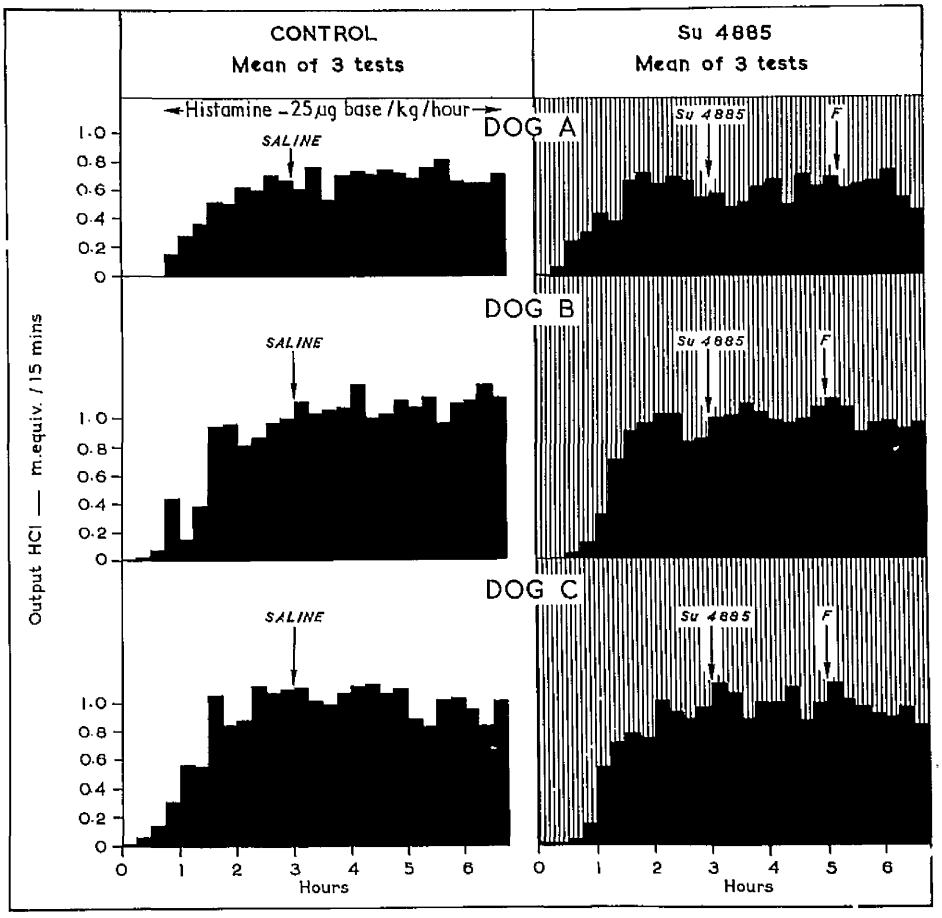


Figure 13 : Effect of intravenous injection of metyrapone bitartrate (15 mg. per kg. body weight) on the output of acid from Heidenhain pouches stimulated by a continuous subcutaneous infusion of histamine acid phosphate (25 µg. base per kg. body weight per hour). 100 mg. cortisol was injected intravenously at point 'F'.

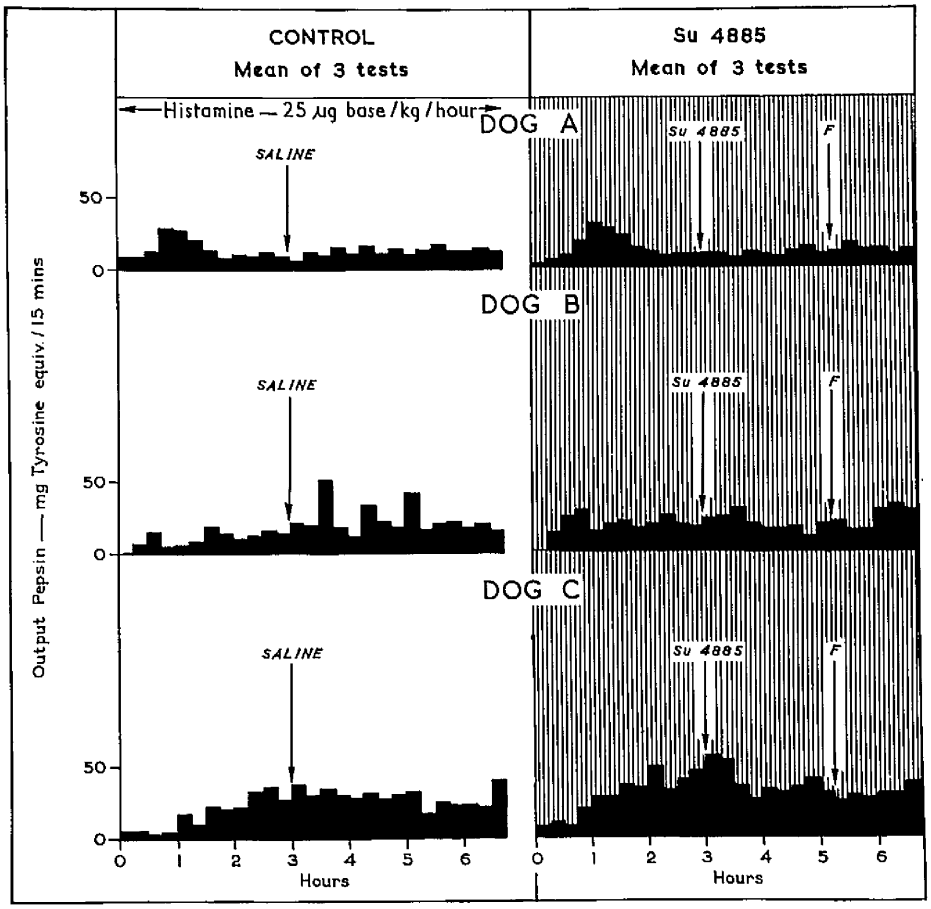


Figure 14 : Effect of intravenous injection of metyrapone bitartrate (15 mg. per kg. body weight) on the output of pepsin from Heidenhain pouches stimulated by a continuous subcutaneous infusion of histamine acid phosphate (25 µg. base per kg. body weight per hour). 100 mg. cortisol was injected intravenously at point 'F'.

experiment on one dog. The output of acid from the stomach pouches was not affected by the intravenous infusion of metyrapone bitartrate 10, 30 or 60 mg. per kg. body weight per hour during the period 2½ hours to 5 hours after the start of the histamine infusion. The injection of metyrapone at a rate of 120 mg. per kg. body weight per hour did not influence the pattern of acid response to histamine in 2 of the dogs but had profound effects in the third animal (Figure 15). Half-an-hour after the start of the metyrapone infusion (120 mg. per kg. body weight per hour), there was a slight fall in acid output from the pouch and exactly 60 minutes after starting the metyrapone the dog collapsed with severe hypotension. The infusion was stopped and intravenous cortisol given with satisfactory improvement in the dog's clinical state. The experiment was not repeated in this dog but no similar or other side-effects were noted when identical doses of metyrapone were administered to the other 2 dogs.

TABLE VII : EFFECT OF METYRAPONE (SU 4885) AND CORTISOL ON SECRETION OF JUICE, ACID AND PEPSIN FROM SEPARATED POUCHES IN THREE DOGS STIMULATED BY CONTINUOUS SUBCUTANEOUS INJECTION OF HISTAMINE ACID PHOSPHATE

Dog	Volume Juice (ml./15min.)						Output Acid (mEq./15 min.)						Output Pepsin (mg.tyrosine/15 min.)					
	Control			Metyrapone			Control			Metyrapone			Control			Metyrapone		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
A	4.4	5.0	5.0	4.9	4.7	4.9	0.603	0.684	0.700	0.653	0.596	0.605	8.7	10.1	11.8	11.2	9.1	11.9
B	7.1	7.3	7.5	6.9	7.3	7.0	0.919	1.076	1.109	0.924	1.018	0.954	12.8	23.6	16.7	17.6	19.2	22.3
C	6.6	6.7	6.1	6.4	7.2	6.5	0.996	1.028	0.929	0.881	1.012	0.930	26.2	29.5	24.0	39.9	37.9	29.1

I = mean of 6 collections before metyrapone

II = mean of 9 collections after metyrapone

III = mean of 6 collections after cortisol

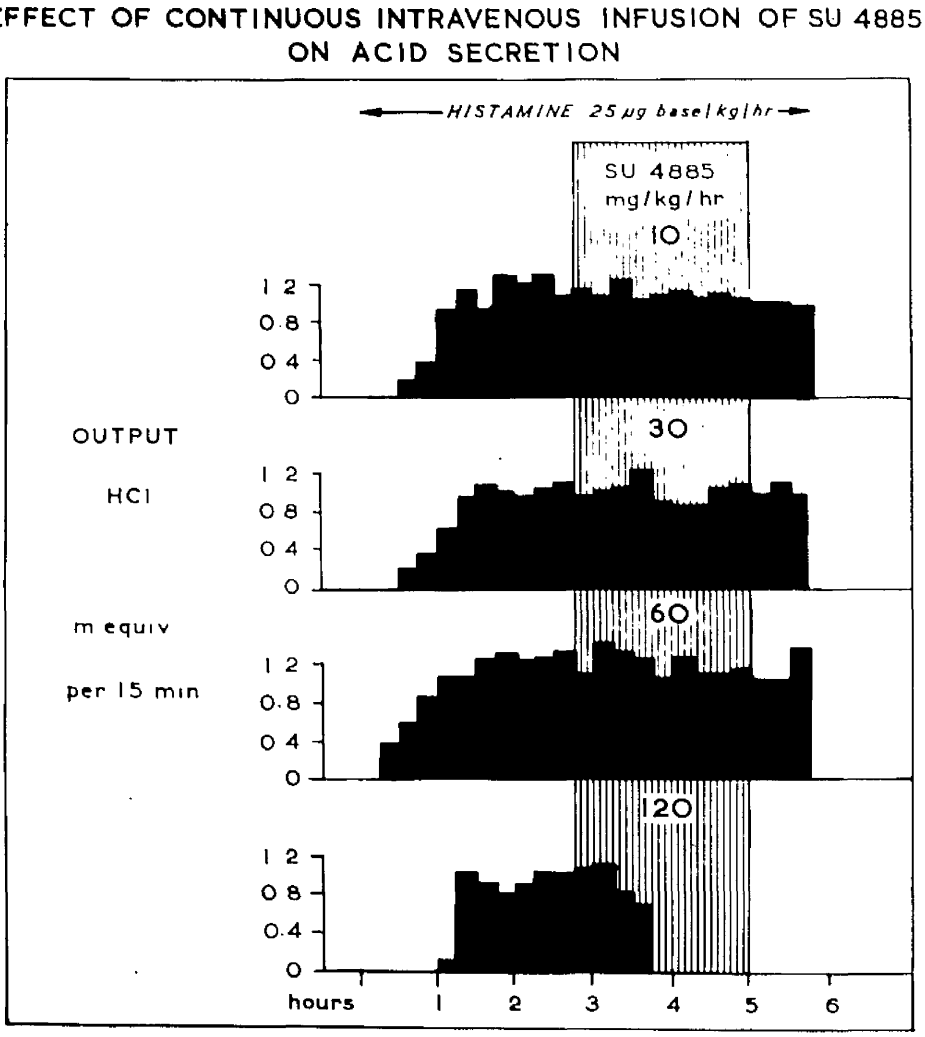


Figure 15 : Effect of continuous intravenous infusion of metyrapone bitartrate, in various doses, on the output of acid from a Heidenhain pouch stimulated by a continuous intravenous infusion of histamine to give secretory rate 40 per cent of maximum.

CONCLUSIONS

Metyrapone (SU 4885) administered intravenously as a single injection was without effect on the immediate secretory response of canine Heidenhain pouches to the ingestion of meat or subcutaneous Mecsthene or histamine.

In none of the 8 dogs was there any alteration in the volume of gastric juice secreted or in the concentration and output of acid after metyrapone (SU 4885) as compared with the saline control experiments.

Continuous intravenous infusion of metyrapone (SU 4885), even in doses sufficient to cause collapse of one dog, was also without significant effect on the secretion of acid from Heidenhain pouches stimulated by a steady continuous subcutaneous infusion of a sub-maximal dose of histamine.

S E C T I O N IX

THE EFFECT OF PROLONGED ADMINISTRATION OF ORAL METYRAPONE ON SECRETION FROM CANINE HEIDENHAIN POUCHES

PROCEDURE

In view of the lack of effect on secretion from Heidenhain pouches shown by short-term administration of metyrapone, the drug was given to 6 further dogs for a minimum of 10 days.

In 4 dogs, tablets of metyrapone base (SU 4885) were given and the 24-hour output of acid and pepsin from their Heidenhain pouches was measured during the 10 days preceeding and following metyrapone administration as well as during the actual test period.

A further 2 dogs were given various doses of cortisone in addition to metyrapone base (SU 4885) and the alterations in pattern of secretion from their Heidenhain pouches was followed by analysing the 24-hour output of juice for sodium, potassium and total chloride content as well as for acid and pepsin. Cortisone was also given alone for 10 days, with control periods before and after, and its effect on the secretion of acid and electrolytes in juice from the pouches was studied.

The significance of the differences in secretion of acid and pepsin during the periods of metyrapone and cortisone administration were assessed using Student's 't'

test and correlations between the concentrations of various electrolytes in juice from the pouches were determined by graphical and statistical methods.

METHODS

Group A

In 4 healthy mongrel bitches with separated pouches of the stomach of Heidenhain type, 24-hour collections of gastric juice were made by attaching a polythene bottle to the cannula draining the pouch. The dogs were fed at the same time each day a standard weighed diet containing equal amounts of meat and cereal.

After a control period of 2-3 weeks, crushed tablets of metyrapone base (SU 4885), in a dose of 100 mg. per kg. body weight, were given in milk each morning for 10-14 days. Daily collections of pouch juice were continued for a further 10-14 days after discontinuing the drug.

Group B

In 2 further mongrel bitches with Heidenhain pouches, the experimental procedure was varied. After a preliminary control period of 15 days, crushed tablets of metyrapone base (SU 4885) were given in their feeds in a dose of 100 mg. per kg. body weight per day for 64 days. For the first 13

days of this period, metyrapone was given alone, then cortisone acetate was added in increments - 5 mg. for 5 days, followed by 10 mg. for 10 days, 50 mg. for 8 days, 100 mg. for 8 days and then metyrapone alone for 10 days. After a further control period of 20 days without drugs, 100 mg. cortisone acetate was administered orally once daily for 10 days and collections were continued for a further 23 days thereafter.

Both metyrapone and cortisone acetate were given orally, once daily with foods.

In both groups, the volume of each 24-hour collection of juice was measured in ml. and the specimen was then centrifuged to separate undissolved mucus and epithelial debris which was discarded. The chemical estimations were carried out in duplicate within 2 hours of collecting the juice.

Free acid was measured by titrating duplicate 5.0 ml. samples with decinormal sodium hydroxide solution using Topfer's solution (p-dimethyl amino-azo benzene) as indicator and total chlorides were estimated on duplicate 2 ml. samples of juice by the mercurimetric method of Schales and Schales (1941) using diphenyl-carbazone as indicator.

Sodium and potassium concentrations in each collection were measured by flame-photometry using external standards.

The concentration of pepsin was estimated by Hunt's (1948) method and the 24-hour output from each pouch expressed in milligrams tyrosine equivalents per 24 hours.

The concentrations of acid and electrolytes were originally expressed in milliequivalents per litre but in certain of the graphs the results are shown in millinormals as being more correct although, to all practical purposes, the two units of measurement are the same.

The means of the logarithms of the outputs of acid and pepsin in the main phases of the experiment for each dog were analysed statistically by Student's 't' test. Correlations between sodium, potassium, chloride and hydrogen ion concentrations were worked out partly on a desk calculating machine and partly on a HEC computer (Glasgow University).

RESULTS

Acid Studies : Group A

In 3 of the first 4 dogs studied, free acid was present in the gastric juice during the control period. In these 3 dogs, the ingestion of metyrapone base (in tablet form) resulted in a reduction in the output of acid from the Heidenhain pouch (Figure 16 and Table VIII). The difference between the means of the logarithms of the outputs of acid during the control period and that in which metyrapone base (SU 4885) was ingested were statistically significant in each animal (Table IX).

One of these 3 dogs (Dog 8) died from peritonitis after retraction of the pouch during the experiment. In the other 2 dogs the acid output returned to the control level when the metyrapone base was discontinued, and the difference between the output of acid during the period of drug ingestion and that following its withdrawal was again significant (Table IX). The fourth dog had no free acid in its gastric juice during any phase of the experiment.

The inhibition of the output of acid was largely due to a reduction in the concentration of acid in the secreted juice (Figure 16) and the slight reduction in the volume of

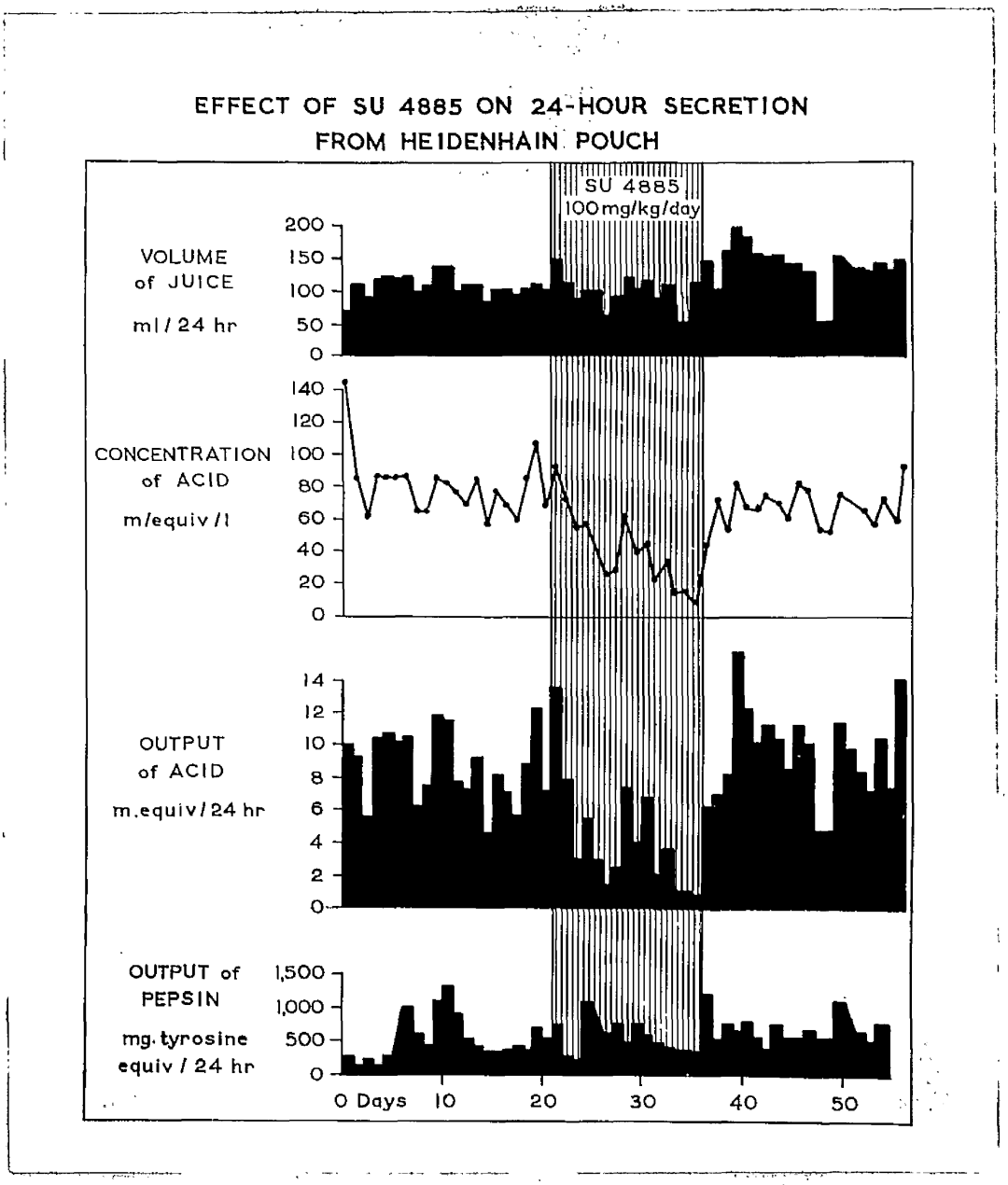


Figure 16 : Effect of oral administration of metyrapone base (SU 4885) (100 mg. per kg. body weight per day) on 24-hour secretion from a Heidenhain pouch.

juice in the 3 dogs which secreted free acid was insignificant (Table X). In the fourth dog (Dog F), which had no free acid in the gastric juice, the volume was increased during the period of metyrapone base administration.

TABLE VIII : EFFECT OF METYRAPONE (SU 4885) ON 24-HOUR SECRETION FROM SEPARATED POUCHES IN FOUR DOGS

Dog	Metyrapone	No. of Collections	Volume Juice (ml/24 hr)	Concentration HCl (mEq/24 hr)	Output HCl (mEq/24 hr)	Output Pepsin (mg.tyrosine eq/24 hr)
D	Before	21	113	77	9.1	517
	During	14	100	39	4.9	528
	After	19	141	64	9.5	659
E	Before	10	48	62	2.9	465
	During	15	47	39	1.9	435
	After	17	53	58	3.4	378
F	Before	21	34	0	0	248
	During	15	50	0	0	505
	After	18	44	0	0	213
G	Before	6	217	107	23.2	488
	During	7	184	84	15.7	763
	After	-	-	-	-	-

TABLE IX : ANALYSIS OF ACID OUTPUTS

Dog	Metyrapone	Mean Log. Acid Output	Mean Difference Log.	P
D	Before	0.932	0.423 ± 0.045 +	<0.001
	During	0.509	0.445 ± 0.046	<0.001
	After	0.954		
E	Before	0.437		
	During	0.267	0.170 ± 0.030	<0.001
	After	0.459	0.192 ± 0.081	<0.05
F	Before	-	-	-
	During	-	-	-
	After	-	-	-
G	Before	1.350	0.169 ± 0.071	<0.05
	During	1.180		

+ = Standard error of means

TABLE X : ANALYSIS OF 24-HOUR VOLUME OF JUICE

Dog	Metyrapone	Mean Volume Juice Dog (ml/24 hr.)	Mean Difference	P
D	Before	2.035	0.053 ± 0.036	>0.1
	During	1.982		
	After	2.142	0.160 ± 0.037	<0.01
E	Before	1.676	0.010 ± 0.027	>0.7
	During	1.666		
	After	1.688	0.022 ± 0.051	<0.6
F	Before	1.519	0.173 ± 0.032	<0.001
	During	1.692		
	After	1.669	0.023 ± 0.027	>0.3
G	Before	2.322	0.065 ± 0.060	<0.3
	During	2.257		
	After	-		

TABLE Xe : ANALYSIS OF 24-HOUR OUTPUT OF PEPSIN

Dog	Metyrapone	Mean Output Pepsin (log. mg. tyrosine eq./24 hr.)	Mean Difference	P
D	Before	2.631		
	During	2.681	0.050 ± 0.093	>0.4
	After	2.822	0.141 ± 0.095	>0.1
E	Before	2.645		
	During	2.608	0.037 ± 0.073	>0.6
	After	2.549	0.059 ± 0.057	>0.3
F	Before	2.347		
	During	2.656	0.309 ± 0.074	<0.01
	After	2.295	0.361 ± 0.077	<0.01
G	Before	2.664		
	During	2.866	0.202 ± 0.077	<0.05

Acid Studies : Group B

In Dogs H and J, there was an even more striking reduction in the output of acid during the period when metyrapone base (SU 4885) was administered by itself (Figures 17 and 18) but the acid concentration and output increased step-wise with incremental doses of cortisone. However, while 50 mg. cortisone acetate daily was sufficient to correct the inhibitory effects of metyrapone on acid secretion in Dog H, the output of acid in Dog J had still not returned to within its normal range when the cortisone was discontinued after 8 days at 100 mg. per day.

Acid output fell once more on stopping the cortisone but the reduction was spread over 4-5 days suggesting a persistence of cortisone action during this time.

Cortisone acetate 100 mg. daily, given on its own, produced a significant increase in the volume of 24-hour secretion in both Dogs H and J as well as in acid concentration and output (Figures 17 and 18) and the return to control levels of acid output from both pouches was delayed for nearly a week after stopping the drug.

