Studies on the relationships between the urinary excretion of iodide and other electrolytes, and the effect of oestrogens on their excretion in the rat.

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SUMMARY

The relationships between the urinary excretion of iodide, sodium, chloride and potassium, and the effect of oestrogens on the urinary excretion of these electrolytes has been studied in the rat.

Acute water and saline-loading experiments have been conducted on female rats where renal tubular reabsorbtion of sodium and chloride has been blocked using chlorothiazide and the aldosterone-antagonist SC-14266. Under these conditions a close correlation exists between iodide, sodium and chloride excretion and it is concluded that iodide and chloride are handled in a similar manner by the kidney. In addition it was found that chlorothiazide is a more powerful chloruretic agent than SC-14266, and that SC-14266 has greater ioduretic properties than chlorothiazide. It is suggested that iodide is preferentially reabsorbed in the distal part of the renal tubule linked to the aldosterone-controlled $Na \rightleftharpoons K$ pump. The acute administration of propylthiouracil in water-loaded female rats produced an ioduresis, naturesis and chloruresis similar to that of SC-14266.

In female rats given water and saline-loads at intervals following a single subcutaneous injection of 400 µg oestradiol benzoate the urinary sodium, chloride and iodide was reduced on the first day after oestrogen treatment. On the second day the

excretion of all these ions was increased, on the fourth day the excretion of these ions became normal but on the sixth day post-oestrogen treatment there was again an increase in the excretion of these ions. However, in similar experiments using male rats there was a continued reduction of sodium, chloride and iodide excretion on these days.

Cyclic variations in urinary electrolyte excretion were found to occur in female rats. A reduced urinary Na:K ratio, sodium, chloride and high potassium excretion occured at proestrous or oestrous indicating an increase in aldosterone activity at these times.

Dietary I^{131} tagging experiments confirmed that a close correlation between the urinary excretion of iodide and sodium chloride exists. It is suggested that a cyclic variation in iodide excretion occurs in the rat oestrous cycle. A reduction in excretion occuring at proestrous and oestrous and an increased excretion at dioestrous.

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CONTENTS

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GENERAL INTRODUCTION

In a study of 184 pregnant women in Aberdeen it was found that seventy per cent had visible and palpable enlargement of the thyroid gland compared to thirty seven per cent of 116 non-pregnant controls (1). These same investigators found that in human pregnancy thyroidal iodine uptake, thyroid clearance rate and renal clearance of iodine were increased, and plasma inorganic iodine levels reduced. The increase in iodine excretion occured early in pregnancy reaching a level twice that found in non-pregnant subjects and returned to normal values by the sixth week post-partum (2, 3). Furthermore, although the glomerular filtration rate increases by fifty per cent by the fifteenth week of pregnancy (4) the increase in iodide clearance is one hundred per cent; iodine loss cannot, therefore, be entirely explained by changes in glomerular filtration rate.

Pregnancy, therefore, results in an iodine deficiency state, partly due to the high renal iodide clearance and the thyroid gland increases in size to compensate. Other workers have shown that urinary iodine excretion is increased (5, 28) reduced (6), and unaltered (7) in human pregnancy; increased in rat pregnancy (8) and the last two days of gestation in the hamster (Mesocricetus auratus) (9).

Accompanying these changes in iodine metabolism in pregnancy are changes in the metabolism of other electrolytes and water (10):

plasma volume (11, 12) and blood volume increase 12, 13); the body accumulates sodium (14, 15, 16), chbride (10), and to a lesser extent potassium (15); there is an increase in total body water (15, 17); an increased renal retention of water (18), and sodium (19) after a water load; an increased retention of water and sodium in the pregnant rat after a saline load (20, 21) and increased appetite for sodium chloride in the gestating rat (22). In these studies on renal sodium retention simultaneous measurements of chloride were not made. The renal tubular reabsorbtion of sodium ions is, however, usually accompanied by chloride ions (23), it could be said therefore that besides the enhanced tubular reabsorbtion of sodium in pregnancy (19) increased chloride reabsorbtion takes place; which would account for the increase in total body chloride found in pregnancy (10).

