UNIDIRECTIONAL SODIUM FLUX IN THE DUODENUM, JEJUNUM, AND ILEUM OF NORMAL AND DESOXYCORTICOSTERONE ACETATE - TREATED MALE, LABORATORY RATS

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by

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RESPECTFULLY DEDICATED TO

THE MEMORY OF

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AND TO

C.J.S.

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CHAPTER I

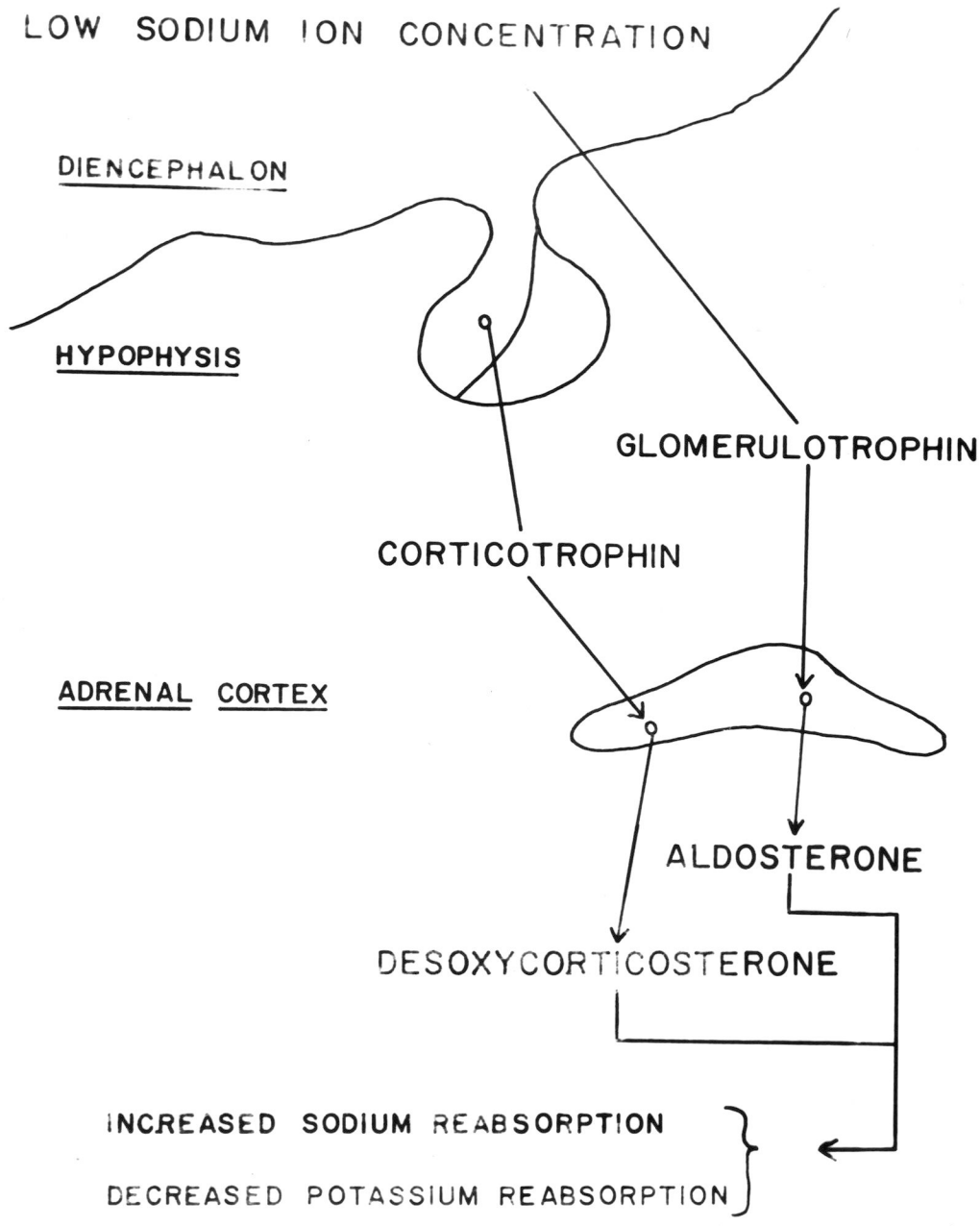
INTRODUCTION

In the animal body the maintenance of a specific electrolyte balance is critical for the well-being of the entire organism. The evolutionary mechanism for this control becomes increasingly complex, reaching a paramount of complexity in the mammals. In the mammalian body the sodium ion balance, one of the main constituents of the electrolyte series, is controlled by a response to the hormones of the adrenal cortex (aldosterone and desoxycorticosterone). The level of the former hormone is thought to be regulated by the direct effect of the sodium ion concentration of the blood on the diencephalon of the brain, while the latter is under the influence of one of the pituitary hormones (corticotrophin). (Figure 1; Guyton, 1961)

The excretion, or conversely, the conservation of sodium ions occurs in four tissues in the mammalian body, i.e., kidney, sweat glands, salivary glands, and intestine (Berger, et al., 1960). Since all these tissues should respond to a hormonal influence in a similar manner, any of these tissues mentioned could be utilized in a study of electrolyte balance equally well, though the intestine better lends itself to the investigation (Berger, et al., 1960).

FIGURE 1. The Interactions of the Sodium Ion Content of the Blood With the Mineralocorticoid Titer.

FIGURE 1


LOW SODIUM ION CONCENTRATION
DIENCEPHALON
HYPOPHYSIS
GLOMERULOTROPHIN
CORTICOTROPHIN
ADRENAL CORTEX
ALDOSTERONE
DESOXYCORTICOSTERONE
INCREASED SODIUM REABSORPTION
DECREASED POTASSIUM REABSORPTION

It has been shown that a decrease in the absorption of sodium occurs in adrenalectomized dogs (Dennis and Wood, 1940), presumably due to the subsequent loss of aldosterone and desoxycorticosterone. It has also been shown that the unidirectional flux of the sodium into and out of the large intestine of the dog was influenced by desoxycorticosterone acetate (DOCA), while a similar effect was not demonstrable in the small intestine of the dog (Berger, et al., 1960). The work of Field, et al. (1955) indicates that there normally is an absorption of sodium from the small intestine of intact dogs.

Thus it has been shown that sodium is absorbed in the small intestine; and it has also been shown that DOCA influences sodium absorption. The effect of DOCA on the sodium absorption in the small intestine is controversial. Therefore, the present study was designed to investigate the relationship between DOCA and sodium retention in the small intestine. A method was devised by which the effect of DOCA on the sodium flux in the small intestine could be measured. Radioactive sodium (sodium-22) was used as a tracer to discern sodium transfer subsequent to DOCA administration, while colorimetric analysis was used to ascertain total sodium. Utilizing this technique, a two fold study was made: 1) to determine the effects of DOCA on the sodium flux in the small intestine and; 2) to determine any possible regional differential in sodium transfer in the small intestine.

