ABSTRACT

IONIC FLUXES IN GASTRIC POUCHES; R. T. Chambers, McGill University M.Sc. Thesis, 1969. **Department of Experimental Surgery.**

Neutral and acidic fluid instillation tests were carried in dog denervated corpus gastric pouches with and without topical treatment of the pouches by saline or urea solutions to delineate the ionic fluxes involved with gastric mucosal competence and acidity regulation.

Results showed a bidirectional chloride ion flux. The flux of chloride from lumen to mucosa was independent of the type of cation but seemed to be constant for the luminal concentration of chloride. Treatment with four molar urea solution results in the loss of the ability of the pouch to contain an acid solution, the gastric mucosal competence. Weaker urea solution treatments result in only an influx of sodium chloride containing fluid into the pouch. The exact mechanism of gastric mucosal competence and acidity regulation could not be delineated but a probable mechanism is suggested.

IONIC FLUXES IN GASTRIC POUCHES. CHAMBERS, R. T.

# IONIC FLUXES IN GASTRIC POUCHES

by

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## SECTION I: INTRODUCTION

### A. Statement of the Problem

The stomach, with its necessity to contain and regulate an acid solution, has presented researchers and clinicians alike with problems in elucidating its physiology and, thus, its pathophysiology. It is only relatively recently that the work of Heidenhain (49) and Pavlov (80) placed the study of gastric physiology on a scientific basis. Since their work many researchers have attempted to define the mechanisms involved in acidity regulation. That this work has met with little success is evidenced by the multiplicity of theories which have been evolved to explain acidity regulation by the gastric mucosa. At a clinical level the failure to accurately define these mechanisms has necessitated a purely empirical approach to the problem of peptic ulcer disease and other alterations in acidity regulation. Of the many theories of acidity regulation the "Exchange Diffusion" hypothesis enunciated by Teorell (92) in 1933 and the "Two-Component" hypothesis of Hollander (59) have gained the widest acceptance. The basis of the "Two Component" hypothesis is the measurement of the chloride content in gastric juice from gastric pouches in dogs (58). This measurement is only of the net chloride ion gain by the gastric juice and does not take into account the possibility that the chloride ion moves in both directions across the gastric mucosa. Several authors (56, 53, 34) have shown, in the in vitro situation, that chloride is able to move across the gastric mucosa bi-directionally. If this bidirectional movement of the chloride ion occurs in the in vivo situation then both the qualitative and quantitative extent of this movement could alter any hypothesis based on a unidirectional movement of the chloride ion. Reference to in vivo studies of

bidirectional chloride fluxes in the gastric pouch of a dog could not be found; thus a part of this study is to determine if such a bidirectional flux of chloride ion occurs in the in vivo preparation and if so to quantitate such fluxes. The study is also designed to qualitate such fluxes, if they occur, at least to the extent that they vary depending on the presence or absence of acid in the gastric lumen. As will be seen Teorell (92) proposed that the acidity regulation was mediated through an exchange of sodium chloride for hydrogen chloride across the gastric mucosa. That this exchange occurs was confirmed by several workers (9, 14, 26, 34, 37, 71, 78, 87, 99) and based on the realization that the gastric mucosa has a unique ability to contain an acid solution, Wlodek (101) extended the "Exchange Diffusion" concept to the broader "Gastric Mucosal Competence" hypothesis. Davenport (30) had shown that the stomach's ability to contain an acid solution could be destroyed by bathing the luminal surface of the mucosa with urea solutions. Thus, a second part of this study is to determine if urea solutions will destroy the gastric mucosal competence and to quantitate and qualitate the nature of the ion fluxes following treatment of the gastric luminal mucosal surface with urea solutions.

B. Theories of Acid Formation

The problem of acid formation is inextricably bound to the problem of acid regulation. Ito (65) notes that direct evidence is not available as to the cellular source of hydrochloric acid in the stomach. There is, however, abundant indirect evidence that the parietal cell is responsible for hydrochloric acid production. The earliest of this evidence comes from Ebstein and Grützner (36) who in 1874 advanced the concept that the parietal cell, being rich in chloride salts, liberated free acid from these salts by some unknown process. In 1881 Langley (67) provided

histological evidence for this conclusion and suggested that the parietal cell should therefore be known as the "oxyntic", the acid-forming, cell. Using histochemical techniques Fitzgerald (39) in 1910 and Collip (15) in 1920 showed the presence of acid in the intracellular canaliculi. This was confirmed by Dawson and Ivy (32) in 1926. In 1953 Kay (66) demonstrated that large doses of histamine resulted in the stomach producing acid at maximal levels. This technique was used in 1960 by Card and Marks (10) to demonstrate that the maximal acid output by the stomach is directly proportional to the total number of parietal cells in the mucosa. With electron microscopic observations Rohrer and his colleagues (89) showed that, following histamine injection, the parietal cells became more prominent and complex. They interpretated this as reflecting an increase in the surface area of the canalicular membrane. At the same time there was a decrease in the number of cytoplasmic vesicles, and those vesicles remaining became oriented about the canaliculi. The non-stimulated parietal cell contains large numbers of cytoplasmic vesicles distributed generally. The indirect evidence cited above has led most investigators to accept the parietal cell as the probable site of formation of hydrochloric acid in the stomach.

The accurate study of the intercellular mechanisms involved in the secretion of hydrochloric acid is even more difficult than the identification of the cell involved. Most of these studies have been done either under in vivo methods where the gastric juice collected has been assumed identical with secretion or under in vitro conditions using electromotive cells which may or may not have any relationship to mucosal function in the intact animal. The assumption that the gastric juice collected under in vivo conditions represents secretion has plagued gastric physiology from its outset. The only assumption which is valid, when studying gastric juice collected from

subjects, is that it represents recoverable interluminal contents. The recoverable interluminal contents are a function of secretion and subsequent modification of that secretion by a variety of factors. These modifying factors could include such things as mixing with other secretions or modification of the ionic content of the juice by the mucosa through such mechanisms as exchange diffusion. Equally the findings of the electrophysiologists can be stated only as representing the behavior of the mucosa under the conditions of the experiment. The experimental conditions are usually so extreme that it is difficult to accept the assumption that the mucosal membrane is not altered by them. However, with these reservations in mind, the information from these experiments has led to the development of several concepts of the intercellular mechanisms involved in acid formation in the vertebrate stomach.

One of the earliest attempts to explain these intercellular mechanisms was that of Davenport and Fisher (23) in 1940. They referred to their ideas as being incomplete but suggested that under the influence of carbonic anhydrase the intercellular carbon dioxide reacted with water to form carbonic acid, which is almost completely dissociated. They felt that the hydrogen ion was then actively secreted and that the chloride ion followed the hydrogen passively. Their evidence for the passive diffusion of the chloride ion was that bromide could be substituted for it. This reasoning assumes that if the chloride ion is actively secreted the mechanism involved is highly specific and able to distinguish between the halogens.

In 1950 Rehm (84) suggested that the secretion of hydrochloric acid by the gastric mucosa was dependent upon the potential difference across the membrane. Separating which of the two, secretion or the potential difference, comes first is very difficult; however, he was able to demonstrate this effect only in the presence of a gastric stimulant,

histamine, and only by applying a large external current across the membrane. Further, Hogben (57) has shown that hydrogen ion secretion can occur in the absence of any potential difference across the membrane.

The most widely accepted model today is that of the "Redox Pump" developed by E. J. Conway (16, 17) to explain the intercellular mechanisms of gastric acid secretion. This model is so widely accepted today that, as Hogben (57) warns:

> "one might be inclined to conclude that it rests on a firm foundation of experiment."

The basis of the model is the oxidation by an electron acceptor of the hydrogen from a substrate molecule at the outer surface of the cell membrane bordering the lumen. The electron acceptor, believed to be an iron containing cytochrome-like compound, then moves to the inner surface of the cell membrane where it, in turn, is oxidized. These reactions can be written:

outer surface:

$$4\ \text{H-(substrate)} + 4\ Fe^{+++} \longrightarrow 4\ H^{+} + 4\ \text{(Substrate)} + 4\ Fe^{++}$$

inner surface:

$$O_2 + 4\ Fe^{++} + 2\ H_2O \longrightarrow 4\ Fe^{+++} + 4\ OH^{-}$$

The hydrogen ions would then be released from the outer surface of the cell membrane into the canaliculi. The thermodynamic considerations of this model are very complex and, as the complete reactions involved in the model are unknown, they are only assumptions based on incomplete information. It would seem that every molecule of oxygen consumed could produce only four hydrogen ions assuming one hundred percent efficiency of the system. In an elegant study (24) Davenport has shown that only two hydrogen ions are secreted for every oxygen molecule taken up by the cells. This would suggest that, if oxygen is directly involved in the reactions as shown, the system is fifty percent efficient. The gradient for the hydrogen ions between the

blood and the gastric lumen is approximately $1:10^6$ and Conway (16) has shown that the minimum caloric cost of transfering a mole of hydrogen ion across this gradient is 10,000 calories. The combustion of glucose yields approximately 100,000 calories per mole of oxygen. Thus the maximum efficiency for the overall process of acid secretion by the stomach should be about twenty percent. However, using Davenport's data and not considering such factors as entropy we have shown an efficiency of about fifty percent assuming oxygen to be directly involved in the acid. This suggests that either the "Redox Pump" is thermodynamically impossible or that it must be modified so that oxygen is only indirectly involved in the production of the hydrogen ions. In fact, Davies and Ogston (31) as well as Heinz and Öbrink (52) have shown that oxygen need not be directly involved.

The mechanisms of chloride ion transport seem to be the same as or closely related to acid formation mechanisms. Using an isolated gastric mucosa preparation Bannister (4) showed that chloride was required in the external medium in order for efficient acid secretion to occur in relation to oxygen consumption. When sulphate was substituted for the chloride the acid formation was sharply inhibited while the oxygen consumption remained constant. Alonso and Harris (2) inhibited acid formation completely when they substituted glucuronate for chloride in the external medium. Durbin (35) extended this work to modify the redox model such that it worked in concert with a chloride transport mechanism. In this model the chloride ion forms a neutral complex with an unknown substance in the transporting membrane. The kinetics for this reaction resemble Michaelis-Menten kinetics ... that is, the reaction velocity is dependent upon the nutrient chloride ion concentration such that if the concentration of chloride (substrate) is less than the Michaelis constant the steady state rate of appearance of the product is directly proportional to the concentration of chloride. If

the concentration of chloride is greater than the Michaelis constant for the reaction then the rate of appearance of the product is independent of chloride concentration and is equal to the reaction constant for the enzyme ($k_3$). Thus the equation may be written:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

where E is the enzyme site, S the substrate, X an intermediate enzyme-substrate complex and P the product. The Michaelis constant for the reaction would be: $\frac{k_2 + k_3}{k_1}$.

Many other explanations of the intercellular mechanisms involved in the production of acid by the stomach have been offered and subsequently rejected. The most that can be said to be known is well stated by Hogben (57):

> "What is certain is that the gastric epithelium does actively transport both the hydrogen and chloride ions. It is by no means certain that there are two quite separate mechanisms responsible for their transport, let alone the possibility that they are transported by different cell types."

The third factor which must be considered is the variability of the composition of the acid secretion. In 1824 Prout (83) identified "muriatic" (hydrochloric) acid in gastric juice and from then until Pavlov became involved with rate and origin of secretion most of the gastric work focused on attempts to identify organic acids in the stomach in addition to the hydrochloric acid. There is no evidence available today which suggests that any acid other than hydrochloric is responsible for the acidity of the stomach. On the basis of indirect evidence it has been assumed that the hydrochloric acid is secreted at a constant composition. This concept was first advanced by Heidenhain (50) and then Pavlov (80).

Hollander (58, 59) reported confirmation of this concept based on the extrapolation of the regression line plotting the hydrogen ion concentration against the concentration of "neutral chloride". This calculated value assumes that there is only a unidirectional flux of chloride ion into the lumen and that there is no loss of hydrogen ion across the mucosa of the stomach. It is an arithmetical value arrived at by subtracting the hydrogen ion concentration of the luminal juice from the chloride ion concentration of the luminal juice. Hollander believed this to be a rectilinar relationship and extrapolated the line to the point of zero "neutral chloride" at which point he believed there was only a pure hydrochloric acid secretion. This point was at 167 milliequivalents per litre of hydrogen ion concentration and this he took as the pure and constant composition of parietal cell secretion. Aside from the original assumptions upon which this concept was based the regression line was plotted from data over a narrow range of hydrogen ion concentration. Thus Riddell et al. (87), by expanding the range of hydrogen ion concentration over which the data was derived, found that the relationship was not rectilinear but curvilinear, possibly fitting a cubic parabola. This would necessarily invalidate the figure of 167 milliequivalents of hydrochloric acid as representing pure parietal secretion; however, it is difficult to accept the basic assumptions behind the data used to plot the regression lines. Careful analysis of the work suggesting a constant composition of the hydrochloric acid secreted results in the conclusion that there is no direct evidence for or against the concept of a constant composition. Most indirect evidence would suggest that the acid does have a constant composition when secreted but this is certainly not acceptable as a definitive conclusion.

C. Theories of Acidity Regulation

As has been suggested acidity regulation is closely bound to acid formation; however, if hydrochloric acid is secreted by the gastric mucosa at a constant composition then consideration must be given to the mechanisms which result in the observed hydrogen ion concentration in the juice recovered from the lumen of the stomach. Again there is little conclusive evidence in this field and this is attested to by the number of theories proposed to explain acidity regulations. The two major schools of thought, the Dilution-Neutralization school and the Exchange Diffusion school are given the most consideration with mention of some of the other schools which have had short periods of prominence. No attempt is made to give a historical review of all the theories which have at one time or another been proposed.

i) The Less Accepted Schools of Thought

The problem of acidity regulation physiology begins in 1785 with the discovery by Carminati (11) that the stomach contents were acidic. Originally it was believed that the acidity was independent of digestive physiology and simply a product of fermentation of food in the stomach. The later work of Prout (83), Heidenhain (49, 50) and Pavlov (80) established the nature of the acid and that it was a product of the stomach in its role in digestive physiology. The part of Pavlov's work which is best known today is discussed later under the Dilution-Neutralization school but a pupil of his, Boldyreff (7), was the strongest early proponent of the concept of duodenal regurgitation as the means of regulating the gastric acidity. He states that Pavlov's work had shown that there was no diluting juice or neutral secretion in the stomach and that the chief factor in lowering the gastric acidity was the alkaline pancreatic juice. Pavlov (80) was fascinated by the accuracy with which the acidity was regulated and the

mechanisms suggested by Boldyreff, for example pyloric spasm and increasing or decreasing pancreatic secretion with subsequent regurgitation, seem insufficient to provide this degree of accuracy. Certainly there are few proponents of this thinking today.

Rosemann (90) by sham feeding dogs found that the concentration of chloride in gastric juice was constant. From this he proposed a mechanism which combined the formation and the regulation of acidity. Basically the Rosemann hypothesis involves the removal of a solution of sodium chloride from the blood and the subsequent conversion of a variable proportion of the cation from sodium to hydrogen by the parietal cell. Presumably the influence of the vagus, gastrin and histamine would be directed at the parietal cell to vary the proportions of cation converted as was necessary. This idea of the constancy of chloride in gastric secretion was supported by the work of Cooke and Grossman (18) although they did find a slight increase in chloride ion concentration when hydrogen ion concentration rose above 150 milliequivalents per litre. Rosemann's original paper reports 25 experiments on one dog and in one of these experiments the gastric juice was hyperosmotic relative to the blood. The major criticism of this work is that it assumes only a unidirectional flux of chloride. Much of the work of the electrophysiologists, including that of Forte (41) who reviews the earlier work and describes three components of the chloride ion flux across isolated gastric mucosa, suggests that the movements of chloride across the mucosa is not as simple as the assumption behind Rosemann's hypothesis.

A varient of Rosemann's ideas is the intreguing "Gastron" hypothesis proposed by Hirschowitz (54). He suggests that the gastric gland is a functional unit and that the pepsin secreting chief cells, which are most numerous at the base of the gland, secrete a sodium chloride solution which, as it passes up through the midportion of the gland, is

modified by the parietal cells which exchange sodium for hydrogen. It is difficult to exclude this theory with the available data. Teorell's data (92) and concept of exchange diffusion is consistent with this view and much of the data of other investigators including Hollander (58, 59) can be fitted to Hirschowitz's views. The major importance of this view is that it emphasizes that different physical chemical events can be occurring at different levels as one proceeds from the base of the gastric gland to the surface epithelium. Much of the confusion as to interpretation of the data available may well be due to the fact that the data represents the net change resultant from two or several interactions at different levels.

ii) The Dilution-Neutralization School

The origins of this school of thought related to regulation of gastric acidity come from the work of Pavlov (80). Pavlov believed that the gastric juice as it came from the gastric glands had a constant acidity and that after the secretion of the acid it flowed over an alkaline mucous membrane to become neutralized to a greater or lesser degree dependent upon the rate at which the acid juice was being produced. His work was done on innervated gastric pouches of dogs and thus free of contamination of the gastric juice by saliva or duodenal contents. He dwells, at some length, on the importance of the collection of pure gastric juice for analysis and did not believe that dilution played a significant role. This theory was extended by Hollander in the years following 1930 (58, 59, 61, 62, 64) and developed into what has become known as the "Two Component" theory. Thus Hollander divides the gastric secretions into a purely parietal cell component which he believes is hydrochloric acid at a fixed concentration of 167 milliequivalents per litre and all other gastric secretions which he refers to as the non-parietal component. Hollander notes (61) that the term non-parietal or alkaline component does not represent a real physiological entity but rather

is a convenient expression which includes all the gastric secretions exclusive of acid. Thus, the mucus secretion, the desqumated cells, the pepsinogen secretion, any transudate of interstitial fluid which might occur and a neutralizing fluid which is reported to contain, at the maximal limit, 120 milliequivalents per litre of sodium chloride and 45 milliequivalents per litre of sodium bicarbonate. Most authors suggest less bicarbonate but the above value, quoted by Fisher and Hunt (38) from the work of Ihre, is the largest found in the literature. Hollander has complicated his terminology slightly by also writing of two-components of the mucus portion of the non-parietal secretion (62). Thus according to him the mucous barrier consists of a non-vital layer of viscous material secreted by the mucus epithelium as well as a cellular component subjacent to the mucous layer. The major criticism of the mucous playing a significant role in acid regulation, and even protection of the stomach tissues from the acid, come from Bonis (8), Mitchell (74), Heatley (48) and Glass (44). These authors note that hydrogen ion is almost freely diffusible through the mucous and that there is little neutralizing ability in mucous. Hollander (62) discusses the slight ability to neutralize acid; however, he felt that the mucus had some contribution to make to neutralization even though it was obviously inadequate to explain all of the apparent neutralization of the gastric juice. The neutralizing fluid is believed to be primarily responsible for the acidity regulation. The major problem with this concept is that no one has been able to demonstrate bicarbonate in gastric secretions under normal conditions. Altamirano (3) reported the measurement of a "pure" alkaline secretion from gastric mucosa under in vivo conditions using large doses of acetylcholine. This secretion, an ultrafiltrate of plasma, did contain bicarbonate ion; however, the methods under which it was obtained leaves its validity in doubt. Obrink (76) could not explain the acidity reduction in his experiments

by the "Two-Component" secretions unless the alkalinity was very high or the volume estimations were incorrect. Glass (45) reviewed the whole problem of the "alkaline constituent" and concluded that there were at least four different non-parietal component secretions and that each was complex in composition. He held the view that the mucous secretion from the cardiac and pyloric glands contained bicarbonates. His evidence for this view is not given. That there is a secretion by the gastric mucosa other than the parietal acid secretion, a secretion which could be called the "non-parietal" secretion, is evident from most work. The presence of calcium, magnesium and small amounts of albumin certainly suggests that there is a transudate, mucus is definitely present in gastric juice and there are other indicators of a "non-parietal" secretion. The question which is not answered is the degree of significance of this secretion. Linde (69) found that most of the volume of gastric secretion was of parietal cell origin; thus, the "non-parietal" secretion must have a very small volume which would have only slight diluting effects. Mucin is not a powerful neutralizer of acid and there is no proof of the existence of a bicarbonate secretion of the strength necessary to account for the degree of acidity reduction usually seen, nor have investigators (92, 68) been able to demonstrate the presence of carbon dioxide as would be expected from such a neutralization.

iii) The Exchange Diffusion School

In 1933 Teorell (92) brought forward the concept of acidity regulation by simple diffusion of the hydrogen ion across the gastric mucosa from the lumen to the blood. These ideas were extended in 1939 (93) and his hypothesis fully enunciated in 1947 (94). In his early work he believed that the process was very simple and attempted to show that hydrogen ion was lost and sodium ion was gained across the gastric mucosa by simple diffusion. He

likened the process to the diffusion of ions across a cellophane membrane. By 1939 it had become apparent to him that the process was much more complicated and he suggested that the ion exchange was a more active process which involved a direct sodium for hydrogen exchange. As with Hollander and his supporters Teorell believed that there was a constant composition to the acid secretion; however some of the work done in his laboratory (68) caused him to qualify that statement to the degree that there was a constant composition of acidity at constant rates of secretion. However, the work leading to this conclusion was done using a glycol marker which may have also acted as a buffer and may have resulted in an abnormal secretion of acid due to the immediate buffering of any acid secreted. This criticism is valid at least at the low secretion rates considered. When Teorell was first enunciating his ideas a physical chemist, Guggenheim (47), working with liquid-liquid junction cells first suggested the possibility of "ion pair" interdiffusion of sodium and hydrogen in the presence of the common chloride anion. This suggestion that the diffusion of molecules was possible simplified the consideration of diffusion constants for such cells and thus led Teorell to reexamine his work and suggest (94) that the diffusion across the gastric mucosa of hydrogen for sodium was definable under these conditions and thus enunciated his hypothesis on a mathematical basis. From this base he suggested that the acidity, sodium and total chloride concentration was dependent only on the rate of secretion of the acid and that the sodium varies inversely with the acid and that the increase in acidity is accompanied by a slight increase in total chloride gain. They also showed that the larger the volume introduced into the stomach in installation experiments the less the decrease in acidity and the increase in sodium ion concentration. From these theoretical concepts derived from a mathematical exposition of his exchange diffusion hypothesis he demonstrated that the observed ion

concentration changes in the gastric juice in the absence of significant volume changes could only be explained by the active exchange diffusion of hydrogen chloride for sodium chloride as the means of acidity regulation. That there is a back diffusion of hydrogen ions in exchange for sodium ions has been shown by several investigators. Terner (95) showed that it occurs across frog gastric mucosa in the in vitro situation while Elliot (37), Lindner (70), Overholt (78) and Chapman (12) have shown that it occurs in the human stomach with Davenport (26), Cope (19), Wlodek (99), Adair (1) and many others supporting Teorell's findings in the dog. One of the major stumbling blocks to the acceptance of Teorell's hypothesis was the idea of a molecular exchange. Very little of the data substantiates this idea and most investigators now refer to the process of exchange diffusion as being an ionic exchange. Elliot (37) concluded that from fifty to eighty percent of acidity regulation was due to exchange diffusion while Lindner (70) suggested that exchange diffusion along with dilution and neutralization played significant roles in acidity regulation. The two major schools of thought related to acidity regulation are shown in Figure 1.

D. The Gastric Mucosal Competence Hypothesis

Teorell's hypothesis was extended and given clinical significance by Wlodek in his enunciation of the gastric mucosal competence theory (101). That the person having a gastric ulcer usually had low or normal levels of acidity in his overnight gastric juice while the person having a duodenal ulcer had excess acidity has been known by clinicians for many years. This apparent paradox has confused the clinicians and it was Davenport (28) who first suggested that the low acidity levels observed in the gastric ulcer patient might be due to the loss of the ability to contain an acid solution. This idea did not consider the pathophysiological derangements; that is,

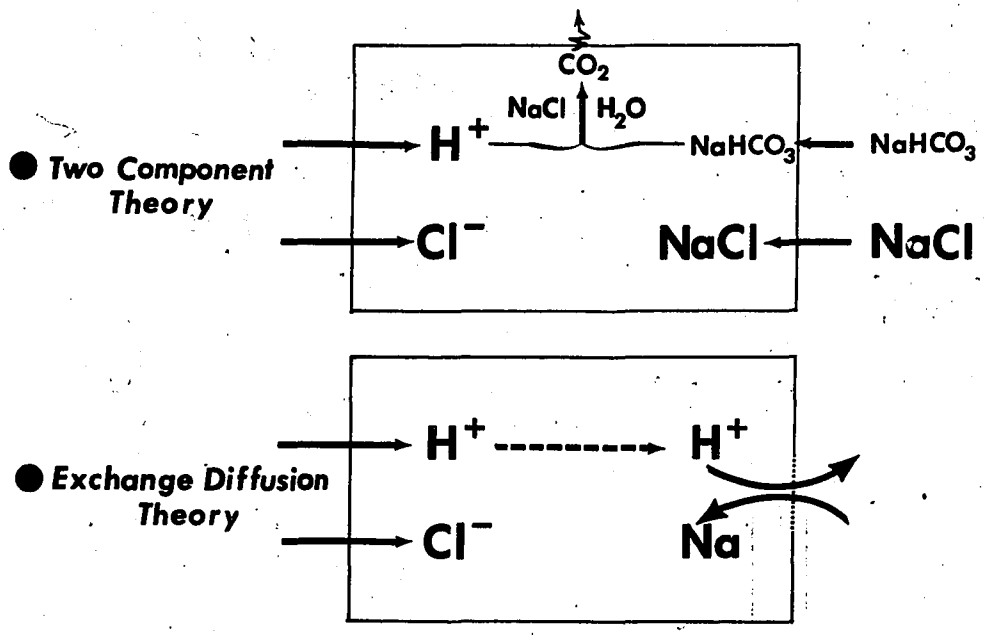


FIGURE 1. The Two Major Theories of Acidity Regulation.

which came first the lost ability to contain the acid solution or the ulcer. Wlodek and Leach (100) demonstrated that there was an increased loss of hydrogen ion in exchange for sodium ion across the ulcer bed. However, the ulceration in these pouches did not lead to a progressive and general impairment in the mucosal barrier thus the author concluded that the deficiency in the barrier was not the result of the ulceration but could be a primary etiological event in their pathogenesis. From this point Wlodek refined the concept (101) into the gastric mucosal competence theory. This concept is that there is an active metabolic process which exchanges hydrogen ion for sodium ion as the mechanism of regulation of gastric acidity. There is a broad population of efficiency of this mechanism giving a normal spectrum of the rate of exchange (Fig. 2), or, in other words, the degree to which the stomach can contain the acid solution, and an abnormal spectrum.