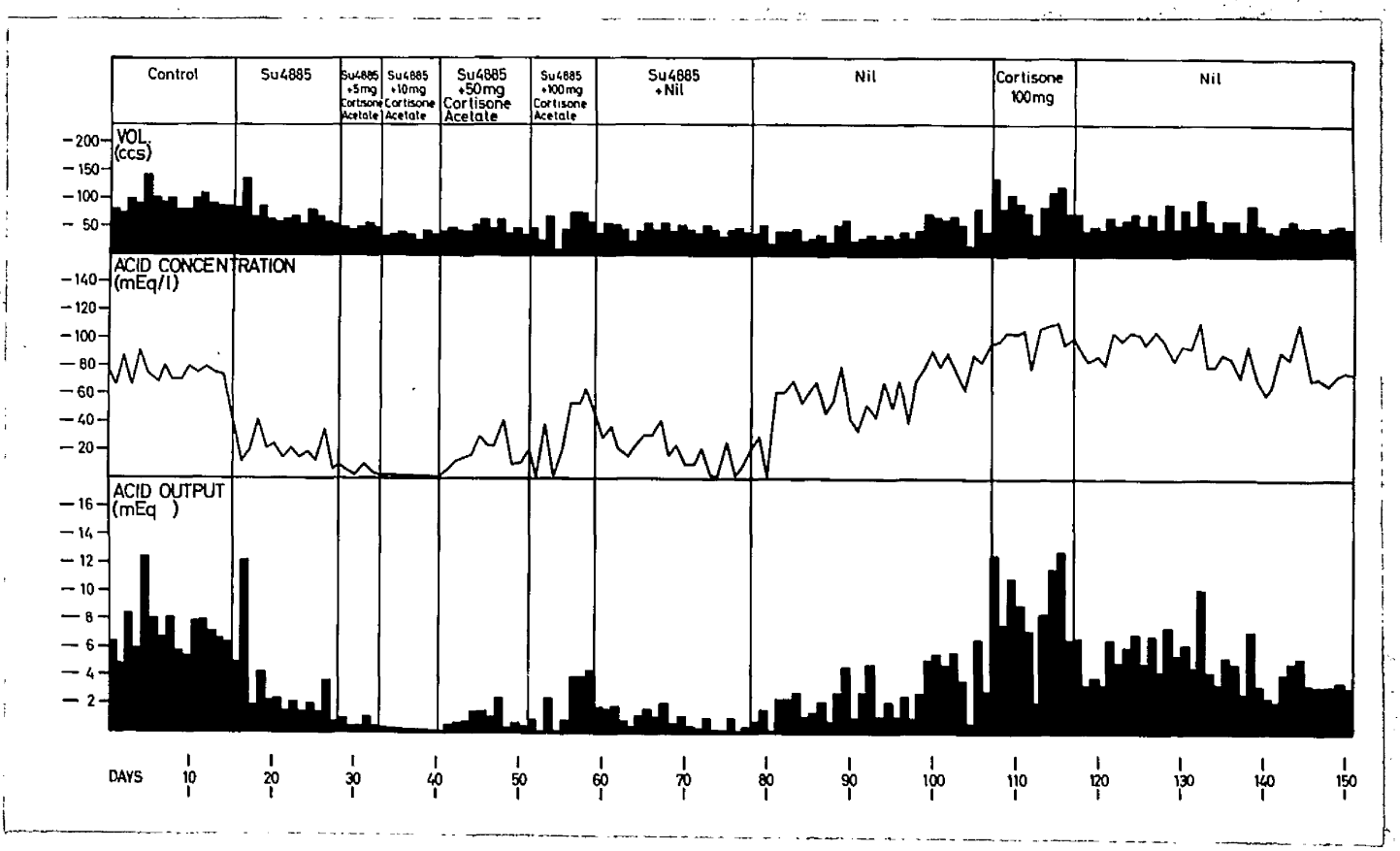


Figure 17 : Effect of prolonged oral administration of metyrapone base and cortisone on 24-hour volume of juice, concentration and output of acid from a Heidenhain pouch (Dog H).

Note: SU 8874 was the preparation used from day 59 to day 78 and not SU 4885 as stated in the caption.

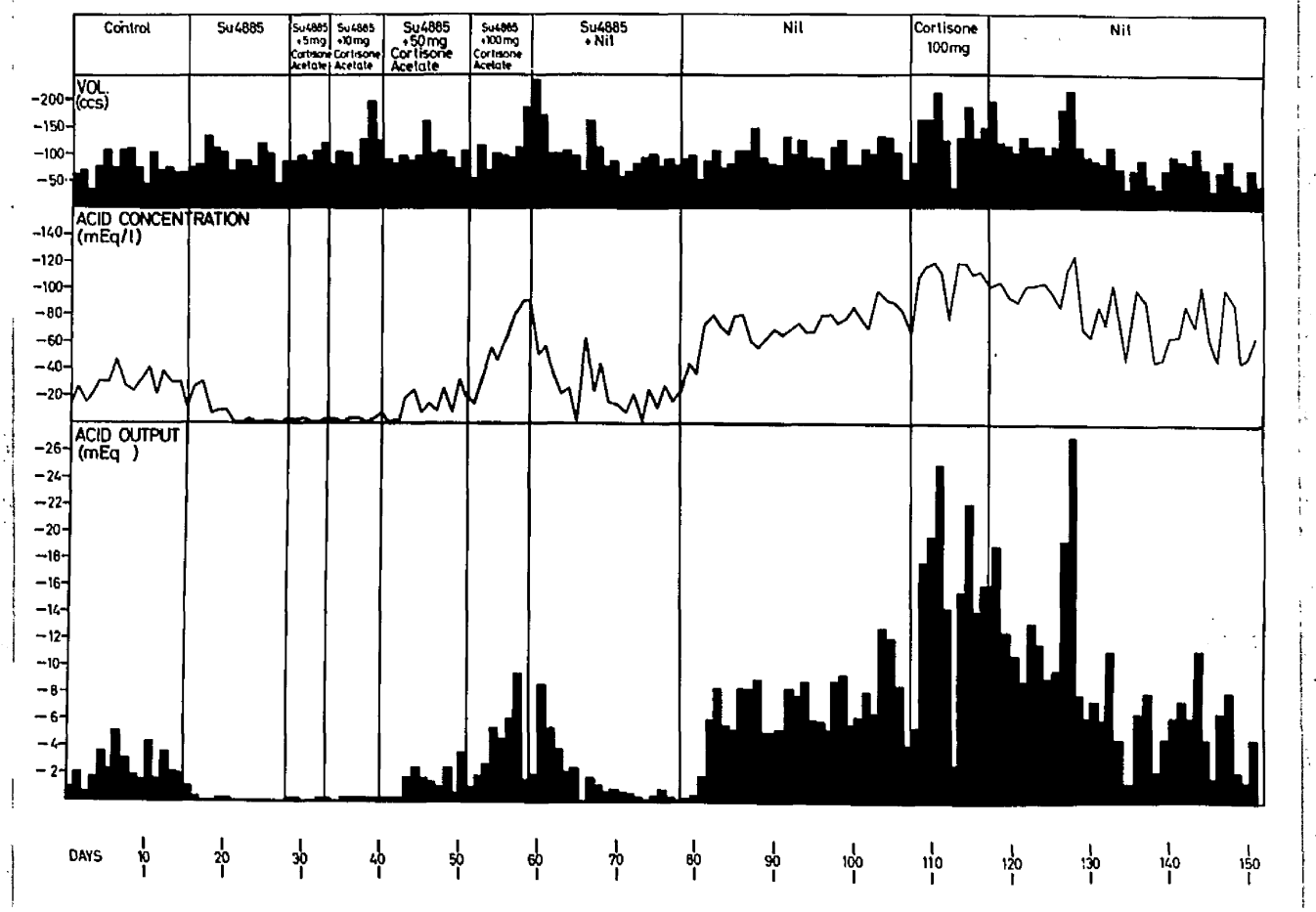


Figure 18 : Effect of prolonged administration of metyrapone base and cortisone on 24-hour volume of juice, concentration and output of acid from a Heidenhain pouch (Dog J).

Note: SU 8874 was the preparation used from day 59 to day 78 and not SU 4885 as stated in the caption.

Both metyrapone and cortisone had to be given to the dogs for several days before their full effect on secretion of acid from the Heidenhain pouches was observed. The means of the volumes of 24-hour collections of juice from the pouches of dogs N and J for each phase of the experiment are shown in Tables XI and XII, along with the means of the 24-hour outputs of acid and pepsin. The concentrations of acid for the same periods are included in Tables XIII and XIV, along with their standard errors and the complete protocol from which these results have been calculated is to be found in the Appendix.

The difference between the means of the logarithms of the outputs of acid during the first metyrapone period and the first control period is statistically significant in both dogs individually (Table XV) and the mean logarithmic difference of output of acid between the phase when 100 mg. cortisone was given alone and the preceeding and succeeding control periods is also significant (Table XV). The volume of 24-hour collections was not significantly effected by metyrapone but cortisone caused a significant increase (Table XVI).

While the reduction in acid output during metyrapone administration was undoubtedly highly significant in all 6

animals tested (dogs D, E, F, G, H, J), P log $<$ 0.05 (Tables IX and XV) acid secretion was not suppressed completely. Even the specimens of juice which gave no titratable acidity with Topfer's reagent (end-point pH 3.5) were all below pH 7.0. It is probable that the reaction of these specimens was maintained above pH 3.5 by the presence of dissolved mucus and other buffer systems. Subsequent experience gained in titrating a considerable number of samples of gastric juice of similar initial pH from other dogs to an end-point of pH 7.0 indicates that the amount of hydrochloric acid present in any of the above 'achlorhydric' samples would be unlikely to exceed 20 mEq./l.

When the means of the 24-hour outputs of acid during the period of combined metyrapone and cortisone administration are plotted against dose of cortisone (Figures 19 and 20), a significant correlation emerges. Various transformations were applied to the data, for example logarithmic, reciprocal, etc., in an attempt to improve the linearity of the correlation, but without success. The spread of outputs at any particular dose level of cortisone, as shown by the standard deviations on these graphs, is due to the lag-period after starting cortisone, before its effect on acid secretion becomes apparent, superimposed on the normal variability in

TABLE XI : DOG H: MEAN 24-HOUR OUTPUTS OF JUICE, FREE HCl AND PEPSIN FROM HEIDENHAIN POUCH. EFFECT OF METYRAPONE AND CORTISONE

	Control	Metyrapone	Metyrapone plus cortisone 5 mg.	10 mg.	50 mg.	100 mg.	Metyrapone	Control	Cortisone 100 mg.	Control
No. of Collections	15	13	5	10	8	8	19	29	10	34
Vol. ML/24 hr.	93.5 ±4.20*	71.5 ±6.47	48.0 ±2.15	35.7 ±1.93	47.2 ±3.62	49.0 ±8.78	40.9 ±2.08	38.11 ±3.32	88.4 ±9.51	54.9 ±13.19
Acid mEq/24 hr.	7.19 ±0.49	3.17 ±0.85	0.47 ±0.11	0.17 ±0.06	1.04 ±0.26	2.04 ±0.63	0.88 ±0.15	2.45 ±0.34	8.87 ±1.04	4.77 ± 0.31
Pepsin mg.tyrosine equiv/24 hr.	1741** ±319.4	2258 ±244.1	2165 ±369.4	1447 ±153.1	1625 ±233.6	1844 ±87.5	1396 ±133.7	1039 ±120.3	1106 ±224.8	1373 ±113.3

* = Standard error of the mean

** = Eight 24-hour collections

TABLE XII - DOG J: MEAN 24-HOUR OUTPUTS OF JUICE, FREE HCl AND PEPSIN FROM HEIDENHAIN POUCH

EFFECT OF METYRAPONE AND CORTISONE

	Control	Metyrapone	Metyrapone plus cortisone 5 mg.	10 mg.	50 mg.	100 mg.	Metyrapone	Control	Cortisone 100 mg.	Control
No. of Collections	14	13	5	10	8	8	19	29	10	32
Vol. ml./24 hr.	80.87 ±8.98*	91.7 ±5.69	98.0 ±7.00	109.6 ±11.46	100.0 ±9.99	102.3 ±13.99	102.1 ±9.93	93.6 ±14.29	135.3 ±15.90	91.7 ±7.63
Acid mEq/24 hr.	2.77 ±0.49	0.55 ±0.19	0.21 ±0.09	0.37 ±0.12	2.04 ±0.35	5.94 ±1.86	4.28 ±1.14	6.95 ±0.49	15.10 ±2.14	6.26 ±1.00
Pepsin Tyrosine equiv/24 hr.	3886** ±575.5	8167 ±948.0	7970 ±140.6	6954 ±979.9	4804 ±494.5	4235 ±524.1	3998 ±297.2	3662 ±358.9	2489 ±478.0	3018 ±255.8

* = Standard error of the mean

** = Eight 24-hour collections

TABLE XIII : DOG H: MEAN CONCENTRATIONS OF FREE HCl, SODIUM, POTASSIUM AND CHLORIDE IN 24-HOUR COLLECTIONS FROM POUCH : EFFECT OF METYRAPONE AND CORTISONE

	Control	Metyrapone	Metyrapone plus cortisone 5 mg.	10 mg.	50 mg.	100 mg.	Metyrapone	Control	Cortisone 100 mg.	Control
No. of Collections	15	13	5	10	8	8	19	29	10	34
Acid mEq/l.	76.07 ±1.88*	39.46 ±6.18	9.60 ±1.94	4.70 ±0.86	20.38 ±3.95	35.25 ±7.68	20.21 ±3.15	58.59 ±1.69	98.40 ±2.86	84.71 ±2.45
Sodium mEq/l.	64.05 ±2.16	80.01 ±3.92	86.14 ±3.64	105.30 ±2.78	101.71 ±3.49	85.80 ±5.52	101.61 ±6.98	69.09 ±3.34	42.52 ±8.33	50.21 ±1.59
Potassium mEq/l.	8.04 ±0.15	5.37 ±0.23	4.74 ±0.13	4.79 ±0.13	4.88 ±0.13	4.23 ±0.23	5.33 ±0.14	9.78 ±0.22	9.09 ±0.32	9.23 ±0.13
Chloride mEq/l.	155.4 ±1.04	156.2 ±1.46	163.4 ±2.20	147.7 ±2.72	148.7 ±1.62	151.1 ±3.62	147.0 ±2.17	153.3 ±1.57	163.4 ±3.60	156.1 ±1.46

* = Standard error of the mean.

TABLE XIV : DOG J: MEAN CONCENTRATIONS OF FREE HCl, SODIUM, POTASSIUM AND CHLORIDE IN 24-HOUR COLLECTIONS FROM POUCH : EFFECT OF METYRAPONE AND CORTISONE

	Control	Metyrapone	Metyrapone plus Cortisone 5 mg.	10 mg.	50 mg.	100 mg.	Metyrapone	Control	Cortisone 100 mg.	Control
No. of Collections	14	13	5	10	8	8	19	29	10	32
Acid mEq/l.	31.88 ±3.11*	6.08 ±2.29	2.4 ±0.98	3.64 ±1.28	20.55 ±3.34	51.44 ±9.87	28.88 ±5.35	69.68 ±3.90	106.6 ±5.56	82.44 ±3.75
Sodium mEq/l.	96.39** ±2.08	105.54 ±3.18	98.34 ±1.88	117.92 ±3.39	109.21 ±3.36	75.96 ±9.03	98.38 ±5.35	60.71 ±3.56	35.36 ±5.56	54.94 ±2.88
Potassium mEq/l.	7.0** ±0.09	4.55 ±0.21	3.76 ±0.05	4.47 ±0.29	4.74 ±0.08	5.60 ±0.35	5.51 ±0.21	8.43 ±0.34	9.01 ±0.43	8.35 ±0.15
Chloride mEq/l.	151.7** ±1.50	155.2 ±3.26	157.9 ±1.43	152.3 ±2.70	149.6 ±1.77	145.5 ±5.58	150.2 ±1.97	148.4 ±5.37	162.7 ±6.91	155.5 ±1.25

* = Standard error of the mean

** = Eight 24-hour collections

TABLE XV : ANALYSIS OF 24-HOUR ACID OUTPUTS

Dog	Period	Mean log. Acid Output	Mean Difference log.	P
H	First Control	0.844	0.397 ± 0.079*	<0.001
	Metyrapone	0.448		
J	First Control	0.299	0.401 ± 0.123	<0.01
	Metyrapone	0.102		
H	Second Control	0.294	0.615 ± 0.12	<0.001
	Cortisone 100 mg.	0.909		
	Final Control	0.648	0.261 ± 0.072	<0.001
J	Second Control	0.805	0.308 ± 0.082	<0.001
	Cortisone 100 mg.	1.112		
	Final Control	0.817	0.296 ± 0.142	<0.05

* = Standard error of Means

TABLE XVI : ANALYSIS OF 24-HOUR VOLUME OF JUICE

Dog	Period	Mean log. Vol. (ml/24 hr)	Mean Difference log.	P
H	First Control	1.9652	*0.1657 ± 0.109	>0.1
	Metyrapone	1.7995		
J	First Control	1.883	0.064 ± 0.052	>0.1
	Metyrapone	1.947		
H	Second Control	1.532		
	Cortisone 100 mg.	1.916	0.384 ± 0.085	<0.001
	Final Control	1.729	0.187 ± 0.051	<0.001
J	Second Control	1.966		
	Cortisone 100 mg.	2.091	0.125 ± 0.056	<0.05
	Final Control	1.865	0.226 ± 0.469	<0.001

* = Standard error of means

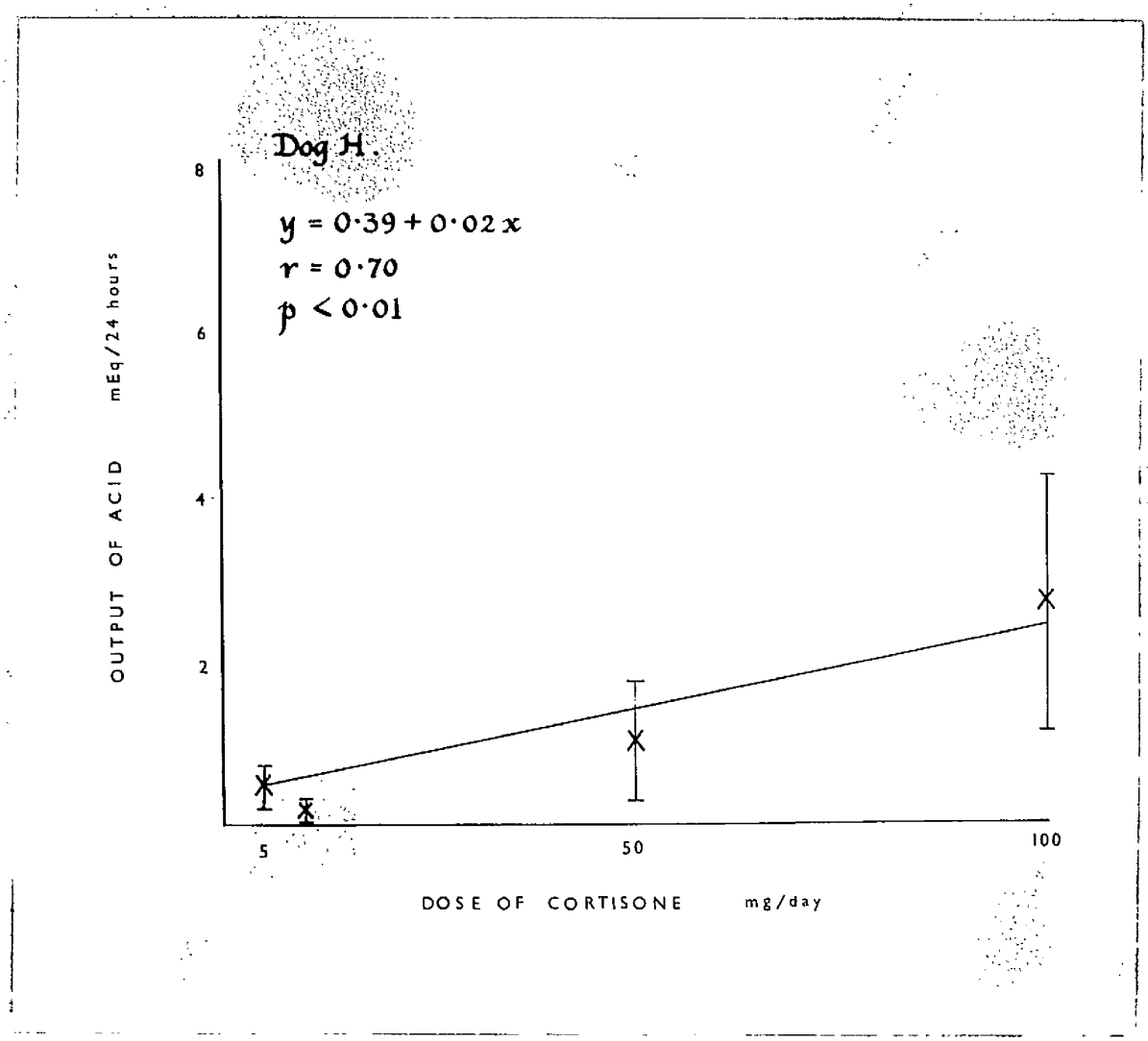


Figure 10 : Mean daily acid output ± one standard deviation plotted against dose of cortisone given along with metyrapone (100 mg. per kg. body weight per day) (dog H).

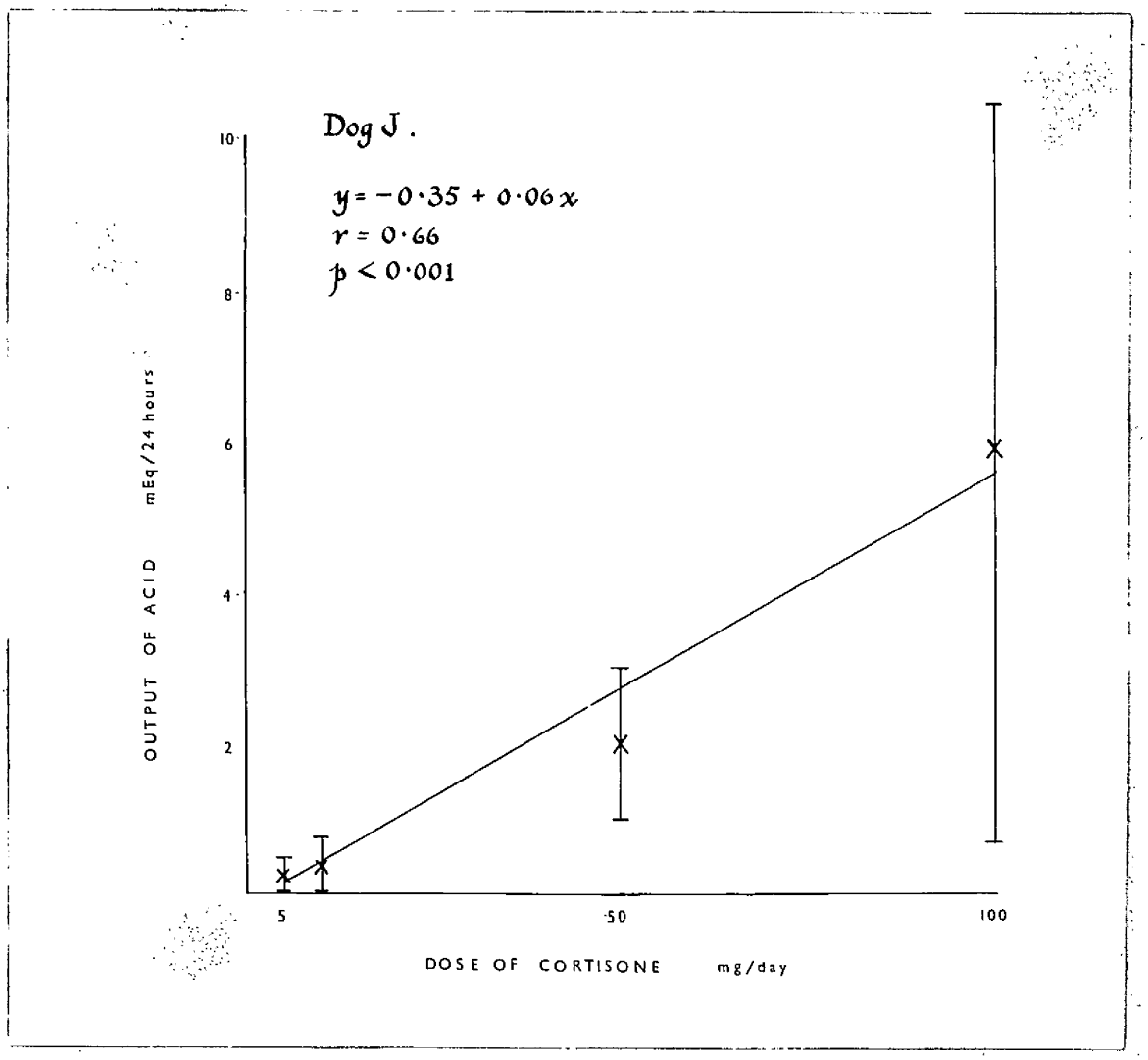


Figure 20 : Mean daily acid output ± one standard deviation plotted against dose of cortisone given along with metyrapone (100 mg. per kg. body weight per day) (dog J).

output of Heidenhain pouches. The difference in slope of the regression lines in the 2 dogs is merely an expression of the difference in pouch size. It is of course not permissible to extrapolate these regression lines to where they intercept the y-axis, but the relationship between acid output and dose of cortisone does suggest that, were the adrenals to be blocked completely, acid secretion might well cease. It should be noted that the doses of cortisone are not absolute values. Metyrapone was not blocking cortisol production completely round the clock, so that, at the low levels of added cortisone, the amount of cortisol passing into the circulation may have distorted the relationship between acid output and administered cortisone.