Since terminology varies with the investigator, the terms "unidirectional influx", "unidirectional outflux" and "net outflux" deserve definition. "Unidirectional influx" will refer to the one-way

passage of electrolyte (sodium ions) from the circulating blood supply to the intestinal lumen. The sodium ions which travel from the intestinal lumen to the circulating blood will be referred to as the "unidirectional outflux". The change in the sodium content of the intestinal lumen without regard to the above mentioned fluxes is defined as the "net outflux".

CHAPTER II

MATERIALS AND METHODS

Male rats of the Holtzman strain weighing from 200 to 250 grams were used in this investigation. For each region of the small intestine studied, twenty rats were paired according to weight, and allowed to become adjusted to their new environment for two weeks prior to actual experimentation. Male rats were used to avoid any possible interaction of the cyclic blood titer of estrogen, which is present in the female of the species, on the sodium flux (Turner, 1960).

The previous study involving DOCA (Berger, et al., 1960), utilized a small number of animals (6) prepared with chronic Thiry fistulas as the experimental population. The current study involves the use of terminal, paired experiments with isolated in vivo intestinal sections on a total of sixty animals; twenty animals being used in each of the areas of study, i.e., duodenum, jejunum, and ileum.

In each of the three experimental groups, there were ten controls and ten treated animals; each pair, consisting of a control and a treated animal, was given feed (Purina Laboratory Chow, Appendix A) and water ad lib. The treated animals were given intramuscular injections (25 gauge by 1 inch needle) of DOCA (Appendix A) at a rate of two milligrams per kilogram of body weight (Berger, et al., 1960) at twenty four hour intervals for three consecutive injections. Prior to the third injection, both animals (control and treated) were taken off feed and allowed only water for a period of twenty four hours; this insured the removal of chyme from the upper gastrointestinal tract.

Five hours subsequent to the third injection, the experimentation was begun. The same procedure was followed for each pair of animals studied.

The experiments were conducted under sodium pentobarbital (Nembutal, Abbott) anesthesia (Appendix A), given intraperitoneally (26 gauge by ½ inch needle with a 45° bevel) at a rate of 35 milligrams per kilogram of body weight (Appendix B). Once in surgical anesthesia, the abdominal walls were reflected to expose the abdominal cavity. The pyloric region of the stomach was then located; a Dieffenbach serrefine clamp was placed approximately six centimeters distal to the first. In this manner an isolated section of duodenum was made available for investigation.

In securing isolated jejunal and ileal sections, the same general procedure was followed with these exceptions: Rather than use the pyloric portion of the stomach as a landmark in isolating the jejunal section, the first loop of the small intestine was used as the end of the duodenum and the clamps placed distal to this point. The caecum of the large intestine and its ileocaecal valve were used as the points of reference in locating the ileum, and the two clamps were placed proximal to this point. The jejunum comprises about three-tenths of the small intestine, and the ileum comprises an additional six-tenths of the total length. Thus, a great margin of safety is present when using these anatomical structures as reference points in locating the various portions of the small intestine.

After the animals were properly prepared, 0.3 c.c. of sodium-22 (Appendix A) in physiological saline was injected (27 gauge by 1 inch needle) into the lumen of the isolated section. After a period of ten

minutes (Appendix B), the intestinal section with the attached clamps was removed *in toto* from the animal. The intestine, together with its contents, was then counted in a deep-well scintillation counter (Appendix C). Following this the contents of the section were removed, and the intestinal section *per se* was washed in running water for a period of five minutes (Appendix B). The washed section was counted again and this count subtracted from the total counts of the intestinal section with its contents. This adjusted count was then taken to equal the total counts of the contents of the intestinal lumen at the end of the experimental period. An aliquot (0.01 c.c.) of the intestinal contents was then analyzed for total sodium by a colorimetric determination (Appendix C, D), and the microequivalents of sodium present were calculated (Appendix E).

To check the possibility of occlusion of the blood supply to the isolated intestinal section by the described technique, the clamps were inserted, as described, in a nonexperimental animal of the same strain (Plate 1); then 0.2 c.c. of India ink (Appendix A) was injected into the femoral artery. After a time interval of three minutes, the entire animal, including the isolated intestinal section, was perfused to an equal degree with the black residue (Plate 2), indicating that circulation was still intact.

A two-way flux of sodium exists in the gastrointestinal tract. Sodium ions from the circulating blood traverse the mucosa and enter the lumen of the intestine and vice versa, depending on the physiological condition of the organism. By the use of a radioactive tracer (sodium-22), the amounts of the two fluxes can be determined (Appendix F).

Any change in the amounts of total sodium (radioactive and non-radioactive) in the lumen of the isolated intestinal section would, of necessity, have to be attributable to either the movement of ions into or out of that section via the blood vessels since a mechanical obstruction exists at each end of the section study. Therefore, the net change or net outflux of a given intestinal section would be equal to the amount of the total sodium injected minus the amount of total sodium recovered divided by the period of time that the sodium was left in the section. Symbolically it would be:

$$\text{net outflux} = \frac{N_1 - N_o}{\text{Time}}$$

Where: N_1 = total sodium injected into the lumen

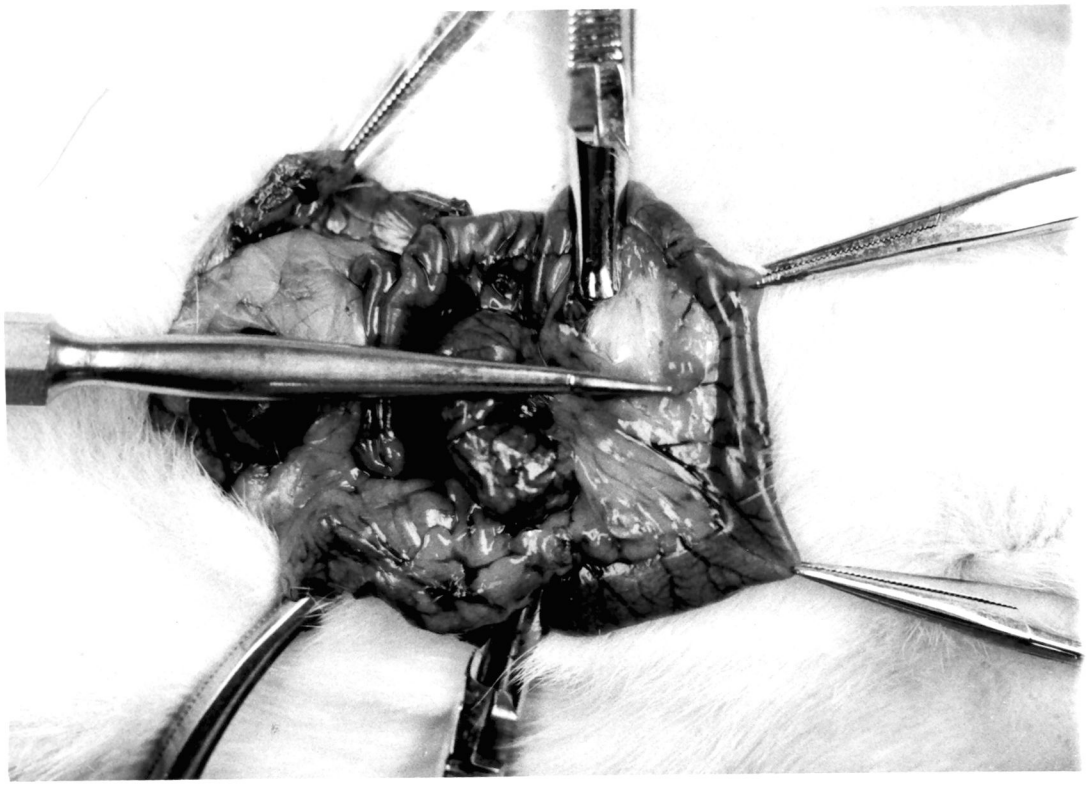
N_o = total sodium recovered from the lumen