Abnormal rates of exchange can be greater or less than the normal rates. Thus, the person who develops a gastric ulcer is believed to have a deficiency in the ability to contain an acid solution at some point in his gastric mucosa. There is then a rapid loss of hydrogen ion across the gastric mucosa in exchange for the sodium ion with the resultant of a gastric ulcer. The pain of the gastric ulcer is believed, by Wlodek, to be caused by a localized chemical peritonitis secondary to the hydrogen ion loss from the lumen of the stomach. This situation is illustrated in Figure 3.

An abnormally increased competence of the gastric mucosa would result in a decreased exchange of hydrogen for sodium ion and higher than normal levels of acidity would be retained in the stomach. Thus the chyme passed to the duodenum will be highly acidic and the duodenum damaged by it. This is illustrated in Figure 4.

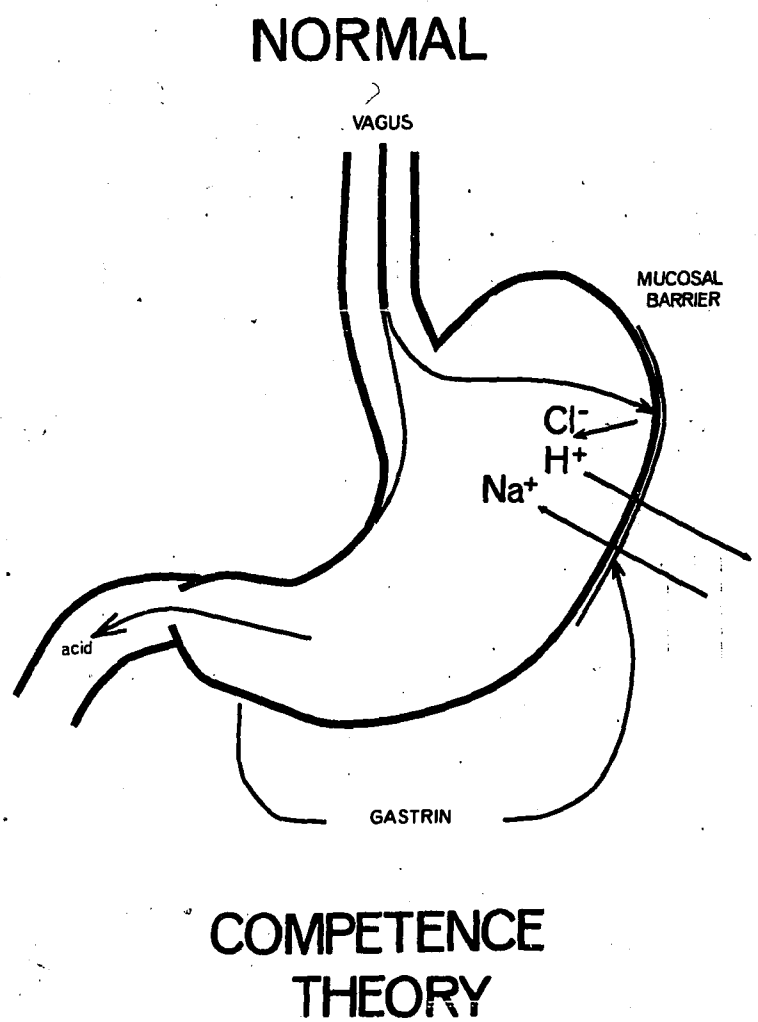


FIGURE 2. The Normally Competent Stomach

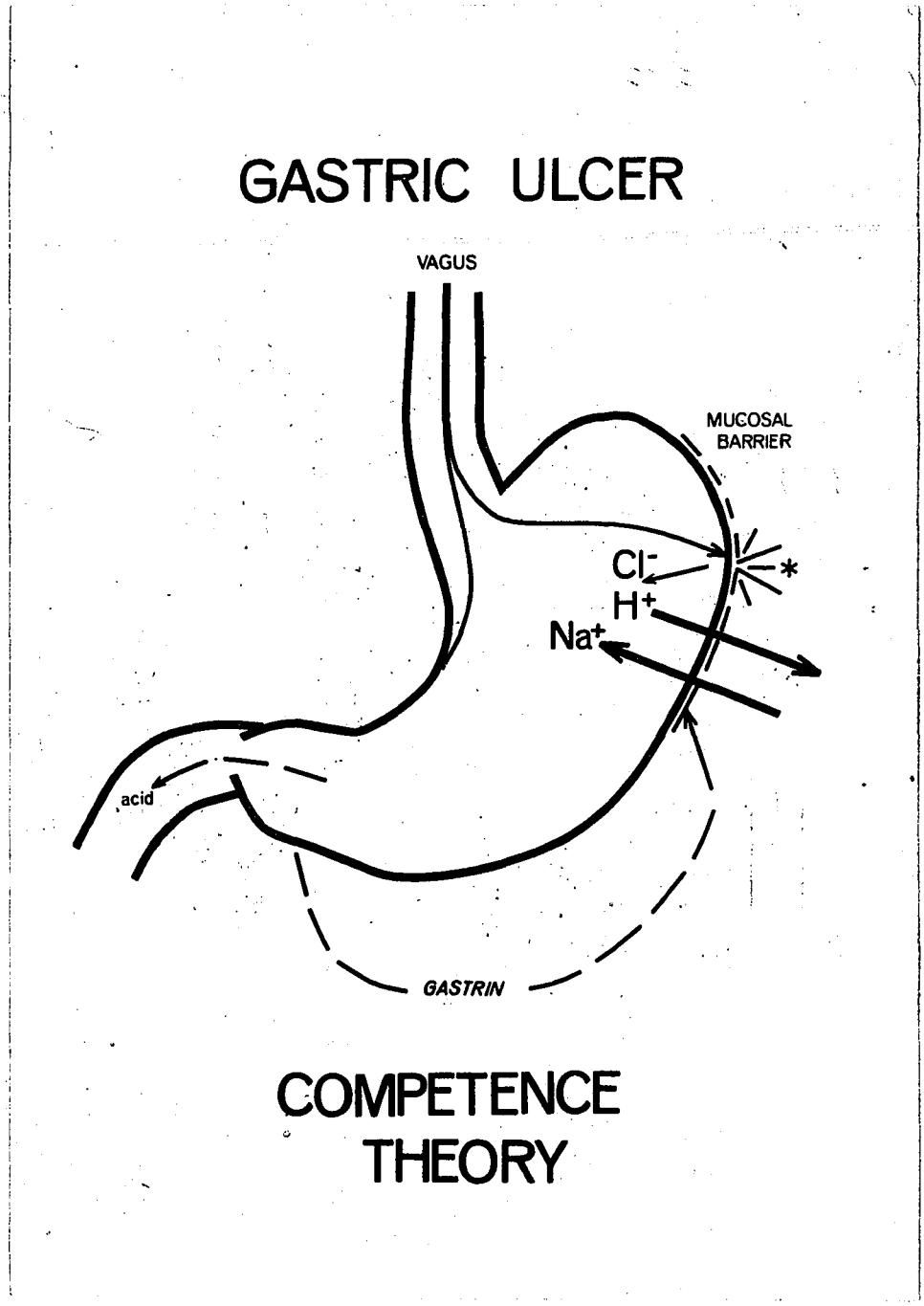


FIGURE 3. Decreased Competence Resulting in a Gastric Ulcer

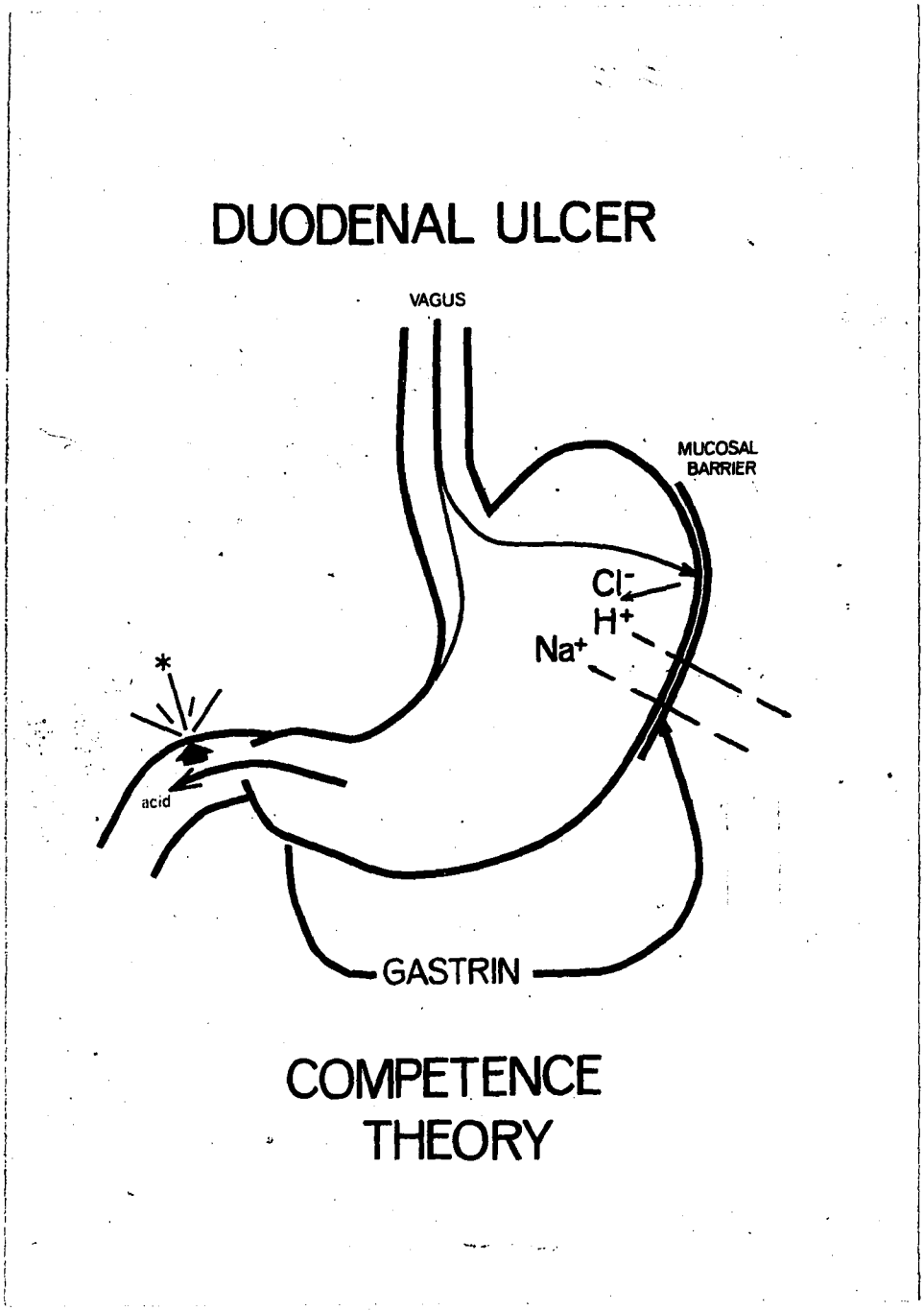


FIGURE 4. Increased Competence Resulting in a Duodenal Ulcer

From his own work and that of other investigators Wlodek believes that gastrin acts to increase the mucosal competence. He does not speculate as to the nature of the intercellular mechanisms which regulate the competence. That the ability to contain an acid solution can be destroyed by topical applications of a variety of solutions has been shown by several investigators. Davenport showed that detergents and urea (30) destroyed the acid containing ability of the stomach and Overholt (79) showed this effect with salicylate.

There is, as yet, no real consenses as to the mechanisms of regulation of acidity. Some investigators support the concepts of the Dilution-Neutralization school, others support the Exchange Diffusionists while yet others contend that both mechanisms are operative. The evidence for each of these views is mostly indirect; thus, there must be a more complete definition of the fluxes across the gastric mucosa and the mechanisms involved before rejecting any of these schools. For this reason Wlodek's hypothesis is useful as an investigative tool and probably useful clinically when dealing with peptic ulcer patients. It will probably be modified as there develops an increased understanding of the fluxes and mechanisms involved.

E. Fluxes Across Gastric Mucosa

i) Water

Most workers (14, 20, 29, 86) agree that water moves in both directions across the gastric mucosa and that this movement is at a rate which is only about one tenth that of the movement of sodium. Gamble (42) suggested in 1928 that the contents of a gastric pouch were isotonic with the blood plasma. This has been confirmed many times since but many workers have assumed that this meant that the secretion was then isotonic with the plasma. With the exception of some authors, notably Bornstein (9), few

believe in any association between the fluxes of water across the gastric mucosa and osmotic pressure gradients. In fact, Davenport (29) notes that the gastric mucosa is remarkably resistant to osmotic gradients. Reitemeier (86) found that water moves from the lumen to the blood across the gastric mucosa of a healthy human at a rate of two to three millilitres per 100 millilitres per minute and that this movement was independent of sodium movement. Code (14) found that water moved from the lumen of the stomach rapidly and the rate was not changed by secretion. As there is little net change in the volume, in fact Pavlov found water to be a mild stimulant to gastric secretion (80), thus there is a small net gain of water, then there must be a flux in the reverse direction equal to the flux of water out of the pouch. Wlodek (102) has found little change in the volume or composition of distilled water placed in a gastric pouch for half hour periods except those predicted from a basal secretion. Cope (20) found no difference in the water fluxes in an antral pouch when compared to a body pouch, a situation markedly different from that seen with sodium ion, and the half of the water in a pouch crossed the mucosa every twenty minutes. In summary there is a very rapid exchange of water across the gastric mucosa with little or no net movement except when the mucosa is stimulated to secrete. The net movement of secretion is accomplished by increasing the blood to lumen flux without changing the lumen to blood flux. Neither the significance of this rapid exchange nor the mechanisms by which it is accomplished are understood.

ii) Potassium

Hollander (64) points out that none of the hypothesis referring to regulation of gastric acidity suggest the possibility that the potassium ion concentration in the gastric juice would be above that found in the plasma; however, there exists many reports indicating that the potassium ion concentration of gastric juice from normal stomachs varies within the range of five to ten milliequivalents per liter. This opens to question the

interpretation of Villegas (96) that in the isolated frog gastric mucosa the net potassium ion flux through the mucosa was due to passive diffusion. The potassium ion does move in both directions across the gastric mucosa but only its flux from the mucosa to the lumen is changed by the addition of histamine (14). In the same study Code also showed that the acidification of the contents of the gastric pouch did not cause a change in the lumen to mucosa flux of potassium. It was his impression that the fluxes of water and potassium from the lumen to the mucosa were independent of each other and independent of the sodium-hydrogen exchange. This was confirmed by Moll (75). To some extent this work is at variance with that of Crane and Davis (22) who found that the rate of transport of potassium in both directions was reversably increased up to sevenfold when histamine was added to the nutrient solution but that this increase was not dependent on the institution of acid secretion. Grey and Bucher (46) found that the potassium concentration in histamine stimulated gastric juice was constant despite wide variation in the rate of secretion and acidity. They interpreted this in the light of the Dilution-Neutralization School by saying that the potassium concentration of both the acid and alkaline components were equal. Hollander (64) disagreed with this view, finding a variation of the potassium ion concentration of the gastric juice after only slight modification of the experimental technique - but he could not correlate this variation with the volume-rate of secretion or the acidity. He concluded that the flux of potassium from lumen to mucosa was independent of secretion and he was reinforced in this view by 'Obrink and Waller (77). When the gastric mucosa is intact and not secreting the fluxes across it are small (14) but when the mucosa is damaged the net movement of potassium from mucosa to lumen is increased as much as tenfold (25, 26). Davenport (27) felt that the small flux of potassium from the intact non-stimulated mucosa was mostly intracellular potassium with possibly a small amount being extracellular. In the case of the secreting mucosa or the damaged mucosa he believes the potassium is both extracellular and

intracellular in origin. That potassium seems important for acid secretion is apparent from the work of Rehm (85) who found that the removal of potassium ion from the chloride ion free external solution in his in vitro experiments resulted in a decrease in the hydrogen ion secretion to zero. The importance of potassium to secretion was also emphasized by Gilder and Moody (43) who found a high correlation between potassium concentration and hydrogen ion concentration in their experiments and concluded that this potassium was from the parietal cell and therefore associated with acid secretion. Thus there is little that is understood about the potassium ion fluxes across the gastric mucosa. Certainly the presence of a pool of potassium in the mucosal wall (29) complicates the interpretation of the data and may be the reason why slight changes in the experimental method results in data which is contrary to that of another investigator looking at the same problem.

iii) Sodium

Davenport (29) describes two barriers to the sodium ion entering the mucosal cell. There is a barrier to sodium entering from the interstitial fluid which is believed to be at or near the basement membrane and there is a second barrier to sodium at the mucosal surface apparently regulated by the hydrogen ion content of the lumen. He does not amplify the nature of these barriers. It would now seem that the small net movement of sodium into the lumen is the resultant of two unidirectional fluxes but the factors influencing these fluxes are not understood. Davenport (29) describes two components of the mucosa to lumen sodium flux; that output of sodium which occurs in exchange for hydrogen and the output of sodium which occurs associated with chloride. He felt that there was a lumen to mucosa flux of sodium and that it was only about one-fifth of the opposite flux. The lumen to mucosa flux was influenced by the hydrogen ion concentration in the lumen, being greatest when the lumen was alkaline or neutral. Reitemeier (86) on the other hand

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did not find any significant lumen to mucosa flux of radiosodium. Code (13, 14) and his group support Davenport's statements and found that there was a mean rate of movement of sodium from lumen to mucosa of 0.44 percent per minute when the contents of the stomach were neutral but none when the lumen was acidic. Cope (19) found a small but significant absorption of radiosodium from the lumen in the presence of acidic gastric contents but that there was two to three times as much absorbed when the contents were neutral. They also found that the antrum absorbed one hundred times as much per unit surface area as did the mucosa of the oxyntic area. Moll (75) using whole stomach preparations in rats supported the idea of absorption of sodium from acidic gastric contents. Thus it would seem that there are two unidirectional fluxes of sodium across gastric mucosa and that the lumen to mucosa flux while always occurring is regulated in degree by some mechanism in relation to the hydrogen ion content of the luminal juice. Aside from the interpretation placed on the relationship between hydrogen ion concentration and sodium ion concentration of the luminal juice, as discussed under acidity regulation, there is question as to the mathematical form of the relationship. Hollander and Colcher (63) found a rectilinear relationship between the two concentrations but this was determined only over the acidity concentration range of 70 to 155 milliequivalents per litre hydrogen ion with most of the values falling in the 120 to 155 milliequivalents per litre range. Milton (73) working with unstimulated cat stomachs found that the relationship was rectilinear in the range of hydrogen ion concentration of 0 to 120 milliequivalents per litre. However, Riddell (87) found that this relationship over the whole range was curvilinear. This work can be criticised on the basis that it was done on the whole stomach, not isolated pouches which differentiate between antrum and oxyntic areas. The meaning of this, relationship if it is rectilinear could be interpreted as supporting a 1:1 exchange diffusion of sodium for hydrogen as proposed by Teorell (92) and supported

by many workers including Bornstein (9) or, as Hollander has (64), supporting the concept of a neutralizing fluid. It is important to note that as there are two unidirectional fluxes of sodium such a relationship reflects only the net change of sodium in the gastric fluid.

iv) Chloride

The fluxes of chloride ion across the gastric mucosa seem to be related to the genesis of potential differences across the mucosa and to the secretion of hydrogen ion; however, as will be seen below, the exact nature of these fluxes and their relationship to one another are unknown. Much of the difficulties attendant with understanding the chloride ion fluxes seem to be a result of attempting to fit all of the data into one particular mechanism; thus, the work of Forte (41) in defining three components to any unidirectional flux of chloride represents a concept which may make the nature of the chloride ion fluxes more comprehensible. Certainly that there may be three components to a chloride flux across gastric mucosa and that the net movement of chloride represents, at least in the in vitro situation, the difference between two opposite unidirectional fluxes (29, 34, 53) makes the interpretations of the earlier workers, which where based on the net chloride gain by the gastric juice being representative of the total chloride entry into the juice, open to question.

Using a variety of in vivo methods with dog gastric pouches or rat stomachs many authors have attempted to define associations between the hydrogen and the chloride ions in the recovered gastric juice. Rosemann (90) in some of the earliest work found that the total chloride of gastric juice was a constant value; this gave him the basis on which he stated his concept of acidity regulation already discussed. Hollander (58) rejected this view as he found that the concentration of chloride slowly increased with increasing acidity and thus supported the Pavlov (80) concept that the difference between

the total chloride concentration and the hydrogen ion concentration represented a neutralizing fluid of "neutral chlorides". More recently Lindner (70) using instillation experiments found that there was a progressive fall in the recoverable chloride ion over a one hour period which was exceeded by the fall in hydrogen ion and based on this difference he concluded that mechanisms other than dilution must be involved in acidity regulation. Adair and Wlodek (1) instilling an acid solution in Pavlov pouches following insulin hypoglycemia and gastrin found no significant difference in the net chloride gain between these two forms of stimuli but a greater number of hydrogen ions remaining in the pouch after the gastrin stimuli. This would suggest that the transport mechanisms of the two ions are different. The fundamental criticism which can be directed against most of this in vivo work is that the interpretations are based on the assumption that the net chloride gain by the pouch or the stomach represents all of the chloride which entered. The in vitro studies which will be discussed suggest that this assumption is incorrect; however, there does not seem to be any work reported in the literature of attempts to measure fluxes of chloride into the pouch and out of the pouch in the in vivo situation.

Much of the in vitro work has been done by Heinz and/or Durbin (33, 34, 51, 53). They found (53) that the flux of chloride ion from nutrient solution to secretory solution increased sharply with an increase in hydrogen ion secretion, without an appreciable change in the sodium flux in that same direction. In some similar work they concluded that the rate of hydrogen ion production depended on the chloride concentration on the nutrient side and that thiocyanate inhibited acid secretion acting in a competitive manner for the chloride transport system (35). They also found that lowering the sodium chloride concentration in the secretory solution caused a proportional drop in the secretory to nutrient flux of chloride and

sodium. The chloride flux from nutrient to secretory also showed a proportional drop but the sodium ion flux did not change. Metabolic inhibitors decreased the chloride ion fluxes in both directions without changing the sodium fluxes. From this work they suggested that an unknown substance acted as a carrier substance which was shared by the chloride ion fluxes in both directions (34, 53). They also concluded (33) that there were two separate chloride ion movements, one involved with acid secretion and one involved with active chloride transport. They believe that carbonic anhydrase may be important in the mechanisms of active chloride ion transport. The problem with the carrier mechanism which they suggest is that it seems to have very little specificity, so little that it will carry virtually any anion. This lack of specificity contrasts with any of the known ion transport systems and while this does not make it impossible it does make it unlikely. Durbin (34) gets around this by suggesting that the anion may function only as a counter ion for the carrier without actually combining with it. Thus, he suggests, the rate of return of the charged carrier would be limited by the mobility of the accompanying counter ion in the carrier region. Thus we see the competitive inhibition of thiocyanate (35) or the bromide flux which is even greater than the chloride flux (51).

Hogben (55, 56) has also approached the problem of the chloride ion fluxes using in vitro methods. He recognized definite fluxes in opposite directions across the gastric mucosa and noted that the absolute magnitude of these fluxes were great with a distinct assymmetry of the fluxes. At the spontaneous potential across the cell the nutrient to secretory flux was greater than the flux in the opposite direction, although the reverse would be expected from the measured potentials. He also recognized two distinct movements of chloride, that due to hydrogen ion secretion and that due to exchange diffusion. He suggested that less than seventy percent of the membrane conductivity could be explained by passive diffusion and that this

represented a flux of about 1.4 microequivalents per hour; thus, a high proportion of the chloride transport had to be due to exchange diffusion. It would seem that Hogben had already in his papers defined the three components of the chloride ion transport across the gastric mucosa but it remained for Forte (41) to quantitate them. He suggested that about seventy-five percent of the total ionic current could be explained by passive leak, presumably through the pores that the membrane physiologists have mathematically predicted exist, and thus the greater amount of chloride ion transport was due to hydrogen ion secretion and exchange diffusion. He also showed that the hydrogen ion secretion was not dependent upon the chloride ion concentration in the lumen (secretory) solution but that decreased rates of hydrogen ion secretion resulted in decreased rates of chloride exchange. Thus, it is apparent that the concepts of the earlier workers involving chloride ion only in a passive sense or only considering the net movement of chloride can not be accepted in the light of our increasing sophistication. However, even with the degree to which the chloride ion fluxes along with the other fluxes across the gastric mucosa have been deliniated there is no consensus of opinion as to the interpretation of this data. Berkowitz and Janowitz (5) have suggested that the ion fluxes demonstrated may well mean that both exchange diffusion as well as dilution-neutralization occur to regulate the gastric acidity. It is equally conceivable that the other theories suggested may play a role or that as yet not enough is known and as more data is evolved the mechanisms of gastric acidity regulation will become apparent.

## SECTION II: EXPERIMENTAL METHOD

### A. Experimental Animal

The tests were carried out on five mongrel dogs with gastric pouches. The dogs weighed between 17 and 22 kilograms and maintained that weight through the test period. The dogs were only used if they were completely free from any disease and only if they regained their preoperative weight. There were no tests carried out on the animals until at least five weeks had elapsed since the gastric pouch operation. The animals were maintained on standard kennel rations with potassium chloride tablets given to supplement the diet. The animals were judged healthy on the basis of normal conjunctive, normal cardio-respiratory system and normal bowel movements with a subjective note of their behavior. These criteria can be criticised on the basis that the animals hemoglobin and serum electrolytes were not checked at regular intervals. The criteria for selection of the five animals can be criticised in that there was no randomization. Those animals which came into our kennel population healthy and remained so for a period of two weeks following immunization were selected for operation on a non-randomized basis and those animals who were able to regain their preoperative weight, whose pouches did not leak and who showed a free flow of fluid to and from the pouch were then selected for the testing.