It is this chloride retention that is possibly responsible for the enhanced iodide excretion in pregnancy. It has been suggested that in the rat (24) and the human (25) the renal tubules are less permeable to iodide than to chloride, and that these two anions compete for reabsorbtion in the tubule. Stimulating tubular anion reabsorbtion would then result in a preferential uptake of chloride while increasing the excretion of iodide. There is indirect evidence in the literature to support the hypothesis that "increasing chloride reabsorbtion increases iodide excretion": Cushing's disease in the human is associated with a high renal iodide clearance (26), and administration of cortisone (27) or aldosterone (29) in the rat increases renal

iodide loss. In more detailed investigations, however, iodide clearance in the human was found to be independent of the clearance of chloride, sodium and potassium, but was closely related to the glomerular filtration rate (30). Other workers found a close correlation between iodide and potassium excretion in the human (26, 28) while in the dog, iodide clearance is determined by the rate of chloride excretion (25, 31). There appears, therefore, to be a certain amount of conflicting evidence on the relationship between iodide excretion and that of chloride, possibly due to the use of different species in these studies.

The increased salt and water retention, characteristic of pregnancy, has been attributed in the rat to an increased aldosterone activity (21). Although an increased aldosterone excretion occurs in human pregnancy (32) the cause of the water and electrolyte retention in the human is still obscure. Oestrogens, which increase in pregnancy (33a) are known, however, to increase the renal absorbtion of sodium, chloride, and water in a number of non-pregnant species (34-42), oestrogens however, are but one of a number of hormones suggested for causing the water and electrolyte retention of pregnancy. The possible link between iodide and chloride excretion has been mentioned and it would seem logical, therefore, in any attempt to explain the changes in iodide excretion in pregnancy, to examine the effects of oestrogens on the renal handling of iodide.

Most of the literature dealing with the effects of oestrogen on iodine metabolism is concerned with the effect on thyroidal iodide uptake and the thyroid: serum iodide ratio, either after oestrogen treatment or during the oestrous cycle. The consensus of opinion in this work is that exogenous oestrogen treatment increases both the absolute iodide uptake by the thyroid and the thyroid: serum iodide ratio in rats and mice; and that values for, iodide uptake by the thyroid and thyroid: serum iodide ratio in both these species show a cyclic variation associated with the oestrous cycle. Both these values are greatest at oestrous in the rat and highest at proestrous in the mouse (43 - 64).

It has been suggested that these rhythmic alterations in the thyroid function during the oestrous cycle are mediated by changes in levels of oestrogen and progesterone; high oestrogen levels at oestrous stimulating thyroidal iodide uptake and high progesterone levels in the luteal phase of the cycle depressing thyroid function (64). To the suthor's knowledge no measurements of renal iodide clearance in the oestrous cycle have been carried out. Moreover, the literature on the effect of exogenous oestrogen on iodide excretion is both sparse and conflicting. Reports that oestrogen administration does not alter (43, 65), increases (66, 67) and decreases(44, 45) renal iodide loss

occur in the literature. It is interesting to note, however, that associated with the increased oestrogen levels of puberty, occurs an elevated renal clearance of iodide (5, 28). In all the work done on the effect of oestrogens on electrolyte excretion, no simultaneous study of both iodide and chloride have been carried out.

It is possible from this brief literature revue to propose that the increased urinary iodide excretion of pregnancy, reported by some investigators, may be due to the increased renal retention of chloride; and furthermore, that the elevated blood oestrogen levels found in pregnancy are responsible for these changes in iodide and chloride metabolism.

There is, however, a certain amount of controversy in the literature concerning the way the kidney handles iodide, particularly the relationships between iodide excretion and the excretion of other electrolytes such as chloride. Also, the work on the effect of oestrogens on renal iodide excretion is not extensive and is conflicting; and while oestrogens are generally known to increase renal sodium chloride retention, no simultaneous study of the effect of oestrogens on both renal iodide and chloride excretion have been made.

It is not the intention of this thesis to examine iodide metabolism in pregnancy per se, but rather an attempt will be made to establish the mechanism whereby iodide is excreted by the kidney and the influence of oestrogens on this process in the normal rat. From such studies information may be provided to suggest the cause for the high renal iodide excretion to be found in pregnancy.

The objectives of this work then are:

(a) To examine urinary iodide excretion in the rat and to see what relationship the excretion of this ion has with sodium, chloride and potassium.

(b) To study the effect of exogenously administered oestrogen in the rat on the renal excretion of iodide, sodium, chloride and potassium.

(c) To examine urinary electrolyte excretion in the rat oestrous cycle where a natural cycle of endogenous oestrogens occurs.

GENERAL METHODS AND MATERIALS

Housing of rats

Wistar rats were used throughout and as a great many of the experimental procedures involved the use of radioiodide tracer techniques all experiments were performed in a separate part of the animal house. All rats were maintained in this separate unit for at least two weeks prior to being used for any experiment, to acclimate to the environmental conditions.