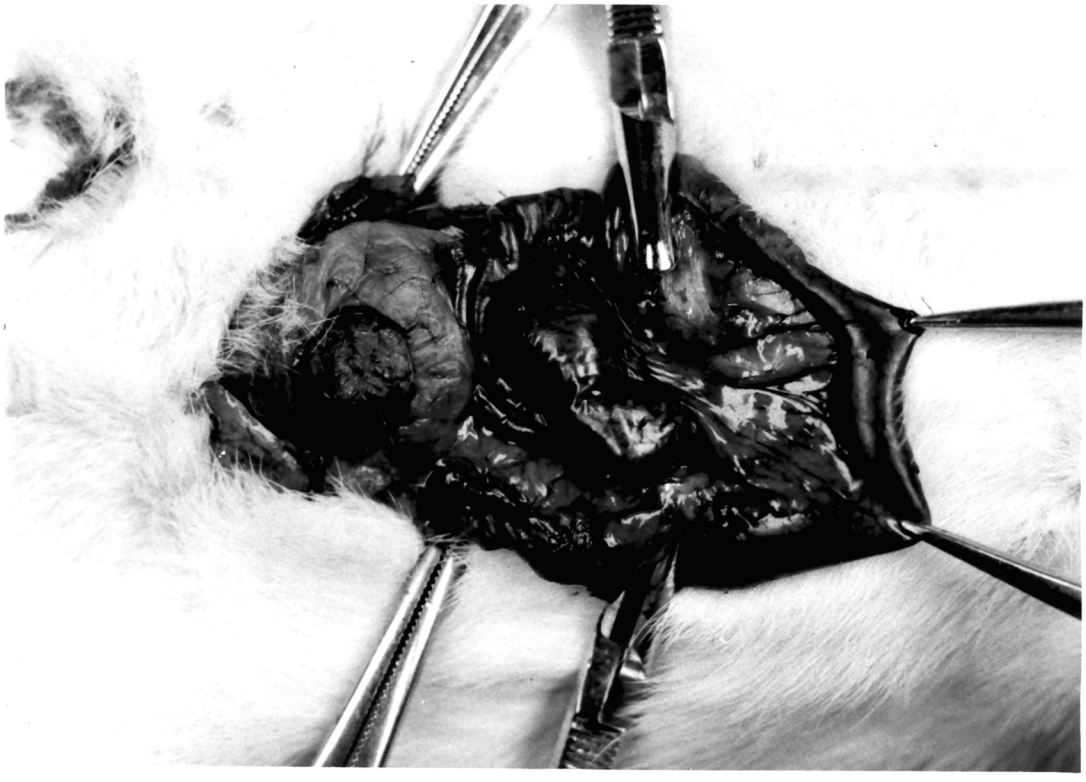
As was previously stated, the main object of this study was to determine the effects of DOCA on the unidirectional fluxes of sodium into and out of the small intestine; therefore, the net outflux *per se* is of little or no use.

The radioactive sodium isotope permits the selective measurement of the sodium ions leaving the lumen of the isolated section. The rate at which the radioactive sodium leaves the lumen of the intestinal section was determined by obtaining the difference between the injected and the

PLATE 1. Isolated Section of Rat Intestine.

PLATE 2. Isolated Section of Rat Intestine After an Intraarterial Injection of India Ink.





recovered sodium-22 and dividing this quantity by the duration of time that the sodium-22 remained in the lumen. At the outset, this would appear to equal the unidirectional outflux of sodium; however, it must be taken into consideration that not only is sodium-22 leaving the section, but some of this sodium may return via the circulation to the lumen of the gut. In addition to this, the rate at which the total sodium changes must be taken into account.

The net outflux of the sodium-22, as was previously stated, is equal to the sodium-22 injected into the lumen minus the sodium-22 recovered from the lumen divided by the time interval. In order to calculate the unidirectional outflux, this net outflux of sodium-22 is divided by the ratio of sodium-22 to the non-radioactive sodium of a specific time interval. Since the time the sodium-22 is in the lumen of the small intestine is small; and, since the ratio of the non-radioactive sodium to sodium-22 is constantly changing, the average of this ratio at the beginning and at the end of the run is taken to give an approximation of the ratio throughout the time interval. This formula takes into account the fact that the system is in dynamic equilibrium.

Graphically it can be shown as:

$$\text{unidirectional outflux} = \frac{\frac{N_1^x - N_o^x}{\text{Time}}}{\frac{1}{2}\left(\frac{N_1^*}{N_1} + \frac{N_o^*}{N_o}\right)}$$

Where: N_1 = total sodium injected into the lumen

N_o = total sodium recovered from the lumen

N_1^* = sodium-22 injected into the lumen

N_o^* = sodium-22 recovered from the lumen

Having determined the net outflux and the unidirectional outflux, the unidirectional influx can be obtained by taking the difference between these two quantities.

$$\text{unidirectional influx} = \text{unidirectional outflux} - \text{net outflux}$$

All values obtained from the foregoing procedures were submitted to an analysis of variance and "t" test on the IBM 1620 Data Processing System.

CHAPTER III

RESULTS

The unidirectional influx of sodium by sections in the rat small intestine is as follows:

Duodenum:

In order to determine the effects of DOCA on the unidirectional influx of sodium in the duodenum, twenty animals were used: ten controls and ten treated (operations performed five hours subsequent to the third intramuscular injection of DOCA). Sodium ion concentration decreased in the duodenal lumen of the treated animals as a result of the increased outflux in that region. The mean control value of the unidirectional influx of sodium in the duodenum was 13.85 microequivalents per minute, while the mean DOCA-treated value of the unidirectional influx of sodium was decreased to 7.97 microequivalents per minute (Table I). Thus, it can be assumed that the DOCA had a significant effect ($p > 0.001$) on the unidirectional influx of sodium in the duodenum of the rat (Table IV).