### B. The Gastric Pouch

The history of the use of the gastric pouch technique in experimental studies has been reviewed by Hollander (60). The original intention was to use innervated pouches for these studies. Accordingly gastric pouches were made using a modification of the Perry (82) technique. The technique was modified such that the pouch was made of corpus of the stomach and not cardia; thus, this could be done through a midline abdominal

incision and did not necessitate a transthoracic approach. Both the anterior and posterior vagal nerves were preserved. The pouches were drained with stainless steel modified Gregory cannulas. There were many problems with this technique and with the health of our animal population; thus, as a compromise, it was elected to build the simpler denervated pouches (Heidenhain type) and it was possible to get a population of five healthy animals on which the experiments could be carried out. Wlodek (102) has found no difference in the basal secretory characteristics of the denervated versus the innervated gastric pouch. It has been pointed out by Ritchie et al (88) that there are histological differences between the denervated pouch and its parent stomach in that there is a decrease in the parietal cell mass with an increase in the number of mucous neck cells present in the denervated pouch. These differences as well as the fact that the pouch is denervated to an unknown degree as the sympathetic supply along the short gastric arteries is believed to remain intact make it impossible to equate directly the function of the mucosa in the pouch and that of the parent stomach. Equally it is impossible to equate the functional characteristics of the dog stomach to that of the human even though there are similarities on a gross level in the function and enzyme systems of the two mucosae. Thus it is apparent that the findings of this study relate only to the mucosal function in denervated gastric pouches in dogs. From these results certain inferences may be drawn with regard to the function of the human stomach; however, these inferences must be qualified in the above manner.

C. Test Solutions

The test solutions instilled into the gastric pouches were of a standard composition excepting that one was acidic while the other was neutral. The standard solution contained polyethylene glycol labeled with carbon-14 at a concentration of about 15,000 decays per minute; cold

polyethylene glycol at a concentration of 1 gram per litre; chloride-36 as sodium chloride at a concentration of about eight thousand decays per minute; 14.2 grams per litre of Mannitol and 100 milliequivalents of chloride. The chloride was balanced with a cation, either 100 milliequivalents per litre of hydrogen ion for the acidic test solution or 100 milliequivalents per litre of sodium ion for the neutral test solution. It is important to note that the concentration of chloride is the same in both solutions.

The use of volume markers in instillation experiments have been tried many times before. Some of the most successful of these have employed the dye phenol red as in the work of Berkowitz (5) and Lindner (70). Wlodek and Leach used polyethylene glycol as a volume marker (98) and then this was refined by Adair and Wlodek (1) who employed polyethylene glycol labeled with carbon-14. The alternative to using a volume marker is the direct measurement of the fluid from the pouch or stomach and this has proved to be inaccurate when dealing with volume changes of under two millilitres. The use of cold polyethylene glycol has given recovery rates of 99% to 100.9% (98) and 99% to 101% (1). This variation in recovery of the polyethylene glycol of plus or minus one percent allows very accurate calculation of volume shifts based on the degree of dilution or concentration of the isotope counts. The cold polyethylene glycol was added to the test solution as this resulted in a better percentage recovery of the labeled marker thus more reproducible results.

The degree of dilution of the chloride-36 isotope in the pouch, knowing the net change of total chloride, permits the calculation of the loss from the pouch. The calculations involved are discussed in subsection F under the heading "Mathematical Interpretation of Scintillation Data".

Mannitol was added to the test solutions as an osmotic agent in order to make this work comparable to previous work where this had been

considered an important factor. It is now felt that this is not only an unnecessary part of the test solution but that its presence may well change the characteristics of any "basal" studies. Thus the control studies which are taken as "basal" values must be interpreted on the basis of mannitol having been present. When comparing the difference in ionic fluxes between using a neutral or acidic or pre-versus post- treatment of the pouch with urea the mannitol is not as important as it was present at the same concentration in all of these studies.

D. Pouch Treatment Solutions

Once the concept of the stomach's unique ability to contain an acid solution was grasped it became obvious that an understanding of the mechanisms behind this fact might be elucidated by destroying that ability and studying the ion fluxes following such destruction. There are natural differences between dogs in their ability to contain an acid solution (29) but some of the first work using a chemical attack on the mucosa was done by Forte (40) who used the calcium chelating agent ethylenediaminetetra-acetate and found that the junctional complexes between oxyntic cells opened (91) and the mucosa became highly permeable to sodium. Davenport (26) has shown an increased loss of hydrogen ion from pouches treated with eugenol and Overholt (79) has used salicylate in the rat stomach to show the same thing. Webster (97) found that saline depleted the surface epithelium of its mucus but did not measure ionic fluxes after such treatment.

Davenport used salicylic acid and acetic acid (25, 27) as well as detergents and urea (30). In these studies urea has been utilized to treat the gastric pouches in order to study the ionic fluxes after such treatment. Urea (30) is a mucolytic agent but it is also a metabolic antagonist, both of these effects are probably secondary to urea's ability to destroy hydrogen bonding. Studies by Mavrias and McArdle (72) have shown that an eight percent solution of urea will depress tissue respiration in liver and

intestinal epithelium tissue slices and smears. This is reversible using eight percent solutions of urea but not reversible using twelve percent solutions. Figures 5, 6, 7 and 8 show the histologic changes seen in the dog's gastric pouch epithelium four hours after histamine stimulation with and without subsequent treatment with four urea solution.

Figure 5 illustrates the normal appearance of dog gastric pouch mucosa following histamine stimulation after using the general stain hematoxylin and eosin. Figure 6 is the same tissue employing a periodic acid Schiff stain combined with alcian as a demonstration of the mucin. The alcian is generally recognized as being specific for mucin (81) although there is much question as to whether it reacts with the uronic acid groups or the sulphated groups on mucosubstances. Under the conditions of pH and electrolyte concentration of the reagents most authors seem to agree that in the stomach it could stain only mucin. The periodic acid Schiff reaction of course acts on any 1:2 glycol groups and thus covers a wide variety of carbohydrate substances. Thus the dark line of dye covering the epithelium as seen in Figure 6 probably represents mucin. Figure 7 illustrates what happens to the tissue four hours after histamine stimulation followed by treatment with four molar urea solution topically. The surface epithelium remains intact but there is an intense polymorphonuclear infiltration of the whole gastric gland area suggesting an immediate and severe inflammatory reaction in response to the urea. It is also evident from figure 8 that the mucin is almost wholly lost to the surface epithelium and only a small amount remains covering the epithelium of the necks of the gastric glands. Thus, from this histologic evidence and from the tissue respiration studies of Mavrias and McArdle quoted it is suggested that the resultant of the treatment of the gastric pouches with urea solution is both mucolysis and metabolic inhibition of the tissues.

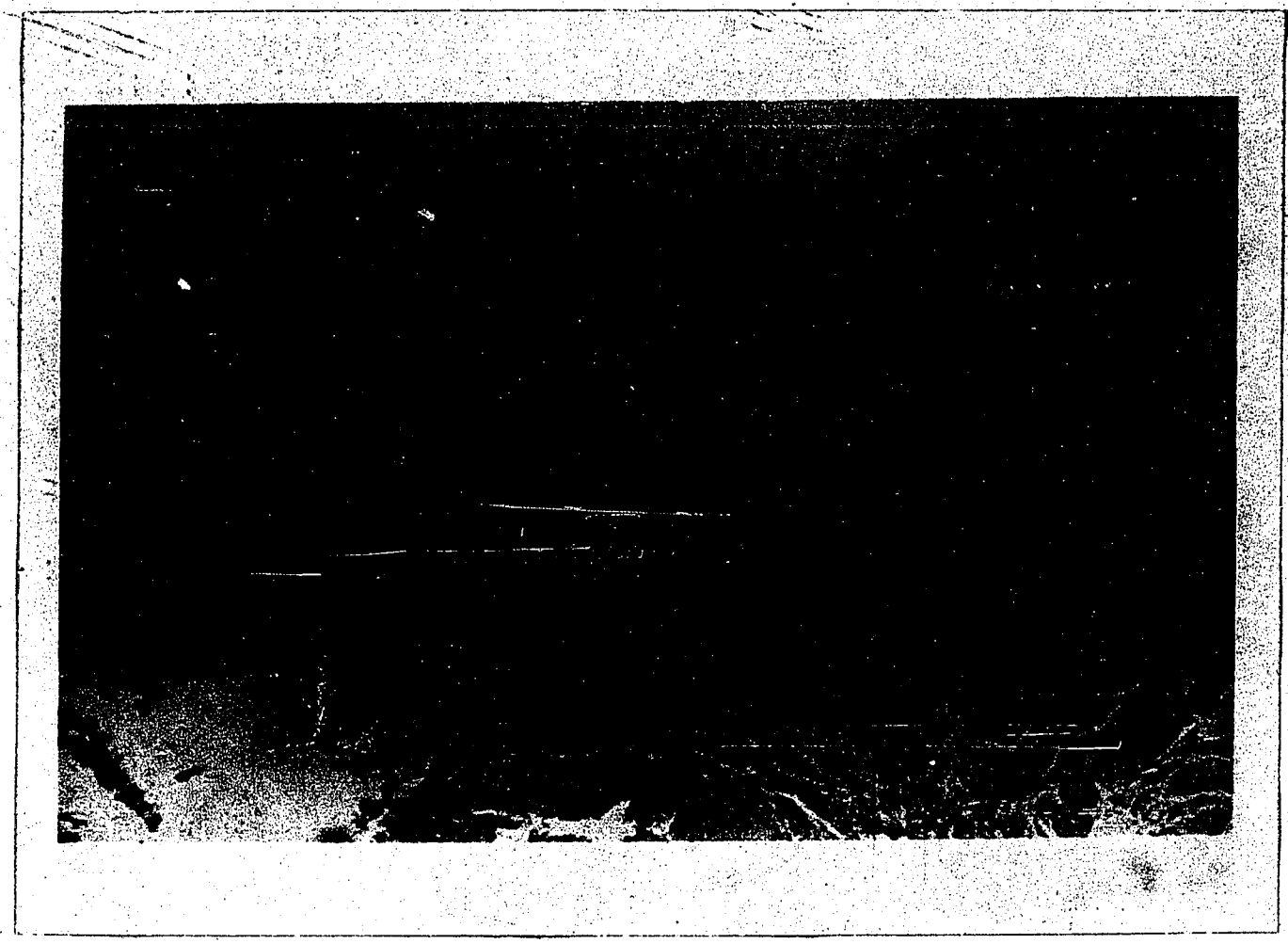

FIGURE 5. Normal Dog Gastric Pouch Epithelium Following Histamine Stimulation Stained with Hematoxylin and Eosin; Magnified x 60.

Fig. 5.2. Normal oesophageal mucosa showing stratified squamous epithelium with mucoid and eosinophilic cytoplasm. x 60.

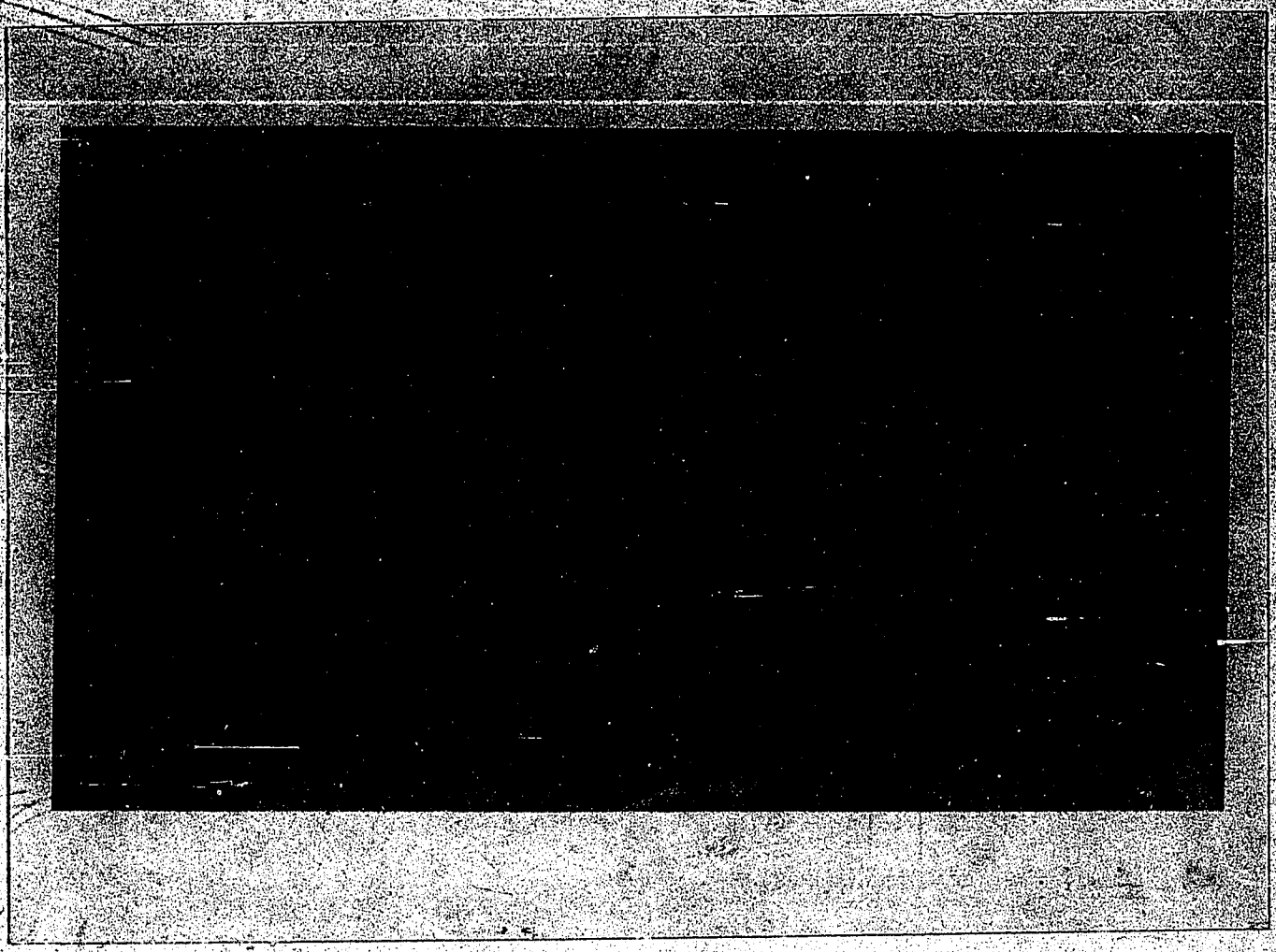

FIGURE 6. Normal Dog Gastric Pouch Epithelium Following Histamine Stimulation Stained with Periodic Acid Schiff and Alcian; Magnified x 60.

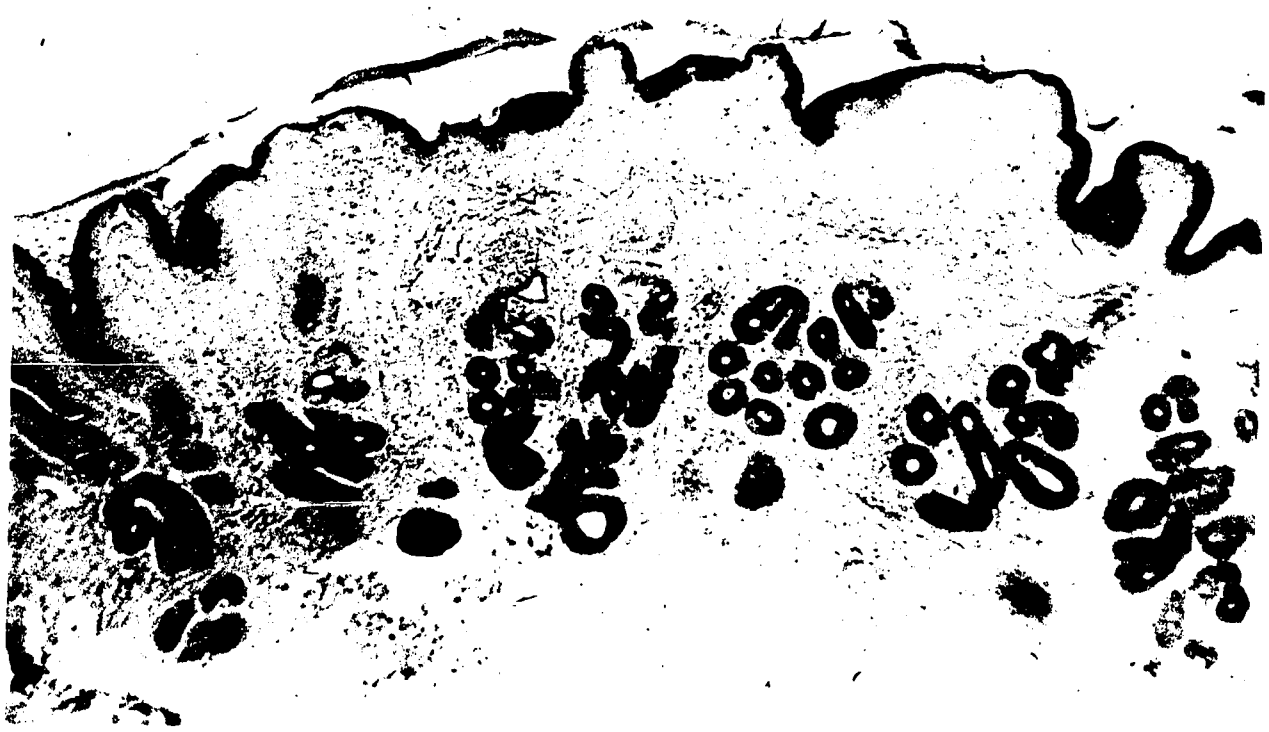

FIGURE 6. Normal Dog Gastric Mucosa Parietal Cells Following Histamine Stimulation Stained with Periodic Acid Schiff and Alcian Blue. Magnified x 60.

FIGURE 7. Dog Gastric Pouch Epithelium After Histamine Stimulation Followed by Molar Urea Treatment Stained with Hematoxylin and Eosin; Magnified x 60.

FIGURE 6. Rat gastric fundic epithelium after histamine stimulation followed by four hour urea treatment fixed with periodic acid-Schiff and Alcian blue, pH 2.5. x 60.

E. Isotopes and Scintillation Counting

Two isotopes were employed in this work and concentrations of both were determined by liquid scintillation counting techniques.

As discussed the volume marker was polyethylene glycol labeled with carbon-14. Carbon-14 emits beta radiation with a particle intensity of one hundred percent and a half life of five thousand seven hundred and seventy years. As such it is an isotope which is readily adaptable to liquid scintillation counting with a high degree of accuracy.

The second isotope employed was chloride-36 which is also a beta emitter and has a particle intensity of ninety-eight percent showing a very slight degree of orbital electron capture. It has a half life of three hundred thousand years and with these characteristics is also a good isotope for liquid scintillation counting.

The basic theory behind liquid scintillation counting is reasonably straight forward (6). A known amount of the isotope containing solution is added to a scintillation liquid and the photon emissions from the scintillation liquid which occur secondary to each radioactive decay occurring are detected by a photoelectric cell which then registers these emissions as electrical impulses which can be counted with each electrical impulse representing one decay. The solution is counted for a known period of time and one then gets a count representing decays per minute. However, this assumes one hundred percent efficiency of the scintillation liquid and counting mechanisms. In fact there is some quenching of the count due to imperfect efficiency of the counting mechanisms, the scintillation liquid and the absorption of photons by other materials in the sample liquid itself. In this work a Packard Tri-Carb 4000 series liquid scintillation counter with automatic standardization counts for each sample was used. This eliminated the problem of machine inefficiency. A two channel technique was used where

by the counting of carbon-14 was done entirely on one channel without overflow into the second electronic channel and the chloride-36 was counted on a second channel accepting an overflow which could always be measured and was never greater than twenty percent. Thus, the isotopes could be counted together and the counts corrected to decays per minute using quench curves which were determined at the window size and gain settings for the two channels by quenching known amounts of the isotopes with picric acid.

The scintillation liquid employed contained 125 grams of 2,5 diphenyloxazole and 2.5 grams of 1,4-bis-2-(4-Methyl-5-phenyloxazole)-Benzene per litre of concentrate. This was diluted to a working solution containing 40 millilitres of the concentrate. This scintillation liquid has been employed in this laboratory for some time now on the advice of the Packard Instrumentation Co. and it has been found to give accurate results.

F. Mathematical Interpretation of the Scintillation Data

The calculation of residual volume and volume secreted utilizing the carbon-14 labeled polyethylene glycol is as follows:

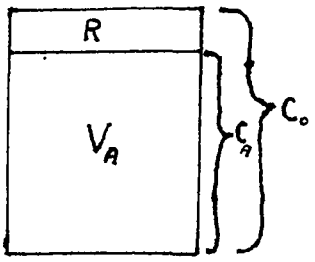


Fig. 1

At 0 time

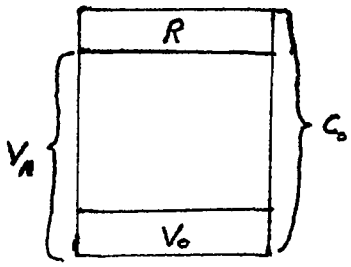


Fig. 2

Between 0 time and 30 min.

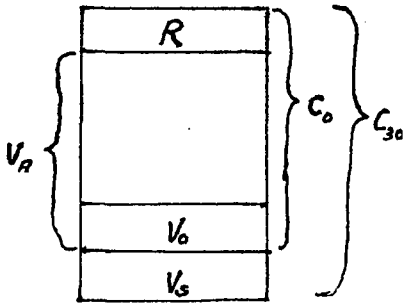


Fig. 3

At 30 min.

$C_A$ = the known number of d.p.m. in test fluid added to pouch per 500 ul.

$C_o$ = the number of d.p.m. counted in 0 time sample/500 ul.

$C_{30}$ = the number of d.p.m. counted in 30 min. time sample/500 ul.

$R$ = residual volume

$V_A$ = the volume added to the pouch

$V_o$ = the volume removed for testing at 0 time

$V_s$ = the volume secreted into the pouch

Residual Volume Calculation (R)

total number of counts added = the total number of count in pouch when fluid added is mixed with residual volume.

$$\therefore V_A C_A = C_o (R+V_A) \quad \text{(see Fig. 1)}$$

$$\therefore R = \frac{V_A (C_A - C_o)}{C_o}$$

Volume Secreted Calculation ($V_s$)

total number of counts in pouch at 0 time = total number of counts in pouch at 30 min. time

$$\therefore C_o (R+V_A-V_o) = C_{30} (R+V_A-V_o+V_s) \quad \text{(see Fig. 3)}$$

$$\therefore V_s = \frac{(C_o - C_{30}) (V_A C_A - V_o C_o)}{C_{30} C_o}$$

Thus the volume in the pouch at 30 min. time ($V_{30}$)

$$V_{30} = R+V_A-V_o+V_s$$

The sodium chloride-36 is used to determine the unidirectional flux of chloride out of the pouch. The calculation is as follows:

$C_o$ = the number of d.p.m./500 ul of $Cl^{36}$ in 0 time sample

$C_{30}$ = the number of d.p.m./500 ul of $Cl^{36}$ in 30 min. time sample

$V_1$ = the volume in the pouch after the 0 time sample

$V_{30}$ = the volume in the pouch after 30 min. elapsed

$E_o$ = the number of microequivalent per c.c. in the 0 time sample

$E_{30}$ = the number of microequivalent per c.c. in the 30 min. time sample.

The steps in determining the formula are:

a) Calculate the total number of counts present in the pouch:

at 0 time = $C_o V_1$

at 30 time = $C_{30} V_{30}$

b) Subtract total number of counts at 30 min. time from total number of counts at 0 time to determine counts lost.

$\therefore$ counts lost = $C_o V_1 - C_{30} V_{30}$

c) Determine the number of c.c. of chloride containing fluid these counts represent:

$$= \frac{\text{number of d.p.m. lost}}{\text{average d.p.m. per 500 ul sample}}$$

$$= \frac{C_1 V_1 - C_{30} V_{30}}{\frac{C_1 + C_2}{2}}$$

d) Determine the number of milliequivalents of chloride the fluid represents

= number of c.c. fluid lost x average mEq Cl/cc

$$= \frac{C_1 V_1 - C_{30} V_{30}}{\frac{(C_1 + C_2)}{2}} \quad x \quad \frac{(E_1 + E_2)}{2}$$

$$= \frac{(C_1 V_1 - C_{30} V_{30})\ (E_1 + E_2)}{C_1 + C_2}$$

G. Analysis of the Ion Concentrations

Both the sample taken at zero time and the thirty minute sample were analyzed for hydrogen, sodium, potassium and chloride ion concentrations.

i) Hydrogen Ion

The concentration of hydrogen ion in any sample was determined by the titration of one millilitre of the sample against a solution of 0.01 normal sodium hydroxide using a Micro-Metric syringe burette. The endpoint of the reaction was taken as pH 7.0 measured using a Radiometer pH meter with

glass-calomel electrode. The result was expressed as microequivalents per millilitre of test solution.

ii) Sodium and Potassium Ion

The concentrations of sodium and potassium ions in the sample were measured simultaneously using a Instrumentation Laboratories Flame Photometer Model 143. This reaction depends upon a complete burn of the sample in a propane flame after dilution of the sample with a lithium standard. The spectrum of the flame is then separated into that component due to sodium, that due to potassium and that due to lithium using three separate filter-photocell circuits. The machine is then calibrated to give a readout of the concentrations of sodium and potassium in microequivalents per millilitre depending upon the intensity of the emission of light in the appropriate spectrum for the ion when compared with the known lithium sample's spectrum intensity. The machine is balanced using samples of known concentrations of all three ions.

iii) Total Chloride Ion Concentration of the Sample

The total chloride concentration of the sample was measured after the technique of Cotlove (21) using a Buckler-Cotlove Chloridometer. This involves the coulometric generation of silver ions which precipitates the chloride present in the sample as silver chloride. This reaction continues until all of the chloride in the sample has been precipitated whereupon the further generation of silver ion activates an ampherometric indicator circuit stopping a time relay. The time relay is calibrated to give a direct readout of chloride concentration in microequivalents per millilitre. The major criticism of this method is that any halogen, or in fact any substance which will irreversibly combine with silver ion, will be measured. However, there is no evidence that gastric juice contains halogens other than chloride in significant quantities.