Besides being a necessary safety measure, the separation of the experimental animals from the rest of the animal house had an advantage in that conditions were quieter and only the author had access; thus unnecessary stress to the rats was avoided. It has been shown that noisy animal house conditions gives rise to adrenal stimulation and an increase in blood corticosterone levels (68).

Room temperature was maintained at 22 $\pm$ 3°C. and animals received a mixture of natural and artificial illumination from 7.30 a.m. - 7.30 p.m., humidity was not controlled. Stock rats were housed four per cage and received water and a standard 41 B diet ad libitum.

Metabolism cages

The experimental work can be divided into two main categories: short-term and long-term.

The short-term experimental procedures involved the collection of urine from rats, after a water or saline load, for a period of four hours and the metabolism cages used in these experiments were of a simple design. Each cage consisted of a stainless steel meshed basket

(6½" x 6½" x 7") with the inside corners fitted with wire mesh to give a roughly circular inside to the basket, and the basket attached to a polythene funnel (7" diameter). Such a unit could be clamped over a glass funnel and measuring cylinder for urine collection. A large rat (400 g.) could sit inside such a cage comfortably and because of the compactness of the cage, loss of urine by adhesion to the sides of the funnel was reduced. Faeces were retained by the mesh of the basket but occasional contamination of urine did occur. No solid or liquid food was given to rats when in these cages.

Before any experiment involving the use of these cages, rats were acclimatised to them by spending two four-hour periods inside the units some days before they were used experimentally.

The long-term type of experiment required the daily collection of urine for a number of weeks. The Howells, Wright and Harrison metabolism cage was used in such experiments (one rat per cage). This type of unit gave an excellent urine faeces separation, and by giving the food as a paste, as a rule, no contamination of the urine with food particles occured.

Renal Iodide Clearance (RCI-)

Renal iodide clearance measurements were performed using an established I^{131} tracer technique (69) and the calculation:-

$$RCI^{-} = \frac{U \times \log. A - \log. B}{(A - B) \times 0.4340 \times T}$$

where RCI^{-} = Renal clearance of I^{131} - cc blood/min

U = Total urinary I^{131} of collection period-Counts/min.

A = I^{131} in first blood sample - Counts/min/cc

B = I^{131} in second blood sample - counts/min/cc.

T = Time of urine collection period - min.

0.4340 = A Constant.

Because whole blood in A and B was used for I^{131} counting, renal iodide clearance values are-cc whole blood/min which is therefore not a "true" clearance measurement.

Electrolyte determinations

Urinary sodium and potassium values were determined using an "Eel" flame photometer and chloride in urine samples measured by an "Eel" chloride meter. I^{131} was counted on a Nuclear Enterprises - Gammamatic instrument.

Drugs and other chemicals used

1.	Oestradiol - 3 - benzoate	Sigma Chemical Co.
2.	6-Propyl - 2 - thiouracil	Sigma Chemical Co.
3.	Arachis oil	
4.	ABIDEC - a soluble aqueous solution of fat-soluble and water-soluble vitamins. (A,D,B_1, B_2, B_6, C, Nicotinamide)	Parke, Davis & Co.
5.	SC - 14266. An Aldosterone-Antagonist (water soluble)	G.D. Searle & Co.
6.	Chlorothiazide B.P.	Merck, Sharp & Dohme Ltd.
7.	NaI^{131} carrier free in isotonic saline	Radiochemical Centre, Amersham.

Fluid loading of rats

Water and saline-loading of rats was achieved by using a Jacques No.3. catheter fitted with a large luer syringe needle. The catheter was inserted into the stomach, a syringe with the loading fluid attached to the syringe needle, and the fluid introduced.

Ovariectomised Rats

Ovarieectomised rats were purchased.

Diets

1. "Low iodine" test diet (formulated per Remington-diet No.347).

   Nutritional Biochemicals Co. Cleveland, Ohio, U.S.A.

   Composition (Manufacturer) :-

(a)	Yellow Corn meal (grown in iodine deficient area)	78%
(b)	Wheat gluten	18%
(c)	Brewers yeast U.S.P.	2%
(d)	$Ca\ CO_3$	1%
(e)	Na Cl	1%

   Further Analysis :-

(f)	Sodium	0.3820%
(g)	Potassium	0.1175%
(h)	Iodine	$< 100\ \mu g.\%$

2. 41 B diet

(a)	Sodium	0.575%
(b)	Potassium	0.840%
(c)	Iodine	$418\ \mu g.\ \%$

Expression of results and statistics

In the short-term experiments the urinary excretion of the following: sodium, potassium, chloride, urine volume, and both parameters of iodide (I^{131}) measurement were expressed as percentage of body weight. Similarly thyroidal iodide (I^{131}) uptake was expressed as a percentage of body weight.