Pepsin

The output of pepsin, as is normal in denervated pouches, was low in these studies. One dog showed an insignificant reduction in daily output of pepsin while on metyrapone (dog E) and 4 (dogs D, F, G, H) showed no significant alteration in output. The sixth animal (dog J) exhibited a significant increase of 110 per cent in mean 24-hour secretion of pepsin while on metyrapone. Cortisone 100 mg. daily for 10 days caused a mean reduction of 35 per

cent in the daily output of pepsin in the 2 dogs to which it was given (dogs H and J). This reduction is significant at the 5 per cent level.

Electrolytes

Electrolyte estimations were carried out on collections from dogs H and J only. The concentrations of sodium, potassium, chloride and acid in the juice from the gastric pouches of dogs H and J are shown in Figures 32, 33 and 34, and the correlations which exist between the various ions is indicated. The complete data from which these graphs have been compiled are included in Appendix A and are summarised in Tables XIII and XIV, where the means of the concentration of sodium, potassium, chloride and acid during the various phases of the experiment are recorded along with their standard errors.

A highly significant negative correlation ($p < 0.001$) exists between sodium and hydrogen ion concentration. The slope of the regression line for Na/H is similar for both dogs, although the intercept on the y-axis differs. Statistical tests confirm that the electrolyte values observed belong to the same population and so it is permissible to combine the results from both dogs into a single

graph. Significant correlations also exist between the concentrations of potassium and hydrogen ions and between the concentrations of chloride and hydrogen ions (Table XVII), and the linear regression equations are in sufficient agreement to allow the combination of results from the 2 dogs.

Inspection of the electrolyte results and Table XVII indicates that the main electrolyte constituents of gastric juice are most closely related to hydrogen ion concentration.

The electrolyte concentrations found in pouch secretion from dogs H and J during (1) the initial control period, (2) metyrapone alone and (3) cortisone alone phases of the experiment were plotted graphically (Figures 21, 22, 23, 24) to see what relationship the various ionic concentrations of electrolytes bore to one another. In every case the control values lie in the middle of the graph while metyrapone results tend to lie to the left and cortisone results to the right. A highly significant correlation exists between H^+ and Na^+, H^+ and K^+, H^+ and Cl^+ which is remarkably similar in each of the three phases of the experiment, suggesting that both cortisone and metyrapone influence gastric secretion by a similar mechanism but in directly opposite directions.

TABLE XVII : CORRELATION COEFFICIENTS

DOGS H AND J

	Dog	H^+	Na^+	K^+	Cl^-
H^+	H	$^+1.000$	$^-0.940$	$^+0.776$	$^+0.417$
	J	$^+1.000$	$^-0.928$	$^+0.896$	$^+0.367$
Na^+	H	$^-0.940$	$^+1.000$	$^-0.746$	$^-0.457$
	J	$^-0.928$	$^+1.000$	$^-0.835$	$^-0.382$
K^+	H	$^+0.776$	$^-0.746$	$^+1.000$	$^+0.223$
	J	$^+0.896$	$^-0.835$	$^+1.000$	$^+0.281$
Cl^-	H	$^+0.417$	$^-0.457$	$^+0.223$	$^+1.000$
	J	$^+0.367$	$^-0.382$	$^+0.281$	$^+1.000$

Number of pairs: Dog H ... 151

Dog J ... 141

$p < 0.001$ in every case.

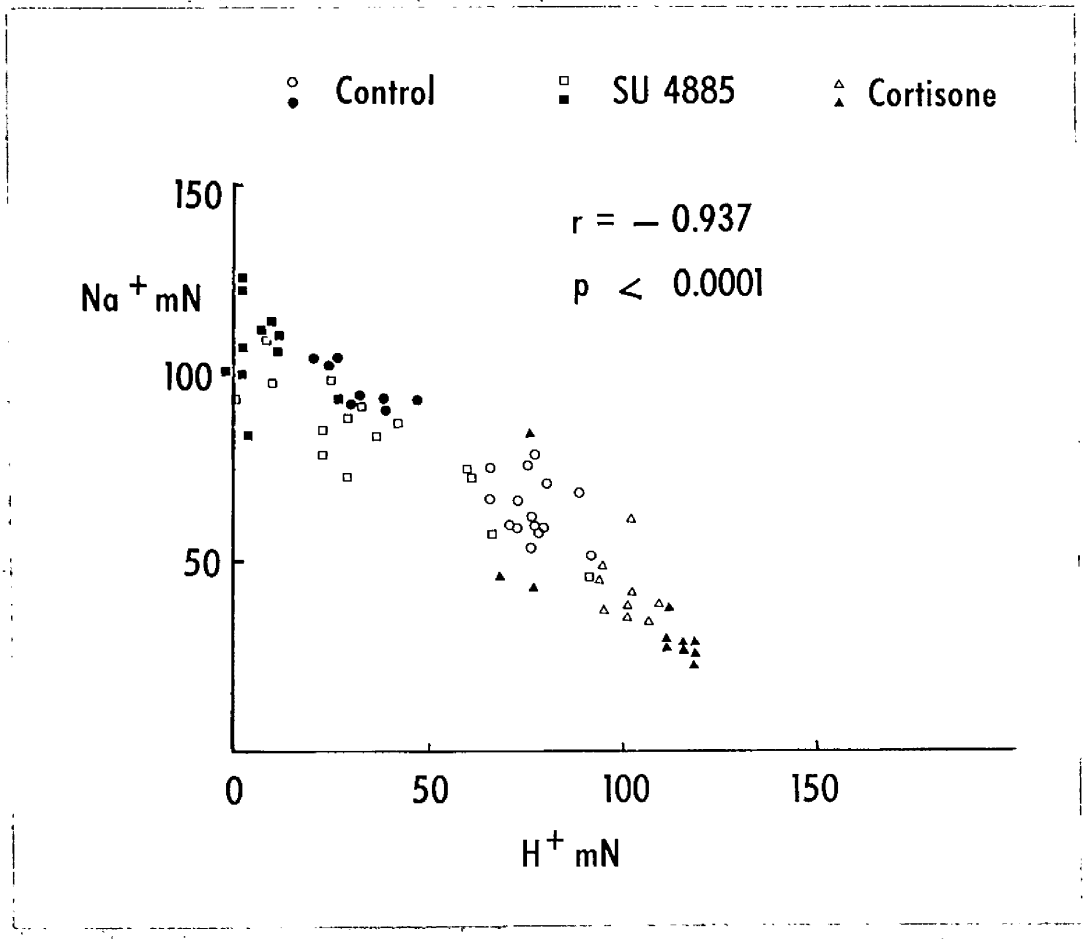


Figure 21 : Correlation between sodium and hydrogen ion concentration in Heidenhain pouch secretion during the administration of metyrapone and cortisone.

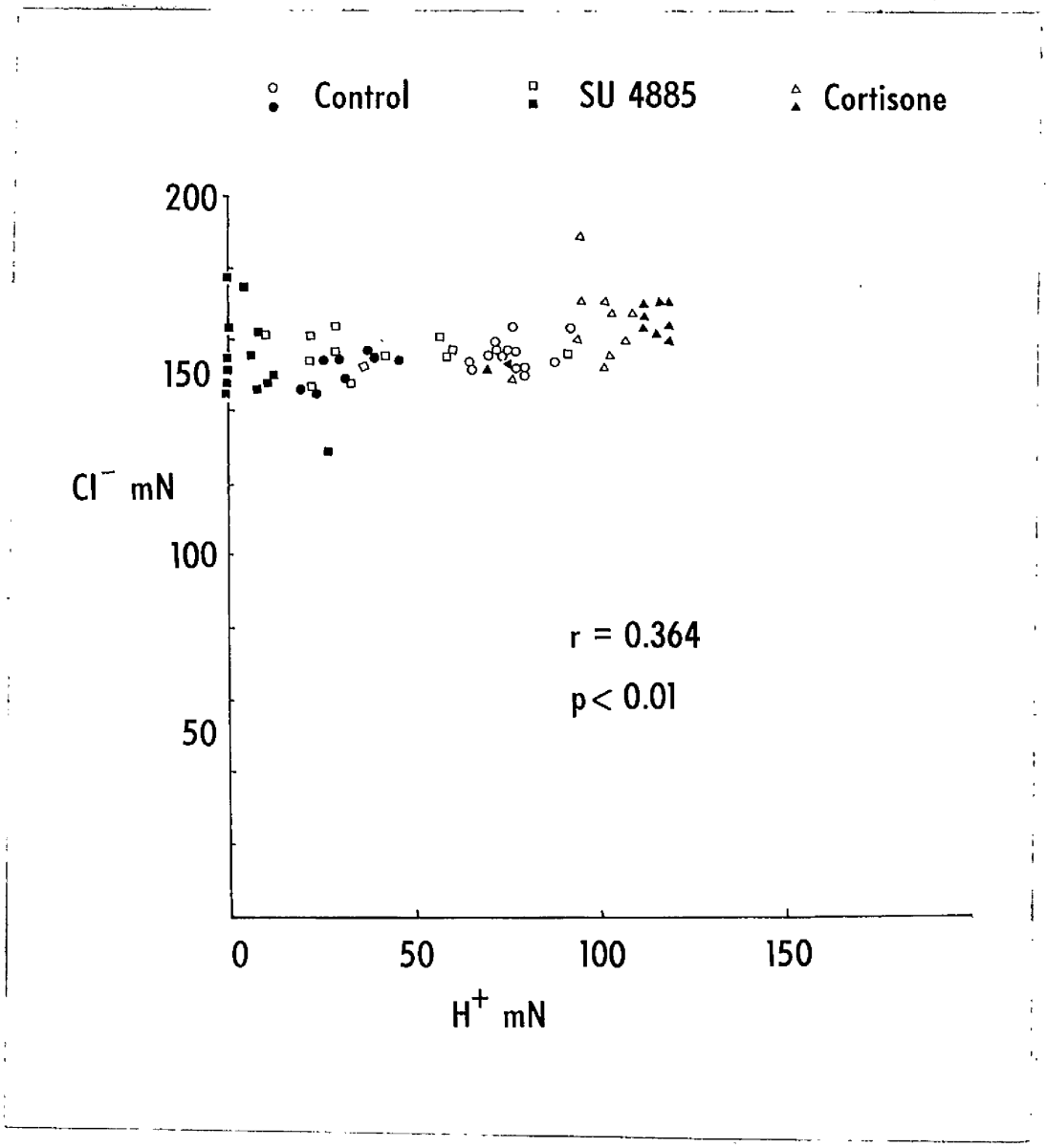


Figure 22 : Correlation between chloride and hydrogen ion concentration in Heidenhain pouch secretion during the administration of metyrapone and cortisone.

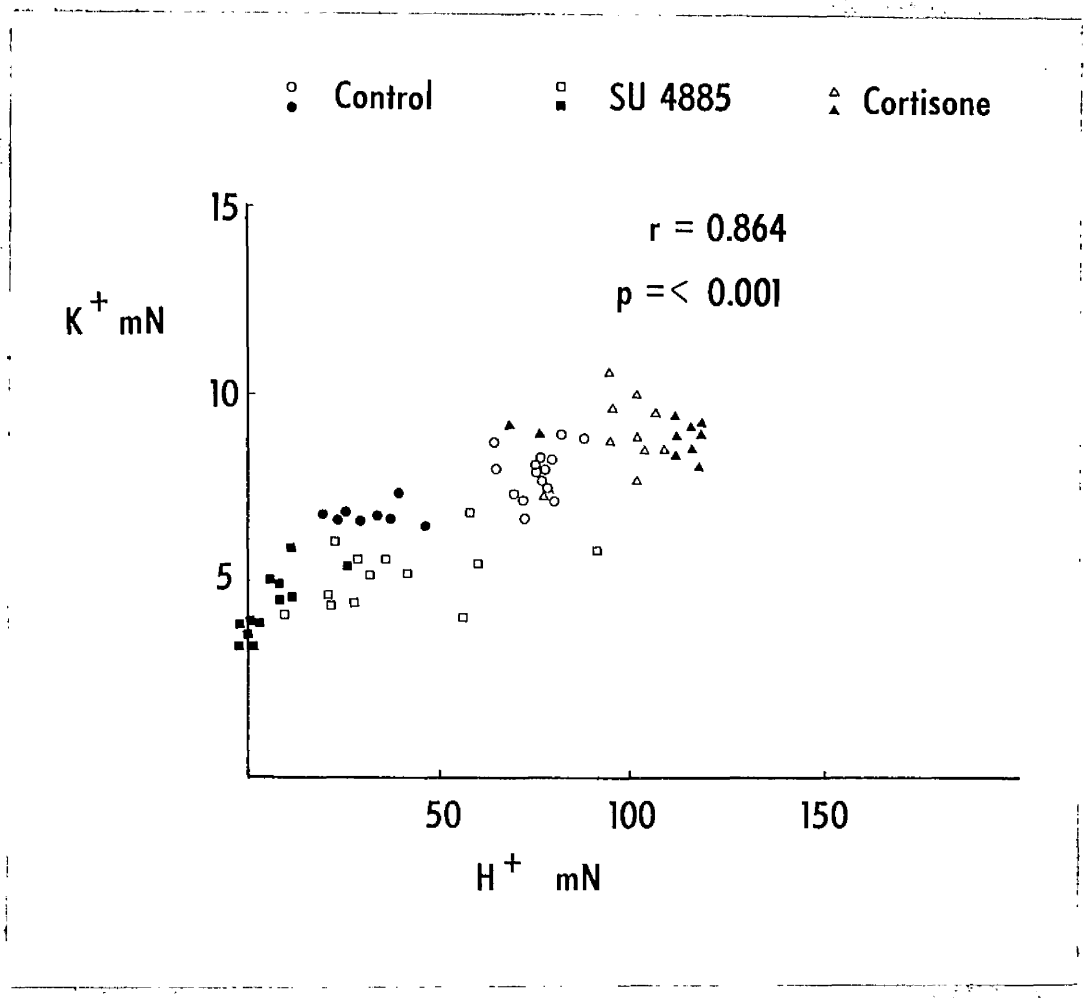


Figure 23 : Correlation between potassium and hydrogen ion concentration in Heidenhain pouch secretion during the administration of metyrapone and cortisone.

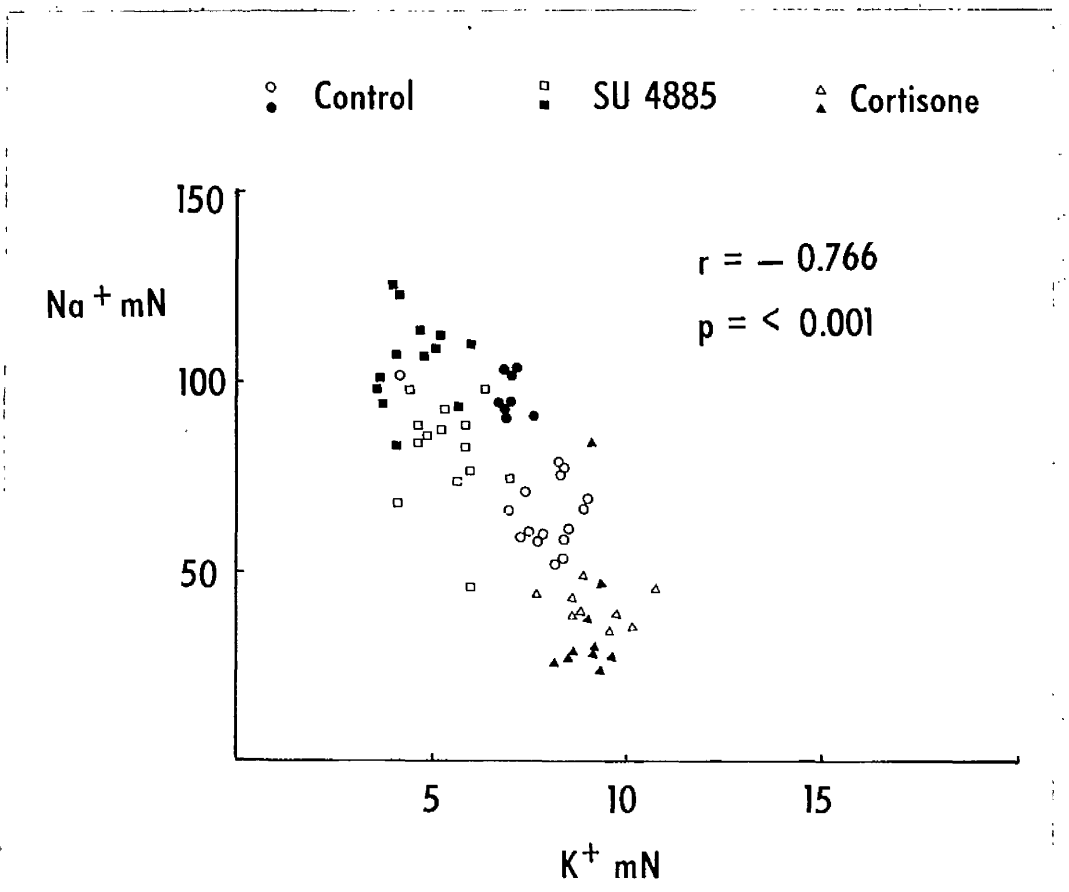


Figure 24 : Correlation between sodium and potassium ion concentration in Heidenhain pouch secretion during the administration of metyrapone and cortisone.

The failure of the sum of the cations to balance the chloride concentration in the samples of gastric juice is due to the fact that only the free, or dissociated, hydrochloric acid was measured by titrating to a pH of about 3.5 (the end-point of Topfer's indicator). In all samples there is also a variable amount of HCl which is buffered by the presence of mucus and other substances in gastric juice.

CONCLUSIONS

Metyrapone is a potent inhibitor and cortisone a potent stimulator of gastric acid secretion in dogs. Both drugs are active when given orally once daily but take several days to achieve their maximum effect. Since the correlations which exist normally between the electrolyte constituents of gastric juice are not disturbed, it is unlikely that the cells of the gastric mucosa have their limiting membranes damaged but rather that intra-cellular enzyme systems are affected. An alternative hypothesis, since only the parietal component is influenced, is that cortisone increases and metyrapone decreases the total number of parietal cells in the stomach.

S E C T I O N III

RESPONSE OF CANINE HEIDENHAIN POUCHES TO VARIOUS DOSES OF HISTAMINE FOLLOWING PROLONGED ORAL METYRAPONE

PROCEDURE

It has been shown in Section II that metyrapone decreases the response of Heidenhain pouches to a balanced meat diet. An alternative stimulus frequently employed in gastric investigation is histamine which, when given by injection, is a very potent provoker of acid, but not of pepsin, secretion in the dog. When histamine is administered by continuous intravenous infusion, after half to one hour the concentration of histamine in the plasma approaches a steady level which is proportional to the rate of injection. Acid production in the stomach also levels out at this time and remains fairly constant for as long as the histamine infusion is continued (Öbrink, 1948). The response of a Heidenhain pouch depends to a large extent on the dose of histamine used and by measuring the output of acid from a pouch after various amounts of histamine, a dose-response curve can be plotted. Code and his colleagues (1949) have shown that the response of pouches to histamine remains steady over long periods and may be used to assess the stimulatory or inhibitory effect of drugs alleged to influence gastric secretion.

Continuous intravenous infusions of various doses of histamine were given to 4 dogs with Heidenhain pouches and the output of acid plotted against histamine dosage so as to obtain a dose-response curve for each dog. The studies were repeated, using the same range of histamine dosage, while the dogs were receiving metyrapone base orally and again while on oral cortisone. Final control tests were carried out after a further 10-14 days when the effect of the cortisone had worn off.

Histamine dose-response curves were plotted for each phase of the experiment and were compared graphically. The results were also analysed statistically by carrying out an analysis of variance between the acid outputs obtained during administration of the 2 drugs and the control tests.

At the time that the experiments in this section were begun, tablets of metyrapone base were no longer available. Gelatine capsules (capsules 'A') containing the dihydrochloride salt of metyrapone in powder form were used initially but, when no significant effect was noted on the 24-hour output of acid from the Heidenhain pouches, gelatine capsules containing fresh metyrapone base dissolved

in oil were substituted. It was thought that the metyrapone powder might have deteriorated with storage and no histamine tests were performed during the period that the dogs received this particular batch of the drug.

METHOD

General Plan

Separated pouches of the stomach of Heidenhain type were constructed in 3 healthy mongrel bitches and one dog (dogs L, M, N, O) of 12.5 kg. to 14 kg. body weight. The experiments were carried out in a different city from those in the preceeding sections but the dogs' diet, amount of exercise in the open air and living conditions were similar and were subject to the same rigorous control. Pouch secretion was collected, as previously, into a polythene bottle which, however, was emptied twice daily - at 6 hours and 24 hours after the daily meal - in order to obtain specimens of juice with a wider range of acid and electrolyte concentrations.

The volume of each specimen was measured in ml. and samples were then centrifuged in order to separate undissolved mucus and debris which was discarded. Duplicate 2 ml. aliquots of spun juice were titrated electrometrically

with N/10 NaOH to an end-point of pH 7.0. Chloride concentrations were measured in duplicate on an EEL chloride meter and sodium and potassium by flame-photometry.

All the acid and electrolyte results were transferred to punched cards and fed into a DEUCE computer in order to obtain correlation coefficients between all the electrolytes, taken in pairs. Linear regression equations were worked out for each pair of electrolytes in each individual dog and also for the combined results from dogs H, J, L, M, N and O.

The 24-hour output of acid from each pouch was adopted as the index of gastric secretory activity and, after a control period of 2-3 weeks, metyrapone dihydrochloride powder (capsules 'A') in gelatine capsules was administered orally in a dose of 100 mg. per kg. body weight once daily before the daily meal for 20 days. Metyrapone base dissolved in oil and contained in orange-coloured gelatine capsules was now substituted for the dihydrochloride salt and was given in the same dose once daily by mouth for 28 days. Thereafter, cortisone acetate was administered by mouth in a daily dose of 100 mg. for 21 days followed by 300 mg. daily for 13 days. The experiment concluded with a final control period of 21 days during which the dogs received no drugs.

Histamine Tests

The histamine infusion tests were begun at the same time each day, approximately 18 hours after the last daily meal, when secretion from the pouches was insignificant and usually contained no free acid. The dogs were trained to stand on a bench for the duration of the experiment which lasted about 4 hours. Histamine acid phosphate was dissolved in sterile normal saline and made up in a dilution such that a fixed body-weight dose of histamine base could be continuously injected each hour by means of a Palmer constant speed injection apparatus. The histamine solution was given intravenously via polythene tubing introduced percutaneously through a hollow needle into the dog's cephalic or saphenous vein.

Only one level of histamine dosage was employed in any one day and the dose was doubled each day until no further increment in acid output occurred.

Gastric juice was collected, from the cannula draining the dog's pouch, into graduated centrifuge tubes which were emptied every 15 minutes. The volume of juice was measured in millilitres and its acidity estimated by recording the pH of each sample, using a glass electrode coupled to an E.I.L.

23A pH meter, and then titrating duplicate 2 ml. aliquots with N/10 NaOH to an end-point of pH 7.0. The product of volume (ml.) and acid concentration (mEq./l.) divided by 1,000 gave acid output from the pouch in milliequivalents (mEq.).

The 15-minute output of acid from the pouch usually levelled off after 1½ hours to 2 hours and collections were then continued for a further 1½ hours. The four consecutive 15-minute specimens which, added together, gave the greatest output of acid, was taken as the maximum response of the pouch under test to that particular dose of histamine. This 'maximum hour' acid output was plotted against histamine dosage on semi-logarithmic graph paper.

The initial control series of histamine tests were carried out only after the 24-hour collections of juice from each dog's pouch showed that gastric secretion had settled down to a steady level following the trauma involved in the construction of the pouches.

No histamine tests were done while the dogs were on metyrapone dihydrochloride powder in gelatine capsules (capsules 'A') as the 24-hour outputs of acid from the pouches during this phase of the experiment, unlike our

findings in Section II of this thesis, had not shown any significant alteration.

A full series of histamine tests was done on all 4 dogs beginning 14 days after starting pure metyrapone base and was repeated during both periods of cortisone administration.

The 'maximum hour' acid outputs obtained with each histamine dose level during the various phases of the experiment were compared statistically using an analysis of variance to assess the significance of the differences observed.