H. Protocol Outline and Controls

An animal being used in an experiment was fasted overnight. The fast period varied between fifteen and twenty hours. During this period and until the experiment was concluded care was taken that the animal would not eat, see or smell food. This included careful washing of the hands of persons handling the animals and the removal of all food from any room the animal was in. The persons who handled the animals during the experimental periods were at no time permitted to feed the animals to prevent any psychic association of that person with food.

On being brought from the animal quarters to begin an experiment the dog was placed in a Pavlov stand and a fifty millilitre reservoir was attached to the modified Gregory cannula via a polyethylene tubing. All attachments were water tight. The pouch was then washed with a mannitol solution (18 grams of mannitol per litre) so that all residual secretions were removed and to assure that there was free flow from the reservoir to the gastric pouch. A sample of wash was saved for isotope counting to assure that there was no build up of isotope within the pouch with repeated experiments. A blood sample was taken for isotopic counting to provide a baseline for that day and to assure that there was no buildup of isotope within the animal with repeated experiments.

The initial fifty millilitre sample of test solution was then instilled into the reservoir and allowed to run into the pouch. This was mixed between pouch and reservoir twice and a zero sample of ten millilitres taken for analysis. The reservoir was then set so that the top of the liquid in it was ten centimeters above the level of the pouch. One half hour later the pouch and reservoir were drained completely and the volume of liquid obtained measured. A sample of the liquid was saved as the thirty minute sample for analysis. The pouch was then washed with the mannitol wash solution and a sample of the wash saved for isotope counting. This

procedure was repeated four times and then 50 millilitres of the pouch treatment solution, either urea solution or saline, was instilled for a further half hour. The control or "sham" treatment was with a saline solution of concentration 100 milliequivalents per litre while the urea solutions were used at concentrations of one, two and four molar. As has been noted saline solution at this concentration was found to deplete the surface epithelium of its mucus; however, there is no true "sham" treatment solution as even distilled water will clear some of the mucus from the surface epithelium and may act as a mild stimulant to secretion (80). Following the pouch treatment a second series of four instillations of the test solution for a one half hour period was carried out. The isotopic and ion concentration analysis were then carried out on each of the eight zero and eight thirty minute samples immediately. Occasionally the samples were saved overnight in a cold room to do the sodium and potassium analysis and occasionally the pipetted samples in the scintillation liquid had to be held for as much as two days before time was available on the scintillation counter. Neither of these exceptions to the rule of immediate analysis should cause any alteration in the results obtained.

Each animal acted as its own control as a series of saline "sham" treatments were done to each animal before any other tests. In addition each individual experiment had a series of four instillations of the test solution prior to any treatment. All experiments were done having had the pouch rinsed with mannitol solution so that residual secretion was removed and any residual volume left in the pouch was mannitol wash solution. Between each half hour instillation test the pouch was rinsed with the mannitol wash solution to rinse out any residual isotope which might build up and this wash counted to assure that such build up was not occurring. The criticism of

using a mannitol wash in this way is that the mannitol may stimulate secretion.

At the end of the experiment a blood sample was taken for isotopic counting to insure that no carbon-14 had crossed the gastric mucosa. The animal was then returned to the animal quarters.

A major criticism of this work was that the same animals were used for repeated experiments and that the experiments proceeded from using saline treatment solution to the series using one molar and then two and finally four molar urea solution treatments. This was done due to the difficulties encountered in getting animals with gastric pouches which had free flow to and from the reservoir and no leakage. There was no attempt made to randomize the experiments as to the treatment solution used as the four molar urea solution was known to destroy the gastric pouch while urea solutions of less concentration caused damage which was histologically reversible. The gastric pouches appeared normal histologically five days after treatment (102) thus no dog was used again within seven days of an experiment being done.

## I. Statistical Analysis

The results were found to fit a Gaussian distribution and were independent of each other thus the Fisher t-analysis was carried out with the t values reported. Ninety-nine point nine percent confidence limits were accepted as significant.

## SECTION III: THE RESULTS

The results of these experiments are summarized in Tables One through Eight following. As there was no significant variation in the volumes or fluxes of each of the four pre-treatment cycles the mean value of all of the pre-treatment cycles for any one experiment was taken as the base-line or "control" value.

### A. Ionic Fluxes In Non-Stimulated Pouches Without Urea Treatment

In an attempt to define the ionic fluxes in a resting gastric pouch the instillation experiments were carried out as described several times on each of the pouches with both acid and neutral test solutions prior to treatment of the pouch with urea. As described a saline "sham" treatment was carried out. Tables One and Two show there is no statistical difference between the control and the post-treatment values for the volume or any of the fluxes at a level of confidence of 99.9 percent. For this reason a mean of all of the pre- and post-treatment values for acid and neutral test solutions were determined and are summarized in figure 9. To refer to these values as representing "basal" ionic fluxes may not be entirely correct as despite the lack of statistical difference in the values they nonetheless represent a mean taken of cycles which are both pre- and post-treatment of the pouches with saline.

It is also possible that simply instilling the test solution in the pouch may stimulate secretion (80) and that the test solution contains mannitol may increase this tendency.

### B. Volume Changes and Ionic Fluxes Following Pouch Treatments

#### i) Volume Changes

The volume changes following treatment when using both acid and neutral test solutions are summarized in Figures 10 and 11.

TABLE ONE: Acid Test Solution (HCl 100 meq/l) - Saline (NaCl 100 meq/l) Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

11 Experiments (n = 53)

| Cycle | Volume Secreted (ml) | Total $Cl^-$ Flux Out | Net $Cl^-$ Flux In | Net $H^+$ Flux Out | Net $Na^+$ Flux In | Net $K^+$ Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 44 half hour pre-treatment cycles) | 1.41 ± 0.74 | 153.1 ± 94.6 | 129.7 ± 73.4 | 128.2 ± 86.0 | 262.6 ± 83.9 | 9.9 ± 4.5 |
| 30 Minute Pouch Treatment with 50 ml. of NaCl (100 meq/l) | | | | | | |
| 0.5 hours post-treatment | 1.78 ± 0.86 (1.408)* | 79.4 ± 107.3 (2.207)* | 163.0 ± 98.6 (1.227)* | 77.3 ± 48.0 (1.857)* | 266.0 ± 104.0 (0.112)* | 12.3 ± 7.4 (1.342)* |
| 1.0 hour post-treatment | 0.95 ± 0.52 (1.910)* | 138.2 ± 79.1 (0.473)* | 74.4 ± 63.9 (2.250)* | 108.1 ± 54.4 (0.726)* | 202.5 ± 64.1 (2.179)* | 8.1 ± 3.3 (1.223)* |
| 1.5 hours post-treatment | 1.23 ± 0.69 (0.718)* | 114.2 ± 131.2 (1.100)* | 138.1 ± 87.9 (0.320)* | 88.9 ± 95.3 (1.302)* | 221.5 ± 31.4 (1.356)* | 8.3 ± 3.3 (1.087)* |
| 2.0 hours post-treatment | 1.36 ± 0.54 (0.207)* | 142.6 ± 67.1 (0.341)* | 156.2 ± 87.0 (1.012)* | 96.0 ± 44.1 (1.181)* | 232.9 ± 51.0 (1.103)* | 9.2 ± 4.0 (0.463)* |

* Value not significant at 0.01 probability level of significance.

TABLE TWO: Neutral Test Solution (NaCl 100 meq/l) - Saline (NaCl 100 meq/l) Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

9 Experiments (n = 41)

| Cycle | Volume Secreted (ml) | Total Cl Flux Out | Net Cl Flux In | Net H Flux Out | Net Na Flux In | Net K Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 34 half hour pre-treatment cycles) | 1.70 ± 1.22 | 159.2 ± 135.1 | 188.6 ± 108.1 | 3.4 ± 9.2 | 182.9 ± 97.7 | 15.2 ± 7.7 |
| 30 Minute Pouch Treatment with 50 ml. of NaCl (100 meq/l) | | | | | | |
| 0.5 hour post-treatment | 1.69 ± 0.52 (0.093)* | 138.0 ± 72.9 (0.442)* | 159.7 ± 74.6 (0.738)* | 3.6 ± 6.5 (0.072)* | 206.7 ± 84.8 (0.652)* | 14.5 ± 6.9 (0.232)* |
| 1.0 hour post-treatment | 1.53 ± 0.46 (0.401)* | 169.7 ± 112.3 (0.210)* | 215.8 ± 65.8 (0.704)* | 1.6 ± 0.9 (0.553)* | 166.9 ± 64.8 (0.451)* | 11.6 ± 4.9 (1.294)* |
| 1.5 hours post-treatment | 1.78 ± 0.77 (0.183)* | 124.4 ± 79.4 (0.723)* | 187.3 ± 137.7 (0.029)* | 2.8 ± 6.4 (0.167)* | 181.3 ± 116.6 (0.038)* | 13.8 ± 7.9 (0.462)* |
| 2.0 hours post-treatment | 1.69 ± 0.36 (0.234)* | 81.1 ± 123.0 (1.533)* | 188.9 ± 48.0 (0.008)* | 2.1 ± 4.6 (0.387)* | 161.5 ± 73.8 (0.595)* | 12.1 ± 8.5 (1.018)* |

* Value not significant at 0.01 probability level of significance.

TABLE THREE: Acid Test Solution (HCl 100 meq/l) - 1 Molar Urea Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

4 Experiments (n = 17)

| Cycle | Volume Secreted (ml) | Total $Cl^-$ Flux Out | Net $Cl^-$ Flux In | Net $H^+$ Flux Out | Net $Na^+$ Flux In | Net $K^+$ Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 15 half hour pre-treatment cycles) | 1.92 ± 0.58 | 180.3 ± 178.4 | 217.7 ± 117.9 | 154.8 ± 67.8 | 364.1 ± 89.0 | 14.1 ± 6.8 |
| | | 30 Minute Pouch Treatment with 50 ml. of 1 Molar Urea | | | | |
| 0.5 hour post-treatment | 4.60 ± 0.71 (7.393)** | 180.7 ± 74.1 (0.004)* | 435.2 ± 80.8 (3.201)*** | 108.0 ± 46.9 (1.230)* | 440.5 ± 60.0 (1.534)* | 22.1 ± 2.3 (2.193)* |
| 1.0 hour post-treatment | 1.95 ± 0.74 (0.082)* | 238.2 ± 181.6 (0.544)* | 177.7 ± 130.4 (0.557)* | 170.8 ± 77.5 (0.384)* | 331.9 ± 82.4 (0.618)* | 14.4 ± 4.4 (0.079)* |
| 1.5 hours post-treatment | 1.66 ± 0.68 (0.726)* | 220.9 ± 98.1 (0.414)* | 162.5 ± 76.1 (0.840)* | 175.7 ± 76.6 (0.504)* | 310.0 ± 46.8 (1.110)* | 15.3 ± 6.8 (0.297)* |
| 2.0 hours post-treatment | 1.77 ± 0.22 (0.480)* | 245.0 ± 98.5 (0.660)* | 122.7 ± 122.1 (1.345)* | 85.4 ± 24.5 (1.904)* | 229.1 ± 39.8 (1.347)* | 13.2 ± 3.0 (0.244)* |

* Value not significant at 0.01 probability level of significance
*** Value is significant at 0.005 probability level of significance
**** Value is significant at 0.001 probability level of significance

TABLE FOUR: Neutral Test Solution (NaCl 100 meq/l) - 1 Molar Urea Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

4 Experiments (n = 17)

| Cycle | Volume Secreted (ml) | Total $Cl^-$ Flux Out | Net $Cl^-$ Flux In | Net $H^+$ Flux Out | Net $Na^+$ Flux In | Net $K^+$ Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 15 half hour pre-treatment cycles) | 2.26 ± 0.69 | 188.6 ± 115.5 | 310.2 ± 91.9 | 2.6 ± 4.9 | 276.1 ± 90.3 | 22.6 ± 5.3 |
| | | 30 Minute Pouch Treatment with 50 ml. of 1 Molar Urea | | | | |
| 0.5 hour post-treatment | 2.93 ± 0.78 (1.587)* | 182.9 ± 78.3 (0.089)* | 349.8 ± 78.5 (0.747)* | 0.9 ± 2.1 (0.655)* | 283.6 ± 27.6 (0.156)* | 20.2 ± 3.6 (0.803)* |
| 1.0 hour post-treatment | 2.63 ± 0.84 (0.859)* | 183.4 ± 73.1 (0.082)* | 359.4 ± 166.8 (0.740)* | 8.3 ± 12.9 (1.301)* | 255.9 ± 54.2 (0.403)* | 23.8 ± 6.8 (0.368)* |
| 1.5 hours post-treatment | 1.54 ± 0.75 (1.722)* | 157.9 ± 30.0 (0.499)* | 238.7 ± 83.2 (1.333)* | 5.7 ± 5.8 (1.015)* | 207.9 ± 94.9 (1.255)* | 21.2 ± 3.3 (0.468)* |
| 2.0 hours post-treatment | 1.85 ± 0.40 (1.078)* | 218.3 ± 143.1 (0.409)* | 264.4 ± 30.9 (0.928)* | 6.4 ± 5.8 (1.248)* | 215.3 ± 48.2 (1.227)* | 18.5 ± 3.0 (1.574)* |

* Value not significant at 0.01 probability level of significance.

TABLE FIVE: Acid Test Solution (HCl-100 meq/l) - 2 Molar Urea Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

3 Experiments (n = 13)

| Cycle | Volume Secreted (ml) | Total $Cl^-$ Flux Out | Net $Cl^-$ Flux In | Net $H^+$ Flux out | Net $Na^+$ Flux In | Net $K^+$ Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 12 half hour pre-treatment cycles) | 2.17 ± 0.50 | 246.8 ± 91.8 | 249.7 ± 69.4 | 157.7 ± 60.4 | 406.2 ± 48.3 | 18.8 ± 3.0 |
| | | 30 Minute Pouch Treatment with 50 ml of 2 Molar Urea | | | | |
| 0.5 hour post-treatment | 5.94 ± 1.47 (6.841)**** | 218.7 ± 60.6 (0.469)* | 633.1 ± 267.9 (4.098)*** | 308.2 ± 167.9 (2.348)* | 609.5 ± 171.8 (3.326)** | 35.7 ± 1.2 (8.891)**** |
| 1.0 hour post-treatment | 2.30 ± 0.76 (0.334)* | 170.3 ± 86.5 (1.216)* | 287.2 ± 119.2 (0.661)* | 247.9 ± 65.5 (2.118)* | 443.7 ± 48.1 (1.119)* | 26.0 ± 4.1 (3.197)** |
| 1.5 hours post-treatment | 1.91 ± 0.26 (0.812)* | 152.3 ± 68.0 (1.557)* | 207.2 ± 73.6 (0.872)* | 217.7 ± 75.7 (1.358)* | 383.2 ± 55.5 (0.667)* | 14.9 ± 2.4 (1.931)* |
| 2.0 hours post-treatment | 2.72 ± 0.66 (1.481)* | 103.9 ± 48.5 (2.428)* | 269.6 ± 54.4 (0.431)* | 126.9 ± 24.2 (0.806)* | 402.9 ± 87.2 (0.097)* | 20.2 ± 4.2 (0.624)* |

* Value not significant at 0.01 probability level of significance
** Value is significant at 0.01 probability level of significance
*** Value is significant at 0.005 probability level of significance
**** Value is significant at 0.001 probability level of significance

TABLE SIX: Neutral Test Solution (NaCl 100 meq/l) - 2 Molar Urea Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

3 Experiments (n = 12)

| Cycle | Volume Secreted (ml) | Total $Cl^-$ Flux Out | Net $Cl^-$ Flux In | Net $H^+$ Flux Out | Net $Na^+$ Flux In | Net $K^+$ Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 11 half hour pre-treatment cycles) | 1.90 ± 0.58 | 182.7 ± 134.1 | 252.7 ± 85.8 | 1.9 ± 2.3 | 237.4 ± 42.8 | 16.2 ± 5.6 |
| | | 30 Minute Pouch Treatment with 50 ml of 2 Molar Urea | | | | |
| 0.5 hour post-treatment | 5.08 ± 0.64 (6.249)**** | 151.4 ± 205.8 (0.292)* | 507.5 ± 140.8 (3.616)*** | 1.8 ± 1.4 (2.426)* | 554.2 ± 136.1 (6.123)**** | 27.0 ± 9.2 (2.361)* |
| 1.0 hour post-treatment | 1.76 ± 0.51 (0.352)* | 336.1 ± 51.7 (1.799)* | 182.9 ± 87.3 (1.151)* | 0.1 ± 1.0 (1.326)* | 180.7 ± 96.0 (1.379)* | 21.0 ± 5.6 (1.230)* |
| 1.5 hours post-treatment | 1.83 ± 0.13 (0.192)* | 335.6 ± 46.8 (1.800)* | 266.1 ± 86.3 (0.222)* | 3.6 ± 4.2 (0.883)* | 175.6 ± 49.3 (1.984)* | 19.2 ± 3.9 (0.818)* |
| 2.0 hours post-treatment | 1.14 ± 0.49 (1.923)* | 161.1 ± 59.1 (0.251)* | 152.4 ± 54.3 (1.779)* | 5.4 ± 5.2 (1.600)* | 114.5 ± 26.3 (4.383)**** | 17.4 ± 3.2 (0.337)* |

* Value not significant at 0.01 probability level of significance
*** Value is significant at 0.005 probability level of significance
**** Value is significant at 0.001 probability level of significance

TABLE SEVEN: Acid Test Solution (HCl 100 meq/l) - 4 Molar Urea Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

6 Experiments (n = 28)

| Cycle | Volume Secreted (ml) | Total $Cl^-$ Flux Out | Net $Cl^-$ Flux In | Net $H^+$ Flux Out | Net $Na^+$ Flux In | Net $K^+$ Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 24 half hour pre-treatment cycles) | 2.88 ± 1.07 | 176.7 ± 116.3 | 248.2 ± 109.0 | 106.8 ± 92.4 | 337.7 ± 64.3 | 17.7 ± 5.1 |
| | | | 30 Minute Pouch Treatment with 50 ml. of 4 Molar Urea | | | |
| 0.5 hour post-treatment | 5.70 ± 1.16 (5.486)**** | 269.5 ± 92.9 (1.756)* | 475.1 ± 87.3 (4.574)**** | 434.4 ± 82.0 (7.672)**** | 962.4 ± 163.8 (14.207)**** | 40.3 ± 6.7 (8.737)**** |
| 1.0 hour post-treatment | 3.78 ± 0.87 (1.845)* | 216.2 ± 127.8 (0.706)* | 306.4 ± 138.6 (1.066)* | 260.8 ± 139.8 (3.147)*** | 633.5 ± 135.7 (7.494)**** | 27.5 ± 6.9 (3.764)**** |
| 1.5 hours post-treatment | 2.80 ± 1.17 (0.155)* | 204.5 ± 51.9 (0.553)* | 280.7 ± 152.7 (0.578)* | 181.6 ± 111.1 (1.643)* | 535.4 ± 155.5 (5.417)**** | 22.7 ± 5.6 (2.039)* |
| 2.0 hours post-treatment | 3.58 ± 0.48 (1.512)* | 189.6 ± 32.7 (0.260)* | 381.8 ± 79.6 (2.725)* | 133.6 ± 38.8 (0.673)* | 519.7 ± 38.1 (3.145)*** | 21.6 ± 4.6 (1.657)* |

* Value not significant at 0.01 probability level of significance
*** Value is significant at 0.005 probability level of significance
**** Value is significant at 0.001 probability level of significance

TABLE EIGHT: Neutral Test Solution (NaCl 100 meq/l) - 4 Molar Urea Treatment

Values in microequivalents per half hour ± Standard Deviation (t-value)

6 Experiments (n = 28)

| Cycle | Volume Secreted (ml) | Total $Cl^-$ Flux Out | Net $Cl^-$ Flux In | Net $H^+$ Flux Out | Net $Na^+$ Flux In | Net $K^+$ Flux In |
|---|---|---|---|---|---|---|
| Control (Mean of 24 half hour pre-treatment cycles) | 1.84 ± 0.63 | 150.2 ± 113.0 | 254.7 ± 89.8 | 2.1 ± 16.6 | 234.3 ± 63.7 | 17.1 ± 5.1 |
| | | 30 Minute Pouch Treatment with 50 ml. of 4 Molar Urea | | | | |
| 0.5 hour post-treatment | 5.40 ± 2.06 (6.982)**** | 306.5 ± 141.3 (2.777)** | 522.6 ± 236.4 (4.273)**** | 1.5 ± 3.9 (0.086)* | 666.2 ± 255.4 (7.167)**** | 33.5 ± 8.4 (5.878)**** |
| 1.0 hour post-treatment | 2.10 ± 0.29 (0.953)* | 215.0 ± 130.5 (1.176)* | 172.5 ± 73.2 (2.007)* | 0.7 ± 1.8 (0.201)* | 220.5 ± 48.5 (0.479)* | 21.3 ± 4.4 (1.801)* |
| 1.5 hours post-treatment | 2.67 ± 1.03 (2.416)* | 156.7 ± 135.6 (0.117)* | 252.0 ± 78.9 (0.065)* | 0.7 ± 2.7 (0.399)* | 235.7 ± 84.5 (0.044)* | 20.8 ± 4.8 (1.566)* |
| 2.0 hours post-treatment | 1.52 ± 1.02 (0.935)* | 165.3 ± 89.5 (0.295)* | 174.0 ± 96.2 (1.876)* | 1.0 ± 2.4 (0.157)* | 161.1 ± 67.9 (2.402)* | 16.6 ± 4.4 (0.195)* |

* Value not significant at 0.01 probability level of significance
** Value is significant at 0.01 probability level of significance
**** Value is significant at 0.001 probability level of significance

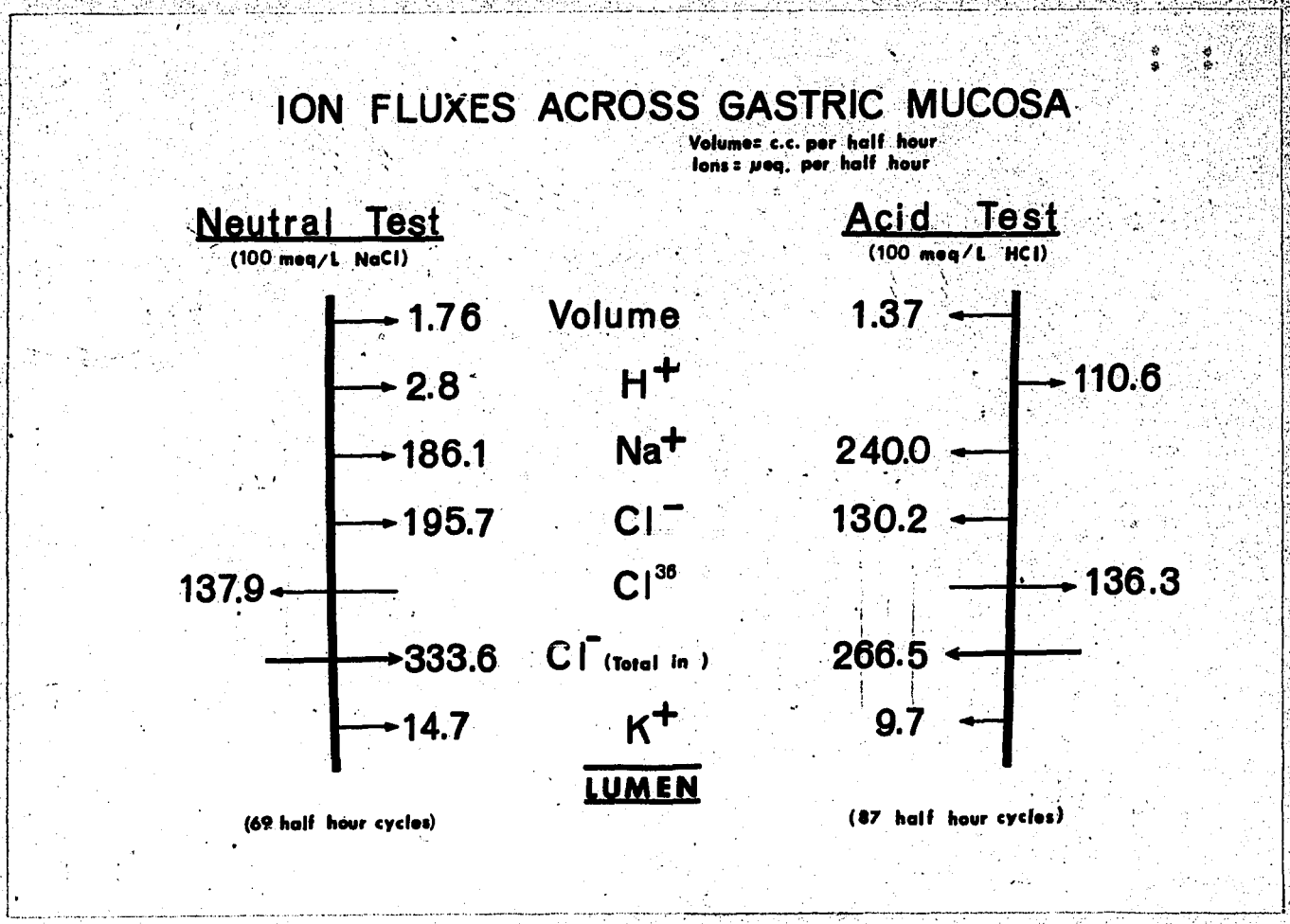


FIGURE 9. Ionic Fluxes Across Resting Gastric Mucosa. The central area between the two vertical bars represent the lumen of the pouch. Arrows from the vertical bar represent a net change while arrows crossing the vertical bar represent a total change.