In group data the mean, standard error, and t values were calculated by computer. In addition to the comparison between control and experimental groups, inter group statistical comparisons were made by the computer. A statistically significant difference between groups was taken as P0-05 or $P < 0.05$.

The best straight line calculations were made by the method of least squares by computer and the correlation coefficients simultaneously determined. A correlation coefficient giving P0.05 or $P < 0.05$ was considered a significant correlation.

IODIDE EXCRETION : I. THE EFFECT OF DIURETICS ON IODIDE EXCRETION

Introduction:

There are two major pathways by which iodine is removed from the body, by urinary excretion and by faecal loss. Most of the iodine found in the faeces is in organic form with very little iodide present, while the iodine found in urine is predominantly as iodide (25, 70).

The origin of faecal iodine products has been extensively studied and has been shown to originate from the bile. Circulating thyroid hormones, the principal one of which is thyroxine, are very rapidly concentrated in the liver (71, 72) where they are metabolised, enter the bile and eventually pass into the small intestine. The nature of these bilary secretions are known to be thyroxine, triiodothyronine, conjugates of thyroxine, and a small fraction of iodide (71 - 74); reabsorbtion of some of this thyroxine occurs in the gut (72). Another source of iodide in the faeces has been shown, in the rat, to be the active secretion of iodide across the intestinal wall against a concentration gradient (75 - 80) Nevertheless, the iodine in the faeces of rats is predominantly in organic form.

The kidney filters iodine through the glomerular membrane as iodide, the passage of thyroxine through the glomerulus being restricted because it is largely bound to plasma proteins (81). Iodine in urine, therefore, is almost (ninety-five percent, or more) entirely inorganic (70, 71, 73, 82, 83). One important factor in determining the amount of iodide excreted by the kidney is the availability of free iodide in

the blood filtered by the kidney (70), and in turn the level of free iodide in the blood depends on the dietary intake of iodine compounds, the rate at which iodide is released by the deiodination of thyroid hormones, and how much of blood iodide is bound to plasma proteins (70). The thyroid is said to compete with the kidney for blood iodide (25) and that the renal excretion of iodide varies inversely with thyroid iodide uptake (75, 84).

The importance of the rate of deiodination in determining renal iodide excretion is exemplified by some work carried out on the thyroidectomised rat maintained on I^{131-} labelled thyroxine (85). In this work it was shown that propylthiouracil administration in these animals reduced the urinary I^{131} levels by fifty per cent, and increased the faecal excretion of I^{131} - labelled compounds; moreover the reduction in urinary iodide excretion was not due to an impaired kidney function. The authors concluded that these alterations in I^{131} excretion were due to inhibition of deiodination by propylthiouracil. When, therefore, blood iodine is mostly in the inorganic form the kidney is the main excretary pathway for this iodine (86), while if blood iodine is chiefly organic the predominant excretary pathway is by the faecal route (71).

A considerable amount of controversy exists in the manner by which the kidney handles iodide, whether for instance the kidney treats iodide in a similar manner to which it handles chloride. It is this intrarenal metabolism of iodide which this work is primarily concerned with.

From an analysis of the considerable amount of literature on the way the kidney handles iodide it is possible to propose four mechanisms which may control renal iodide clearance:

(a) iodide clearance controlled by the glomerular filtration rate;

(b) passive tubular reabsorbtion of iodide similar to that of chloride;

(c) passive reabsorbtion of iodide similar to chloride but renal tubules preferentially reabsorb chloride ion to iodide; and (d) Active secretion of iodide by the renal tubules, similar to the active secretion of potassium.

(a) In a detailed study of renal iodide clearance in the human BRICKER and HLAD (30) deduced that iodide is filtered by the glomerulus and reabsorbed by the tubules predominantly by a passive back-diffusion. These authors also observed that iodide clearance was independent of the clearance of sodium, chloride, potassium and urine flow. For instance, they found that a mercurial diuretic, while increasing chloride, sodium and urine excretion did not increase iodide clearance. Throughout their work they did, however, find a close correlation between the glomerular filtration rate and iodide clearance over a very wide range of filtration rates; and they state that "acute increases and decreases in filtration rate were associated with changes in $C_{I^{131}}$ in the same direction as those of Cin." Other authors have also shown that the glomerular filtration rate can influence the rate of iodide clearance, but they also indicate that other factors such as other electrolytes, are also involved in kidney control of iodide clearance (5, 26).