Jejunum:

Twenty animals were studied. Ten paired experiments were conducted in which sodium transfer was measured. The mean values for the unidirectional influx of sodium in the jejunum were 16.87 microequivalents per minute and 12.24 microequivalents per minute for the control and treated animals, respectively (Table II). Apparently the DOCA accounted for the significant increase ($p > 0.001$) in the absorption of sodium

TABLE I

THE EFFECT OF DOCA ON THE UNIDIRECTIONAL INFLUX OF SODIUM IN THE DUODENUM OF THE RAT

(values* expressed in microequivalents per minute)

CONTROL	TREATED
14.19	6.88
14.47	9.12
14.38	7.13
14.36	9.85
17.44	8.99
10.75	5.67
11.90	5.72
14.74	8.69
12.53	8.75
13.78	8.90
MEAN	
13.85	7.97

* all values are rounded to the nearest hundreth.

from the jejunum of these animals (Table IV).

Ileum:

The mean value of the influx of sodium for the ten control animals was 8.52 microequivalents per minute, while the mean value of the influx of sodium for the ten DOCA-treated animals was increased to 10.25 microequivalents per minute (Table III). The great amount of fluctuation observed (Table III), in both control and treated groups, accounted for the lack of significance (Table IV).

In Figure 2 the unidirectional flux of sodium into the small intestine of the control groups at the three levels of study (duodenum, jejunum, and eleum) is summarized. The analysis of variance indicated highly significant differences between the various sections (Table V).

The unidirectional influx of sodium in the small intestine of the DOCA-treated rats at the various levels of study (duodenum, jejunum, and ileum) is summarized in Figure 3. A high level of significance between the duodenum and the jejunum (Table VI) was indicated by the analysis of variance, a similar significance was not demonstrable between the jejunum and the ileum, and between the duodenum and the ileum.

In Table IV is summarized the effect of DOCA on the unidirectional influx of sodium in the rat small intestine in comparison with the control condition, while Tables I, II, and III give all the values obtained in the duodenum, jejunum, and ileum, respectively. DOCA significantly alters the influx of sodium in the duodenum and the jejunum ($p > 0.001$ and $p > 0.001$, respectively). At the level of the ileum, DOCA did not significantly alter the unidirectional influx of sodium (based on statistical analysis).

TABLE II

THE EFFECT OF DOCA ON THE UNIDIRECTIONAL INFLUX OF SODIUM IN THE JEJUNUM OF THE RAT

(values* expressed in microequivalents per minute)

CONTROL	TREATED
15.58	12.25
16.51	12.10
16.41	14.87
19.03	15.33
20.10	11.10
16.52	10.51
16.49	13.51
19.31	9.23
13.03	13.58
15.71	9.87
MEAN	
16.87	12.23

*all values are rounded to the nearest hundreth.

TABLE III

THE EFFECT OF DOCA ON THE UNIDIRECTIONAL INFLUX OF SODIUM IN THE ILEUM OF THE RAT

(values* expressed in microequivalents per minute)

CONTROL	TREATED
10.17	5.62
13.57	3.72
6.65	14.97
6.97	9.99
12.73	12.23
1.36	4.29
8.24	16.96
5.83	9.33
7.89	13.88
11.80	11.56
MEAN	
8.52	10.25

* all values are rounded to the nearest hundreth.

TABLE IV

THE EFFECT OF DOCA ON THE UNIDIRECTIONAL INFLUX OF SODIUM IN THE RAT SMALL INTESTINE

(Mean values expressed in microequivalents per minute)

SECTION	CONTROL	TREATED	t*	p**
Duodenum	13.85	7.97	7.8872	0.001
Jejunum	16.87	12.23	4.9705	0.001
Ileum	3.52	10.25	0.9372	0.4

* Student's "t"

** significance

TABLE V

COMPARISON BY REGIONS OF THE UNIDIRECTIONAL INFLUX OF SODIUM IN THE RAT INTESTINE IN THE CONTROL CONDITION

(mean values expressed in microequivalents per minute)

SECTION	CONTROL	t*	p**
Duodenum	13.85	3.4387	0.01
Jejunum	16.87		
Jejunum	16.87	6.2456	0.001
Ileum	8.57		
Duodenum	13.85	4.114	0.001
Ileum	8.52		

* Student's "t" test

** significance

TABLE VI

COMPARISON BY REGIONS OF THE UNIDIRECTIONAL INFLUX OF SODIUM IN THE RAT INTESTINE IN THE TREATED CONDITION

(mean values expressed in microequivalents per minute)

SECTION	CONTROL	t*	p**
Duodenum	7.97	5.2715	0.001
Jejunum	12.23		
Jejunum	12.23	1.2524	0.3
Ileum	10.25		
Duodenum	7.97	1.5070	0.2
Ileum	10.25		

*Student's "t" test

**significance

FIGURE 2. The Individual Unidirectional Influx of Sodium in the Duodenum, Jejunum, and Ileum in the Control Condition.

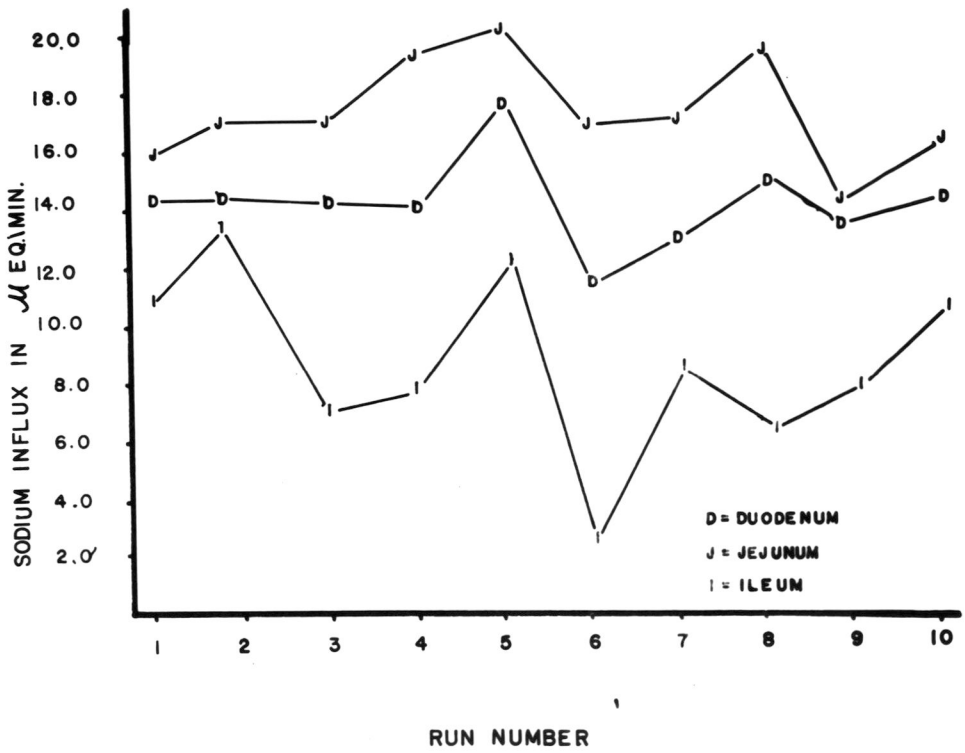

20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
SODIUM INFLUX IN μ EQ\MIN.
D = DUODENUM
J = JEJUNUM
I = ILEUM
1
2
3
4
5
6
7
8
9
10
RUN NUMBER