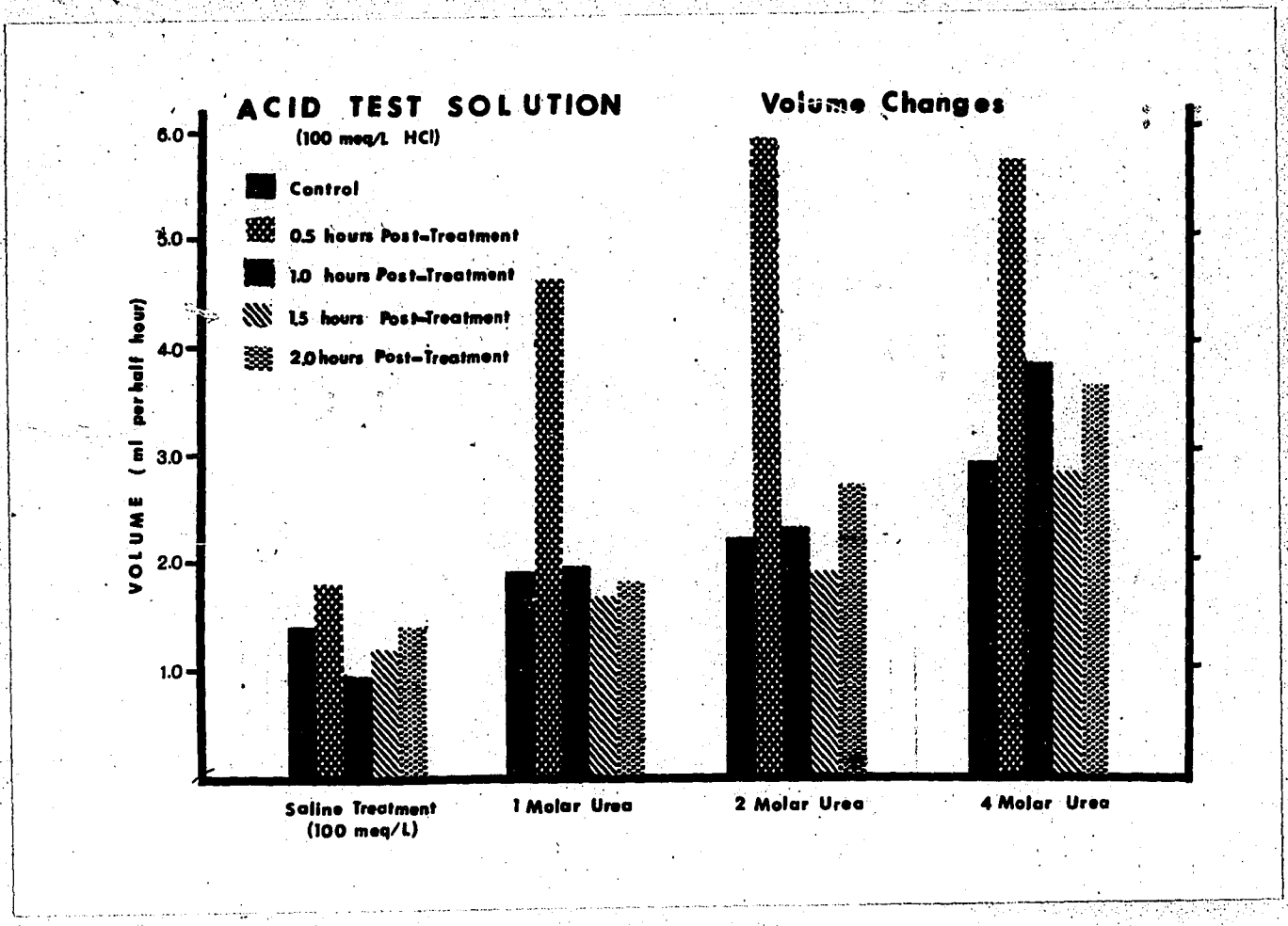


FIGURE 10. Net Volume Changes with Acid Test Solution

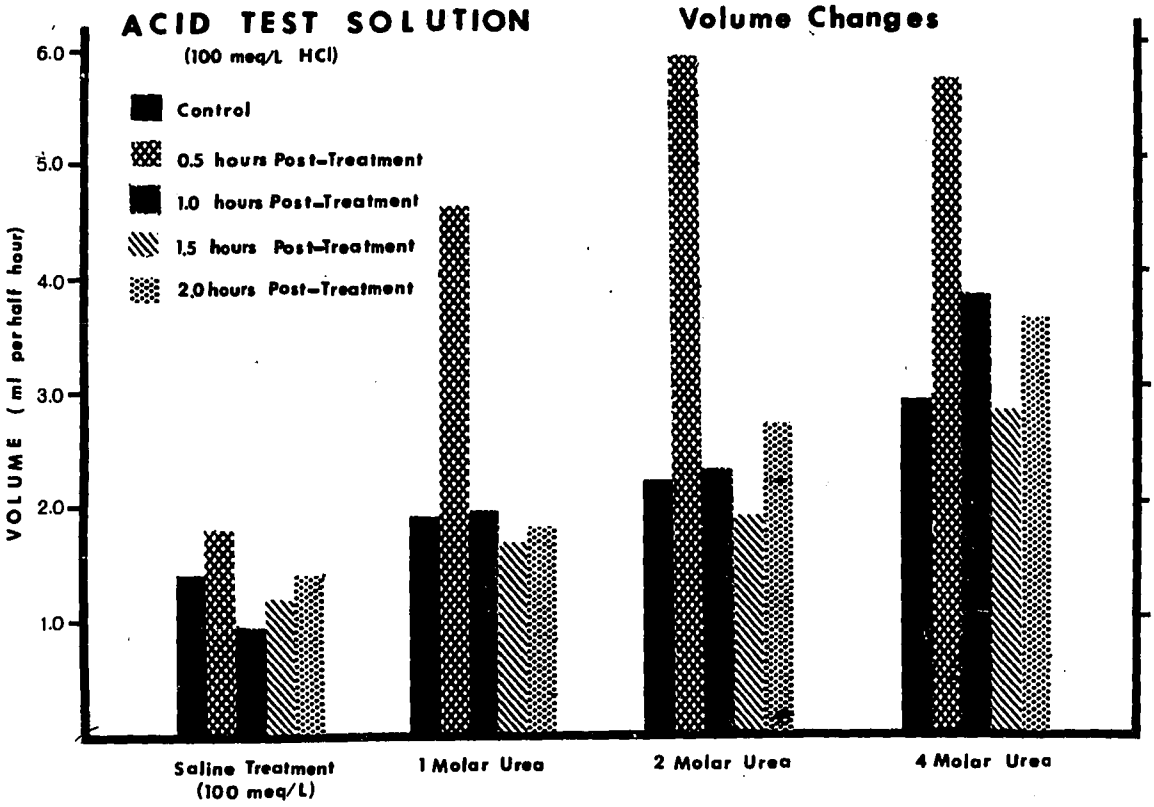


FIGURE 10. Net Volume Changes with Acid Test Solution

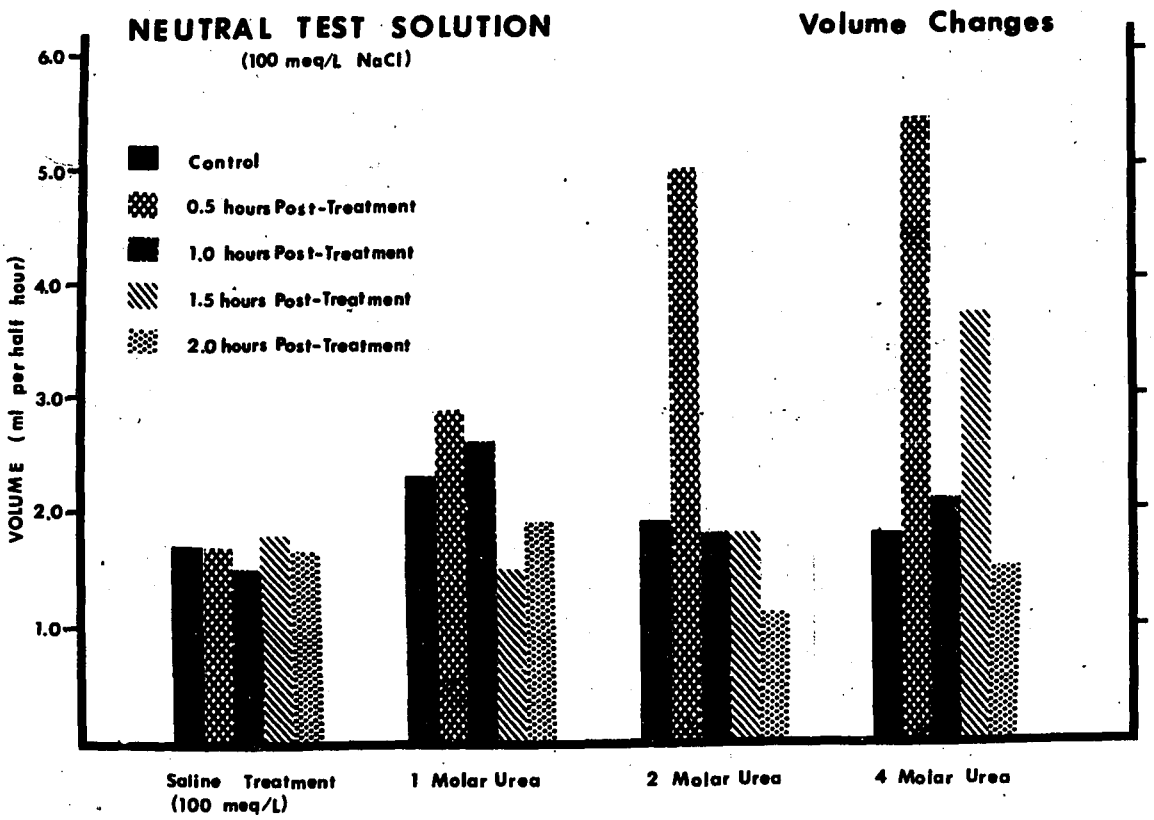


FIGURE 11. Net Volume Changes with Neutral Test Solution

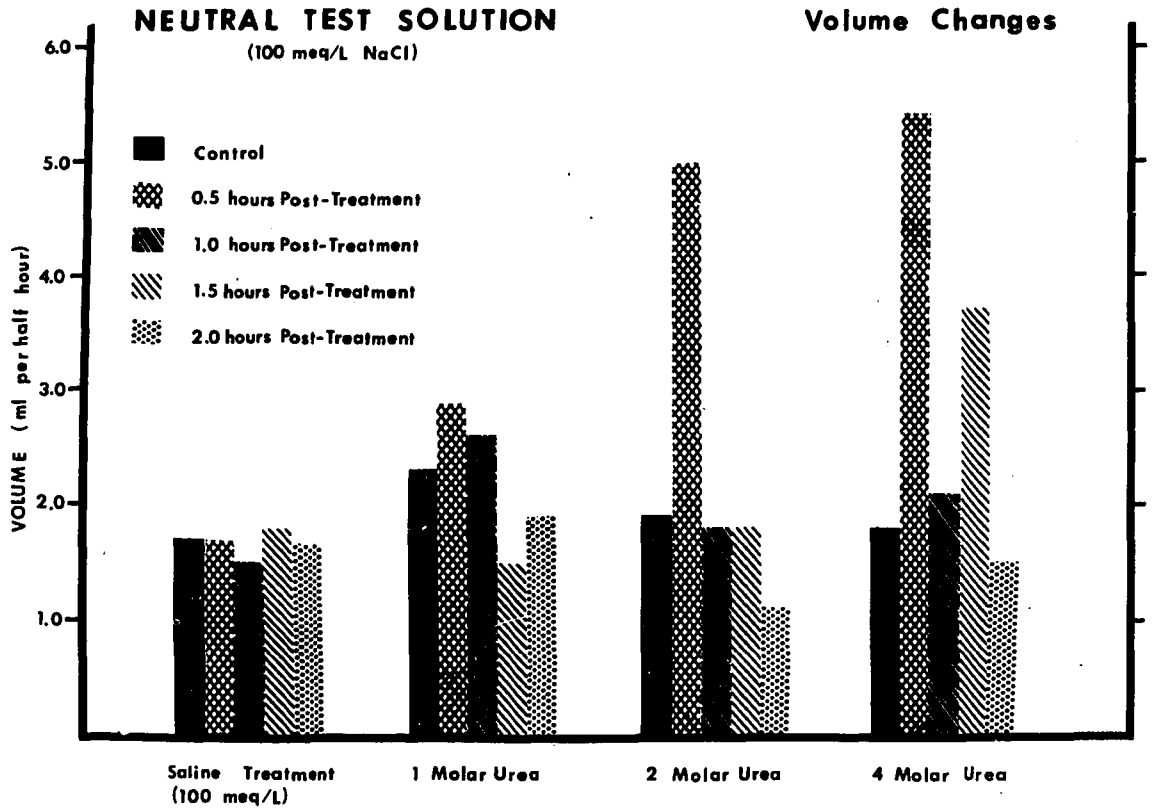


FIGURE 11. Net Volume Changes with Neutral Test Solution

When the pouch was treated with saline there was no change in the volume secreted in any of the half-hour post treatment cycles irregardless of the test solution used. There was a significant increase in the volume in the first half hour post treatment cycle following treatment with 1 molar urea when the acid test solution was instilled. This increase in volume from 1.92 ± 0.58 millilitres to 4.60 ± 0.71 was significant with a 99.9 percent limit of confidence ($t = 7.393$). There was no such significant increase in the volume secreted when the neutral test solution was used. When the pouch was treated with 2 molar urea solution there were significant increases in the volume secreted with both acid test solution (2.17 ± 0.50 ml. to 5.94 ± 1.47 ml.; $t = 6.841$) and neutral test solution (1.90 ± 0.58 ml. to 5.08 ± 0.64 ml.; $t = 6.249$). Similar significant increases in the volume secreted were seen in the first post treatment cycle following four molar urea pouch treatment in both the acid test solution (2.88 ± 1.07 ml. to 5.70 ± 1.16 ml.; $t = 5.486$) and the neutral test solution (1.84 ± 0.63 ml. to 5.40 ± 2.06 ml.; $t = 6.982$). The volume secreted in all cycles following the first half hour post treatment were not significantly different from the control values with either acid or neutral test solutions and for all treatments used.

ii) Hydrogen Ion Fluxes

The hydrogen ion fluxes following treatment when instilling the acid test solution are summarized in Figure 12.

As can be seen from Figure 12 the hydrogen ion loss from the pouch following treatment did not increase following treatment except in the first half hour following treatment with 2 and 4 molar urea. The change seen following treatment with 4 molar urea (106.8 ± 92.4 ueq to 434.4 ± 82.0 ueq.; $t = 7.672$) is the only change which is significant at a 99.9 percent level of confidence.

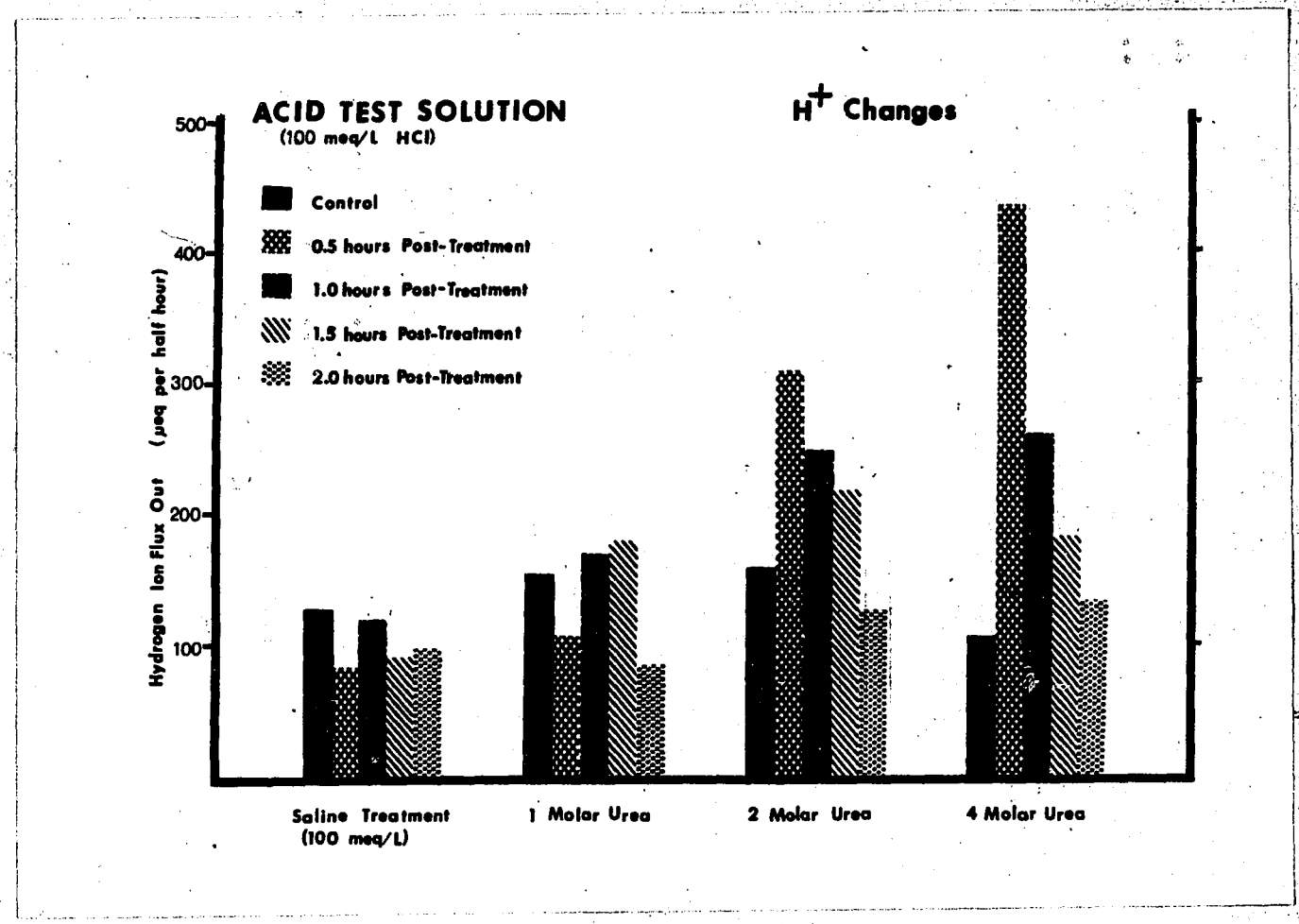


FIGURE 12. Net Hydrogen Ion Fluxes Out of the Pouch with Acid Test Solution

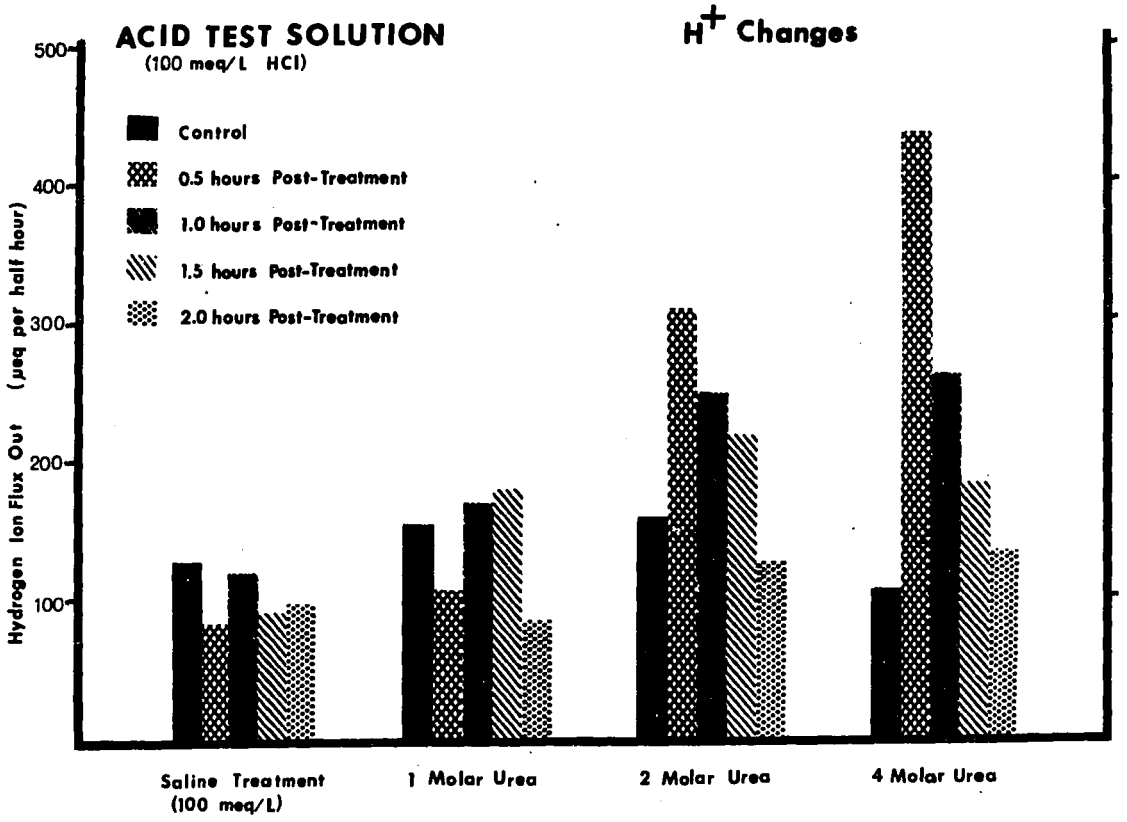


FIGURE 12. Net Hydrogen Ion Fluxes Out of the Pouch with Acid Test Solution

The hydrogen ion fluxes with instillation of neutral test solutions following the treatments can be seen in Tables Two, Four, Six and Eight. None of these changes were significantly different from the control values. In most cases the pouches showed a net gain of one or two microequivalents of hydrogen ion.

iii) Sodium Ion Fluxes

The pouches all gained sodium ion when either acid or neutral test solution was instilled. The changes in these fluxes following treatment are summarized in Figures 13 and 14. As can be seen from these figures there were slight increases in the sodium ion gain in the first half hour post-treatment when the acid test solution was instilled following any urea treatment but this gain was significant only following four molar urea treatment. Following four molar urea treatment similar significant gains in sodium ion flux were seen in the second (0.5 to 1.0 hrs) and third (1.0 to 1.5 hrs) cycles following treatment but not in the fourth cycle (1.5 to 2.0 hrs) (see Table Seven).

When the neutral test solution was used significant increases in the sodium ion flux into the pouch were seen in the first half hour following treatment with two and four molar urea. Curiously there was a significant decrease in the amount of sodium gained by the pouch in the last post-treatment cycle (1.5 to 2.0 hours post-treatment) when the pouch was treated with 2 molar urea. This was not seen following treatment with four molar urea solution. When the neutral test solution was used and there was a significant increase in the sodium ion gain in the first post-treatment cycles the gain in subsequent post-treatment cycles were not significantly different from the control excepting the one significant decrease in gain noted with 2 molar urea treatment.

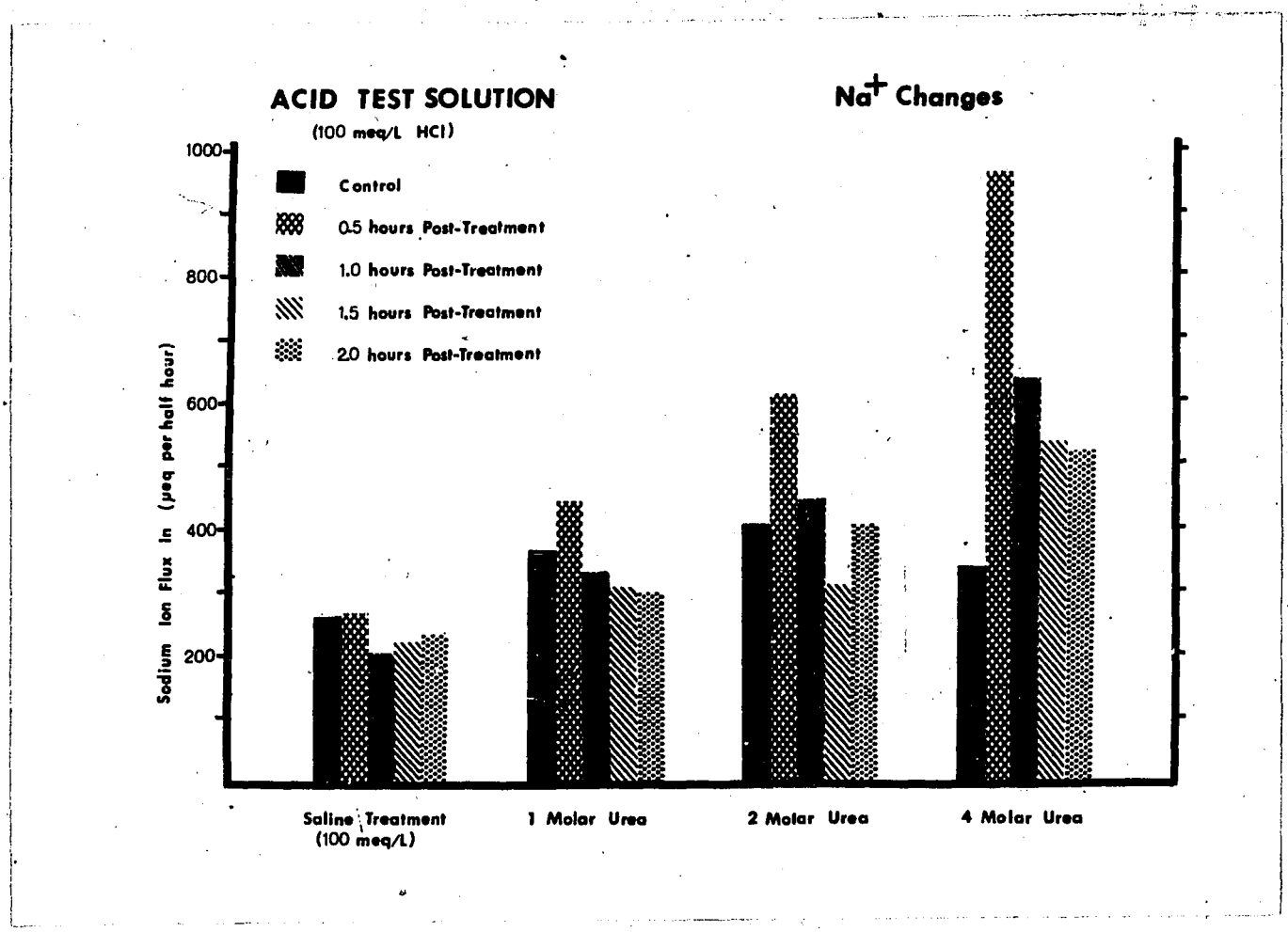


FIGURE 13. Net Sodium Ion Fluxes with Acid Test Solution.