RESULTS

24-hour Output from Pouches

The 24-hour volume of secretion, concentration and output of acid from the stomach pouch in dog L for the complete experiment is shown in Figure 25. Juice secreted dur ing the actual period of a histamine infusion has been excluded in calculating these results, but as there is normally a comperatively insignificant amount of juice secreted from a Heidenhein pouch from 18 hours to 24 hours after a meal, when the histamine tests were conducted, this does not introduce any significant error. Contrary to

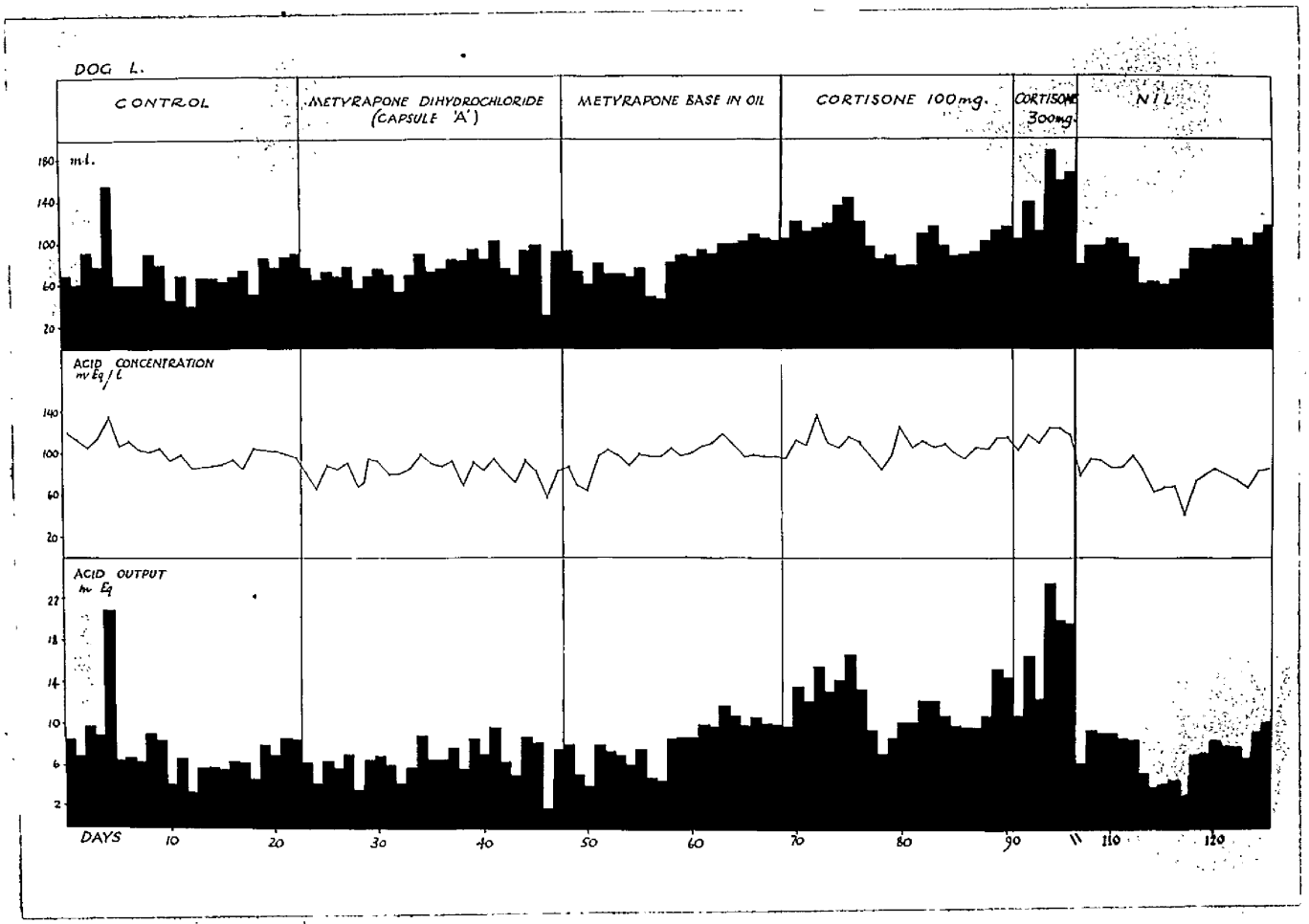


Figure 25 : Effect of prolonged administration of metyrapone dihydrochloride (capsules 'A'), metyrapone base in oil (Metopirone) and cortisone on 24-hour secretion of acid from Heidenhain pouch (Dog L).

the findings reported above, in Section II, prolonged oral metyrapone did not, on this occasion, cause any apparent reduction in secretion from the Heidenhain pouches. The patterns of secretion found in dogs M. N and O were similar to that of dog L and consequently have not been illustrated. Neither metyrapone dihydrochloride in gelatine capsules (capsules 'A') nor metyrapone base in oil in gelatine capsules (Metopirone) reduced the 24-hour output of acid from the denervated gastric pouches in any of the 4 dogs.

During cortisone administration, there occurred an increase in acid concentration and output in all 4 dogs, similar to that seen in dogs H and J in Section II.

While the dogs were receiving 300 mg. cortisone per day, the pouch juice in dogs L, M and O was discoloured by small amounts of altered blood; dog N suffered a brisk haemorrhage of 150 ml. to 200 ml. in the middle of this period and the 24-hour collections had to be interrupted. However, it settled down without alteration in the dose of cortisone.

Immediately following the cortisone phase of the experiment, while the dogs were not on any drugs, all 4 developed pouch trouble. The pouch mucosa prolapsed in

dog L and it had to be reduced under general anaesthesia plus narrowing of the stoma. The cannula slipped out, or more probably was extracted by the dog herself. In dog O, laparotomy plus refashioning of the pouch stoma was required. This animal died two months later from a subphrenic abscess. In dogs M and N, excoriation of the abdomen occurred due to leakage of juice around the cannula, but this healed quickly when the polythene collecting bottles were left off and the dogs allowed to lick the area.

In every case the trouble could be traced to a change in the composition of the pouch secretions while on high doses of cortisone whereby the juice became less viscous and more irritating to the tissues if it leaked around the cannula. The irritation caused the dogs to bite their cannula, pull on it with their teeth and enlarge the size of the stoma leading to further leakage of juice.

Histamine Tests

The pattern of secretion from a Heidenhain pouch following the intravenous infusion of a sub-maximal dose of histamine is shown in Figure 26, and the results of 4 such tests on the same dog using different doses of histamine on 4 different days is demonstrated in Figure 27.

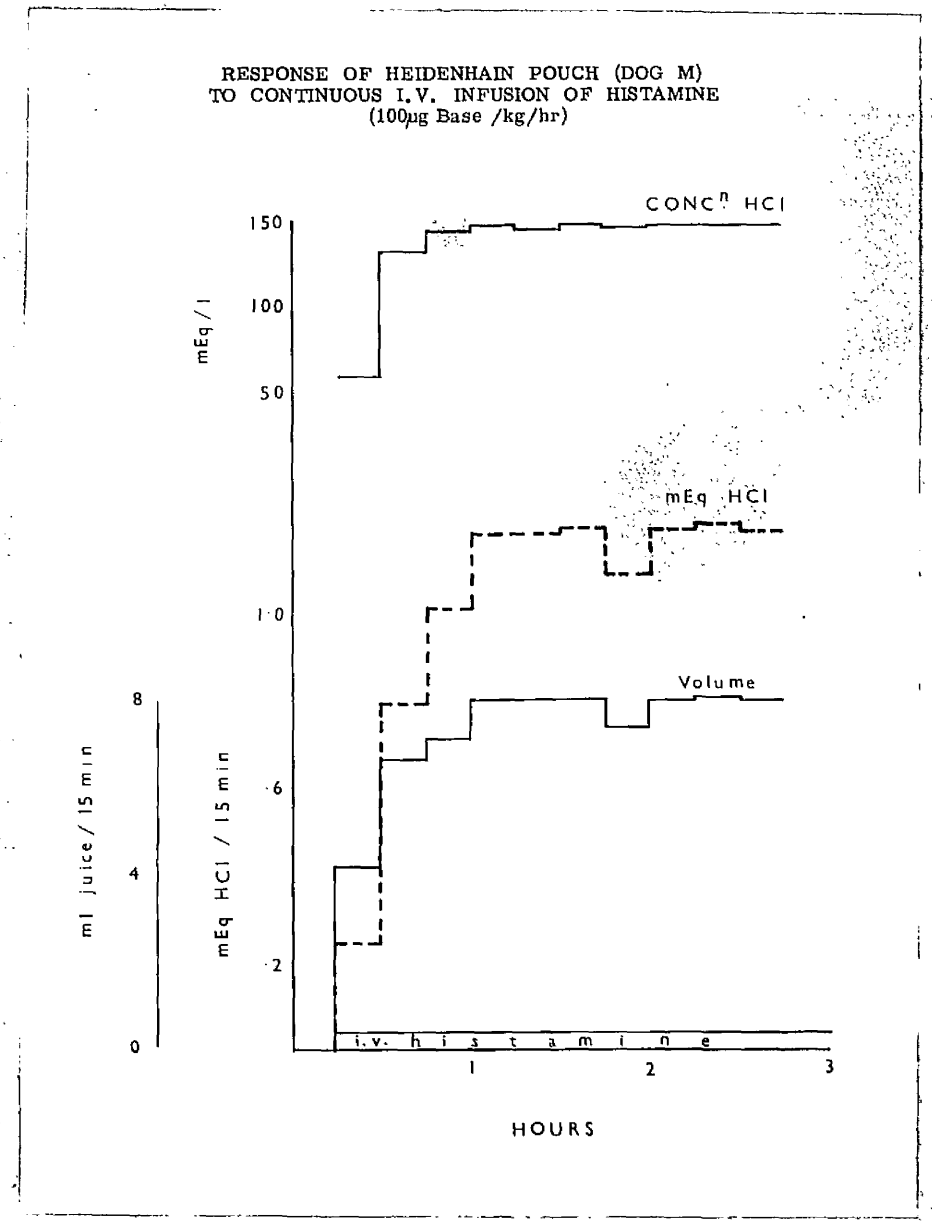


Figure 26 : Typical response of a Heidenhain pouch to a continuous infusion of histamine.

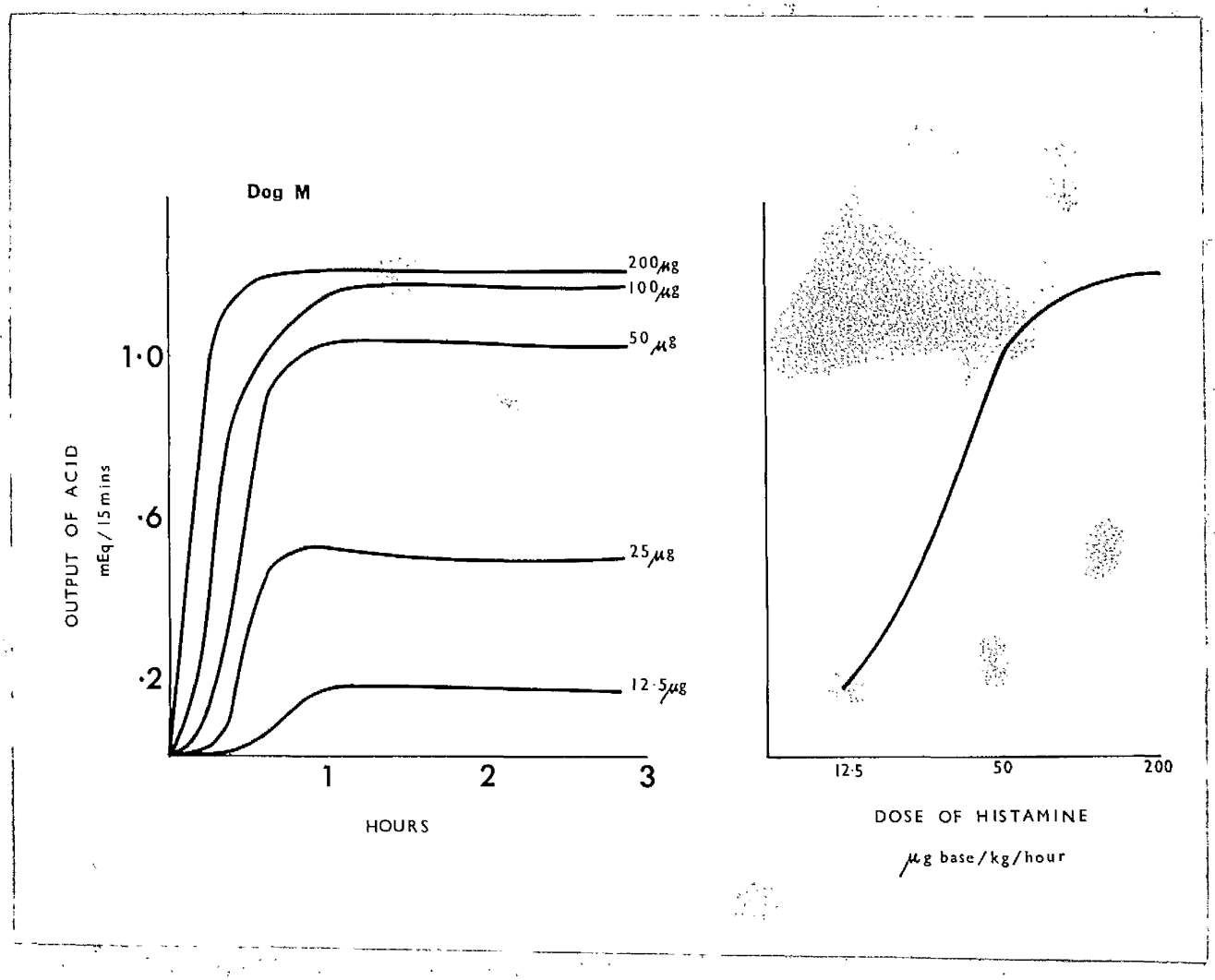


Figure 27 : On the left is shown the output of acid from a Heidenhain pouch stimulated by different doses of histamine. On the right, the meximum hourly output of acid is plotted against the logarithm of the dose of histamine.

Acid output from the pouch rises steadily to reach a maximum level proportional to the particular dose of histamine employed and, when the maximum acid response is plotted against the logarithm of the histamine dosage on semi-logarithmic graph paper, a roughly sigmoid-shaped dose-response curve is obtained. Above a certain dose of histamine, gastric acid secretion actually falls away again.

The histamine responses for the 4 dogs obtained during the different parts of the experiment are shown in Tables XVIII and Figures 28, 29, 30 and 31. The volume, concentration and output of acid in the highest 4 successive 15-minute samples in each individual test are recorded in the Appendix. In all 4 dogs the prolonged ingestion of metyrapone resulted in the histamine dose-response curve being shifted down and to the right. The output of acid from all the pouches was reduced at each level of histamine dosage and in 3 of the 4 animals the maximum acid output was obtained with a lower dose of histamine, the optimum dose in the control series causing a falling-off in acid secretion in the metyrapone tests. In other words, metyrapone caused a reduction in the 'maximum histamine response'.

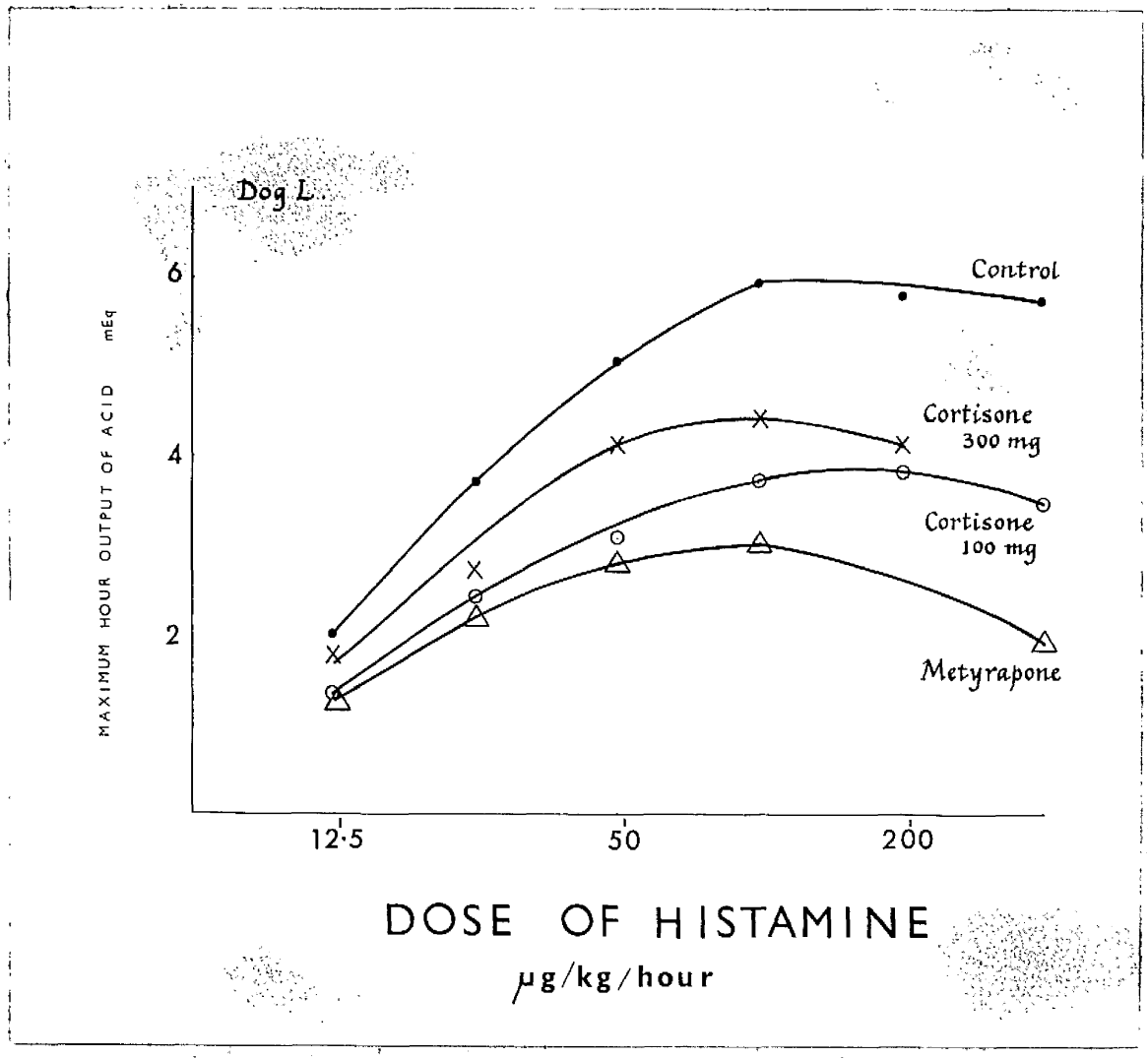


Figure 28 : Histamine dose-response curves (dog L).

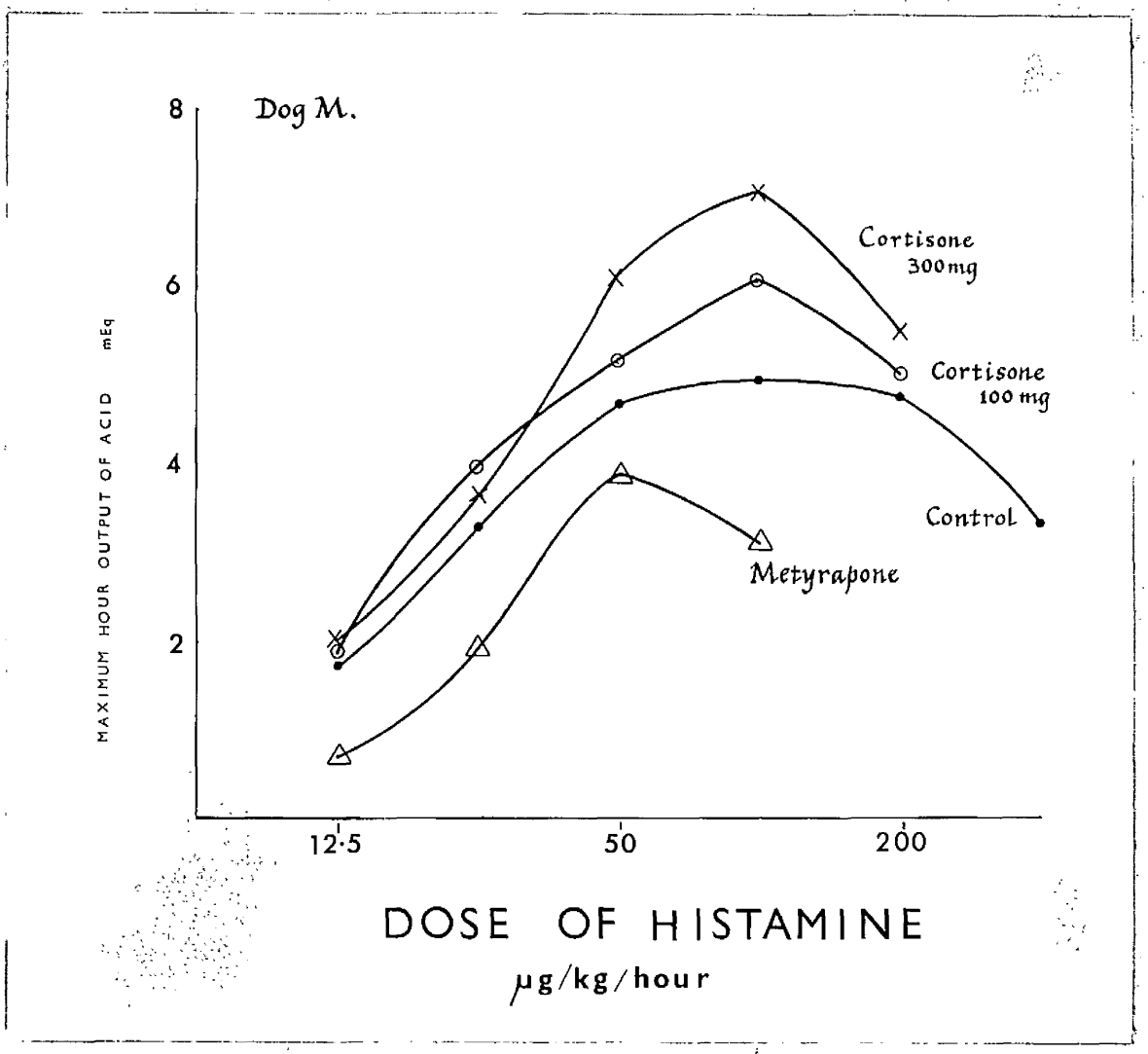


Figure 20 : Histamine dose-response curves (dog M).

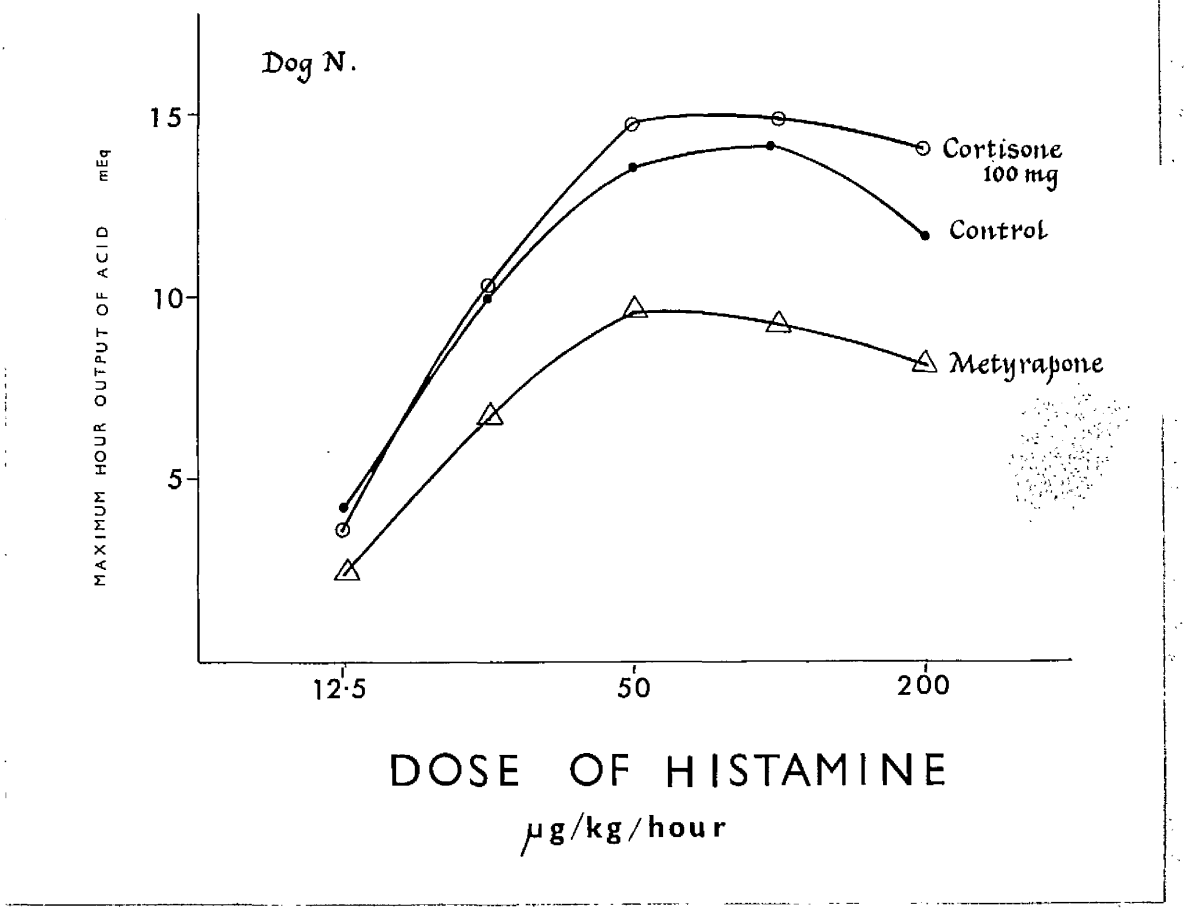


Figure 39 : Histamine dose-response curves (dog N).