FIGURE 2

FIGURE 3. The Individual Unidirectional Influx of Sodium in the Duodenum, Jejunum, and Ileum in the Treated Condition.

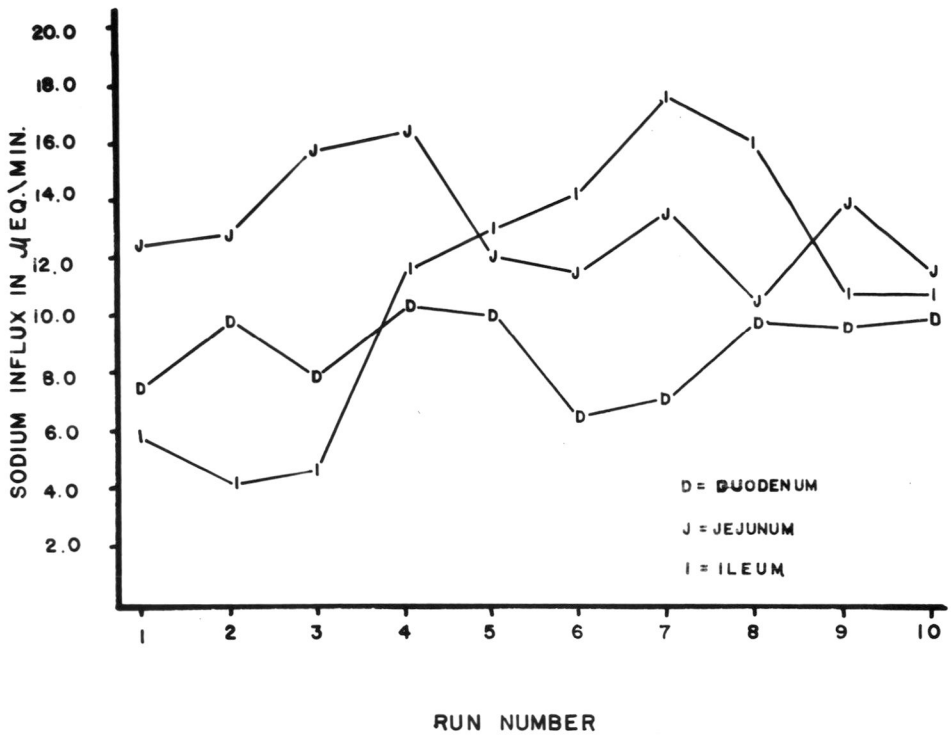

SODIUM INFLUX IN μEQ.\MIN.
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
1
2
3
4
5
6
7
8
9
10
RUN NUMBER
D = DUODENUM
J = JEJUNUM
I = ILEUM

FIGURE 3

CHAPTER IV

DISCUSSION

Sodium ions are definitely retained (absorbed) following the administration of DOCA. Studies on the fecal sodium of rats indicated a decrease in sodium excretion following the administration of DOCA (Berger, et al., 1951). A synthetic mineralocorticoid, 9- α-fluorohydrocortisone, also produced a decrease in the sodium content of dog feces (Poutsiaka, Thomas and Linegar, 1957). Other workers (Davis and Howell, 1953; Davis, Howell and Southworth, 1953; and Davis, Ball, Bahn and Goodkind, 1959) have shown that in the dog the sodium content of the feces is interrelated with the amount of urinary aldosterone.

In man the amount of sodium in the stool following the administration of DOCA is decreased (Relman and Schwartz, 1952). Testing of the sodium content of the feces of individuals who lack the sodium-retaining hormones (e.g. in Addision's disease or after bilateral adrenalectomy) indicates a large excretion of sodium in the stools, which was reduced by the administration of DOCA (Emerson, Kahn and Jenkins, 1953). A small sodium content of the feces is associated with a high urinary aldosterone, i.e., the urinary aldosterone seems to be a function of the fecal sodium present (Duncan, Liddle and Bartter, 1956).

Thus, it has been well documented that sodium retention occurs following the administration of DOCA, and that the sodium level in man, dog, and rat is governed to some extent, at least, by the circulating titer of the mineralocorticoids. This investigation is confined to the regional absorption of sodium from the small intestine.

The current data indicate that there is a definite absorption gradient of sodium in the small intestine of the control groups. The transfer of sodium is greater in the jejunum than in the duodenum or in the ileum. Intramuscular administration of DOCA has a significant effect on the absorption of sodium by the duodenum and the jejunum. This effect of DOCA in the small intestine was implied by the work of Field, et al. (1955) which demonstrated that normally sodium absorption exists in the ileal region of the dog; by Clark (1939) who found that following the ingestion of sodium chloride, more chloride was found in the intestine of adrenalectomized rats than in the normal condition; and by Dennis and Wood (1940) who, working with adrenalectomized dogs, found a decrease in the absorption of sodium from chronic lower ileal Thiry fistulas.

A significant effect of intramuscular administered DOCA on the sodium transfer in the ileum was not supported by the data in this investigation. This duplicates the results reported by Berger, et al. (1960).

The unidirectional influxes recorded in the current research seem to correlate quite well with the physiological activity of the section under study. In the duodenum most of digestion occurs and a quantity of bile and digestive enzymes (pancreatic) enter at this point along with the secretion of large amounts of mucus (Brunner's glands). Little absorption of digested food occurs here. The amount of sodium absorbed was relatively low (Table I), 13.85 microequivalents per minute, as might be expected. The majority of absorption of the digestive products takes place in the jejunum, and its unidirectional influx of sodium was corres-

pondingly elevated, 16.87 microequivalents per minute (Table II). Since the surface area per unit length of the ileum is less than the other two sections (Grim, 1962), it would follow that its absorption rate would be less. This assumption was borne out by the data, 8.52 microequivalents per minute (Table III).