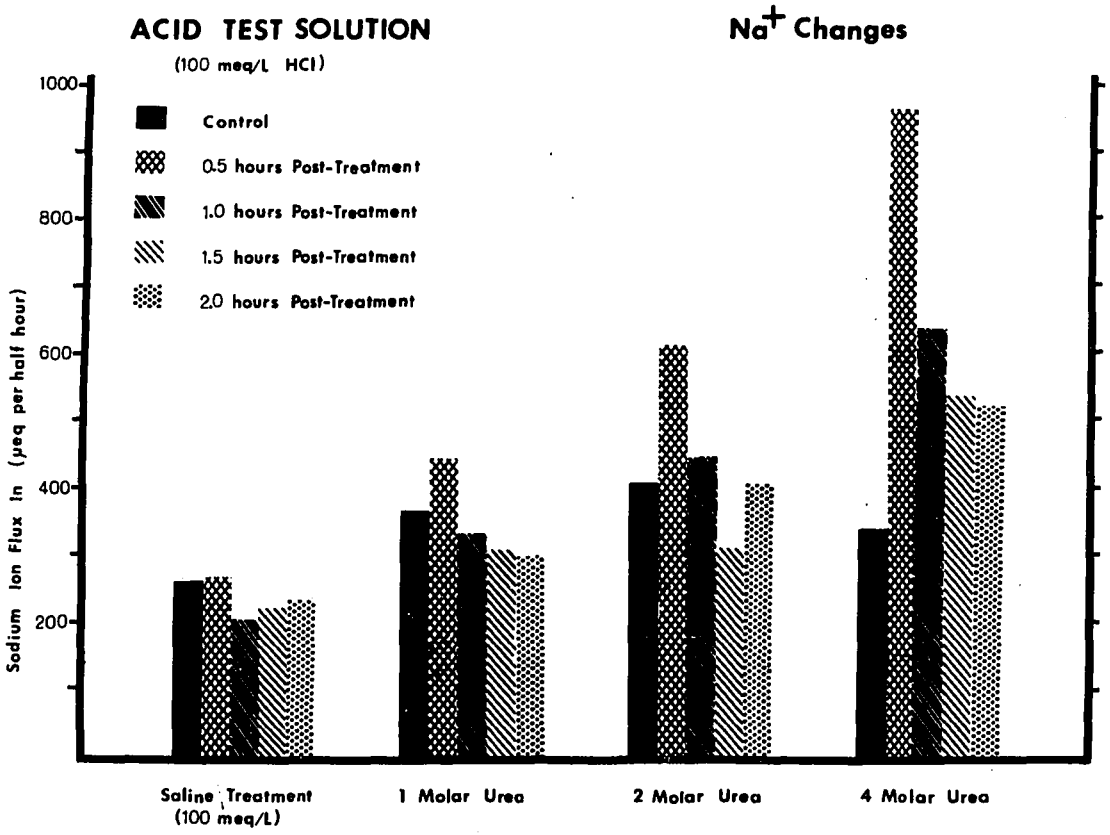


FIGURE 13. Net Sodium Ion Fluxes with Acid Test Solution.

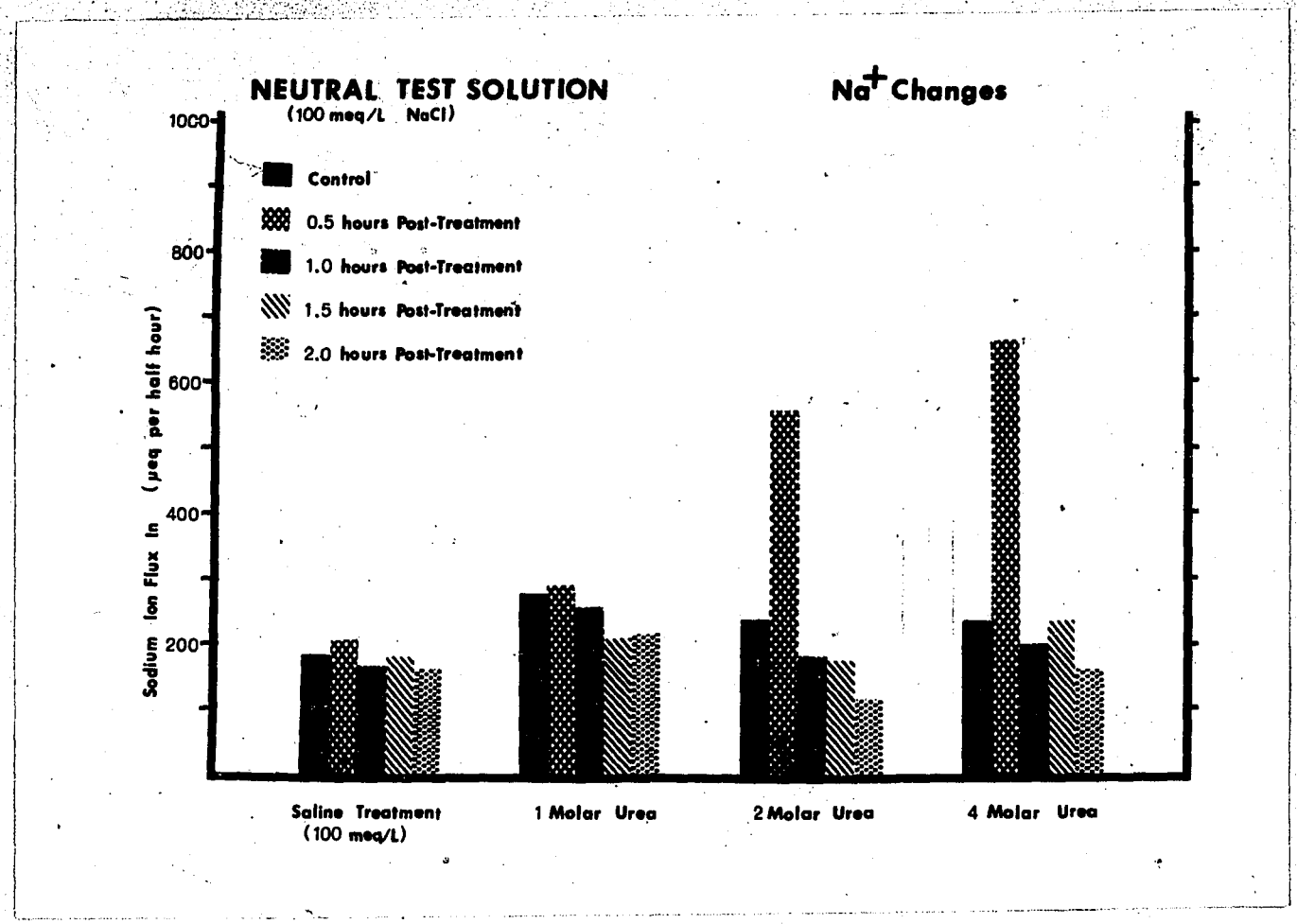


FIGURE 14. Net Sodium Ion Fluxes with Neutral Test Solution

iv) Potassium Ion Fluxes

All of the pouches gained potassium ion with either instillation test solution and following all treatments. There was a significantly increased potassium flux into the pouch using the acid test solution in the first half hour following treatment with 2 molar urea and in the first and second half hours following treatment with 4 molar urea. Using the neutral test solution an increased potassium gain by the pouch was seen only in the first half hour following 4 molar urea treatment. These changes are summarized in Figures 15 and 16.

v) Chloride Ion Fluxes

a) Net Chloride Flux Into Pouch

The net luminal gain of chloride using both the acid and the neutral test solutions is illustrated in Figures 17 and 18. The only significant change seen in the saline or one molar urea treatments occurred in the net chloride gain by the pouch using acid test solution over the first half hour after treatment. Similarily the first half hour after treatment using 2 and 4 molar urea showed significant increases in the chloride gain by the pouch but in all cases there was no significant difference from the control period in the subsequent post-treatment periods.

When the neutral test solution was used (Figure 18) there was a significant increase in the chloride gain by the pouch in the first half hour post-treatment only after treatment with two and four molar urea. In all cases the subsequent periods post-treatment showed no significant variation from the control.

b) Total Chloride Ion Leaving the Pouch

Figures 19 and 20 illustrate the changes in the total chloride lost from the pouch with acid and neutral test solutions following treatment.