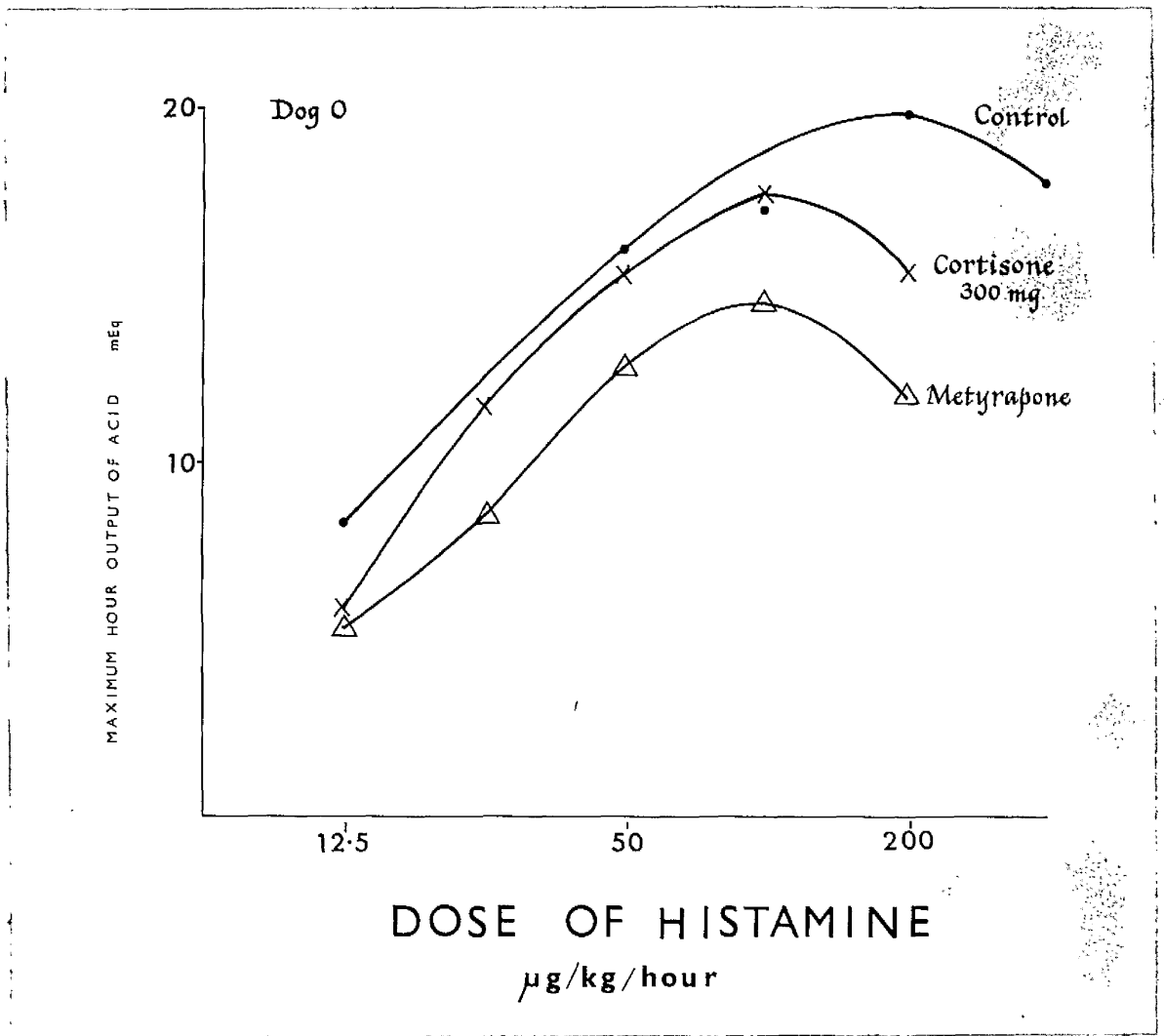


Figure 31 : Histamine dose-response (dog 0).

TABLE XVIII : MAXIMUM HOUR OUTPUT OF ACID (mEq.)

Drug	Dog	Dose of Histamine Base µg/kg body weight/hour			
		12.8	25	50	100
Nil	L	2.018	3.768	5.079	5.929
	M	1.692	3.242	4.638	4.861
	N	4.258	9.097	13.531	14.051
	O	8.353	-	16.068	17.157
Metyrapone	L	1.284	2.198	2.801	2.911
	M	0.702	1.925	3.883	3.145
	N	2.432	6.733	9.580	9.237
	O	5.145	8.569	12.687	14.524
Cortisone 100 mg.	L	1.300	2.441	3.089	3.753
	M	1.906	3.973	5.168	6.050
	N	3.604	10.303	14.741	14.813
	O	-	-	-	-
Cortisone 300 mg.	L	1.815	2.735	4.188	4.431
	M	2.016	3.665	6.636	7.347
	N	5.238	9.469	15.225	15.285
	O	5.850	11.552	15.271	17.564

Exactly opposite effects were seen with cortisone administration in 3 out of the 4 dogs.

While dog M was receiving cortisone acetate 100 mg. daily, its histamine dose-response curve was shifted up and to the left and a further increase in secretory response occurred when it received 300 mg. cortisone acetate daily. The dose of histamine which elicited the maximum output of acid from the pouch was 100 µg. per kg. body weight per hour which was the same as in the control series for this dog.

Dogs N and O did not show a significant increase in acid response to histamine while on cortisone and dog L did not reach even control levels.

The 'maximum hour' output of acid from the gastric pouches in response to histamine (12.5 - 100 µg. base per kg. body weight per hour) for the 4 dogs is shown in Table XVIII.

Statistical analysis of the results of the histamine tests was restricted to those results appearing in Table XVIII. Figures obtained in the final control series of tests were excluded because it appeared that the secretory capacity of the pouches, in at least 2 of the dogs, had

been affected adversely by the pouch trouble which followed the administration of high doses of cortisone. An analysis of variance confirmed that the reduction in acid response to histamine during metyrapone administration was significant ($p < 0.01$). The difference in acid response during treatment with cortisone 100 mg. and 300 mg. daily did not reach significance level.

Inspection of the histamine test data in the Appendix reveals that, during metyrapone treatment the concentration of acid was regularly less than concentration reached in the corresponding control tests. Acid concentrations in the cortisone series tended to run to about control levels. It appears as if metyrapone prevented the pouches from secreting acid at as high a concentration as usual.

Electrolytes

The various electrolyte concentrations obtained in dogs L, M, N and O were plotted against hydrogen ion concentration (Figures 32, 33 and 34). The volume and concentration of the main electrolytes in each collection of juice are given in the Appendix. The exceptionally wide range of values was obtained by taking a number of fasting samples of juice as well as collections at 6 hours and 24 hours after a meal.

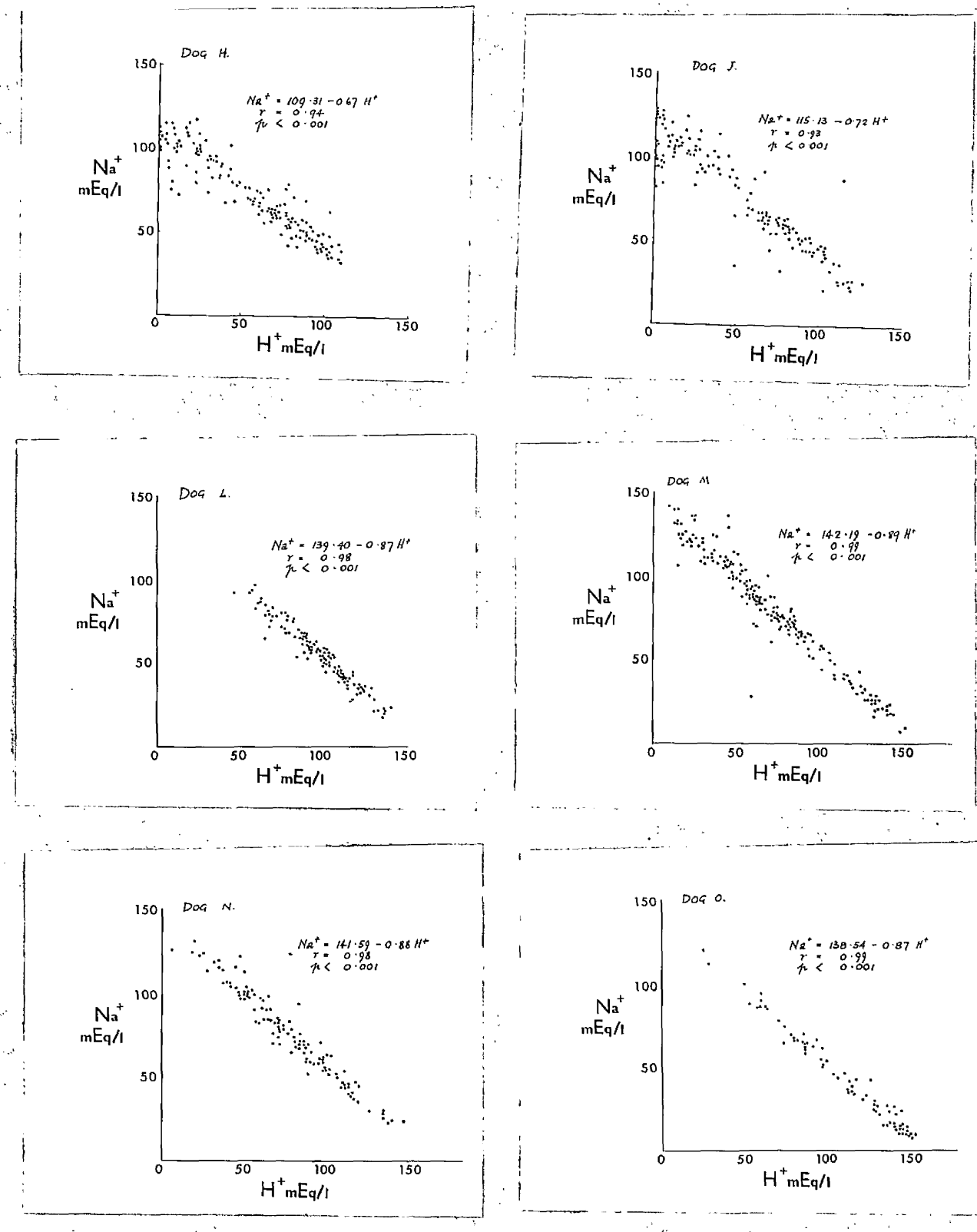


Figure 32 : Concentrations of sodium and hydrogen ions (dogs H, J, L, M, N and O).

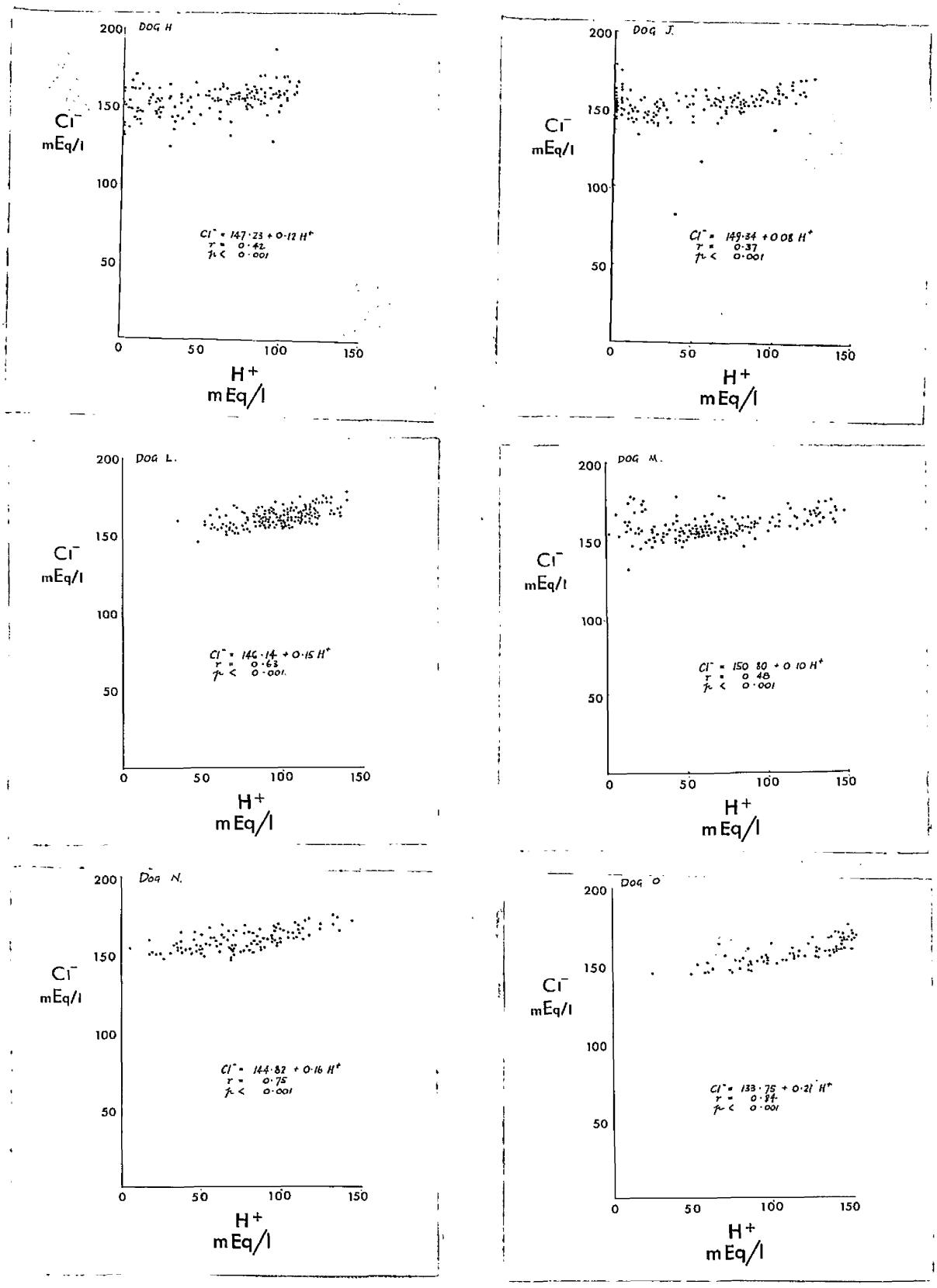


Figure 33 : Concentrations of chloride and hydrogen ions (dogs H, J, L, M, N and O).

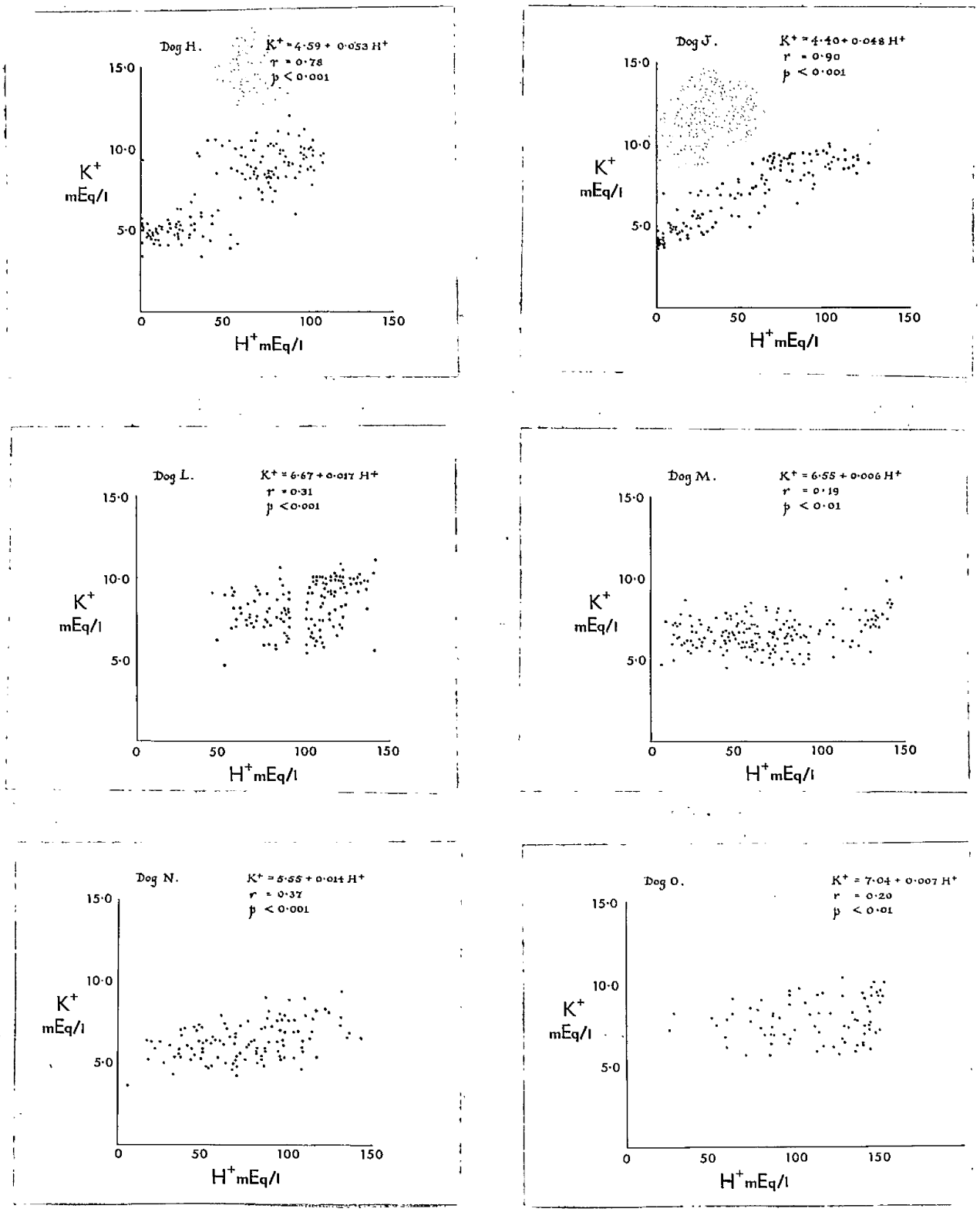


Figure 34 : Concentrations of potassium and hydrogen ions (dogs H, J, L, M, N and O).

Figures for juice collected during histamine stimulation were excluded and do not appear in the graphs. Inspection of the linear regression equations reveals close similarity between these 4 dogs and dogs H and J in Section II, with regard to sodium/hydrogen ion relationship. The reduction in scatter in sodium values at low acidities in dogs L, M, N and O is due to titrating such specimens, which frequently contain much mucus and other buffers, to pH 7.0, instead of merely estimating the amount of free acid present, as was done with dogs H and J.

The correlations between potassium and hydrogen and between chloride and hydrogen ion concentrations differ considerably in Section III dogs from those found in Section II as well as showing significant differences within the group. The reason for these differences is not clear. However, it does appear as if the concentrations of sodium, potassium and chloride all vary with respect to the hydrogen ion concentration.

Although it is not strictly correct to combine the results from the 6 dogs in which electrolytes were done, for reasons just stated, this exercise has been carried out and analysis of the 935 sets of results yielded the following

TABLE XVIX : CORRELATION COEFFICIENTS FOR ELECTROLYTES IN GASTRIC JUICE FOR DOGS L, M, N AND O INDIVIDUALLY AND COMBINED

	Dog	No.	Na^+	K^+	Cl^-
H^+	L	200	-0.979	-0.310	-0.634
	M	211	-0.985	-0.191	-0.484
	N	157	-0.982	-0.378	-0.746
	O	75	-0.989	-0.196†	-0.844
	All 6	935	-0.934	-0.536	-0.560
Na^+	L			-0.342	-0.560
	M			-0.158*	-0.428
	N			-0.420	-0.084
	O			-0.251*	-0.791
	All 6			-0.523	-0.497
K^+	L				-0.359
	M				-0.332
	N				-0.378
	O				-0.330
	All 6				-0.301

$p < 0.001$ except* for * $p < 0.05$

† $p \geq 0.05$

linear regression equations:

$$n = 935$$

$$Na^+ = 125.19 - 0.75H^+$$

$$K^+ = 5.55 - 0.024H^+$$

$$Cl^- = 147.98 - 0.116H^+$$

Inspection suggests that these equations have been weighted by the inclusion of the free acid results from dogs H and J.

While a simple linear relation obviously exists between sodium and hydrogen ion concentration, it may well be that a rather more complicated curvilinear, logarithmic or other relation would provide a more acceptable explanation for the potassium and chloride versus hydrogen ion concentration data.

The lowest sodium content of any sample of gastric juice was 8.0 mEq./l. which corresponded to an acidity of 150 mEq./l. Thus every specimen contained some non-parietal secretion although it was probably minimal in this particular instance.

The amount of mucus in each sample was not measured in these experiments due mainly to the difficulties involved

in separating out and estimating the various fractions which are loosely grouped under the generic label - mucus.

CONCLUSIONS

Metyrapone administered orally to dogs for several days produces a significant reduction in acid response from Heidenhain pouches over a wide range of dosage of histamine while high doses of cortisone generally produce a small but not significant increase in acid response to the same doses of histamine. The sigmoid histamine dose-response curve is shifted downwards and flattened by metyrapone while its gradient and highest peak is increased by cortisone.

If the maximum histamine response is in fact a measure of the parietal cell mass (Marks et al., 1960), then these results suggest that metyrapone decreases the parietal cell mass in the stomach of the dog. However, there is also evidence that the sensitivity of the parietal cells to histamine is affected and that metyrapone interferes with the ability of the parietal cell to secrete acid at its normal concentration.

G E N E R A L

D I S C U S S I O N

There is a wide variety of substances available which affect gastric secretion and in selecting tests for measuring the effect, and mechanism of action, of drugs on gastric function it is essential to choose methods of stimulation which are as physiological as possible and appropriate to the species and type of preparation used; a standard technique giving good reproducibility is also necessary. Histamine is one of the most potent stimuli known for gastric secretion and in the dog its action is almost confined to the oxyntic cells (Gregory, 1962). The secretory response of Heidenhain pouches to continuous infusions of histamine tends to remain constant over many months (Code et al., 1949), and has come to be adopted as the standard bio-assay method for the quantitative evaluation of gastric secretory inhibitors. The collection of juice from canine gastric pouches following a meal of meat is probably a more physiological method and allows the measurement of mixed parietal and non-parietal secretions. Since the Heidenhain pouch is deprived of its vagal nerve supply, pepsin output from the dogs in this work was small

and the parasympathomimetic drug mecothane was used to stimulate pepsin secretion in these experiments designed to test the effect of metyrapone on the secretion of this enzyme.

The experiments in Section I of this thesis show quite clearly that metyrapone in single doses sufficiently large to block 11-β-hydroxylation in the adrenal cortex for at least 4 hours, and possibly much longer, is without effect on the output of acid or pepsin from Heidenhain pouches stimulated to secrete by histamine, meat or the parasympathomimetic drug mecothane. It is apparent therefore that transient fluctuations in the levels of circulating cortisol do not affect gastric secretion. This is in keeping with the absence of effect of corticotrophin and adrenocortical steroid administration on stomach secretion in acute experiments reported by Hirschovits et al. (1957) and Dreiling et al. (1958). Although it has been reported that single injections of cortisone in a dose of 100 mg. potentiate the secretory response of denervated gastric pouches in dogs to histamine and mecothane (Shay, 1959), no such effect was observed in this present study.

In the only previous report in the literature on the effect of an adrenal-inhibitor drug on gastric secretion, Manrique et al. (1958) noted a marked reduction in gastric acid secretion in rats in the few hours following a single intravenous injection of Amphenone B. Although this latter drug shares with metyrapone the property of blocking cortisol formation in the adrenal cortex, it also possesses a number of other actions and it seems clear that the action of Amphenone B on the gastric glands is a direct toxic one and not mediated via cortisol suppression. The close similarity between control and test results in Section I indicates that metyrapone does not have any direct toxic effect on gastric mucosa. A slow cumulative toxic action is not ruled out and will be considered later.

When metyrapone was given for several days, a decisive reduction in the daily output of free acid occurred in most of the dogs. Due to the method of estimating acid concentration by titrating with sodium hydroxide using Topfer's solution (end-point pH 3.5) as indicator, in some of the experiments, it is likely that the reduction in total acidity was less striking, but only to the extent of 10-20 mEq./l. However, the later batches of metyrapone appeared

to exert rather less effect on the 24-hour secretion of acid and the reason for this is not clear. There is no reason to expect any difference in pharmacological action between metyrapone base in tablet form and dissolved in oil - or even its dihydrochloride salt - when all are given orally.

Gelatine capsules were used merely to ease administration of metyrapone, which has an unpleasant bitter taste, but such capsules are quickly digested in the gastro-intestinal tract and are most unlikely to delay or impair absorption to any significant extent. However, metyrapone base is fat-soluble and consequently its absorption is inevitably less predictable than that of a water-soluble preparation. Unfortunately, there is no means available of detecting directly the proportion of each dose of metyrapone which is actually absorbed.