In a previous study (Berger, et al., 1960) an effect of DOCA on the unidirectional influx of sodium in the small intestine was not demonstrated. The fact that an effect was observed in this study was, in all probability, due to the type of experiment performed. In Berger's work the experimental population had chronic Thiry fistulas which, at best, are artifical. By having a section isolated in this manner its normal functions, i.e., digestion and absorption, are impaired. The lack of food (chyme) in this region could quite easily lead to an atrophy or, at least, a partial degeneration of the gastrointestinal lining. A comparable procedure is utilized in medicine to allow for the healing of injured intestinal segments following resections. Although a mechanical obstruction was caused by the Diaffenbach serrefine clamps used in this study, the intestinal lining retained its natural integrity. In Berger's work the use of anesthesia during experimentation was not needed; however, in this study the nature of the procedure necessitated the use of sodium pentobarbital. It is well to note that Code, et al. (1960) have reported that anesthesia with sodium pentobarbital did not affect the rate of "insorption" (unidirectional influx) of either water or sodium in the small intestine of the dog.

When a physiological (isotonic) solution is instilled within the

lumen of an isolated section, no net change of the constituents is expected to occur; however, a new change in the components was noted. The obvious question is: What is the force that causes a flux of sodium when there is no osmotic concentration difference? According to Grim (1962), the mechanism which might explain the net movement under these conditions are as follows: 1) diffusion as a consequence of a concentration difference; 2) bulk flow as a consequence of hydrostatic or osmotic pressure differences; 3) electrolytic transport as a consequence of potential differences; or 4) active transport. Since physiological solutions were utilized, there is no concentration difference; hence, diffusion cannot be the mechanism. On the same basis, no osmotic pressure differences existed, and no hydrostatic pressure was present in the experiment (quantities of physiological saline injected were not sufficient to distend the lumen), therefore, bulk flow can be eliminated. Furthermore, since an isotonic solution was instilled, there can be no significant potential difference across the intestinal wall; consequently, electrolytic transport, though present, is probably minimal. It is probable that active transport is the chief mechanism involved.

The exact chemical mechanism for the action of any hormone is not known; however, certain facts concerning hormone action have been and are being elucidated and working concepts are being formed. In previous work with isolated, in vitro rabbit ileum (Schultz, et al., 1963, 1964), changes in the transmural potential, the short circuit current, and the sodium fluxes were attributed to the active transport of sodium from the mucosa to the serosa. It was also found (Schultz, et al., 1964) that

this active transport was dependent on the presence of intact aerobic metabolic pathways and was inhibited by a low concentration of ouabain in the serosal medium. Thus, the assumption can be made that the sodium is transported via a carrier-facilitated movement and DOCA may be inducing the active transport of sodium by a mechanism similar to that of the insulin-glucose transport theory (Figures 4 and 5).

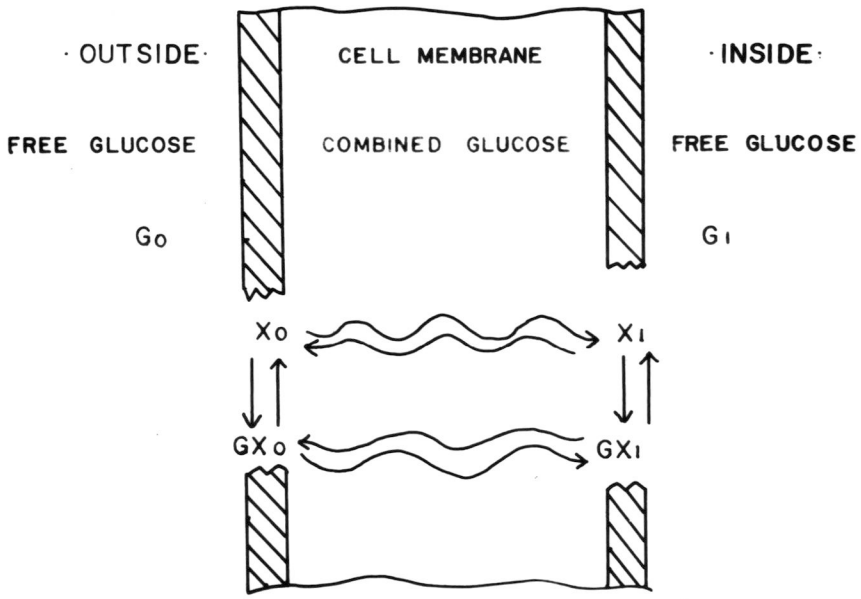
Hormones are capable of causing great changes in cellular activity even when present in small titer. Most hormones have been shown to bind quite tenaciously to proteins, and DOCA is no exception (Westphal, 1957). According to Ling (1962), it is presumed that the association of the hormone with its "target cells" affect these cells through changes in their free energy (F-effects) created by the hormone's adsorption onto the cellular protein and the consequent electron distribution change. This, in effect, creates an activated state which is highly labile for reactions. Thus, it is possible that the adsorption of DOCA onto the cellular protein resulted in the impetus for the increased sodium retention observed.

Cafruny, et al. (1957) demonstrated a decrease of reactivity in response to DOCA in sulfhydryl group-containing protein of kidney tissue (a sodium-regulating tissue). The differences in the fluxes of the three intestinal sections could possibly be explained by the amounts of sulfhydryl groups present in the protein of the duodenum, jejunum, and ileum.

The exact mechanism for the differences in the sodium flux in the small intestine must await the elucidation of the mechanism of selective

FIGURE 4. Diagrammatic Representation of a Carrier Scheme for a Glucose Transport Through the Cell Membrane. (Eisenstein, 1964)

FIGURE 4



G = GLUCOSE

X = UNOCCUPIED "CARRIER"

GX = OCCUPIED "CARRIER"