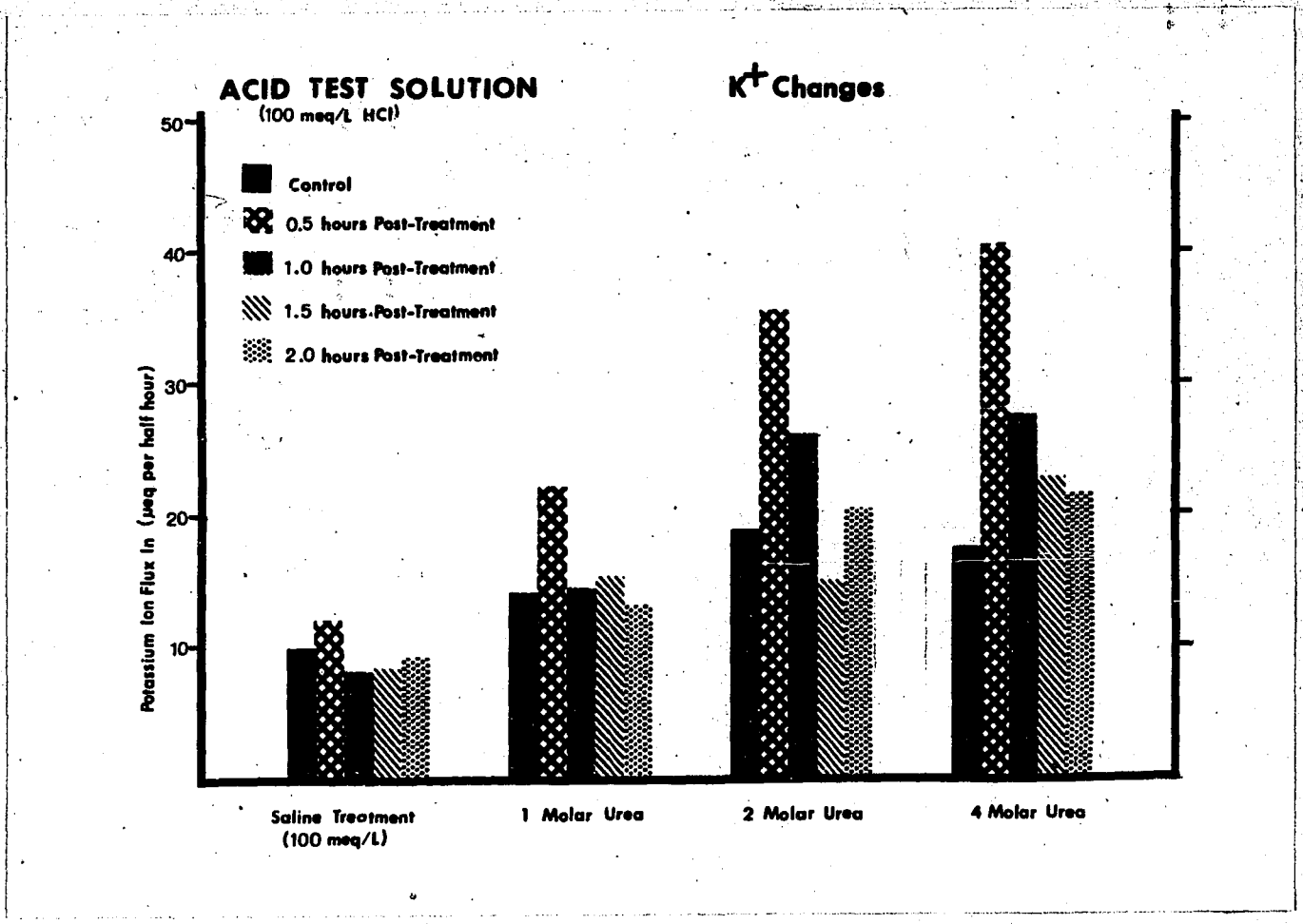


FIGURE 15. Net Potassium Ion Fluxes with Acid Test Solution

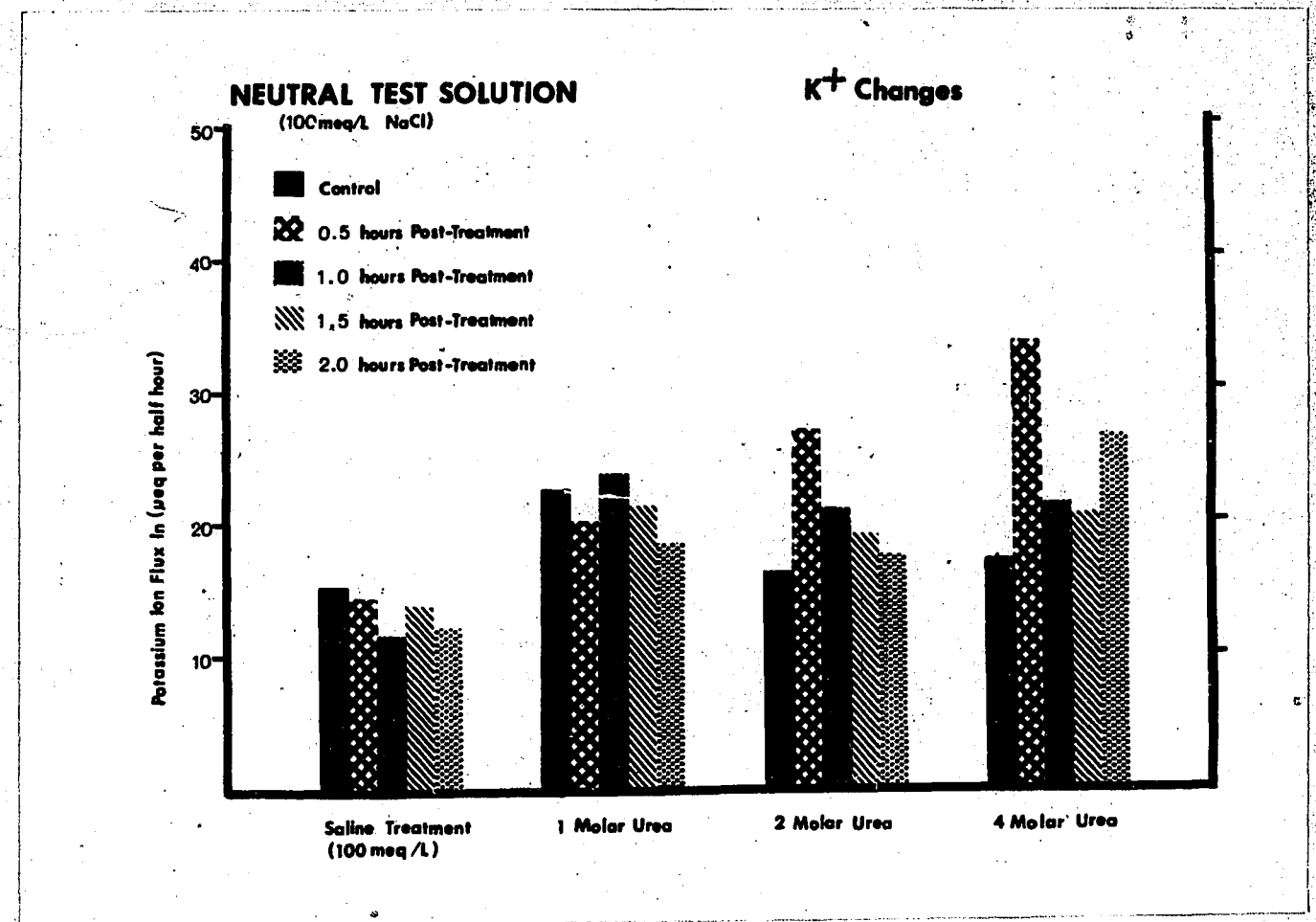


FIGURE 16. Net Potassium Ion Fluxes with Neutral Test Solution

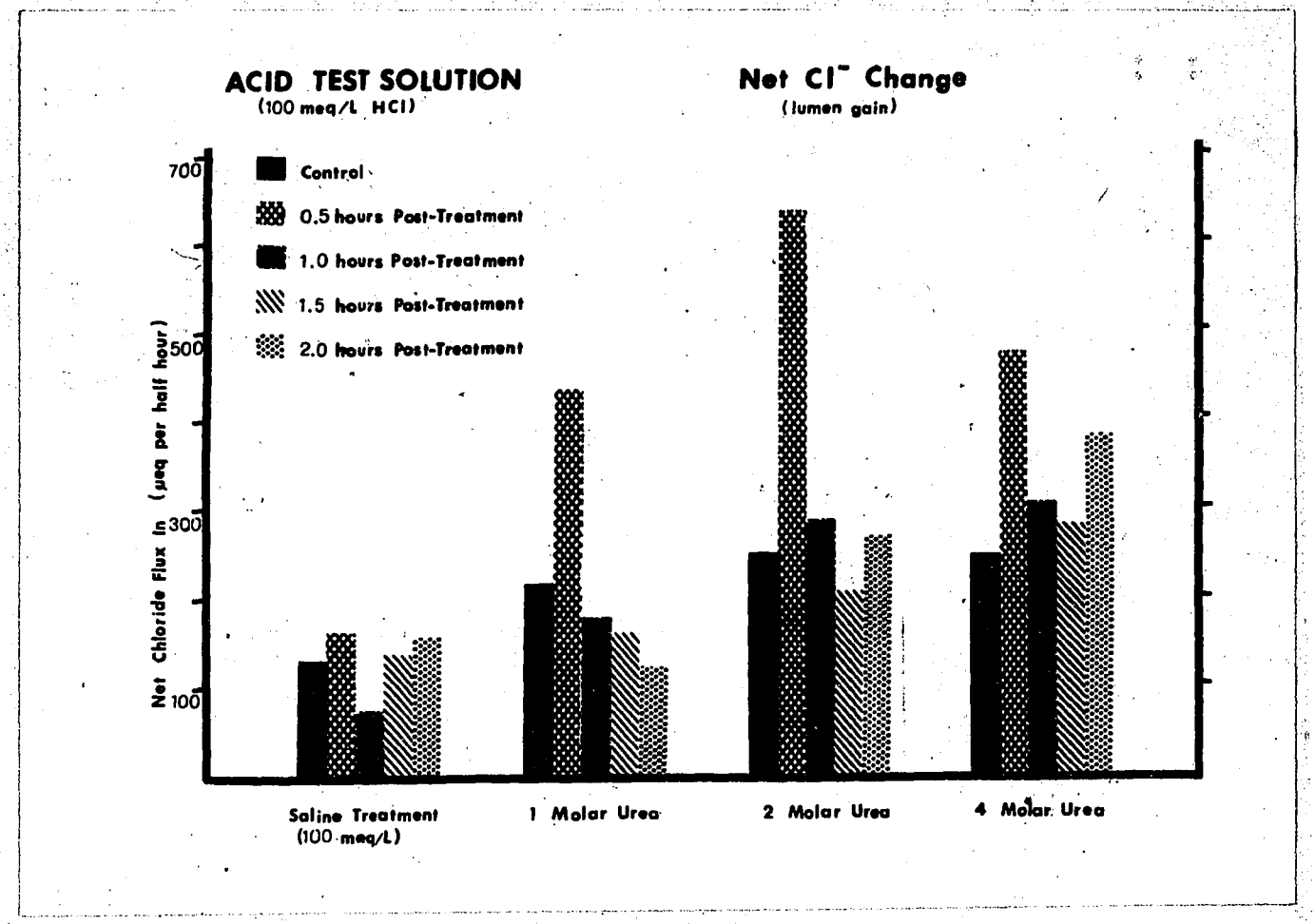


FIGURE 17. The Net Chloride Change Using Acid Test Solution

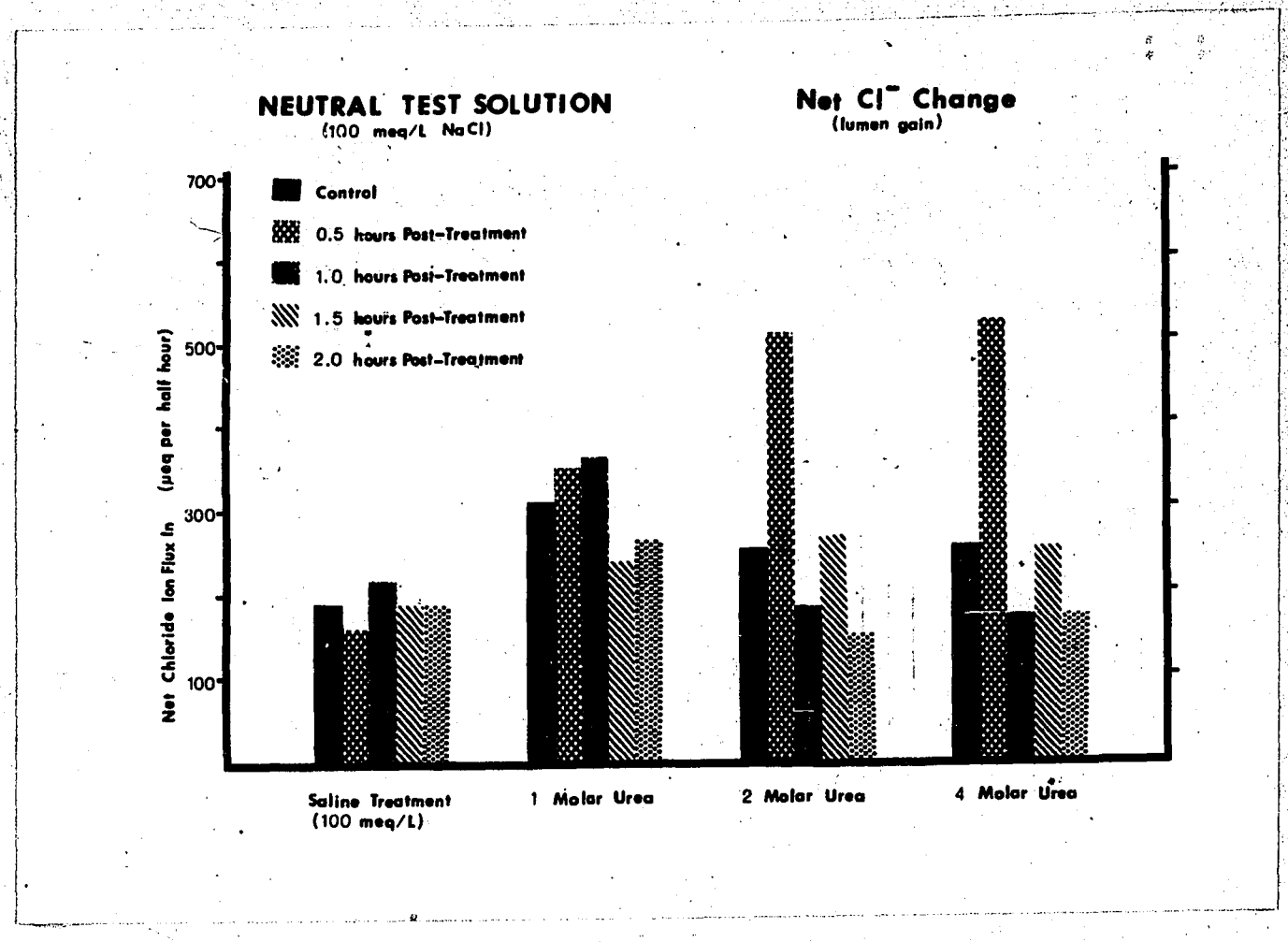


FIGURE 18. The Net Chloride Change Using Neutral Test Solution

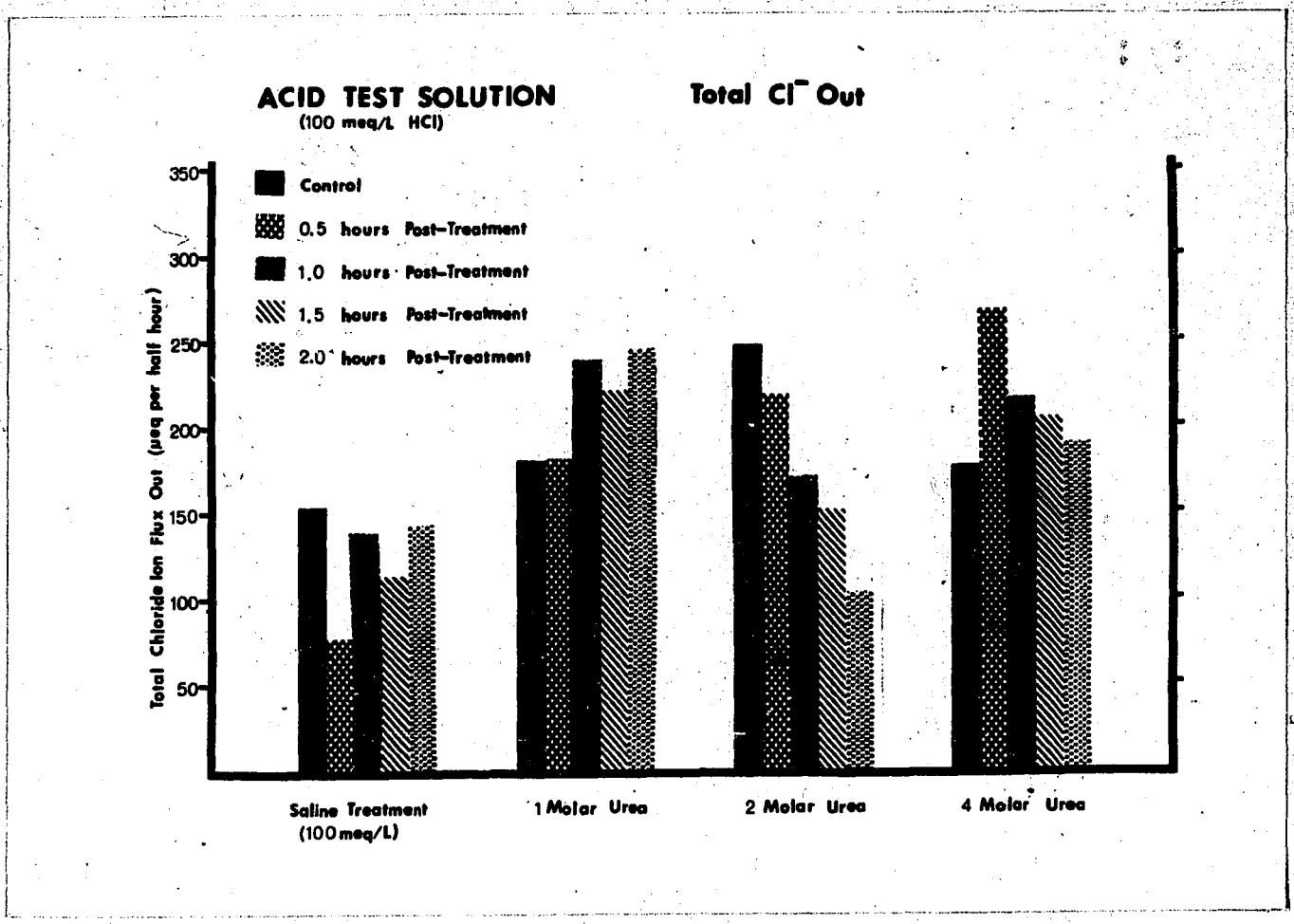


FIGURE 19. Total Chloride Ion Lost From the Pouch Using Acid Test Solution

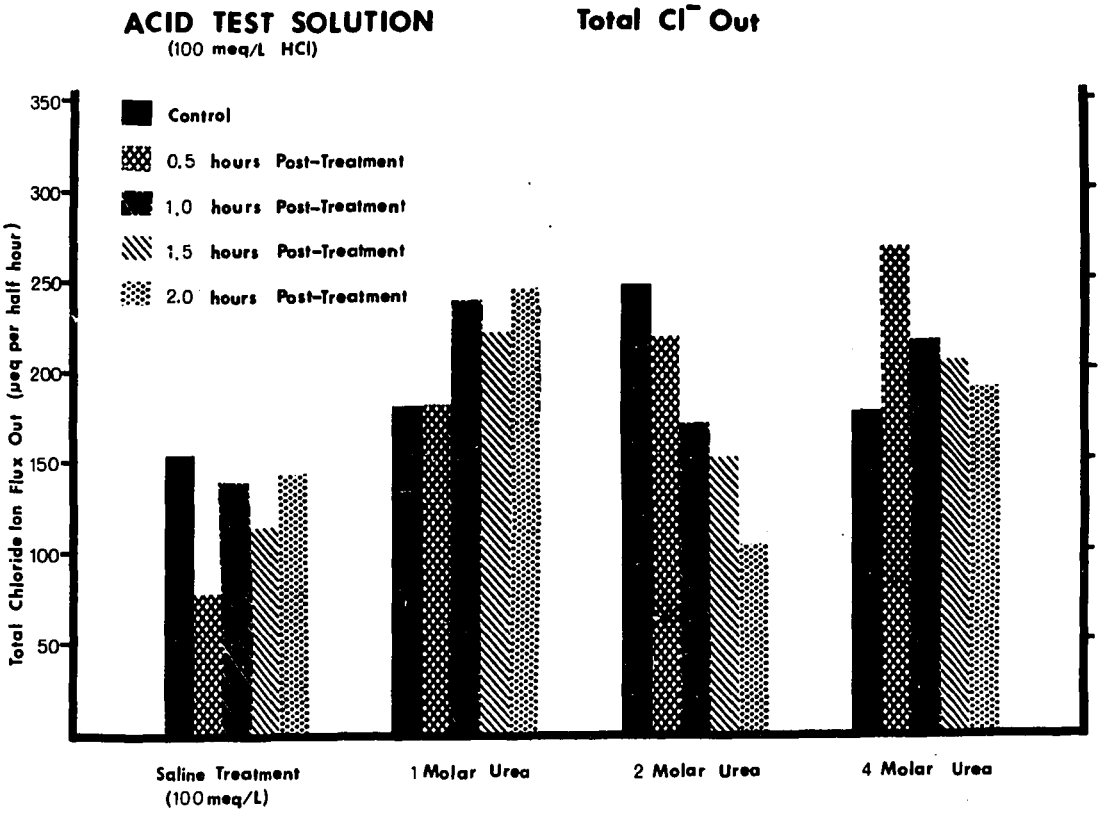


FIGURE 19. Total Chloride Ion Lost From the Pouch Using Acid Test Solution

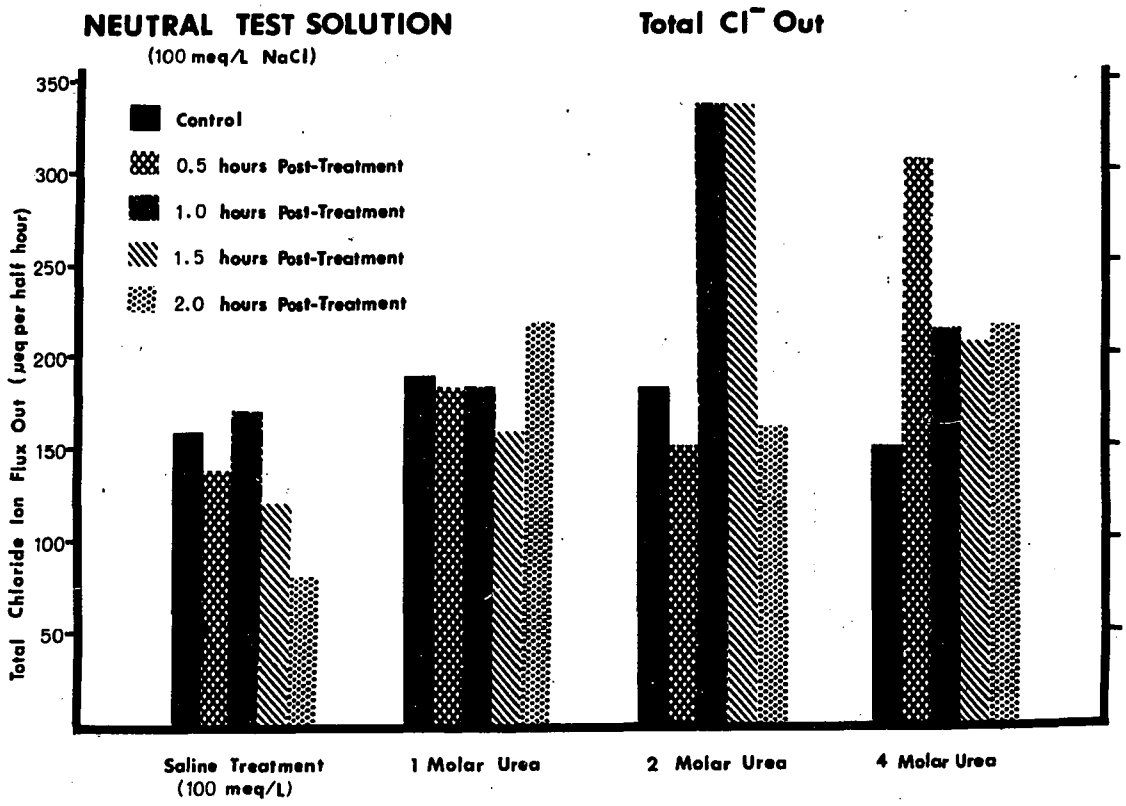


FIGURE 20. Total Chloride Ion Lost From the Pouch Using Neutral Test Solution

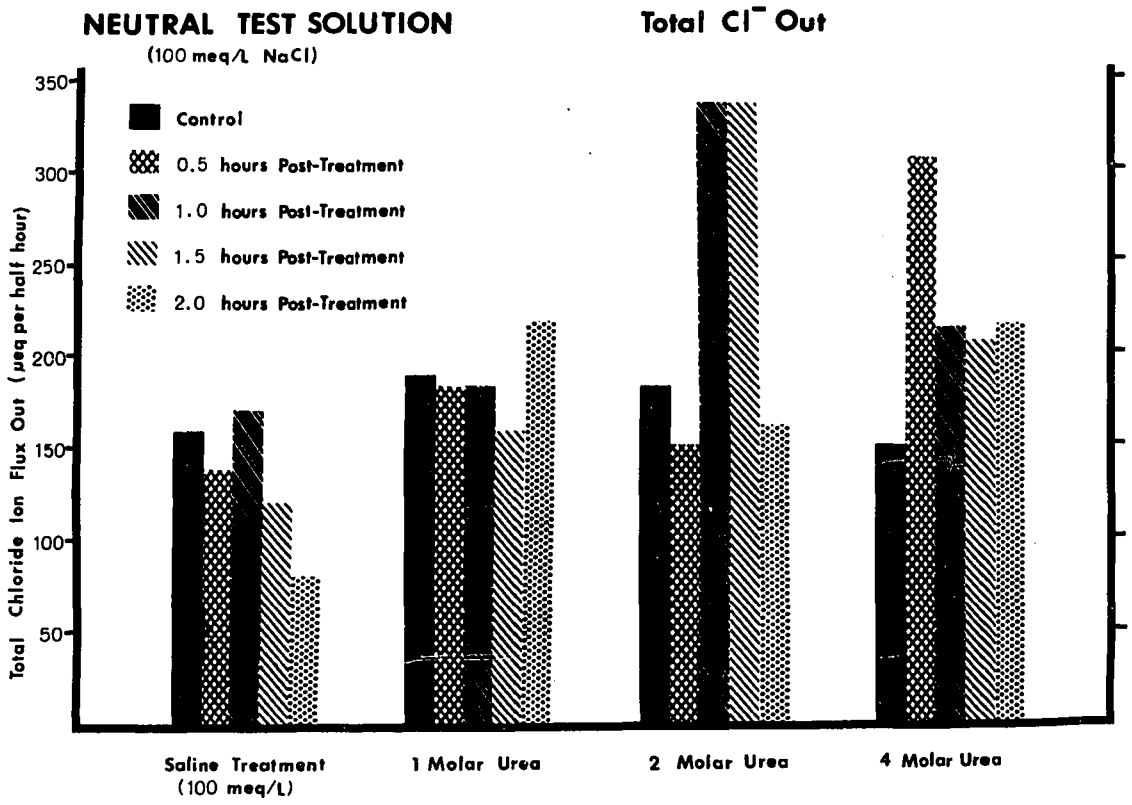


FIGURE 20. Total Chloride Ion Lost From the Pouch Using Neutral Test Solution

None of the changes illustrated represent differences from the control values which are significant at a 99.9 percent level of confidence.

c) Total Chloride Ion Entering the Pouch

The sum of the total chloride entering the pouch and the net chloride ion gained by the pouch must represent the total chloride ion entering the pouch. These sums are represented in Figures 21 and 22.

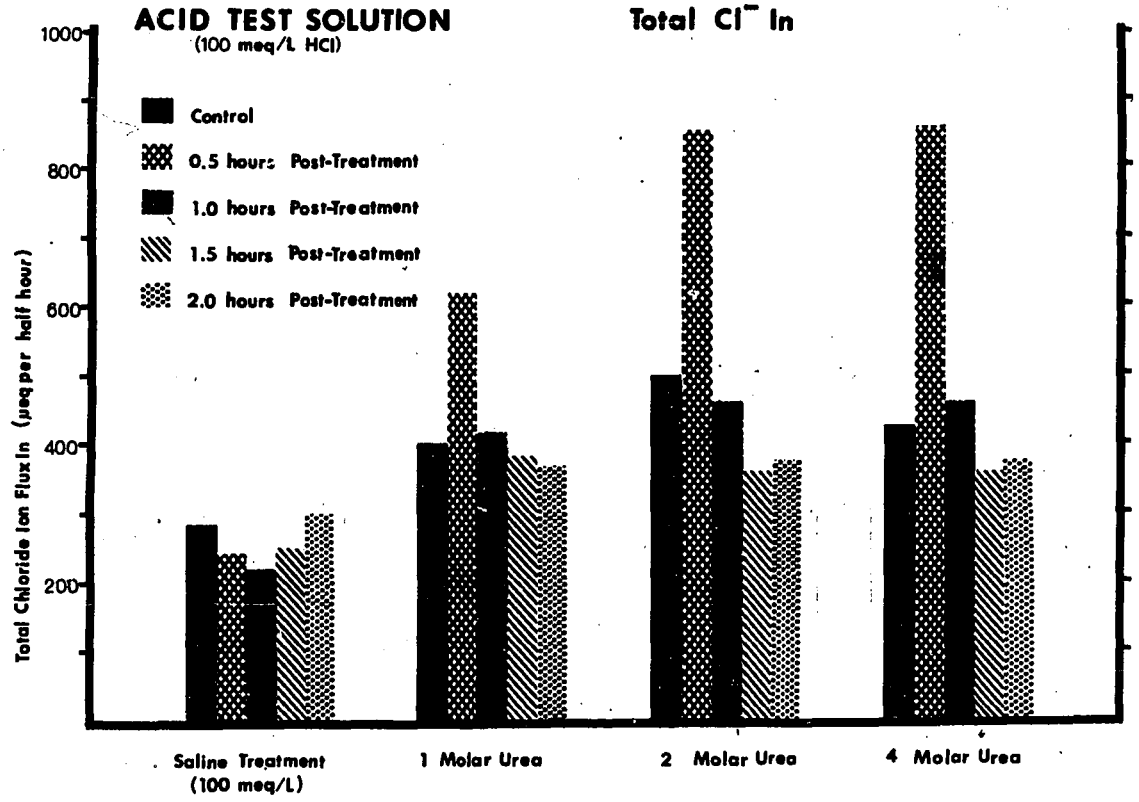


FIGURE 21. Total Chloride Ion Entering the Pouch Using Acid Test Solution

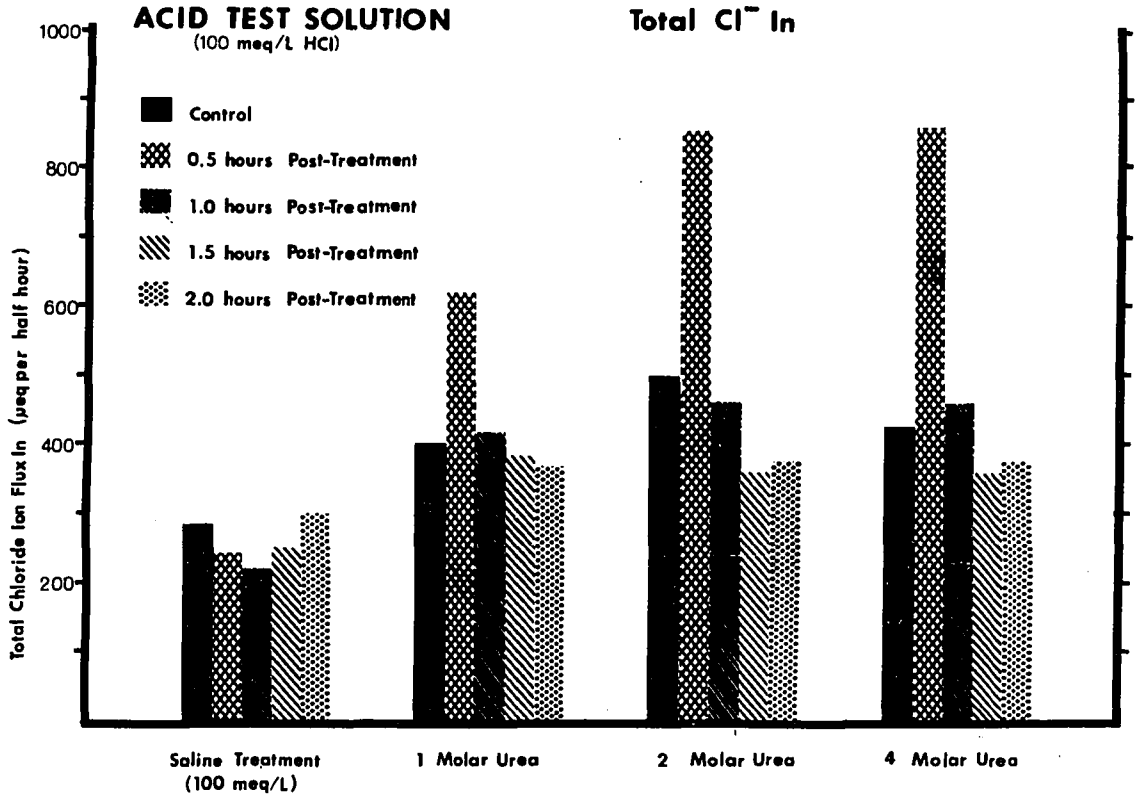


FIGURE 21. Total Chloride Ion Entering the Pouch Using Acid Test Solution

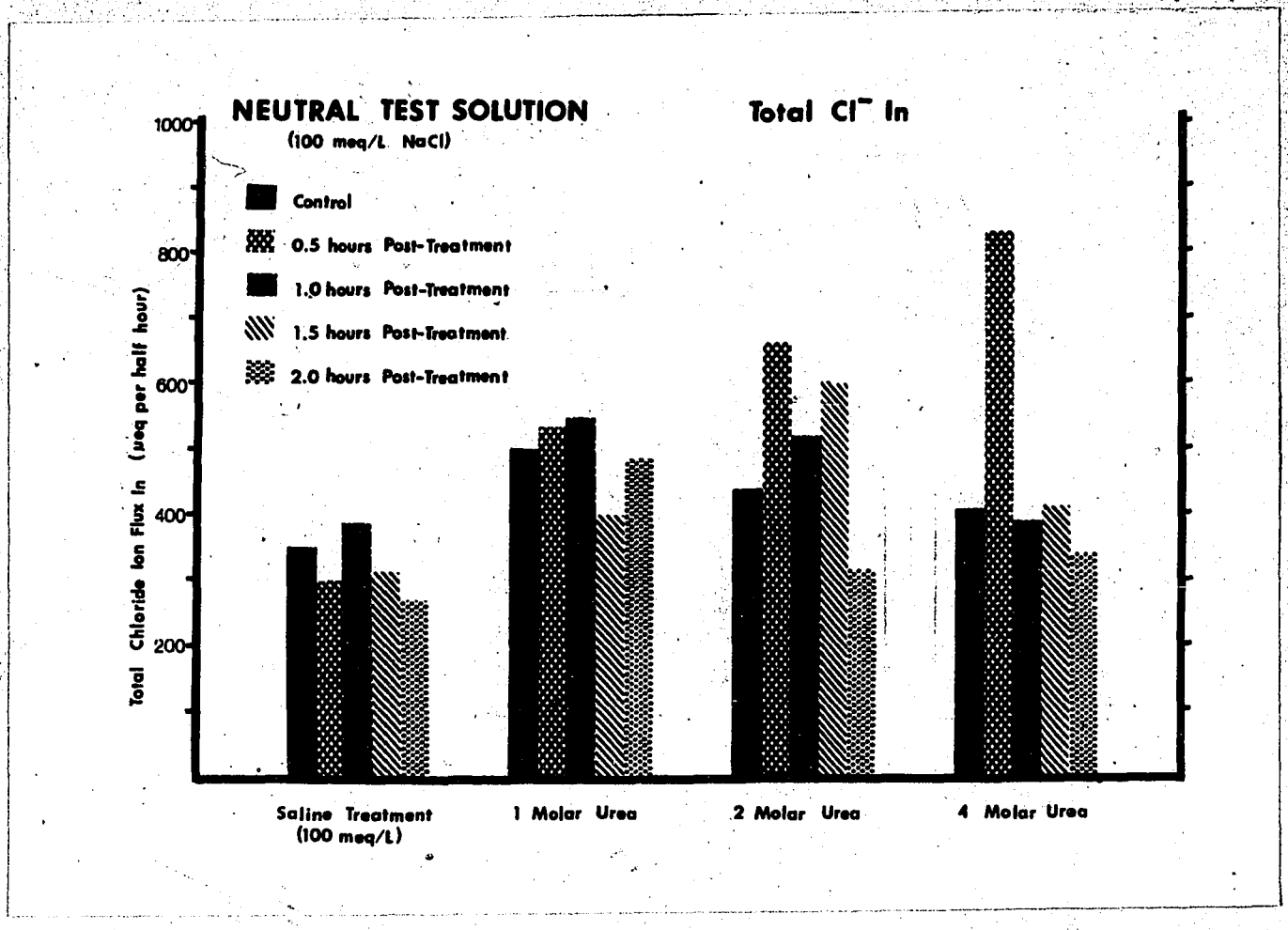


FIGURE 22. Total Chloride Ion Entering the Pouch Using Neutral Test Solution

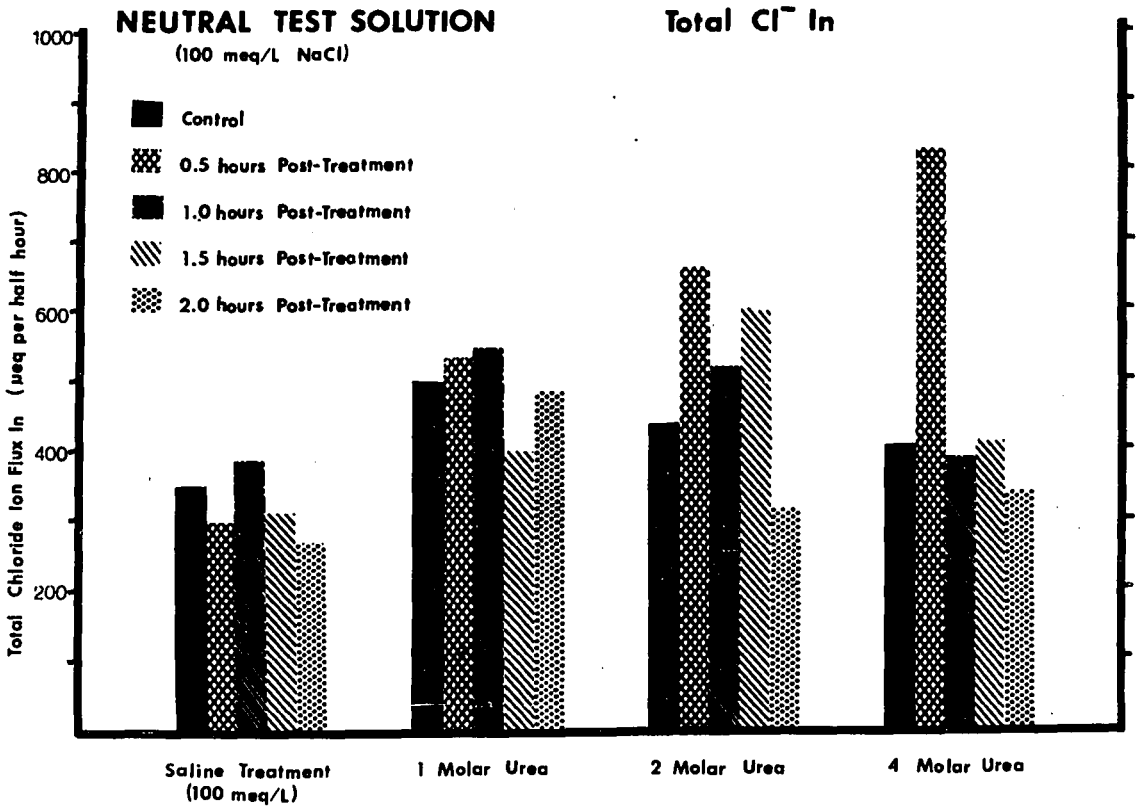


FIGURE 22. Total Chloride Ion Entering the Pouch Using Neutral Test Solution

## SECTION IV: DISCUSSION

### A. Basal Ionic Fluxes

Within the limits of the criteria discussed when the results from non-stimulated pouches before urea treatment were presented these fluxes represent the basal situation in a dog's gastric corpus pouch (see Figure 9).

One of the most obvious findings is that there are bidirectional fluxes of chloride ion across gastric mucosa in vivo. While this has not been shown previously with an in vivo preparation the finding does agree with the in vitro findings of Forte (41), Davenport (29), Heinz and Durbin (33, 34, 51, 53) and other investigators. As has been noted much of the thinking of Pavlov and Hollander is based on measurements of the net chloride gain in a pouch and using this measurement as representative of total chloride input to the pouch. The results which are presented here are from a preparation not dissimilar to that used by these workers and the fact that there is a bidirectional chloride flux leaves the interpretation by Pavlov and Hollander of their data open to question. This may not be as serious a criticism of their interpretation as the data first suggests for it is noted that the flux of chloride out of the pouch is constant at about 136 to 140 microequivalents per half hour irrespective of the degree of acidity of the luminal contents. Thus it is noted that both the neutral test solution and the acid test solution had a similar loss of chloride from the lumen of the pouch. There was only a single chloride concentration used, 100 milliequivalents per litre of test solution and Wlodek (102) has found that the flux of chloride ion out of the pouch varies with the concentration of chloride in the luminal juice. The anion concentration which was measured by Pavlov and Hollander was over a fairly narrow range thus the use of net chloride gain to the pouch as representative of total chloride input would be correct in a relative sense as there would be only slight variation of

the chloride loss from the pouch and the relative changes would thus be the same for net and total chloride gain by the pouch.

There are however other discrepancies in the values seen for the basal ionic fluxes which cannot be explained by the dilution-neutralization school. Fisher and Hunt (38) using Ihre's data reported the largest sodium bicarbonate concentration believed to make up the neutralization fluid. Using this value of 45 milliequivalents per litre (45 microequivalents per millilitre) one calculates that a total of 2.24 millilitres of neutralization fluid would be required to neutralize the 100.6 microequivalents of hydrogen ion lost from the pouch using the acid test solution under basal conditions (Figure 9). If the fluid acted both by dilution and neutralization a greater volume would have been necessary to handle the acid. In fact the volume secreted by the pouch during this period was 1.37 millilitres or slightly more than one half of the volume required according to the dilution-neutralization school of acidity regulation. It should be noted that in his original work Pavlov (80) did not find any increase in volume which would suggest a dilution mechanism.