It is theoretically possible to estimate the degree and duration of suppression of cortisol synthesis following metyrapone by measuring plasma corticoid levels; but the amounts present in peripheral blood in the dog are insufficient to permit their measurement in reasonably-sized samples. The withdrawal of adrenal vein blood, although possible, presents considerable difficulties in survivor

dogs and might have affected the validity of any conclusions drawn from other aspects of the study. Attempts were made to assess approximately the extent to which metyrapone affected the total daily output of cortisol by the adrenals by measuring that proportion of adrenocortical steroid metabolites excreted in 24-hour specimens of urine. Using paper chromatography (Zaffaroni and Burton, 1951), the tetra-hydro derivatives of cortisol and 11-deoxycorticosterone were isolated although technical difficulties prevented quantitative analysis. It did appear however that, in the single daily dose given, although there was a marked reduction in the amount of cortisol secreted, a significant amount was still being produced. The actual time in the 24-hours during which metyrapone was effective could be of some importance, for there is a diurnal pattern of adrenocortical activity with cortisol production reaching a peak during the morning; in Section II, group A dogs were given the drug in the morning and group B in the afternoon without any noticeable difference in effect.

From what is known about the duration of action of metyrapone when given orally to dogs (Chart et al., 1958; Jenkins et al., 1958), it is unlikely that 11-β-hydroxylation

in the adrenal cortex, and consequently cortisol synthesis, would be blocked for more than 8 hours after administration. Partial inhibition of steroid production might persist for much longer though, and there is always the possibility of a compensatory increase in steroid secretion when the block wears off, but there is no evidence of the latter in any reports to date. Eight hours after administration would cover the period of maximum acid secretion after a meal of meat, but the experiments in Section I show quite clearly that the effect of metyrapone on gastric acid output is not observed in the first few hours after stimulating secretion by various means. It may be that it is an alteration in the total 24-hour output of cortisol from the adrenal cortex which is the operative factor.

A more complete suppression of cortisol formation can be obtained by administrating metyrapone every 2 hours (Bmus et al., 1962), consequently efforts were made to develop a sustained-release preparation of metyrapone suitable for once-daily intramuscular injection. However, the problems of producing a non-irritant, sterile, pharmacologically active injection were not completely overcome in a number of presentations tried and this aspect

of the project was not pursued further.

The electrolyte results in Section II show that under both metyrapone and cortisol the various ions maintain their normal physiological relationship to each other. No evidence emerges to suggest that metyrapone damages the gastric mucosa or impairs the permeability of the parietal cell membranes, in which case one would expect a leakage of potassium as well as sodium ions into the gastric tubules.

The observations in Section II that graduated doses of cortisone restored gastric secretion to normal in a step-wise fashion, is additional evidence in favour of the hypothesis that the action of metyrapone on gastric secretion is secondary to, and a consequence of, its action in reducing the amount of cortisol produced in the adrenal cortex and made available to the gastric mucosa. The depression of acid secretion which occurs in Addison's disease in man (Stempien and Bagradi, 1954; Engel, 1955) and following bilateral adrenalectomy in dogs is restored by cortisone in a similar incremental manner (Sigel et al., 1957; Nicoloff et al., 1961). It is not permissible to extrapolate the regression lines in the acid output versus cortisone dosage graphs (Figures 19 and 20) beyond the limits of actual

observations, but these graphs do suggest that, if there is no cortisone, there would be no acid.

The difference in magnitude of acid response to prolonged cortisone administration that occurs from dog to dog depends to some extent on differences in size of pouch, but the observation that 100 mg. cortisone acetate was sufficient to increase the daily output of acid above control amounts in one dog in the long-term metyrapone studies and not in another can only be satisfactorily explained on the basis that, just like man, dogs vary in their individual responses to adrenocortical steroids. A similar variation in cortisone requirements is seen in dogs following surgical adrenalectomy (Nicoloff et al., 1961).

It is also evident from Section II that a certain minimum level of adrenocortical glucocorticoid secretion is required for gastric acid secretion to proceed at normal efficiency. Rapid fixation of cortisol by the body tissues has been shown to occur within 30 minutes of completing an infusion of cortisol (Nelson et al., 1951). From the time-relationships observed in this study it seems likely that the gastric mucosa must take up cortisol from the plasma and store it for some 3 or 4 days, for the acid-suppressing

effect of metyrapone does not become fully manifest until several days after starting to give the drug. Similarly, the increase in acid output which occurs with a course of cortisone continues for up to a week after stopping its administration.

Theoretically, acid gastric secretion in a given time may be considered as the product of the number of secretory units multiplied by the rate of secretion per unit (Card, 1952), and it is also generally believed that parietal cells give an all-or-none response, differing only in their threshold to stimulation according to a normal distribution (Adam et al., 1964). Thus, in the dog when the gastric glands are stimulated to secrete acid at their maximum possible rate by means of injection of the optimum dose of histamine, the resulting acid output is a linear function of the total number of parietal cells present (Marks et al., 1960). A similar correlation probably obtains in man (Card and Marks, 1960).

In the control series of histamine tests in Section III, the dose-response curves, obtained by plotting the maximum one-hour acid output against the logarithm of the corresponding dose of histamine, had the same normal sigmoid character

as those previously reported in the dog (Obrink, 1948; Komarov and Shay, 1958) and in man (Adam et al., 1954). Since the maximum histamine response was significantly reduced while the dogs were on metyrapone and apparently increased by cortisone, it may be argued that the parietal cell masses of these dogs' stomachs were altered by the drugs given. By carrying out parietal cell counts on dogs' stomachs following a course of cortisone, Clarke, Neill and Welbourn (1960) were able to demonstrate an increase of 50 per cent in the number of parietal cells as compared with control animals. This corresponded with a mean increase of 49 per cent in the maximum histamine response. Parietal cell counts were not done in this present work since this would have involved sacrificing some of the dogs during the course of the experiment, which, with the small number of animals available, would have reduced the significance of the final results. Biopsy specimens of gastric mucosa were considered but it was decided that tissue obtained in such a manner did not carry any guarantee of being representative of the gastric mucosa as a whole. The delay of several days before the full effects of metyrapone and cortisol are seen on acid output may reflect the time required for the parietal cells to

decrease or expand in number.

The concept that the parietal cells always secrete acid of a fixed concentration which is subsequently diluted and partially neutralised by the non-parietal component of gastric secretion (Pavlov, 1910; Hollander, 1931) is not fully borne out by the results of the histamine dose-response tests. The maximum concentration of HCl recorded in the metyrepone series were always less than those noted in the corresponding control tests. Since the volumes of the 15-minute collections were also reduced and histamine exerts relatively little effect on non-parietal secretion, it appears that the concentration of acid coming from the parietal cells may have been reduced by metyrepone. The fact that acid concentration was not raised higher in the cortisone series of histamine tests does not influence the significance of this observation, for, as Hollander (1940) has shown, there is a theoretical and physiological limit to the strength of acid which can be secreted by the stomach.

There is considerable interest at the present time in the 'reactivity' of the parietal cell (Stavney et al., 1964). It is felt that the sensitivity of the parietal cell and its ability to respond to stimulation, be it hormonal or nervous,

can be impaired by the action of drugs. Were metyrapone to exert its effect on gastric secretion by reducing the sensitivity of the parietal cells to histamine, one would expect the effect to be overcome by increasing the dose of histamine used in the tests. In fact, increasing the dose of histamine beyond the optimal amounts used in the control tests caused a further reduction in acid output in the metyrapone series.

If the output of pepsin is taken as an index of non-parietal secretion, then the results in Section II show that metyrapone does not cause any consistent alteration in that aspect of gastric secretion, and the acid values recorded are consequently real and not the result of variations in the relative amounts of parietal and non-parietal components. In point of fact, pepsin secretion from Heidenhain pouches, as determined by the chemical analytical methods available, is notoriously variable in amount from day to day, so that only very gross alterations in pepsin output are accepted as being significant.

In most reports, the administration of adrenocortical steroids has not been accompanied by consistent changes in output of pepsin from the stomach (Hirschowitz et al., 1957;

Drye and Schoen, 1958; Clarke, 1960) and experiences in the present investigation are similar. The evidence of a physiological action of adrenocortical steroids on the secretion of pepsin by the stomach is tenuous and appears to be based on an increase in uropepsin excretion observed during steroid therapy (Grey et al., 1951; Krakower et al., 1957). Such changes are probably the result of a lowered renal threshold for pepsinogen rather than a reflection of a raised plasma pepsinogen level consequent on increased gastric secretion of pepsin (Spiro and Mills, 1960).

The possibility that metyrapone exerts a cumulative selective toxic effect on acid secreting mechanisms in the stomach was raised earlier in this discussion. Metyrapone takes several days to achieve its maximum suppression of acid production, but return of secretion to normal occurs more quickly, usually within 3 days. The doses of metyrapone used in this investigation were relatively large pharmacological amounts (100 mg. per kg. body weight per day) and it is not known whether metyrapone blocks any other enzyme systems in the body in addition to 11-β-hydroxylation in the adrenal cortex. The prevention of inhibition of acid secretion achieved by the simultaneous administration of

cortisone along with metyrapone does not support the toxic theory unless both drugs act on the same intracellular enzyme systems, for example by a process of substrate competition.

The site of action of cortisol in the stomach is not known at present, but, elsewhere in the body, cortisol has been shown to play a most important role in cellular processes concerned with the release of energy for metabolism (Bush, 1962; Dixon et al., 1964). One theory of action proposed by Talalay and Williams-Ashman (1958) is that cortisol catalyses transhydrogenation reactions within target cells and so controls the balance of diphosphopyridine and triphosphopyridine nucleotides (D.P.N. and T.P.N.). Cortisol may well act in a similar capacity within the gastric parietal cell where a considerable amount of energy is required to concentrate hydrogen ions a millionfold and then expel them into the gastric lumen.

The electrolyte results in Sections II and III bear out the idea that the initial event in the secretion of hydrochloric acid by the stomach is the extrusion of hydrogen ions into the lumen of the gastric tubules. The concentrations of sodium, potassium and chloride are all closely related to

that of hydrogen ions. Active absorption of sodium by undifferentiated epithelial cells in the stomach of the rabbit foetus has been demonstrated by Wright (1962) and a similar sodium 'pump' probably functions in the dog (Bornstein et al., 1959). Sodium secretion and absorption in the stomach are less well understood than acid secretion. Cortisone may have an action on the reabsorption of sodium from the stomach tubules similar to that which it exerts in the kidney but this seems unlikely in view of the extremely close correlation between sodium and hydrogen ion concentration in gastric juice which would entail a direct exchange of one sodium for each hydrogen ion. Furthermore, such an action of cortisol could not explain the rapid and frequent changes in gastric acidity which occur in the course of the 24 hours.

It has recently been suggested by Hirschowitz (1961) that there is a sodium-rich secretion derived from the cells in the base of the gastric tubules and that this secretion is subsequently modified by the addition of hydrogen ions from the parietal cells in the neck of the tubules. Although it has been stated earlier that the concentration of potassium and chloride ions in the present investigation

followed changes in hydrogen ion concentration more closely than alterations in sodium, such evidence is merely suggestive and does not prove conclusively the priority of hydrogen ions in gastric secretion. Since chloride concentration varies within fairly narrow limits, it is possible that a rapid influx of H^+ ions from rapidly secreting parietal cells upsets the ionic balance within the lumen of the gastric tubule and the most readily available, and mobile, sodium ions require to be actively removed by surface mucus or other, as yet unidentified, cells, in order to restore ionic equilibrium. There is no evidence of a two-way passage of ions across the parietal cell membrane.

The two-component theory of Hollander (1931) was put forward as 'a working hypothesis' and it must continue to serve a most useful function in this respect until much more information is obtained on the intracellular processes involved in the secretion of hydrochloric acid by the stomach. Enough however is known already to state with certainty that the secretions of the adrenal cortex are essential for the elaboration of hydrochloric acid by the stomach in the dog, and the evidence for a similar relationship obtaining in man is very strong.

The present investigation has not thrown any additional light on the problem of peptic ulcer. The deleterious effects which large doses (300 mg. per day) of cortisone had on denervated gastric pouches in several dogs is similar to the experience of other workers in this field (Cooper et al., 1961; Chaikof et al., 1961; Johnson, 1963). The dogs did not show a significant increase in acid secretory response to maximal histamine stimulation while on prolonged treatment with this dose of cortisone and it appears more likely that cortisone impaired the efficiency of the mechanisms responsible for protecting the gastric mucosa. It is obvious on revieving the literature on peptic ulcer that too much emphasis has been placed on gastric hypersecretion in the past and the time has come for a mass deployment of effort into the investigation of gastro-duodenal mucosal resistance.

S U M M A R Y

1. The association of gastro-intestinal symptoms with disorder of the adrenal cortex dates back to Thomas Addison's (1855) original description of adrenocortical insufficiency.

2. Hypochlorhydria was first noted in Addison's disease by von Gravitz in 1907.

3. Test meals of various kinds indicated that approximately 50 per cent of patients with Addison's disease were achlorydric and, in recent times, this has been confirmed by more satisfactory estimates of gastric secretory capacity such as Kay's (1953) augmented histamine test.

4. Chronic peptic ulcer is uncommon in Addison's disease, possibly because of the reduced secretion of acid by the stomach.

5. Treatment of adrenocortical insufficiency by replacement doses of steroids results in a return of acid secretion to normal levels and an increased liability to peptic ulceration.

6. Hypersecretion of adrenocorticoids in Cushing's syndrome is associated with increased gastric acidity but there is no increased susceptibility to peptic ulceration.

7. The actions of corticotrophin and adrenal steroids on gastric secretion have been the subject of many conflicting reports. The particular drug used, the dose, the duration of treatment and the disease treated are some of the factors affecting the results.

8. The physiological mechanism regulating gastric secretion appear to be similar in man and dog but other animals show species differences which prevent the transfer of conclusions reached therein to man.

9. Dogs with separated pouches of the stomach are especially suitable for assessing the effects of drugs on gastric secretion.

10. Single doses of corticotrophin or adrenal steroids have no effect on gastric secretion in rodents, dogs or man.

11. Prolonged treatment with physiological doses of adrenal steroids merely depresses endogenous steroid production via pituitary inhibition of corticotrophin release.

12. Long-term administration of steroids in pharmacological doses increases gastric acid secretion in man and dog.

13. Approximately 7 per cent of patients treated with corticotrophin or adrenal steroids develop peptic ulceration; the incidence in an appropriate control series is between one and two per cent.

14. Steroids reduce the secretion of mucus by the stomach and also alter its composition.

15. 'Steroid ulcers' are due mainly to impaired mucosal resistance and increased secretion of acid is a contributory factor.

16. The experiments in this thesis were performed on dogs with stomach pouches of Heidenhain (vagally denervated) type. The effect of an adrenal inhibitor drug metyrapone and cortisone on gastric secretion was studied.

17. Single injections of pharmacological doses of metyrapone were without effect on secretion from canine Heidenhain pouches stimulated to secrete by (a) a meal of meat, (b) the parasympathomimetic drug mecothane or (c) histamine. Single injections of cortisone had no effect on the gastric response to histamine.

18. Prolonged treatment with oral metyrapone produced a striking reduction in the 24-hour output of acid from stomach pouches in dogs, with return to control levels on discontinuing the drug.

19. The administration of various doses of cortisone along with metyrapone restored gastric acidity in direct proportion to the dose of cortisone used.

20. Cortisol produced by the adrenal cortex is essential for normal gastric secretion, but its exact role is still being debated.

21. It is likely that cortisol acts on enzyme systems within the parietal cell concerned with the production of energy necessary for acid formation.

22. Histamine dose-response curves of acid output were obtained in dogs while on (a) no drug, (b) prolonged oral metyrapone and (c) prolonged oral cortisone (100 mg. and 300 mg. per day). Metyrapone caused a significant reduction in the volume and concentration of acid secreted. Cortisone produced an inconsistent increase in acid output which was not statistically significant.

23. The relationships between sodium, potassium, chloride and hydrogen ion concentrations in gastric juice were not affected by metyrapone or cortisone.

24. A very close negative correlation was observed between sodium and hydrogen ion concentration.

25. Changes in concentration of potassium and chloride ions appear to follow variations in hydrogen ion concentration more closely than they do sodium. This suggests that the primary event in the secretion of acid by the stomach is the extrusion of hydrogen ions into the lumen of the gastric tubules.

26. All the data in these experiments support Hollander's two-component theory of gastric secretion, with the proviso that the concentration of acid produced by the parietal cells may, on occasion, be reduced below the theoretical value by deficiency of cortisol.

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APPENDIX

Dog K

Volume of 24-hour collections of juice from Heidenhain pouch; concentration of electrolytes and pepsin.

Day	1	2	3	4	5	6	7	8	9	10	11
Volume ml	82	73	95	90	140	105	93	102	80	76	100
Acid mEq/l	77	65	88	65	92	76	70	80	72	72	78
Potassium mEq/l	8.2	8.2	9.0	8.9	8.2	7.9	7.5	7.4	7.3	6.9	7.1
Sodium mEq/l	78.0	75.0	69.0	67.0	51.0	60.0	60.0	71.0	58.7	65.9	58.1
Chloride mEq/l	156	153	153	153	163	164	155	150	157	159	152
Pepsin mg.Ty.eq/l	-	-	-	-	-	-	12	18	12	18	26

Day	12	13	14	15	16	17	18	19	20	21	22
Volume ml	104	90	87	85	80	135	67	85	62	58	62
Acid mEq/l	76	79	76	75	59	92	29	60	36	42	23
Potassium mEq/l	8.5	8.4	8.3	8.2	7.0	6.0	5.8	5.6	5.8	5.4	6.3
Sodium mEq/l	61.0	57.8	52.5	75.5	73.8	46.2	87.9	72.6	82.8	87.1	98.0
Chloride mEq/l	155	151	155	155	155	156	156	161	152	155	146
Pepsin mg.Ty.eq/l	17	10	9	51	41	21	26	7	28	38	57

Dog H

Day	23	24	25	27	28	29	30	31	32	33
Volume ml	65	53	80	67	61	55	52	43	49	53
Acid mEq/l	32	29	22	22	57	10	16	6	6	12
Potassium mEq/l	5.3	4.6	4.6	4.8	4.2	4.4	4.9	4.6	4.6	5.
Sodium mEq/l	91.8	73.0	79.0	85.0	65.9	97.0	90.0	89.6	93.3	72.
Chloride mEq/l	170	161	148	148	153	136	138	159	150	138
Pepsin mg.Ty.eq/l	43	43	-	46	26	26	70	43	37	34

Day	34	35	36	37	38	39	40	41	42	43
Volume ml	43	29	36	38	33	23	40	35	38	43
Acid mEq/l	8	7	5	4	4	4	0	0	0	11
Potassium mEq/l	4.4	4.7	5.0	5.4	4.6	4.8	5.1	4.2	4.9	4.
Sodium mEq/l	85.3	84.7	102.7	104.9	114.7	108.4	114.4	107.1	112.6	102.
Chloride mEq/l	170	161	148	148	153	136	138	159	150	138
Pepsin mg.Ty.eq/l	43	29	36	38	33	23	40	35	38	43

Dog E

Day	44	45	47	48	49	50	51	52	53	54
Volume ml	42	42	50	63	47	60	35	44	36	45
Acid mEq/l	12	17	29	23	24	41	9	9	11	22
Potassium mEq/l	5.1	5.4	4.6	5.0	5.0	4.4	5.0	4.4	5.2	4
Sodium mEq/l	100.7	107.9	92.9	95.7	108.6	83.9	112.4	108.6	103.7	95
Chloride mEq/l	146	155	155	148.9	148.9	146.4	148.1	141.3	146.0	147
Pepsin mg.Fy.eq/l	27	27	58	17	19	22	50	46	53	31

Day	55	56	57	58	59	60	61	62	63	64
Volume ml	22	67	6	42	72	72	66	37	53	50
Acid mEq/l	0	37	36	16	53	53	65	46	30	36
Potassium mEq/l	3.4	4.6	3.4	4.1	4.7	3.9	5.2	6.2	6.7	6.
Sodium mEq/l	98.6	90.0	83.2	107.0	70.0	75.5	62.8	77.5	95.0	89
Chloride mEq/l	134.5	141.8	157.4	151.5	157.0	160.0	161.6	156.6	124.4	155
Pepsin mg.Fy.eq/l	20	30	-	55	28	41	27	15	15	20

Dog H

Day	65	66	67	68	69	70	71	72	73
Volume	40	20	37	52	35	53	38	52	43
Acid	21	16	21	30	32	42	17	23	10
Potassium	5.2	5.6	5.3	5.3	5.0	5.9	5.1	5.3	4.8
Sodium	117.4	109.1	96.5	93.7	99.3	100.7	115.0	97.9	100.0
Chloride	144.7	150.6	144.7	143.0	135.4	149.4	151.4	154.0	141.3
Pepsin	28	46	64	42	35	28	48	46	39

Day	74	75	76	77	78	79	80	81	82
Volume	36	48	40	25	39	41	38	35	48
Acid	9	20	0	0	24	0	7	22	32
Potassium	5.0	4.7	5.4	5.2	4.1	5.7	4.8	6.3	7.2
Sodium	98.7	102.1	105.2	109.5	101.4	108.2	114.3	96.6	82.6
Chloride	146.0	151.5	149.8	160.8	154.9	130.8	141.8	143.0	139.7
Pepsin	33	25	34	19	30	55	35	37	10

Dog H

Day	83	84	85	86	87	88	89	90	91
Volume	15	38	38	42	20	22	32	15	51
Acid	0	60	60	66	54	60	67	45	56
Potassium	9.3	8.5	9.6	10.5	8.8	10.1	9.9	8.5	10.0
Sodium	130.0	66.3	61.3	63.8	70.0	66.8	62.4	68.8	68.8
Chloride	137.5	141.8	146.8	153.2	149.0	153.6	154.5	164.6	155.3
Pepsin	26	10	13	12	22	22	28	43	26

Day	92	93	94	95	96	97	98	99	100
Volume	58	20	24	29	24	31	25	36	25
Acid	82	40	34	52	44	67	48	69	34
Potassium	11.1	10.5	9.7	10.5	10.6	8.6	10.2	11.0	9.7
Sodium	47.5	67.5	96.4	76.8	79.8	64.0	78.9	64.4	93.2
Chloride	157.4	153.2	151.5	156.6	137.5	130.5	144.1	154.7	143.7
Pepsin	18	40	52	27	39	47	13	32	42

Dog H

Day	101	102	103	104	105	106	107	108	109
Volume	40	68	65	62	66	50	12	78	35
Acid	68	76	88	79	88	77	62	86	83
Potassium	10.5	10.2	10.9	10.2	12.0	10.3	9.5	9.5	9.9
Sodium	61.3	53.8	45.6	56.9	48.6	53.8	71.3	51.0	55.3
Chloride	157.8	151.6	158.6	158.6	157.0	157.8	164.1	153.9	160.1
Pepsin	34	41	38	29	19	20	33	30	17

Day	110	111	112	113	114	115	116	117	118
Volume	133	80	107	88	70	28	80	110	118
Acid	94	95	103	101	102	77	101	107	109
Potassium	10.8	8.9	8.7	8.9	7.8	7.6	10.1	9.6	8.7
Sodium	45.0	49.1	42.2	37.5	62.4	42.5	35.0	34.4	39.1
Chloride	159.4	187.5	167.2	150.8	154.7	149.2	169.5	159.4	167.2
Pepsin	21	19	5	12	19	47	8	8	6

Dog H

Day	119	120	121	122	123	124	125	126	127
Volume	70	68	40	46	44	65	52	60	70
Acid	98	95	83	84	80	102	97	102	100
Potassium	9.8	9.5	9.7	9.9	10.3	9.9	11.2	10.5	10.0
Sodium	38.0	50.4	41.8	47.9	47.9	38.1	41.1	47.7	40.5
Chloride	169.5	150.8	160.9	154.7	160.1	155.5	146.5	170.3	159.0
Pepsin	4	40	29	24	33	9	19	11	13

Day	128	129	130	131	132	133	134	135	136
Volume	50	68	45	80	44	70	52	95	58
Acid	97	102	97	95	80	97	90	108	77
Potassium	10.0	9.6	9.6	10.1	8.8	9.4	9.1	9.7	8.6
Sodium	42.8	36.2	39.7	41.0	54.0	41.8	47.1	32.5	57.4
Chloride	157.0	160.9	157.8	159.4	155.5	160.1	145.3	164.1	149.6
Pepsin	-	16	14	10	31	18	22	17	26

Dog H

Day	137	138	139	140	141	142	143	144	145
Volume	45	62	61	40	78	50	44	38	48
Acid	77	86	82	71	94	67	57	62	88
Potassium	8.6	8.3	8.2	9.2	8.6	8.5	8.6	8.1	9.1
Sodium	54.5	57.9	57.1	61.9	41.3	57.4	70.0	63.9	50.3
Chloride	161.3	162.1	162.5	159.4	137.5	139.8	153.5	163.3	171.1
Pepsin	39	-	34	55	46	43	42	-	29

Day	146	147	148	149	150	151	152	153
Volume	60	50	50	50	40	48	50	46
Acid	85	108	69	68	63	69	73	72
Potassium	9.2	9.1	8.2	8.9	9.0	8.3	8.4	9.5
Sodium	52.2	44.2	60.6	58.8	55.9	58.6	48.9	65.7
Chloride	170.3	160.1	158.4	152.2	144.2	147.2	139.7	155.9
Pepsin	9	12	32	15	11	10	19	10