o = ON OUTSIDE SURFACE

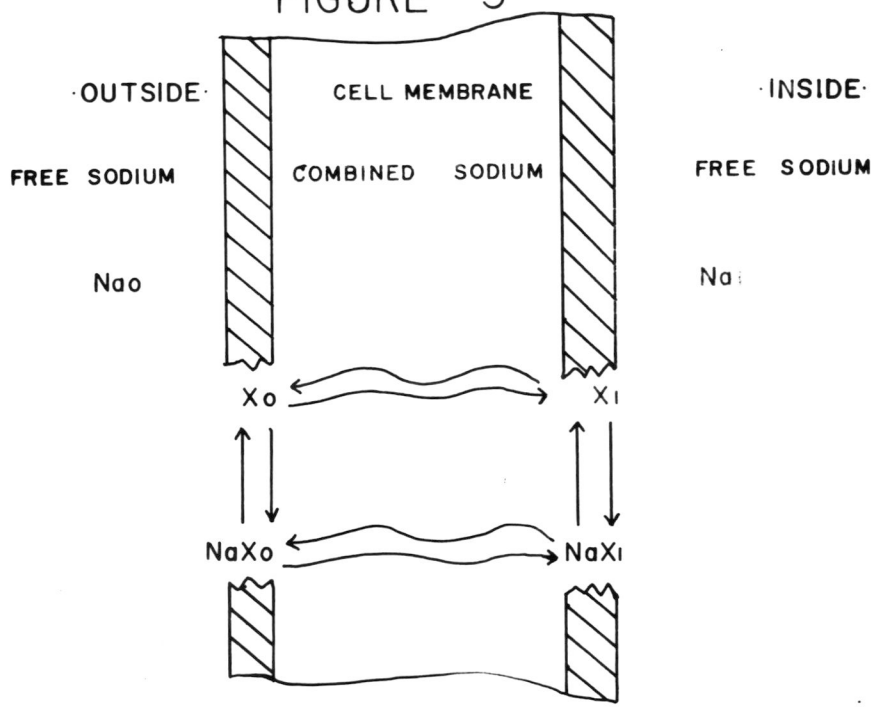
i = ON INSIDE SURFACE

⇄ = REACTION CLOSE TO EQUILIBRIUM

≈ = RATE-LIMITING PROCESS

FIGURE 5. Diagrammatic Representation of a Carrier Scheme for a Sodium Transport Through the Cell Membrane.

FIGURE 5



Na = SODIUM
X = UNOCCUPIED "CARRIER"
NaX = OCCUPIED "CARRIER"
o = ON OUTSIDE SURFACE
ı = ON INSIDE SURFACE
⇄ = *REACTION CLOSE TO EQUILIBRIUM*
≈ = *RATE-LIMITING PROCESS*

membrane permeability, and of the mechanism of hormone action.

CHAPTER V

SUMMARY

In order to determine the effects of desoxycortiosterone acetate on the unidirectional influx of sodium, a technique for the study of *in vivo* isolated intestinal sections was devised. Utilizing this technique, a total of sixty male, albino rats were studied: twenty each in the duodenal, jejunal, and ileal regions.

A definite absorption gradient of sodium was noted in the control condition with the greatest absorption in the jejunum. DOCA was found to significantly decrease the unidirectional influx of sodium in the duodemum and the jejunum, while a similar effect was not demonstrated in the ileum.

A carrier-facilitated movement, similar to that of the insulin-glucose transport theory, is postulated and discussed as a possible mechanism for the action of DOCA and the active transport of sodium.

APPENDIX A

MATERIALS FOR MAINTENANCE AND TREATMENT OF ANIMALS

Purina Laboratory Chow

Crude protein not less than	23.0%
Crude fat not less than	4.5%
Crude fiber not more than	6.0%
Ash not more than	9.0%

Ingredients: Meat and bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, dried beet pulp, ground extruded corn, oat middlings, soybean meal, dehydrated alfalfa meal, cane molasses, animal fat preserved with BHA, vitamin B_{12} supplement, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, brewer's dried yeast, thiamin, niacin, vitamin A supplement, D activated plant sterol, vitamin E supplement, 0.5% defluorinated phosphate, 0.5% iodized salt, 0.075% ferric ammonium citrate, 0.02% manganese sulphate and a trace of zinc oxide.

Obtained from: Ralston Purine Company
Checkerboard Square
St. Louis 2, Missouri

Desoxycorticosterone acetate

Injectable, aqueous macrosuspension five milligrams per c.c. Each c.c. contains: desoxycorticosterone acetate U.S.P. 5 mg.; methocel, 0.1 mg.; sorbitol, 5%; P.V.P., 0.5%; procaine HCL, 0.5%;

disodium EDTA, 0.05%; thiocarbamide, 0.25 mg.; and benzethonium chloride, 0.01%.

Obtained from: Vitamix Pharmaceuticals Incorporated
Philadelphia, Pennsylvania

Nembutal

Sodium pentobarbital injection, 250 milligrams per c.c.

Each 5 c.c. contains: sodium pentobarbital, 250 mg.; alcohol, 10%; propylene glycol, 20%; water for injection, to make 5 c.c.; pH adjusted with sodium hydroxide.

Obtained from: Abbott Laboratories
North Chicago, Illinois.

Sodium-22

In dilute HCL, reduced 1:100 with physiological saline before use. This was equal to 0.003 μC/injection (0.3 c.c.) or approximately 55,000 counts/min., the upper limit of the scaler.

Obtained from: Atomic Laboratories, Inc.
San Ramon, California.

India Ink

Standard draftsman's ink, Higgins American India Ink

Obtained from: Higgins Ink Company
New York, New York

APPENDIX B

DOSIMETRY OF DRUGS USED

Sodium pentobarbital administration:

25 mg. per kg. of body weight.....groggy, reactive

30 " " " " " " .....slowed markedly, mild eye reflex

35 " " " " " " .....surgical anesthesia

40 " " " " " " .....LD_{50}

Duration of time for sodium-22 in the lumen of the gut:

1 min. in the lumen of the gut .....no effect

2 " " " " " " " ..... " "

4 " " " " " " " .....some uptake

6 " " " " " " " .....more uptake

8 " " " " " " " .....maximum uptake

10 " " " " " " " ..... " "

12 " " " " " " " ..... " "

14 " " " " " " " .....diminished uptake

16 " " " " " " " ..... " "

Duration of the running water wash for the intestinal section:

1 min. in running ..... less counts

3 " " " ..... " "

5 " " " ..... no effect

7 " " " ..... " "

10 " " " ..... " "

APPENDIX C

INSTRUMENTS USED

Scintillation counter:

Model 151A Decade Scaler in conjunction with Model DS-200(V) Scintillation Detector.

Obtained from: Nuclear-Chicago Corporation

DesPlaine, Illinois.

Colorimeter:

Bausch and Lomb Spectronic 20 Colorimeter.

Obtained from: Bausch & Lomb Optical Company

Rochester 2, New York.

APPENDIX D

COLORIMETRIC ANALYSIS OF SODIUM

(Snell and Snell, 1949)

Prepare a uranyl zinc acetate reagent by mixing 80 grams of uranyl acetate dihydrate with a solution containing 14 ml. of glacial acetic acid in 427 ml. of water. Into another solution of 7 ml. of glacial acetic acid in 294 ml. of water, stir 220 grams of zinc acetate dihydrate. Heat the two solutions separately on a water bath to dissolve. Mix while hot, cool, and add 0.2 gram of sodium uranyl zinc acetate crystals. To obtain these crystals, add 125 ml. of uranyl zinc acetate reagent to 5 ml. of a 2 per cent sodium chloride solution and filter through a porous porcelain crucible. Wash several times with glacial acetic acid and then with ether, and dry in a desiccator over calcium chloride for 1 hour. Allow the uranyl zinc acetate reagent to stand overnight, store in a dark bottle, and filter immediately before use.