To explain the loss of hydrogen ion from the pouch when testing with the acid test solution by the exchange diffusionists ideas would require a 1:1 exchange of sodium for hydrogen. Thus we would expect that if there was a 100.6 microequivalent per half hour loss of hydrogen ion there should have been a comparable gain of sodium ion. In fact there was a gain of sodium ion to the extent of 240.0 microequivalents per half hour. This is 129.4 microequivalents greater than the predicted gain by the exchange diffusion school. A possible explanation of this discrepancy is that both exchange diffusion and dilution-neutralization are occurring and that the extra sodium input is as sodium chloride or sodium bicarbonate.

It would be impossible from this data to determine to what degree the net change of any ion is due to exchange diffusion or to a neutralization-dilution fluid. If, however, consideration is given to the concept of a carrier mechanism for the exchange diffusion as suggested by Heinz and Durbin (34, 35, 53) then the significance of the constant flux of chloride out of the pouch becomes more obvious. When examining the acid test solution data (Figure 9) it would be expected that the outward flux of 136.3 microequivalents of chloride carried hydrogen ion out of the pouch. The net loss of hydrogen ion measured was only 100.6 microequivalents; thus, suggesting that there is a hydrogen ion secretion of 25.7 microequivalents. The outward flux of 136.3 microequivalents of hydrogen ion is in exchange for sodium ion according to the exchange diffusionists. Thus, it is predicted that 136.3 microequivalents of sodium ion would enter the pouch. In fact there is a net gain of 240.0 microequivalents of sodium ion suggesting that 103.7 microequivalents of sodium entered the pouch unrelated to exchange diffusion. If the 25.7 microequivalents of hydrogen ion is secreted as hydrochloric acid then there is an unexplained 104.5 microequivalents of chloride ion that entered. It seems probable that this 103.7 microequivalents of sodium and 104.5 microequivalents of chloride which entered the pouch unrelated to acid secretion or exchange diffusion are due to the secretion of sodium chloride. Presumably this is a non-parietal secretion; however, the volume of secretion involved is too small to have a dilutional effect and may have been affected by the mannitol present so the function of this secretion is not clear. A similar calculation can be carried out for the neutral test solution data. The problem with this data is that it is unclear how much of the carrier is carrying sodium ion and how much is carrying hydrogen ion. It is known (14, 26) that no sodium is lost from an acid filled pouch but that there is a small flux of sodium from lumen to mucosa in a pouch having

neutral contents. For the purposes of this discussion the figure of 60 microequivalents is adopted as representing the non-parietal sodium chloride secretion for the neutral test data. This is an arbitrary figure which has little justification except for the discussion. Using this figure of 60 microequivalents of sodium as being due to a non-parietal secretion then there is 126.1 microequivalents of sodium which are not explained by a non-parietal secretion and presumably must have entered in exchange for hydrogen ion secreted. There is a net gain of 2.8 microequivalents of hydrogen ion; thus, the total hydrogen ion secretion must be 128.9 microequivalents. If this enters as hydrochloric acid then there is 66.8 microequivalents of chloride ion not accounted for by acid production or exchange diffusion. This agrees reasonably closely with the 60 microequivalents of sodium ion suggested as being due to a non-parietal secretion.

There is another way to juggle these figures. Most authors accept that acid secretion will continue in an acid filled stomach but at a reduced rate when compared to the acid secretion into a stomach of neutral contents. This would be especially true of a gastric pouch which did not include antrum. Many authors also believe that the net gain of chloride by the lumen is due to acid secretion. Considering these two ideas together it is suggested that the chloride input of 130.2 microequivalents per half hour represents hydrochloric acid secreted. Thus a total of 110.6 plus 130.2 or 240.8 microequivalents of acid is lost by the pouch over the half hour period. The exchange diffusionists 1:1 sodium for hydrogen exchange is seen as the net gain of sodium by the pouch of 240.0 microequivalents. When examining the data from the neutral test solution the chloride gain by the pouch of 195.7 microequivalents may represent hydrochloric acid secretion. However, only a net gain of 2.8 microequivalents is measured; thus, there is

a loss of 192.9 microequivalents of hydrogen ion. This suggests a net gain by the pouch of 192.9 microequivalents. The measured gain is 186.1 microequivalents which agrees with the predicted within the bounds of experimental error. Some of the discrepancy between the predicted and the measured sodium values may be due to the flux of sodium out of a pouch with neutral contents which is known to occur (14, 26). Using this interpretation of the data it is interesting to note that the neutral test solution data shows a secretion of hydrogen ion which is 65.5 microequivalents per half hour greater than that seen in the presence of the acid test solution and that it requires an additional 0.39 millilitres of volume to carry out this secretion. If all of the fluid difference represents only pure acid secretion then the concentration of that acid secretion is 168.0 milliequivalents per litre. This is the same value suggested by Pavlov and Hollander as being the constant concentration of pure acid secretion. The 5.0 microequivalents per half hour greater potassium net gain by the pouch when using the neutral test solution as compared with the net potassium gain using an acid test solution fits with the suggestion by Gilder and Moody (43) that potassium secretion is associated with acid secretion.

What might these interpretations mean in terms of a mechanism for acidity regulation. There is truth in Hogben's statement quoted earlier (57) to the effect that gastric epithelium actively transports hydrogen and chloride but it is not known whether or not this is by a single or different mechanism. There is insufficient data in this study to give definitive answers. This study does show that the flux of chloride out of the pouch is constant for the luminal concentration of chloride used, 100 milliequivalents per litre, irrespective of the cation involved. This strongly suggests the concept of the chloride ion being involved with a carrier mechanism in some way. Wlodek (102) has found that there is a variation in the rate of exchange of sodium for hydrogen which depends upon

the rate of chloride transport from the lumen to the mucosa and that this rate is different for different concentrations of chloride in the lumen. The rate increases with an increasing luminal chloride concentration. Code and his group (14) showed a relationship between the percentage of sodium absorbed from the stomach and the ratio of sodium to hydrogen ions in the luminal contents. Until the concentration of sodium ions exceeds the concentration of hydrogen ions by a ratio of 10:1 there is little or no movement of sodium from gastric contents to blood. This suggests a competition between sodium and hydrogen for the carrier mechanism with hydrogen being moved preferentially. Davenport (26) had similar results. Thus, there would appear to be a carrier mechanism which exchanges sodium for hydrogen across the gastric mucosa and which, when the luminal concentration ratio of sodium to hydrogen exceeds 10:1, can exchange sodium for sodium. The rate at which this exchange mechanism works is governed by the concentration of the chloride ion in the luminal contents. The chloride ion is directly involved in the carrier mechanism or functions as a counter ion. From this work and the work of others including Code (14), Davenport (26) as well as Heinz and Durbin (34, 35, 53) this would appear to be the most likely mechanism although there is no proof that in fact the various components of the fluxes are in fact those suggested earlier.

What of the second interpretation of the fluxes suggested. There are no grounds upon which to make the assumption that all of the chloride input into the pouch is due to acid secretion. While many investigators have come to this conclusion many others have reached the opposite conclusion. The whole interpretation is based on this assumption and none of the data in this study supports such an assumption. The interpretation itself suggests that there is no association between exchange diffusion and chloride ion movement which leaves a flux of chloride ion of about 140

microequivalents per half hour out of the pouch that is unaccompanied by any cation to provide the electrical neutrality required. Even if this flux represented the potential difference across the mucosa it would have to be followed eventually by a cation. The potential difference may well be due to this flux of chloride out of the pouch; however, the first interpretation involving a carrier mechanism allows this as well as eventual electrical neutrality.

There is a third possibility. The "Gastron" concept proposed by Hirschowitz (54) is an intreguing one and the fluxes seen may represent the net changes of a variety of ionic fluxes occurring at different levels throughout the "Gastron". If this is correct the whole picture is so complex that it is impossible to deliniate one flux from another using the investigative tools available today.

As discussed the available evidence today is most suggestive of acidity regulation occurring through a carrier mechanism which exchanges sodium for hydrogen with chloride functioning as a counter ion. There does seem to be a non-parietal secretion of about 100 microequivalents per half hour of sodium chloride but the volume involved is too small for this to have any significant effect on acidity regulation.

B. Ionic Fluxes Following Pouch Treatments

Davenport (30) used urea to destroy "the barrier which resists the tendency of $H^+$ to diffuse from the lumen into the mucosa ..." It is implied that this "barrier", which Wlodek (101) refers to as the stomach's competence, is an active metabolic process. Urea destroys hydrogen bonding and by this mechanism is known to be mucolytic. The work of Mavrias and McArdle (72) referred to previously suggests that urea also acts as a metabolic inhibitor. In addition to these actions it also seems to set up an intense inflammatory response in the gastric mucosa as seen in the histological sections presented

earlier. Thus, it becomes difficult to define which of the effects give the results one is attempting to interpret. Webster (97) showed that a saline solution of about the concentration used here cleaned the mucus off the surface epithelium. As discussed there is no significant variation in the volumes or ionic fluxes before and after treatment of the pouches with saline; this would suggest that the effects seen with urea are not due to its mucolytic effect, at least not at the surface epithelial level.

The changes seen with urea treatment are progressive as the concentration of urea increases. The first changes are seen with the one molar urea pouch treatment when using the acid test solution. The most obvious change is an increase in volume from 1.92 millilitres to 4.60 millilitres. No such volume change is seen using neutral test solution and a one molar urea treatment. At a 99.0 percent level of confidence there is a significant increase in the net chloride gain by the lumen from 217.7 microequivalents to 435.2 microequivalents. There is no increase in the concentration of any particular cation to match this change and there is no loss of hydrogen ion from the pouch. Thus it would seem that the competence of the pouch is still intact after treatment with a one molar urea solution but that there is a mucosal response resulting in chloride containing water exuding from the surface. This suggests an increase in the "passive leak" component of the chloride fluxes described by Forte (41) and by his concepts would represent simply mechanical damage. This is consistent with the results of Mavrias and McArdle (72) who showed no inhibition of the respiratory enzymes with low concentrations of urea.

More pronounced changes are seen following treatment with two molar urea solution. Mavrias and McArdle (72) did begin to see a slight metabolic inhibition effect at this concentration of urea. When the acid test solution is used it is found that there is a volume increase even greater than that seen with one molar urea treatment (see Figure 10) significant at the 99.9

percent level and that this is accompanied by a net increase in chloride significant at the 99.5 percent level (see Figure 17). In addition to these changes there is an increase in the net potassium gain by the pouch which is significant at a 99.9 percent level of confidence. There is no significantly increased loss of acid from the pouch apparent; although, there was an increase in the net loss of acid from 157.7 microequivalents to 308.2 microequivalents ($t = 2.348$; $n = 13$) which one has only between 95.0 and 97.5 percent confidence of significance. This is compatible with the possibility of a very slight inhibition of the competence of the stomach. The meaning of the potassium outflux could be one of a loss of potassium from the surface epithelial cells due to their destruction by the urea solution or may, if the work of Gilder and Moody (43) is correct, represent acid secretion. This means that at least some of the net chloride increase may be due to acid secretion and that the apparent net hydrogen ion loss from the pouch is actually much greater than the results determined would indicate. If this were in fact the case one would expect a significant increase in the net sodium gain by the pouch (78, 100) but the increase seen with 2 molar treatment in the first half hour post-treatment on Figure 13 is significant with only between 99.0 and 99.5 percent confidence ($t = 3.326$; $n = 13$). This suggests that the net sodium flux seen is, like the net hydrogen ion loss seen, probably not significant or only a slight early change. This argument would tend to nullify the idea of the urea treatment solution having acted as a secretagogue which was not apparent due to the increasing loss of competence of the stomach. It is most probable that the volume, chloride and potassium changes seen with acid test solution in the first half hour following treatment with two molar urea represents only surface cellular damage and not a loss of competence of the stomach. As with the changes seen with the one molar test solutions all fluxes return to control levels for the periods subsequent to the first half hour post-treatment.

The first changes seen when using the neutral test solution were seen following treatment with two molar urea solution. These changes (Figures 11, 14 and 18) were similar to those seen with the acid test solution after one molar urea treatment excepting that the increased sodium flux into the pouch in the first half-hour following treatment with two molar urea using a neutral test solution was significant at a level of 99.9 percent confidence. This is still compatible with the urea having caused surface damage and an outpouring of sodium chloride containing exudate as a part of the inflammatory response due to the urea. It is interesting that the pouch is relatively more susceptible to this type of damage when it contains an acid solution as opposed to a neutral solution. The inflammatory response seen histologically after four molar urea treatment may thus be due to the leak of hydrogen ions back across the mucosa and not directly an effect of the urea. This fits in with the view that for a peptic ulcer to develop there must be acid in the stomach.

There is one change which cannot be explained, the sodium flux significantly decreased in the 1.5 to 2.0 hours post-treatment half hour cycle when using neutral test solution after treatment with two molar urea solution. This is probably an artifact due to the fact that there were only three experiments in this series. As will be discussed under validity of the experimental model, all of the results using two molar urea pouch treatment are open to question as there were only three experiments done on two dogs for the series with acid test solution and for the series with neutral test solution.

The damage done to the pouch is histologically maximal with four molar urea treatment and at this concentration of urea Mavrias and McArdle showed a definite respiratory enzyme inhibition (79). As is apparent in the results (Figure 12) this concentration of urea treatment solution is the first one which causes a decrease in mucosal competence. There is a

significant outpouring of hydrogen ion from the pouch lumen to the mucosa (confidence level: 99.9%) in the first half hour following treatment. This effect is not seen in subsequent cycles. This suggests that the effect is rapidly reversible with the removal of the urea solution. Mavrias and McArdle found (72) with their lower concentrations of urea solution the inhibition of the respiratory enzyme systems was rapidly reversible. It is implied that the effect seen on the loss of competence of the stomach was thus due to metabolic inhibition as the inflammatory response would not be so rapidly reversible. Figure 13 indicates that there was an increase in the net gain of sodium by the pouch for up to one and one half hours following treatment. In the first half hour some of this would be due to the exchange of sodium for hydrogen ion (78, 100), but the later change would be due to cellular damage alone. This argument falls down when one looks at the net chloride gain to the pouch (Figure 17) as there is a significant (level of confidence: 99.9%) increase in the net chloride gain but this is reversed in the second and subsequent half hours following four molar urea treatment. When the acid test solution is used following the pouch treatment with four molar urea solution there is a significant (level of confidence: 99.9%) increase in the net potassium gain by the pouch for the first two half hour periods post-treatment but not with subsequent cycles. These changes could be due to the destruction of the surface cells and are reversed because there is no further cellular destruction after the urea is removed or may represent an inflammatory reaction which is rapidly reversed following removal of the urea. There were no histological studies done shortly after urea treatment; however, it is known that the pouch mucosa appeared completely normal histologically five days following treatment with the four molar urea solution. Figure 10 also shows a significant (level of confidence: 99.9%) increase in the volume immediately post-treatment with four molar urea but this was also reversed rapidly. Interestingly there was at no time a significant change in the flux

of chloride out of the pouch. It has already been noted that this outflux of chloride is independent of the secretory state of the pouch and seems to be dependent upon the luminal concentration of chloride alone. It is believed to be actively metabolically mediated. Does this mean that the metabolic pathways involved employ enzymes which are not affected by urea? This is unlikely, as urea exerts its effect by breaking hydrogen bonding and as proteins all enzymes contain hydrogen bonds. More likely is the idea, similar to the concept of Hirschowitz (54), that the exchange occurs at a level in the gastric gland much below the surface epithelium and the urea does not exert an effect at this level following an instillation which is only one half hour long. This would suggest that the site of the metabolic pathways involved with competence would be at or close to the surface of the lumen. The other possibility is that the chloride flux from lumen to mucosa is purely passive and is not metabolically mediated.

When the pouch was tested with a neutral solution following four molar urea treatment there was a significant (confidence level: 99.9%) increase in the volume in the first half hour cycle post-treatment (Figure 11) and this was accompanied by a significant increase (confidence level 99.9%) in the net chloride gain (Figure 18), net sodium gain (Figure 14) and net potassium gain (Figure 16) in the first half hour period post-treatment. All of these changes are reversible by the second half hour cycle post-treatment. It is impossible to interpret these results. The competence may or may not be destroyed. From table eight it is apparent that the pouch continued to gain a small amount of hydrogen ion and this would suggest that the competence was not destroyed. This could only be proven by trapping the hydrogen ions in a buffer fluid under the same conditions but even then the buffer fluid could significantly alter the secreting characteristics of the mucosa. If the competence of the pouch was not destroyed using four

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molar urea and a neutral test solution then the changes may be purely due to destruction or damage done to the surface cells in the pouch by the urea.

C. Validity of the Experimental Model

The criticisms of this work have been discussed under each respective section; however, in the light of this criticism it is necessary to justify the work and the conclusions which were reached. There is little that this work definitely adds to the understanding of gastric physiology. Certainly it does suggest a variety of possibilities all of which need more comprehensive study.

The problem of non-randomization of the studies has been discussed. Ideally the study would have been carried out on a large population of dogs fitted with gastric pouches and any one dog would be used for only one treatment experiment with the selection of animal and treatment being randomized. Pragmatic considerations related to size of animal quarters available, and the health of the animals dictated that only five dogs could be used. As this meant that the animals would have to be used repeatedly it was decided that the saline treatment series would be completed on any one animal first and then the animal gradually progressed from one to two to four molar urea solution treatments. As the pouch epithelium returned to normal histologically within five days it was decided that the arbitrary time lapse of at least seven days would be allowed between experiments on any one animal. In most cases the time lapse between experiments was even greater. This was a necessary compromise between the ideal and the pragmatic and as such is inadequate. However, this approach did suggest some information. It will be seen from Figures 10, 15, 17 and to some extent 13 that the control values for each series of experiments gradually shifted upwards very slightly with continued urea treatments at increasing concentration. Multivariance analysis was not carried out on this; however, it is suggested that this represents more of a tendency than a statistically significant result. Curiously it

was not seen in Figure 12 which represents hydrogen ion losses from the pouches. However, this may represent a chronic effect on the pouch and with more treatments at a greater concentration the competence might eventually be chronically decreased.

The second major problem is the type of pouch used. It would have been more physiologic to have used innervated pouches; however, *pragmatic* problems related to operative survival necessitated the use of denervated (Heidenhain) pouches. Most other investigators have encountered similar problems and have done their studies on the denervated pouch; thus, these results can be directly compared with other work done. There is also some discussion as to the degree of denervation of a Heidenhain pouch. At least the sympathetic supply to the pouch is left intact.

The use of isotopic markers has proven very reproducible. The results obtained with the isotopes (Tables One through Eight; volume secreted and total chloride flux out) were the most reproducible and had the smallest standard deviation. As far as the volume studies were concerned this method is reliable and produces a degree of accuracy not seen in any other method used for studying gastric pouch volumes.

The analysis of ion concentration is likewise very accurate. The manufacturer claims a variability of less than $\pm$ 0.5 milliequivalents per litre for sodium and less than $\pm$ 0.05 milliequivalents per litre for potassium on the flame photometer. Our experience with the instrument duplicated this reliability. The manufacturer claims an accuracy of $\pm$ 0.5% for the chloridometer and our experience was that it was at least this accurate. The analysis of hydrogen ion concentration was accurate to $\pm$ 1.0 milliequivalent per litre when the sample concentration was in the range of 100 milliequivalents per litre. The samples from experiments using neutral test solution gave much less reliable results as larger sample sizes had to

be used with less titrating fluid. All analysis, isotopic and ion concentrations, were carried out in duplicate.

The general protocol and controls employed have been discussed; however, it should again be noted that the instillation of any fluid into a pouch may cause that pouch to secrete or in some other manner alter its physiological responses from normal. It is also true that these results can only be interpreted as meaningful for a denervated gastric pouch of the dog. The results can be extrapolated to the whole stomach or even the human stomach but this extrapolation has no foundation in accuracy.

The definitive conclusions which can be reached from this work are few; however, the results do suggest a variety of possibilities of physiological mechanisms by which the stomach deals with acidity. These have been discussed. It is interesting that the data derived is similar to that found by many investigators before and the real problem is one of interpretation. Knowing that there is a bidirectional chloride flux aids in the interpretation and the ideas of the exchange diffusionists as well as those of Pavlov and Hollander must be modified in the light of this information. It cannot definitely be concluded that there is a pure acid secretion of fixed composition at about 165 to 170 milliequivalents per litre hydrochloric acid and that this acid is subsequently modified by exchange diffusion but this seems to be a likely possibility. Undoubtedly there is a "non-parietal" secretion in the strict terms of Hollanders definition of that secretion; however, it seems unlikely that this non-parietal secretion has much to do with acidity regulation.

D. Unanswered Questions

In 1952 Hollander said (61):

"..... the worker who attempts to present a well rounded picture of this branch of physiology finds that his canvas contains more blank spaces than solid areas of comprehension."

It is obvious from this work that that statement is still very correct. One of the more obvious unanswered questions is the cellular source of the hydrochloric acid secreted. Most investigators believe that this is the parietal cell but this has never been proven. The mechanism of acid secretion has not been proven, nor has the stomach's means of regulating the acidity. While the concept of competence is useful (101) it is not known what metabolic pathways are involved or indeed whether or not this is an active metabolic process. From a clinical standpoint the concept of competence aids in the understanding of disease processes which involve a breakdown in the mechanisms of acid secretion and regulation. Hopefully it will become clear what cells are responsible and what the metabolic pathways involved are. Up to this time few other organs have been as intensively studied with the results giving as poor an understanding of the exact physiology.

## SECTION V: CONCLUSIONS

One of the definitive conclusions of this work is that under the in vivo basal conditions defined there is a bidirectional flux of chloride across the mucosa of a dog's dennervated gastric corpus pouch. The significance of these fluxes of chloride into and from the lumen have been discussed both in terms of the physiologic process this might represent and the fallicies in interpretation of data which represents a net chloride gain as the total chloride input to the lumen. The bidirectional fluxes occur such that there is a net gain of chloride ion by the lumen. The movement of chloride from the lumen is apparently constant for a constant luminal concentration of chloride irrespective of the cations involved.

When comparing the results using the acid test solution as opposed to the neutral test solution, where the anion concentration was equal in both cases and only the cation was different, it is apparent that there was a greater net gain by the lumen of fluid, chloride and potassium when the test solution cation was sodium as opposed to hydrogen. This may represent increased acid secretion in the face of a neutral luminal contents.

If competence is defined as the ability of the stomach to contain an acid solution then this competence was destroyed by treating topically the gastric pouch with a four molar urea solution for one half hour. In the acute experiment this loss of competence is regained within one hour of the treatment of the pouch.

The urea solutions used to topically treat the pouches did damage other than altering the competence of the pouch to contain an acid solution. In the presence of an acidic luminal contents a one molar urea solution produced a transitory increase in net volume gain and net chloride gain. Similar changes were seen with a neutral luminal contents but only after treatment with two molar urea solution. Following treatment of a pouch with two molar urea solution and in the presence of an acid content there

was an increase in the net sodium gain to the pouch. This occurred without an increase in the hydrogen ion loss by the pouch and presumably does not represent an increased exchange diffusion in the face of inhibition of the mechanisms governing competence. When the competence of the pouch is altered, after four molar urea treatment, there is also a variety of other changes which result in shifts in the ionic fluxes of the other ions involved and it is impossible from this work to determine to what degree any one change in the mucosa as a result of the urea has caused the ionic fluxes seen.

## SECTION VI: SUMMARY

This study was carried out in order to delineate the ionic fluxes across the gastric mucosa of denervated dog gastric corpus pouches under basal conditions and following topical treatment of the pouch mucosa with urea solutions of different concentrations. In particular the study sought to delineate the in vivo bidirectional chloride fluxes and the variation in the fluxes which occurred if the luminal contents were acidic or neutral. It was hoped that the qualitation and quantitation of these fluxes would delineate the mechanisms involved in gastric mucosal competence and acidity regulation by the stomach.

The study was carried out using five dogs fitted with denervated gastric corpus pouches. Instillation of acid and neutral test solutions, both containing 100 milliequivalents per litre of chloride ion, into the pouches for half hour periods were carried out. Following four pre-treatment half hour instillations the pouch was topically treated with saline, one molar, two molar or four molar urea solution. This topical treatment was followed by four half hour post-treatment instillations. The fluxes of hydrogen, potassium, sodium and chloride ions as well as the volume changes for each half hour instillation were determined. The bidirectional chloride flux was determined using the isotope chloride-36. Carbon-14 labeled polyethylene glycol was used as a volume marker. Each dog was tested with acid and neutral test solutions with saline, "sham" topical pouch treatment first then progressed to one molar urea treatment, followed by the two molar and then the four molar treatment. One week was allowed between any two experiments with one animal.

The results show that there is a bidirectional flux of chloride ion across the mucosa and that the flux of chloride from lumen to mucosa is constant for a luminal content of 100 milliequivalents per litre of chloride

ion irrespective of the test solution cation. This in vivo finding confirms the in vitro finding of other investigators and opens to question the interpretations of some gastric physiologists who assumed that the net chloride gain by the pouch was representative of the total chloride input into the pouch. The data also suggests that the 1:1 sodium for hydrogen exchange seen by previous investigators occurs and that it is mediated by an exchange carrier mechanism with chloride functioning as a counter ion. The rate of transport by the carrier mechanism seems to be regulated by the concentration of the counter ion, chloride, in the luminal contents.

Treatment of the pouch with urea solution does destroy the ability of the stomach to contain an acid solution, the gastric competence, when a four molar urea solution is used for the topical treatment and when the test solution is acidic. However, before the ability to contain an acid solution is lost there is, with one and two molar urea topical treatments, an influx of sodium chloride containing fluid. The degree to which the changes in the ionic fluxes with four molar urea treatment are due to gastric mucosal competence destruction or to the influx of this sodium chloride containing fluid cannot be assessed. The fluxes were greatest, and presumably the damage greatest, for any concentration of urea treatment when the acid test solution was used as compared to when the neutral test solution was used. This increased damage with acid test solution may have been due to the loss of hydrogen ion across the mucosa from the lumen; although, a significant net loss of hydrogen ion was seen only with the four molar treatment.

While this work adds a little to the knowledge of the ionic fluxes across the gastric mucosa the mechanisms proposed are only speculative. It is not known how the stomach regulates its acidity nor the mechanism which gives it this unique ability to contain an acid solution.

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