Dog J

Volume of 24-hour collections of juice from Heidenhain pouch; concentration of electrolytes and pepsinl

Day	1	2	3	4	5	6	7	8	9
Volume ml	65	76	38	79	110	74	110	115	76
Acid mEq/l	13	25	14	20	31	30	46	26	24
Potassium mEq/l	-	-	-	-	-	-	6.7	7.1	6.9
Sodium mEq/l	-	-	-	-	-	-	93.2	103.9	103.3
Chloride mEq/l	-	-	-	-	-	-	152.9	153.7	145.4
Pepsin mgtyeq/ml	-	-	-	-	-	-	42	44	23

Day	10	11	12	13	14	15	16	17	18
Volume ml	44	108	69	92	65	-	78	80	138
Acid mEq/l	32	39	20	38	30	-	12	27	11
Potassium mEq/l	6.9	7.0	7.0	6.9	6.9	-	6.0	5.7	4.8
Sodium mEq/l	93.6	90.0	102.9	92.8	91.4	-	109.8	93.2	105.7
Chloride mEq/l	149.2	155.0	146.2	156.7	154.6	-	150.0	138.7	149.2
Pepsin mgtyeq/ml	31	57	64	38	56	-	107	56	108

Dog J

Day	19	20	21	22	23	24	25	26	27
Volume	115	105	70	90	90	74	120	100	45
Acid	7.4	8.0	9.4	-	-	4.3	-	-	-
Potassium	5.2	5.1	4.7	4.0	4.1	4.1	3.8	3.8	3.7
Sodium	111.7	108.7	112.9	124.5	122.5	84.3	94.0	100.0	98.8
Chloride	156.2	145.8	161.7	148.3	152.1	175.0	144.6	179.2	154.2
Pepsin	10	74	128	172	129	97	-	90	148

Day	28	29	30	31	32	33	34	35	36
Volume	87	85	95	85	105	120	80	108	100
Acid	0	4.2	3.8	4.0	0	0	4.0	3.6	0
Potassium	4.1	3.8	3.9	3.7	3.6	3.8	3.9	3.9	3.9
Sodium	106.5	96.7	97.3	100.0	104.5	93.2	93.3	122.2	126.1
Chloride	162.5	162.5	156.7	155.0	155.4	160.0	165.0	163.3	158.3
Pepsin	63	157	59	71	65	67	85	78	82

Dog J

Day	37	38	39	40	41	42	43	44	45
Volume	78	130	200	125	90	87	98	85	93
Acid	4.0	4.4	0	3.8	14	4.6	0	18.0	25.6
Potassium	4.3	4.5	4.7	4.2	4.2	5.0	4.1	4.3	4.6
Sodium	127.4	118.3	126.7	124.1	106.3	116.0	118.8	123.6	115.0
Chloride	155.0	149.8	136.3	146.0	147.7	149.4	152.3	157.0	151.5
Pepsin	43	70	69	38	54	71	53	40	50

Day	46	47	48	49	50	51	52	53	54
Volume	160	100	105	92	60	105	52	112	70
Acid	10.0	12.6	30.4	25.4	9.0	33.4	18.7	15.4	35.4
Potassium	4.9	4.6	4.7	5.0	4.8	5.0	4.4	4.7	5.2
Sodium	100.0	110.7	100.0	109.5	120.0	94.9	101.5	107.1	104.3
Chloride	144.3	143.0	151.5	153.2	151.9	144.7	147.3	133.3	140.5
Pepsin	45	54	41	64	46	46	45	26	42

Dog J

Day	55	56	57	58	59	60	61	62	63
Volume	98	95	92	113	186	235	170	100	100
Acid	55	48	64	83	92	93	50	57	38
Potassium	5.0	5.6	6.2	6.4	7.3	7.6	6.9	7.3	6.1
Sodium	74.9	66.3	62.8	46.7	44.1	43.8	81.5	79.4	114.2
Chloride	116.4	146.4	163.8	153.2	163.3	166.3	166.3	159.1	160.4
Pepsin	43	49	75	45	26	18	23	17	22

Day	64	65	66	67	68	69	70	71	72
Volume	104	95	65	160	115	75	84	57	65
Acid	21	25	0	60	24	44	16	11.8	8.2
Potassium	5.6	5.5	4.5	5.8	5.6	5.7	5.2	4.5	4.9
Sodium	101.9	90.9	122.8	87.8	83.6	101.3	109.3	103.4	106.4
Chloride	148.5	146.0	140.1	153.2	140.5	158.7	154.9	140.5	143.0
Pepsin	56	68	66	32	28	54	54	42	45

Dog J

Day	73	74	75	76	77	78	79	80	81
Volume	80	91	100	77	89	78	87	95	50
Acid	20.2	0	24	11.2	27.2	18.2	22.6	44.6	36.5
Potassium	4.9	4.7	5.5	4.9	4.5	5.0	5.9	6.9	7.3
Sodium	110.5	109.1	106.8	104.9	95.9	115.7	101.4	89.1	100.0
Chloride	141.3	144.3	147.7	149.4	149.0	143.9	141.3	151.5	150.2
Pepsin	37	45	34	56	69	37	42	24	23

Day	82	83	84	85	86	87	88	89	90
Volume	85	105	75	80	105	105	145	90	-
Acid	72.5	80	73	67	80	80	63	56	-
Potassium	7.8	8.4	9.0	8.5	9.4	8.8	9.1	8.8	-
Sodium	63.8	51.9	62.5	66.1	56.0	57.3	64.4	70.0	-
Chloride	158.7	153.2	158.3	157.0	153.2	156.6	156.6	162.5	-
Pepsin	18	39	40	40	23	39	41	29	-

Dog J

Day	91	92	93	94	95	96	97	98	99
Volume	75	128	98	125	90	90	67	110	125
Acid	65	67	69	72	68	68	79	81	76
Potassium	9.0	9.3	9.2	9.3	8.9	9.0	9.3	9.4	9.1
Sodium	91.8	65.8	55.0	55.3	64.6	63.1	58.8	57.5	58.8
Chloride	155.7	156.6	147.3	152.3	150.8	148.4	147.6	154.7	157.8
Pepsin	62	59	35	46	47	21	43	40	74

Day	100	101	102	103	104	105	106	107	108
Volume	70	74	105	96	130	127	96	50	80
Acid	78	87	77	69	100	95	91	86	69
Potassium	9.2	8.1	8.8	8.6	9.5	9.4	9.0	9.5	9.4
Sodium	55.0	48.8	60.0	63.5	38.8	44.4	45.4	51.6	46.3
Chloride	154.7	157.0	147.6	148.4	157.8	155.5	157.0	154.7	150.8
Pepsin	43	45	38	3	36	32	46	26	39

Dog J

Day	109	110	111	112	113	114	115	116	117
Volume	160	160	210	123	35	130	105	125	145
Acid	111	116	118	116	76	119	119	111	111
Potassium	9.0	8.7	8.2	9.2	9.1	9.1	9.3	8.5	9.6
Sodium	37.5	27.8	25.0	27.5	84.0	27.5	22.9	28.3	26.8
Chloride	166.4	169.9	163.3	160.9	152.3	170.3	159.4	169.1	164.1
Pepsin	21	8	24	19	31	12	24	8	6

Day	118	119	120	121	122	123	124	125	126
Volume	195	120	115	100	130	112	114	95	112
Acid	102	105	94	90	102	102	103	98	87
Potassium	8.8	9.2	9.6	9.4	10.0	9.9	8.9	9.6	8.9
Sodium	21.7	33.2	49.7	45.4	43.0	45.4	47.1	46.1	50.7
Chloride	163.3	160.9	161.7	160.1	161.7	154.7	168.0	155.5	512.7
Pepsin	22	56	40	28	26	26	18	23	-

Dog J

Day	127	128	129	130	131	132	133	134	135
Volume	180	215	110	95	87	80	110	68	30
Acid	107	125	73	65	85	77	102	66	48
Potassium	9.1	8.9	8.7	7.8	7.8	7.8	8.5	7.2	7.7
Sodium	37.3	25.7	60.3	66.3	55.9	60.3	40.5	67.2	35.0
Chloride	159.4	171.1	154.7	151.6	158.6	160.5	156.2	145.3	141.8
Pepsin	22	18	11	53	32	34	32	9	-

Day	136	137	138	139	140	141	142	143	144
Volume	65	88	45	35	70	-	45	50	75
Acid	100	92	48	48	64	-	55	90	73
Potassium	8.6	8.1	7.7	7.0	7.6	-	7.0	8.3	8.4
Sodium	47.8	51.5	88.2	84.0	68.6	-	66.3	51.8	62.0
Chloride	137.5	157.8	154.3	159.4	155.5	-	150.0	153.1	155.5
Pepsin	22	57	65	68	65	-	-	39	40

Dog J

Day	145	146	147	148	149	150
Volume	95	55	45	55	70	72
Acid	113	58	62	62	78	64
Potassium	8.5	7.4	8.0	8.0	7.7	7.0
Sodium	88.2	68.6	66.5	62.8	62.6	58.3
Chloride	154.7	156.8	153.4	153.8	153.4	141.3
Pepsin	44	39	20	22	9	41

Dog L — Volume of collections of juice from Heidenhain pouch; concentration of electrolytes.

Sample	1	2	3	4	5	6	7	8	9	10
Volume ml	70	60	80	12	78	75	80	60	60	60
Acid mEq/l	120	114	121	3	114	140	131	108	111	104
Potassium mEq/l	10.8	9.8	10.0	7.0	10.0	11.0	9.7	10.0	10.0	10.0
Sodium mEq/l	33	30	28	119	39	16	24	46	40	46
Chloride mEq/l	170	168	165	150	167	177	174	167	157	165

Sample	11	12	13	14	15	16	17	18	19	20
Volume ml	90	80	45	60	9	40	40	37	30	32
Acid mEq/l	101	105	93	107	34	85	119	116	51	121
Potassium mEq/l	10.9	9.8	9.1	10.0	9.4	9.5	9.8	10.2	4.7	9.6
Sodium mEq/l	52	48	59	46	115	72	33	37	95	39
Chloride mEq/l	165	167	165	166	159	167	171	163	156	161

Dog L

Sample	21	22	23	24	25	26	27	28	29	30
Volume	32	36	32	75	31	20	42	45	35	42
Acid	56	120	64	84	121	51	117	71	118	70
Potassium	8.9	10.0	9.1	10.6	10.4	9.0	10.1	9.2	9.8	9.4
Sodium	91	94	89	65	31	103	41	81	35	83
Chloride	156	165	162	163	161	158	160	167	157	168

Sample	31	32	33	34	35	36	37	38	39	40
Volume	38	50	47	44	27	51	65	32	42	15
Acid	117	84	111	76	103	69	65	109	70	67
Potassium	9.9	9.9	10.0	9.0	9.0	8.6	7.4	8.2	7.4	7.7
Sodium	35	65	58	72	49	83	79	39	70	72
Chloride	169	166	174	164	162	156	155	158	151	153

Dog L

Sample	41	42	43	44	45	46	47	48	49	50
Volume	54	32	47	13	43	22	47	23	53	70
Acid	88	110	76	59	69	114	86	101	88	87
Potassium	8.0	7.4	7.3	7.0	7.2	7.4	7.3	8.0	7.8	6.7
Sodium	67	44	80	83	76	36	65	45	56	61
Chloride	160	163	157	153	152	161	160	157	158	168

Sample	51	52	53	54	55	56	57	58	59	60
Volume	26	28	27	42	36	55	19	55	28	48
Acid	114	47	108	69	117	86	89	90	101	79
Potassium	7.8	6.2	7.4	6.9	8.0	7.3	6.1	7.0	6.9	7.1
Sodium	135	193	44	78	44	68	66	63	52	68
Chloride	157	145	158	156	166	158	165	158	155	152

Dog L

Sample	61	62	63	64	65	66	67	68	69	70
Volume	25	60	95	29	57	46	58	22	55	21
Acid	99	90	92	99	75	116	78	96	78	76
Potassium	7.1	7.5	7.2	7.2	7.0	7.0	7.9	7.7	8.1	7.4
Sodium	48	61	56	54	72	37	79	57	68	71
Chloride	160	156	155	159	156	158	159	156	153	153

Sample	71	72	73	74	75	76	77	78	79	80
Volume	50	50	45	100	32	38	55	24	70	17
Acid	71	122	62	83	57	109	67	85	87	75
Potassium	7.6	7.5	7.8	8.6	8.1	9.1	9.1	7.4	8.1	5.9
Sodium	78	32	87	66	94	39	81	70	65	80
Chloride	154	160	154	158	154	157	157	162	157	164

Dog L

Sample	81	82	83	84	85	86	87	88	89	90
Volume	58	12	50	17	66	72	45	27	17	53
Acid	67	63	65	89	100	103	123	56	78	91
Potassium	7.6	6.0	7.1	6.3	7.2	7.4	8.3	6.9	5.9	6.8
Sodium	80	89	85	65	50	56	33	93	76	63
Chloride	154	156	150	165	158	165	161	154	160	158

Sample	91	92	93	94	95	96	97	98	99	100
Volume	9	68	50	48	83	30	60	15	73	95
Acid	60.5	105	96.5	96	104	109	91	82	103	106
Potassium	5.3	7.1	7.2	6.6	6.3	6.3	5.8	5.7	6.1	6.6
Sodium	93	54	57	58	53	48	63	76	54	45
Chloride	155	159.5	160.5	163	159.5	163	159	163	159	154

Dog L

Sample	101	102	103	104	105	106	107	108	109	110
Volume	90	100	100	28	75	110	30	75	19	85
Acid	109	118	110	100	94.5	98	103	94	82	99.5
Potassium	6.0	6.5	5.7	5.4	5.6	6.4	6.3	5.9	5.9	5.9
Sodium	43	37	45	59	62	54	50	61	75	56
Chloride	158	616.5	159.5	161	154	160	164	158	162	154

Sample	111	112	113	114	115	116	117	118	119	120
Volume	30	75	40	82	43	70	68	47	120	45
Acid	105	89	120	108	121	100	136	85.5	110	130
Potassium	5.9	6.4	7.1	6.8	7.0	7.5	8.1	7.8	8.4	10.0
Sodium	54	65	32	45	35	50	22	65	42	22
Chloride	158.5	156	162.5	161	156	157.5	163.5	156	164	170

Dog L

Sample	121	122	123	124	125	126	127	128	129	130
Volume	92	73	72	47	75	62	35	38	47	10
Acid	92	140	91	124	101	121	59	113	61	83
Potassium	7.5	10.3	8.6	9.1	9.0	8.2	7.4	9.1	8.1	7.6
Sodium	58	24	60	36	58	37	97	39	87	54
Chloride	164	172	154	170	169	172	166	165	160.5	155

Sample	131	132	133	134	135	136	137	138	139	140
Volume	80	80	25	111	48	70	100	88	90	33
Acid	99	127	106	112	123	93.5	109.5	101	97.5	128
Potassium	9.0	9.6	9.6	9.6	9.4	8.7	8.9	8.5	8.8	9.9
Sodium	52	31	48	41	35	61	44	50	52	35
Chloride	161	173	170	161.5	170	166	163	157	159	171

Dog L

Sample	141	142	143	144	145	146	147	148	149	150
Volume	60	54	50	136	125	46	60	73	68	58
Acid	93	117	91.5	115	117	121	90	135.5	100	132
Potassium	7.8	8.9	8.3	9.0	9.1	9.4	8.1	9.3	8.7	10.2
Sodium	65	28	59	41	43	35	52	22	50	22
Chloride	166	164	160.5	163	167	163.5	158.5	167	162	162.5

Sample	151	152	153	154	155	156	157	158	159	160
Volume	55	104	85	85	77	60	108	45	36	35
Acid	89	136	111	135	111	130	111	118	28.5	87.5
Potassium	7.9	9.8	9.6	9.8	9.3	9.7	8.8	8.1	8.7	7.8
Sodium	67	20	41	18	42	28	42	50	111	63
Chloride	161	162	160	167	158	163	158	173	147	156

Dog L

Sample	161	162	163	164	165	166	167	168	169	170
Volume	64	34	65	50	56	42	58	53	35	40
Acid	101	94	93	109	67	110	71	118	68	112
Potassium	9.1	9.0	9.1	8.4	8.0	8.5	8.0	8.5	8.0	7.9
Sodium	59	63	59	48	83	41	83	35	79	41
Chloride	166.5	166	168	161	159.5	166.5	157	162.5	165.5	164

Sample	171	172	173	174	175	176	177	178	179	180
Volume	22	22	41	25	37	30	37	17	59	41
Acid	36.5	103	41.5	99	45	97.5	46	83	29	105
Potassium	8.1	9.1	8.0	9.1	8.8	8.9	8.7	8.2	8.8	9.4
Sodium	115	45	101	44	99	48	101	66	107	45
Chloride	158	161	154	160	153	157	148	157	143	160

Dog L

Sample	181	182	183	184	185	186	187	188	189	190
Volume	55	47	43	56	49	51	55	34	65	51
Acid	49.5	111	114	64	113.5	118.5	32	102	49	131
Potassium	8.9	9.1	9.5	8.5	9.7	10.2	9.0	9.3	9.0	10.1
Sodium	93	40	39	87	42	33	111	50	95	21
Chloride	148	162	160	153	159	163	149	160.5	148	156.5

Sample	191	192	193	194	195	196	197	198	199	200
Volume	60	58	105	79	67	60	58	89	50	62
Acid	42	113	97	92.5	90	89	93	93	112	86
Potassium	9.4	10.0	9.7	9.7	9.4	10.0	9.1	9.9	8.8	8.1
Sodium	99	39	54	56	56	64	59	54	38	70
Chloride	144	157.5	157	160	158.5	162	154	153.5	157	164

Dog M

Volume of collections of juice from Heidenhain pouch; concentration of electrolytes.

Sample	1	2	3	4	5	6	7	8	9	10
Volume ml	60	55	65	70	40	72	50	90	80	60
Acid mEq/l	63	60	71.5	64	98.5	80.5	59	101	55	79
Potassium mEq/l	6.6	5.9	4.7	6.4	6.6	6.6	5.8	6.8	6.7	6.0
Sodium mEq/l	79	71	74	83	56	74	87	57	97	72
Chloride mEq/l	159	149	153	165	167	162	149	158	161	159

Sample	11	12	13	14	15	16	17	18	19	20
Volume ml	60	55	16	45	17	40	29	40	26	42
Acid mEq/l	41	52	14	24	66	16	93	25	88	24
Potassium mEq/l	5.8	6.7	7.2	7.1	5.6	6.4	6.1	6.2	6.4	6.8
Sodium mEq/l	105	103	131	133	85	122	65	124	65	135
Chloride mEq/l	153	161	161	165	166	177	160	128	157	169

Dog M

Sample	21	22	23	24	25	26	27	28	29	30
Volume	18	40	83	23	30	20	45	20	46	21
Acid	83	14	44	74	9	50.5	23	57	13	60.5
Potassium	6.2	7.2	7.8	7.5	7.4	7.2	7.7	6.9	7.2	7.1
Sodium	74	139	109	73	141	106	135	89	131	83
Chloride	156	173	162	153	152	159	174	154	155	165

Sample	31	32	33	34	35	36	37	38	39	40
Volume	53	22	36	62	20	40	25	50	14	42
Acid	17	78	15	22	62	30	75	29	45.5	19
Potassium	7.2	7.2	7.1	5.6	5.6	5.9	6.3	6.3	6.4	6.0
Sodium	124	70	124	117	84	107	68	120	103	126
Chloride	176	164	156	155	159	149	158	155	155	154

Dog M

Sample	41	42	43	44	45	46	47	48	49	50
Volume	16	16	45	23	47	80	20	50	34	67
Acid	52	55	19.5	72.5	14	28	70	30	81	37.5
Potassium	6.0	5.8	6.1	5.6	5.0	5.8	5.9	6.2	5.5	6.2
Sodium	87	83	120	74	106	108	60	111	65	108
Chloride	156	162	154	160	131	145	151	149	155	153

Sample	51	52	53	54	55	56	57	58	59	60
Volume	28	81	35	73	19	70	25	67	105	95
Acid	70	58	85.5	42	60	47	80	43	30	51
Potassium	6.0	7.0	6.6	6.5	6.2	6.7	6.4	6.7	6.2	7.2
Sodium	83	93	63	108	94	99	71	111	115	97
Chloride	161	153	158	160	152	155	162	148	158	152

Dog M

Sample	61	62	63	64	65	66	67	68	69	70
Volume	22	53	20	68	27	51	15	21	53	80
Acid	72.5	32	62	51	81	30	48	60	38.5	21
Potassium	6.7	6.4	6.6	7.9	7.4	7.2	6.7	7.2	7.6	8.7
Sodium	77	111	93	101	69	115	114	87	111	121
Chloride	156	153	156.5	152	152	151	154	156	156	157

Sample	71	72	73	74	75	76	77	78	79	80
Volume	57	19	45	17	73	17	50	13	60	15
Acid	56	54	29	50	47	56	16	45	35	44
Potassium	8.3	7.4	7.5	6.3	7.5	6.1	6.4	5.6	7.0	6.2
Sodium	92	95	121	113	107	97	130	99	111	106
Chloride	156	157	156	159	157	158.5	146	149	148	156

Dog M

Sample	81	82	83	84	85	86	87	88	89	90
Volume	46	70	30	19	45	21	72	70	80	58
Acid	27	36	73	71	34.5	74	49	55	63	42.5
Potassium	6.7	6.8	6.6	5.4	6.9	5.5	6.6	6.1	5.7	5.3
Sodium	117	115	81	78	113	76	98	89	85	106
Chloride	149	152.5	160	155	150.5	156	149	150.5	152	150

Sample	91	92	93	94	95	96	97	98	99	100
Volume	30	50	9	50	72	71	18	50	74	55
Acid	90	13	25	14	59	66	57	22	70	44.5
Potassium	4.8	6.0	5.9	6.6	5.0	4.9	5.2	6.2	5.2	4.5
Sodium	65	139	122	131	87	80	92	120	76	107
Chloride	157	161	148	159	151.5	154	152	144	150	148.5

Dog M

Sample	101	102	103	104	105	106	107	108	109	110
Volume	28	50	94	30	66	40	61	44	34	66
Acid	85	34.5	74	93	54	108	62.5	119	57	131
Potassium	4.8	5.6	5.9	5.1	5.8	5.2	7.1	6.1	7.9	7.1
Sodium	59	109	76	65	104	39	84	34	87	26
Chloride	145	149.5	149.5	158.5	152.5	157	153.5	156	154.5	164

Sample	111	112	113	114	115	116	117	118	119	120
Volume	28	115	37	65	56	35	65	75	88	20
Acid	55	105	131	115	133	46	124	114	140	68
Potassium	7.2	7.2	7.4	9.3	7.1	7.5	7.4	8.1	7.5	8.0
Sodium	94	54	24	41	28	108	34	41	22	100
Chloride	152.5	163	166	169	173	152	168	172	175	154

Dog M

Sample	121	122	123	124	125	126	127	128	129	130
Volume	62	42	15	83	98	40	98	53	90	59
Acid	132	68	75	118	137	59	134	85	107.5	85
Potassium	7.1	8.2	8.2	8.2	7.8	8.5	7.6	7.4	7.2	6.8
Sodium	24	77	68	36	21	89	21	64	47	67
Chloride	169	156.5	155	163.5	174	158	166	157.5	164.5	155

Sample	131	132	133	134	135	136	137	138	139	140
Volume	57	42	66	30	40	26	48	35	47	31
Acid	101	93	130	18	108	46.5	82.5	79.5	114	47
Potassium	5.5	5.3	5.5	7.8	6.4	7.7	6.5	6.0	6.2	6.2
Sodium	44	52	29	118	40	99	72	68	39	93
Chloride	155	149	167	167	161	145	157.5	152.5	157.5	147.5

Dog H

Sample	141	142	143	144	145	146	147	148	149	150
Volume	47	20	54	30	43	20	73	37	72	25
Acid	123	60	127.5	82	135	63	132	88	140.5	75
Potassium	5.9	7.7	7.4	7.0	7.0	8.0	8.0	7.2	8.5	8.
Sodium	30	91	26	79	26	87	16	65	18	76
Chloride	158.5	155	157.5	159	163	159	166	153.5	162	154

Sample	151	152	153	154	155	156	157	158	159	160
Volume	47	25	80	38	97	42	64	45	76	32
Acid	142	90	139.5	94.5	148	105.5	141.5	101	142.5	77
Potassium	8.2	6.5	9.8	7.1	10.0	7.4	8.7	6.8	8.4	6.
Sodium	24	61	20	61	8	50	18	57	18	76
Chloride	169.5	160	165.5	163	168	157.5	160	160	168	154

Dog H

Sample	161	162	163	164	165	166	167	168	169	170
Volume	30	65	143	70	75	52	35	32	45	18
Acid	127	127	133	132.5	125	84	118	87.5	72	140
Potassium	7.2	8.0	7.6	7.5	6.8	5.0	5.8	5.4	6.7	10.
Sodium	32	32	20	26	31	69	37	66	81	26
Chloride	166.5	165	161	166	160.5	163	162.5	164	161.5	174.