To 1 ml. of sample add 5 ml. of freshly filtered reagent. Mix well and let stand. At 5-minute intervals, add seven 0.3-ml. portions of 95 per cent ethanol. Mix after the first five additions by rolling the tube between the hands. Wash down the sides of the tube with the last two additions and let them layer on the surface. Finally centrifuge at 2000 rpm. for 10 minutes, decant, invert, and drain for five minutes. Wipe the mouth of the tube dry and agitate the precipitate with 2 ml. of a mixture of 30 ml. ethyl acetate diluted to 100 ml. with glacial acetic acid. Wash down the wall of the tube, centrifuge, and complete

as before. Wash the precipitate and the wall of the tube with 5 ml. ether. This time in completing, drain for only one minute as the precipitate may otherwise dropout. Repeat the ether wash and evaporate the last traces of ether by putting in a warm place for 5 minutes.

These precipitates are dissolved and the color developed in the following manner:

Prepare a reagent by dissolving 2 grams of alizarin per liter of 95 per cent ethanol. Store this at 40°C for 24 hours and filter. Dissolve the precipitate in water and dilute to about 90 ml. in a 100-ml. volumetric flask. Add 7 ml. of the reagent solution and dilute to volume. Read the transmittance with a 570-mu filter. The zero setting on the instrument is taken with 7 ml. of reagent and 40 ml. of 95 per cent ethanol diluted to 100 ml. The plot obtained is a straight line, showing that Beer's law applies, but does not pass through the origin because the reagent itself is colored.

APPENDIX E

CALCULATION OF MICROEQUIVALENTS OF SODIUM

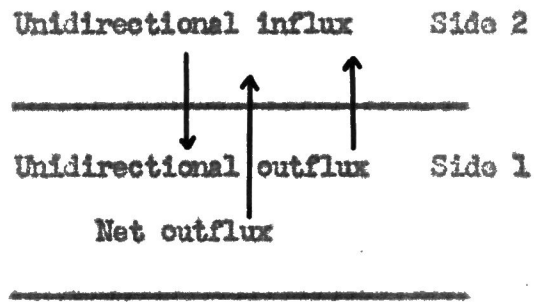
In studying the alterations in the sodium concentration of the lumen of the small intestine it does not matter how much the ions weigh but rather how many ions there are. The microequivalent system of terminology is an important tool in studying fluxes, for it is virtually impossible to follow the electrolyte shift when they are expressed as milligrams per cent. Dividing the number of micrograms of a monovalent substance by its atomic weight gives the number of combining power or microequivalents. The rule for this conversion can be expressed:

$$\frac{\text{micrograms}}{\text{atomic weight}} \text{ times } 10 = \text{microequivalents/liter}$$

APPENDIX F

DERIVATION OF FORMULAE

(Snell, et al., 1965)



The net flux of a constituent across a membrane is the result of a difference between two unidirectional fluxes proceeding simultaneously.

Suppose:

Ji is the diffusion flux

i is the moles of substance

diffusing/ unit area/ unit time

subscript 12 is diffusion from side 1 to side 2.

subscript 21 is diffusion from side 2 to side 1.

Then: $Ji = Ji_{12} - Ji_{21}$

or net outflux is equal to unidirectional outflux minus the unidirectional influx.

The flux of i from 1 to 2, Ji_{12}, involves molecules of i originating in 1 and should be proportional to the number per unit volume in side 1, ci_1.

Then: $Ji_{12} = (Pi^m)\, ci_1$

Where: Ji_{12} = unidirectional outflux

Pi^m = permeability coefficient

ci_1 = concentration of i in side 1

The flux of i from 2 to 1, Ji_{21}, involves molecules of i, which originate in 2 and therefore should be proportionnl to ci_2.

Then: $Ji_{21} = (Pi^m)\, ci_2$

Where: Ji_{21} = unidirectional influx

Pi^m = permeability coefficient

ci_2 = concentration of i in side 2

This division of the flux into two unidirectional fluxes is a purely artifical device unless some method is found by which it can be distinguished from which side the molecules originate. In addition to labeling the molecules, the method that is used should not otherwise alter the molecules behavior. Radioactive isotopes fulfill these criteria.

The isotopic concentration on side 1 can be designated as ci^* (e.g. counts/cm^3) and then the specific activity may be defined as

$\alpha\, i_1 = ci_1^*/ci_1$

Where: ci_1 = moles/cm^3

The rate of isotopic flux from 1 to 2, Ji^*_{12}, is then:

$$Ji^*_{12} = Ji_{12}\,(ci^*_1/ci_1)$$

or isotopic outflux is equal to the unidirectional outflux times the specific activity;

or solving for the unidirectional outflux

Then: $Ji_{12} = Ji^*_{12}/(ci^*_1/ci_1)$

By substituting the fluxes in the opposite direction (2 to 1), the unidirectional influx can be calculated:

$$Ji_{21} = Ji^*_{21}/(ci^*_2/ci_2)$$

APPENDIX G

SAMPLE OF RAW DATA

Duodenal section, experimentation conducted on January 12, 1965:

	CONTROL	TREATED
Background count:	1,618 counts/min	1,735 counts/min
Total counts injected:	61,373	59,429
Total counts recovered with intestinal section:	19,475	27,692
Total counts of washed intestinal section:	3,121	4,000
Total counts recovered:	14,736	21,957
Background at the finish:	1,700	1,644
Total sodium injected:	119.74 μEQ	119.74 μEQ
Total sodium recovered:	119.74 μEQ	106.91 μEQ
Net outflux:	0.0 μEQ/min	1.28 μEQ/min
Unidirectional outflux:	14.47	10.40
Unidirectional influx:	14.47	9.12

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J. Robert Mannino UNIDIRECTIONAL SODIUM FLUX IN THE DUODENUM, JEJUNUM, AND ILEUM OF NORMAL AND DESOXYCORTICOSTERONE ACETATE TREATED MALE, LABORATORY RATS. (Under the direction of Everett C. Simpson) Department of Biology, July 1965.

The purpose of this study is to investigate the relationship between desoxycorticosterone acetate (DOCA) and sodium retention in the small intestine. A method was devised by which the effect of DOCA on the sodium flux in the small intestine could be measured. Radioactive sodium (sodium-22) was used as a tracer to discern sodium transfer subsequent to DOCA aministration, while a colorimetric analysis was used to ascertain total sodium. Utilizing these two techniques together with isolated, in vivo intestinal sections, a two fold study was made 1) to determine the effects of DOCA on the sodium flux and; 2) to determine any possible regional differential in sodium transfer in the small intestine.

It was found that a definite absorption gradient of sodium was noted in the control condition with the greatest amount of absorption in jejunum. DOCA was found to significantly decrease the unidirectional influx of sodium in the duodenum and jejunum, while a similar effect was not demonstrated in the ileum.

A possible mechanism, similar to the glucose-insulin transport theory, is postulated and discussed for the action of DOCA and the active transport of sodium.