Sample	171	172	173	174	175	176	177	178	179	180
Volume	25	17	83	38	45	32	42	32	40	25
Acid	81	125	105	110	41	92	16.5	87	43	92
Potassium	8.3	9.9	8.3	8.6	8.0	7.2	7.9	7.2	8.0	7.
Sodium	76	30	55	50	105	65	119	167	115	63
Chloride	158.5	162.5	164	168.5	157	159.5	147	163	160.5	162.

Dog H

Sample	181	182	183	184	185	186	187	188	189	190
Volume	44	22	46	24	44	24	41	32	43	22
Acid	51	80	22	85.5	18.5	79	21.5	88	31	74
Potassium	8.0	7.9	7.8	7.2	8.5	7.2	8.4	7.4	7.9	6.9
Sodium	99	67	114	61	131	65	129	61	115	79
Chloride	162.5	154	159	157	157	157	161	158	156	160.5

Sample	191	192	193	194	195	196	197	198	199	200
Volume	57	21	44	42	50	56	54	34	41	33
Acid	24	75	22.5	92	118	64	118.5	107	28.5	103.5
Potassium	9.1	7.3	8.4	7.7	8.4	9.8	8.0	7.4	9.0	7.4
Sodium	120	76	117	54	35	85	39	48	113	48
Chloride	151	158	157	157	164.5	152	160	162	154.5	157.5

Dog M

Sample	201	202	203	204	205	206	207	208	209	210
Volume	53	25	56	46	54	53	58	49	46	30
Acid	28	69	30	106	68	48	55	57	55	111
Potassium	8.7	7.0	8.9	8.1	8.0	8.0	8.2	7.6	6.9	7.
Sodium	117	81	115	45	81	98	87	91	85	39
Chloride	153	154.5	151	164	150.5	154	154	155.5	143.5	154

Sample	211
Volume	49
Acid	52
Potassium	7.1
Sodium	89
Chloride	148

Dog H — Volume of collections of juice from Heidenhain pouch; concentration of electrolytes.

Sample	1	2	3	4	5	6	7	8	9	10
Volume ml	60	46	60	36	35	35	70	56	50	50
Acid mEq/l	33	116.5	53	36.5	43	50	111	115	111	19
Potassium mEq/l	4.3	5.4	4.7	5.4	5.2	6.2	7.6	7.7	7.9	6.
Sodium mEq/l	120	45	102	116	116	104	46	47	41	131
Chloride mEq/l	156	162	156	138	153	158	165	171	168	151

Sample	11	12	13	14	15	16	17	18	19	20
Volume ml	26	72	52	60	55	90	48	90	48	65
Acid mEq/l	116.5	16.5	94	17.5	91	32	87	38	104	37
Potassium mEq/l	6.4	8.2	7.6	6.2	7.2	6.3	7.2	6.7	7.6	7.
Sodium mEq/l	35	131	61	129	58	116	61	107	50	107
Chloride mEq/l	173	159	160	152	159	153.5	154	151.5	162.5	154

Dog N

Sample	21	22	23	24	25	26	27	28	29	30
Volume	120	27	80	56	95	71	150	63	102	65
Acid	50	101	40	96	44.5	98	78	111	48	104
Potassium	7.2	8.8	7.1	8.0	7.2	7.9	7.7	8.9	7.3	6.
Sodium	99	63	107	59	101	63	74	44	97	52
Chloride	150	165	153	167	159	169	155	164	155	159.

Sample	31	32	33	34	35	36	37	38	39	40
Volume	102	56	140	152	34	84	50	75	50	85
Acid	46.5	96	63	41	59	18	98	25	87	22
Potassium	7.0	7.0	7.1	6.1	5.4	5.2	6.4	6.3	5.8	5.
Sodium	97	61	85	105	97	125	56	124	70	123
Chloride	152	168	156	152	158	149.5	157	150	157.5	149.

Dog H

Sample	41	42	43	44	45	46	47	48	49	50
Volume	44	105	65	95	200	160	63	100	49	105
Acid	95	36	98	31	59.5	89	96	44	86	50
Potassium	5.8	5.6	6.1	6.1	6.4	6.3	5.4	5.9	4.9	5.
Sodium	71	114	55	119	92	65	62	103	75	101
Chloride	158	153	157	151	154.5	159.5	164	150	166	153

Sample	51	52	53	54	55	56	57	58	59	60
Volume	51	80	52	95	39	99	85	70	75	41
Acid	85	34	83	27	68	43	55.5	95	49	76
Potassium	5.4	5.4	5.0	5.0	5.0	5.4	5.9	6.1	6.0	5.
Sodium	69	115	72	123	85	100	100	65	99	77
Chloride	162	155.5	158	147	153.5	148.5	154.5	162	147.5	155.

Dog M

Sample	61	62	63	64	65	66	67	68	69	70
Volume	90	185	60	127	96	90	135	70	115	20
Acid	57.5	113	102	71	125	120	71.5	109	50	70
Potassium	5.8	5.6	6.1	6.1	6.3	6.7	7.3	6.0	5.9	5.4
Sodium	91	42	46	79	31	37	79	54	101	91
Chloride	148.5	160	157.5	150.5	158	162	154.5	170	153	162

Sample	71	72	73	74	75	76	77	78	79	80
Volume	152	177	165	194	239	150	105	177	225	81
Acid	82	99	70	96	70	85	87	52	69	108
Potassium	5.9	5.4	4.8	5.3	4.4	5.6	5.9	4.8	5.3	4.7
Sodium	70	55	78	61	79	72	52	98	85	48
Chloride	156.5	157	152.5	159	148.5	154	158	148	147	160

Dog H

Sample	81	82	83	84	85	86	87	88	89	90
Volume	173	175	167	170	51	123	111	135	118	95
Acid	70	85	74.5	82	109	107	144	97	132	63
Potassium	5.4	5.3	5.3	5.8	5.8	7.0	6.2	7.7	7.4	7.
Sodium	80	65	76	76	46	44	24	54	28	91
Chloride	154.5	155.5	152	164	166	164	172	155	176	162

Sample	91	92	93	94	95	96	97	98	99	100
Volume	120	76	100	103	82	145	163	115	81	135
Acid	132	61	124	87	123.5	89	132	77	114	79.
Potassium	9.4	8.0	8.2	9.0	8.3	8.2	7.9	7.6	7.0	6.
Sodium	26	85	30	60	30	59	30	65	37	68
Chloride	175	152	170	153.5	166.5	155	170	164	160	153

Dog II

Sample	101	102	103	104	105	106	107	108	109	110
Vloume	276	160	177	145	157	34	95	39	96	53
Acid	109	136	99	66	68.5	88.5	30.5	76	29.5	82
Potassium	6.5	6.9	7.0	6.7	6.6	7.6	6.8	7.3	6.6	7.
Sodium	44	24	52	76	74	59	112	72	111	68
Chloride	160.5	166	160	158.5	147.5	162	150	161	152	161

Sample	111	112	113	114	115	116	117	118	119	120
Volume	120	65	40	42	85	139	122	161	168	170
Acid	32	91	87	71.5	46.5	86	68	63.5	78	74.
Potassium	7.2	8.1	9.1	8.4	5.8	6.3	6.1	6.3	6.8	6.
Sodium	115	61	65	81	97	71	77	81	70	72
Chloride	148	161	161.5	158.5	152.5	158	153.5	152	155.5	154

Dog M

Sample	121	122	123	124	125	126	127	128	129	130
Volume	180	136	177	116	134	102	98	97	101	101
Acid	82	75.5	94	66	60	70.5	44	51.5	61	50
Potassium	7.2	7.3	7.2	6.5	6.8	7.2	6.4	6.7	6.8	5
Sodium	69	70	58	76	91	78	101	92	86	90
Chloride	156	156	157	152	157.5	156	153	151	149	148

Sample	131	132	133	134	135	136	137	138	139	140
Volume	98	102	90	95	104	112	88	137	198	139
Acid	63	54	51	49	59	79	55	79	113	71.5
Potassium	6.5	6.3	6.2	6.4	7.0	7.4	6.6	7.9	8.9	7.8
Sodium	74	83	89	87	82	63	91	66	31	72
Chloride	149.5	149	153	151	151.5	155	151	154	153.5	153

Dog M

Sample	141	142	143	144	145	146	147	148	149	150
Volume	283	96	130	97	131	91	180	85	71	115
Acid	105.5	47	61	33.5	60	27.5	100	33	37	78
Potassium	8.7	7.5	8.0	6.6	8.3	6.7	8.2	6.1	5.3	7.
Sodium	42	97	85	117	89	117	50	117	108	74
Chloride	153	150	152.5	155	151.5	153	160.5	154.5	150	155.

Sample	151	152	153	154	155	156	157
Volume	125	108	120	96	98	89	89
Acid	25	57	90	28	35.5	32	62
Potassium	5.2	6.0	5.3	5.8	5.5	5.1	5.4
Sodium	121	93	57	119	113	109	83
Chloride	151.5	153	152	153.5	149	150	152.5

Dog 0 — Volume of collections of juice from Heidenhain pouch; concentration of electrolytes.

Sample	1	2	3	4	5	6	7	8	9	10
Volume ml	92	40	168	113	180	98	25	130	71	90
Acid mEq/l	135.5	72.5	141.5	86.0	90.0	120.0	25.5	135.0	49.0	113.
Potassium mEq/l	7.8	8.5	8.6	8.0	7.9	8.2	7.2	7.8	7.9	8.
Sodium mEq/l	17	75	11	59	63	31	121	16	101	39
Chloride mEq/l	156.0	145.5	160.0	150.5	151.5	154.5	144.0	158.5	144.0	153.

Sample	11	12	13	14	15	16	17	18	19	20
Volume ml	108	110	119	75	26	125	55	140	64	65
Acid mEq/l	95	81	84	139	85	143	128	143	58	108
Potassium mEq/l	6.4	6.9	5.7	6.4	6.9	8.3	7.4	7.2	7.5	8.
Sodium mEq/l	55	67	71	15	65	15	27	15	89	46
Chloride mEq/l	151.5	154.0	159.5	159.0	150.0	167.0	160.0	162.5	149.5	153.

Dog O

Sample	21	22	23	24	25	26	27	28	29	30
Volume	208	75	65	169	55	80	100	93	206	235
Acid	96	137.5	58.5	127	86	140	77	79	113	111
Potassium	8.7	8.2	7.8	9.2	8.2	7.5	8.4	7.3	7.1	7.4
Sodium	52	16	91	30	65	13	70	67	35	37
Chloride	155	159	150.5	154.5	163	159.5	151.5	152	154.5	157

Sample	31	32	33	34	35	36	37	38	39	40
Volume	147	232	243	243	200	270	185	260	218	212
Acid	149	99	144	93	147	140	54	131	58	135
Potassium	7.2	7.2	6.0	6.9	7.0	7.0	6.2	7.3	6.8	5.9
Sodium	10	54	24	67	12	22	95	22	87	27
Chloride	171	163	169	154	175	170	145	164	144.5	166

Dog0

Sample	41	42	43	44	45	46	47	48	49	50
Volume	232	185	259	230	224	97	270	230	266	300
Acid	139	136	112	70	85	125	86	122	86	95.5
Potassium	6.3	6.3	5.9	5.7	6.4	5.7	6.9	6.2	7.4	6.7
Sodium	26	24	42	78	65	42	61	33	62	61
Chloride	170	167	160	154.5	146	167	150	163	145	153

Sample	51	52	53	54	55	56	57	58	59	60
Volume	278	123	151	136	124	120	125	200	105	178
Acid	115.5	128	73	143	79	144	62	146	127	140
Potassium	6.2	6.4	7.7	7.5	9.0	9.7	9.1	10.0	10.3	9.0
Chloride	158	154	145	163.5	146.5	160	147	165	160	159

Dog 0

Sample	61	62	63	64	65	66	67	68	69	70
Volume	123	74	81	64	70	200	65	100	15	115
Acid	102	148.5	96	152	96	139	112	149	28	148.5
Potassium	9.7	9.5	9.3	10.0	9.5	9.4	9.3	9.2	8.2	8.8
Sodium	46	10	50	10	50	16	35	10	113	10
Chloride	150	159	150.5	168	151.5	157;5	155.5	165	138	169

Sample	71	72	73	74	75
Volume	85	240	175	265	166
Acid	115	147	105	149.5	144
Potassium	9.4	9.4	8.8	9.2	9.2
Sodium	35	14	44	8	16
Chloride	155	167	152	166	165

Dog I — Histamine Tests: Maximum 4 consecutive 15-min. Collections.

Dose of Histamine	Control			Metyrapone			Cortisone 100 mg.			Cortisone 300 mg.		
	Vol. ml.	Conc. mEq/l	Output mEq	Vol. ml.	Conc. mEq/l	Output mEq	Vol. ml.	Conc. mEq/l	Output mEq	Vol. ml.	Conc. mEq/l	Output mEq
12.5 µg.	3.2	142	0.451	2.5	136	0.340	2.5	128	0.320	3.3	138	0.455
	3.0	142	0.526	2.2	137	0.301	2.5	128	0.320	3.3	138	0.455
	3.5	140	0.490	2.2	135	0.297	2.8	125	0.350	3.4	141	0.479
	3.2	142	0.551	2.6	133	0.346	2.5	124	0.310	3.0	142	0.426
25 µg.	6.0	158	0.948	4.0	146	0.584	4.1	147	0.603	4.5	151	0.680
	6.0	156	0.936	3.8	147	0.559	4.4	147	0.647	4.5	151	0.680
	6.0	157	0.942	3.6	147	0.529	3.6	147	0.529	4.5	151	0.680
	6.0	157	0.942	3.6	146	0.526	4.5	147	0.662	4.6	151	0.695
50 µg.	8.5	160	1.360	4.4	152	0.669	5.0	149	0.749	6.8	152	1.034
	7.5	160	1.200	5.0	150	0.750	5.4	150	0.810	7.0	152	1.064
	7.6	163	1.239	4.5	153	0.689	5.2	150	0.780	6.8	152	1.034
	8.0	160	1.280	4.5	154	0.693	5.0	150	0.750	6.9	153	1.056
100 µg.	8.6	162	1.393	4.5	148	0.666	6.0	148	0.888	7.5	148	1.110
	9.4	162	1.523	5.0	149	0.745	6.5	150	0.975	7.2	151	1.087
	9.0	162	1.458	5.0	151	0.755	6.6	150	0.990	7.5	152	1.140
	9.6	162	1.555	4.9	152	0.745	6.0	150	0.900	7.2	152	1.094
200 µg.	8.9	162	1.442				5.8	153	0.887	7.7	153	1.178
	8.0	162	1.296				6.1	152	0.927	7.0	154	1.078
	9.5	161	1.530				7.0	152	1.064	7.0	154	1.078
	9.5	162	1.539				6.5	152	0.988	7.2	154	1.140
400 µg.	9.4	155	1.457	3.7	147	0.544	6.3	147	0.926			
	9.0	155	1.395	3.0	150	0.450	6.2	148	0.918			
	8.9	159	1.415	3.2	150	0.480	5.9	148	0.873			
	9.4	161	1.513	3.0	150	0.450	5.4	148	0.799			

Dog M — Histamine Tests: Maximum 4 consecutive 15-min. Collections.

Dose of Histamine	Control Vol. ml.	Control Conc. mEq/l	Control Output mEq	Metyrapone Vol. ml.	Metyrapone Conc. mEq/l	Metyrapone Output mEq	Cortisone 100 mg. Vol. ml.	Cortisone 100 mg. Conc. mEq/l	Cortisone 100 mg. Output mEq	Cortisone 300 mg. Vol. ml.	Cortisone 300 mg. Conc. mEq/l	Cortisone 300 mg. Output mEq
12.5 µg.	2.5	143	0.358	1.7	112	0.190	3.2	140	0.448	4.0	141	0.564
	3.4	145	0.493	1.6	112	0.179	3.4	135	0.459	3.8	141	0.536
	2.8	145	0.406	1.5	112	0.168	3.3	139	0.459	3.2	143	0.458
	3.0	145	0.435	1.5	110	0.165	3.8	142	0.540	3.2	143	0.458
25 µg.	5.0	145.5	0.728	3.5	136	0.476	7.6	146	1.110	6.4	147	0.941
	6.0	146	0.876	3.5	138	0.483	7.0	147	1.043	6.2	148	0.918
	5.5	146	0.803	3.5	138	0.483	6.5	146	0.949	6.0	148	0.888
	5.7	146.5	0.835	3.5	138	0.483	6.0	146	0.876	6.2	148	0.918
50 µg.	7.5	160	1.200	6.5	143	0.930	9.1	148	1.347	10.6	152	1.611
	7.5	161	1.208	7.0	143	1.001	8.8	148	1.302	10.4	152	1.581
	7.0	163.5	1.145	6.7	143	0.958	8.7	148	1.288	10.6	152	1.611
	7.0	155	1.085	7.0	142	0.994	8.2	150	1.230	11.4	152	1.733
100 µg.	8.5	151	1.284	5.6	148	0.829	10.0	151	1.510	12.0	152	1.824
	8.0	151.5	1.212	5.2	146	0.759	10.0	151	1.510	11.8	153	1.805
	8.0	154	1.232	5.2	146	0.759	10.0	152	1.520	11.3	153	1.729
	7.5	151	1.133	5.5	145	0.798	10.0	151	1.510	13.0	153	1.989
200 µg.	8.3	154	1.278				9.0	149	1.341	9.2	146	1.343
	7.5	153.5	1.150				7.9	148	1.169	9.2	147	1.352
	7.5	158	1.185				8.0	149	1.192	9.2	147	1.352
	6.5	155	1.008				8.9	150	1.335	10.0	146	1.460
400 µg.	5.5	152	0.836				8.7	150	1.305			
	5.5	154	0.847				9.2	150	1.380			
	4.5	156	0.702				8.9	150	1.335			
	5.5	159	0.785				9.0	150	1.350			

Dog K — Histamine Tests: Maximum 4 consecutive 15-min. collections.

Dose of Histamine	Control Vol. ml.	Control Conc. mEq/l	Control Output mEq	Metyrapone Vol. ml.	Metyrapone Conc. mEq/l	Metyrapone Output mEq	Cortisone 100 mg. Vol. ml.	Cortisone 100 mg. Conc. mEq/l	Cortisone 100 mg. Output mEq	Cortisone 300 mg. Vol. ml.	Cortisone 300 mg. Conc. mEq/l	Cortisone 300 mg. Output mEq
12.5 µg.	8.0	141	1.128	5.0	133	0.665	6.8	137	0.932	9.0	147	1.323
	7.5	142	1.065	4.0	131	0.524	6.5	137	0.891	8.7	147	1.279
	7.5	140	1.050	5.5	130	0.715	7.0	137	0.959	9.2	147	1.352
	7.2	141	1.015	4.0	132	0.528	6.0	136	0.822	8.7	147	1.279
25 µg.	16.0	157	2.512	11.2	146	1.635	17.6	150	2.640	15.7	152	2.386
	16.0	156	2.496	11.4	147	1.676	17.0	150	2.550	15.2	152	2.310
	16.0	156	2.496	11.7	147	1.720	17.2	150	2.580	16.1	152	2.447
	16.2	154	2.493	11.5	148	1.702	17.0	149	2.533	15.2	153	2.326
50 µg.	21.7	159.5	3.381	16.0	148	2.368	22.7	159	3.609	23.9	156	3.728
	21.1	159	3.355	16.0	148	2.368	23.8	159	3.784	25.0	156	3.900
	21.2	161	3.413	15.5	148	2.294	23.5	159	3.737	25.3	156	3.947
	21.0	162	3.382	17.0	150	2.550	23.0	157	3.611	23.1	158	3.650
100 µg.	23.0	162.5	3.641	16.0	143	2.288	23.3	155	3.612	26.0	154	4.004
	22.5	161	3.623	16.5	142	2.343	24.5	156	3.822	23.5	155	3.643
	21.0	158	3.318	16.1	144	2.318	23.0	157	3.611	24.8	154	3.819
	21.5	161	3.462	16.0	143	2.288	24.0	157	3.768	24.8	154	3.819
200 µg.	18.9	158	2.986	12.1	148	1.791	22.4	152	3.405	24.3	154	3.742
	17.0	158	2.686	14.0	148	2.072	24.1	152	3.663	23.1	154	3.557
	18.9	160	3.024	14.0	150	2.100	23.6	152	3.587	23.3	154	3.588
	18.0	161	2.898	14.5	150	2.175	22.0	153	3.366	24.0	155	3.720
400 µg.	15.8	162.5	2.568	10.0	136	1.360						
	17.4	162	2.819	9.0	142	1.278						
	17.1	160	2.736	8.0	144	1.152						
	18.6	158.5	2.948	7.2	145	1.044						

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Dog O — Histamine Tests: Maximum 4 consecutive 15-min. collections.

Dose of Histamine	Control Vol. ml.	Control Conc. mEq/l	Control Output mEq	Metyrapone Vol. ml.	Metyrapone Conc. mEq/l	Metyrapone Output mEq	Cortisone 100 mg. Vol. ml.	Cortisone 100 mg. Conc. mEq/l	Cortisone 100 mg. Output mEq	Cortisone 300 mg. Vol. ml.	Cortisone 300 mg. Conc. mEq/l	Cortisone 300 mg. Output mEq
12.5 µg.	13.5	151	2.039	9.0	143	12287				9.5	139	1.321
	14.0	151	2.114	8.4	139	1.168				11.3	140	1.582
	14.5	150	2.175	9.0	140	1.260				9.5	141	1.340
	13.4	150	2.025	10.0	143	1.430				11.4	141	1.607
25 µg.	Dose calculated incorrectly			15.3	146	2.234				19.3	151	2.914
				16.3	146	2.380				18.8	152	2.858
				13.4	143	1.916				18.9	152	2.873
				14.0	145	2.030				19.0	153	2.907
50 µg.	26.4	154	4.066	21.0	153	3.213				27.3	152	4.150
	26.0	154	4.004	20.5	152	3.116				25.3	152	3.846
	25.5	154	3.927	21.6	152	3.283				21.1	151	3.186
	26.4	154	4.066	20.5	150	3.075				26.9	152	4.089
100 µg.	28.1	153	4.299	25.7	148	3.804				28.5	153	4.361
	28.5	154	4.389	22.0	148	3.256				30.0	153	4.590
	28.0	153	4.284	25.1	148	3.715				27.8	154	4.281
	27.0	155	4.185	25.5	147	3.749				28.5	152	4.332
200 µg.	32.0	155	4.960	19.7	152	2.994				24.1	154	3.711
	31.0	154	4.774	19.6	152	2.979				26.2	154	4.035
	30.0	154	4.620	19.0	155	2.945				25.8	156	4.025
	30.0	155	4.620	19.1	152	2.903				23.2	156	3.619
400 µg.	31.0	152	4.712									
	30.0	155	4.650									
	27.8	154	4.281									
	27.5	155	4.263									

NOT DONE

2nd Control

Histamine Tests: Maximum 4 consecutive 15-min. collections.

Dose of Histamine	Dog L Vol. ml.	Dog L Conc. mEq/l	Dog L Output mEq	Dog M Vol. ml.	Dog M Conc. mEq/l	Dog M Output mEq	Dog N Vol. ml.	Dog N Conc. mEq/l	Dog N Output mEq	Dog O Vol. ml.	Dog O Conc. mEq/l	Dog O Output mEq
12.5 µg.	2.6	136	0.354	2.1	100	0.210	8.5	143	1.216	17.7	146	2.584
	2.7	136	0.367	1.8	104	0.187	7.4	144	1.066	17.4	146	2.540
	2.7	137	0.370	1.7	100	0.170	6.6	143	0.944	18.9	146	2.759
	2.8	136	0.381	1.6	98	0.159	8.5	144	1.224	17.5	145	2.538
25 µg.	3.8	138	0.524	4.3	130	0.559	12.0	150	1.800	27.6	151	4.168
	3.7	141	0.522	4.0	133	0.532	12.5	150	1.875	25.9	152	3.937
	3.4	141	0.479	3.7	135	0.500	11.5	151	1.737	27.5	152	4.180
	4.0	141	0.546	3.8	135	0.513	11.4	151	1.721	25.8	152	3.922
50 µg.	5.4	148	0.799	7.1	146	1.037	13.7	151	2.069	30.8	152	4.682
	5.7	149	0.849	7.0	146	1.022	13.7	152	2.082	30.5	153	4.667
	4.9	151	0.740	6.7	147	0.985	13.7	152	2.082	29.3	153	4.483
	5.5	151	0.831	7.0	146	1.022	13.8	152	2.098	30.6	153	4.682
100 µg.	7.6	147	1.117	8.0	149	1.192	19.3	155	2.992	39.6	158	6.257
	6.7	149	0.998	7.4	147	1.088	15.1	155.5	2.348	27.8	154.5	4.295
	7.5	150	1.125	8.0	149	1.192	16.5	156.5	2.582	39.1	155.5	6.080
	7.4	150	1.110	8.1	149	1.207	14.8	156	2.309	33.7	155.5	5.240
200 µg.	7.0	151	1.057	8.4	149	1.252	18.1	156	2.824	34.0	153	5.202
	6.7	153	1.025	8.1	150	1.215	20.0	156	3.120	33.5	153	5.126
	6.7	155	1.039	8.1	150	1.215	15.5	159	2.465	33.4	153	5.110
	6.6	156	1.030	8.0	150	1.200	19.5	159	3.101	32.5	153	4.973