(b) RIGGS found that in the dog iodide is reabsorbed by a passive mechanism and that iodide and chloride clearance are closely related (31), but that in man iodide excretion is independent of changes in chloride excretion (25). Other workers have also demonstrated that these two ions are passively reabsorbed throughout the same portion of the nephron in the dog (87).

Indirect evidence that iodide and chloride are treated by the kidney in a similar manner is afforded by the work of FREGLY (88, 89) who found that hydrochlorothiazide and chlorothiazide fed to rats for a number of weeks, increased thyroid weight, thyroidal I^{131} uptake, and thyroidal I^{131} release; which indicated that the thyroid glands of these rats were under the influence of augmented secretion of TSH from the pituitary gland. In further experiments, FREGLY observed that hydrochlorothiazide increased the renal excretion of administered I^{131} to nearly twice that of control rats and that excretion of chloride was also elevated, as also was sodium and potassium. He concluded that the effect of thiazides on the thyroid gland is indirect and related in fact to the increased renal loss of iodide. Thus thiazides increased iodide as well as chloride excretion which suggests that these two anions are handled the same by the kidney. It is possible, however, that iodide excretion was related somehow to sodium and potassium as the excretion of these ions was also increased. Thiazide treatment in the human is also known to be associated with an ioduresis, though negative reports of the iodurectic properties of thiazides in the human do exist (90).

In one such negative report (101) the authors found that both a mercurial diuretic and chlorothiazide did not alter either thyroidal iodide uptake or urinary iodide excretion even though both diuretics had a marked chloruretic effect, and they agree with the conclusions of BRICKER and HLAD (30). (c). It is reported that in the rat chloride and iodide ions compete for renal tubular reabsorbtion, and that the tubules preferentially reabsorb chloride to iodide (24). It has also been shown that the sea gull can concentrate iodide as well as chloride in the nasal gland but chloride is secreted from this organ to a greater extent than iodide; moreover, the kidney in this bird reabsorbs chloride from the tubules in preference to iodide (91). These authors attribute the differential handling of these ions to the fact that they move through cell membranes at different rates.

If, then, the kidney tubules do preferentially reabsorb chloride to iodide it can be postulated that stimulating the tubular site for chloride reabsorbtion will lead to an increase in iodide excretion. There is in fact much evidence in the literature to support this hypothesis, particularly derived from the work on the effect of adrenal steroids on iodide metabolism.

In the human ACTH, cortisone (92), Cushing's disease and Acromegaly (5, 26) are all known to promote an increase in iodide clearance and all are known to increase renal sodium and chloride retention. Although somatotrophic hormone, the levels of which are elevated in Acromegaly, is not usually considered a major salt-retaining hormone it has been shown to have a similar effect to aldosterone in that it increases sodium and chloride retention and increases potassium excretion (93). Cortisone (27, 94, 95) and aldosterone (29) have been shown to increase

renal iodide loss in the rat, though PARIS et al (96) have shown that in the intact rat cortisone did not increase iodide excretion.

ACTH, Cortisone (94, 97, 98) and Aldosterone (29) do in fact reduce the thyroidal iodide uptake in the rat and the human (92) and it has been stated by MONEY (99) that - "the primary way that cortisone influences the thyroid, if not the only way, is through increased renal excretion of iodide". Undoubtedly the adrenal steriods exert an important affect on thyroid function through their action on iodide excretion, but it is doubtful whether all the effects of steroids on thyroid metabolism are mediated through this raised iodide excretion (96, 98, 100).

While the effects of adrenal steroids on iodide excretion can be explained by the hypothesis that tubular differentiation between chloride and iodide exists, the effects of adrenalectomy on iodide excretion cannot be explained by the same hypothesis. Addison's disease (5, 26, 28) and adrenalectomy in the rat (27, 94) reduce the urinary iodide clearance. The aforementioned hypothesis presupposes that chloride and iodide use the same site in the renal tubule for reabsorbtion, if then, chloride reabsorbtion is halted by adrenalectomy, it would be expected that iodide reabsorbtion would also be prevented. A possible explanation for this reduction in iodide excretion is the reduced glomerular filtration rate found as a consequence of adrenalectomy (33 a, b). (d). It has been stated by some workers that iodide excretion is in many respects similar to that of potassium and that active tubular excretion takes place for both iodide and potassium (26, 28). The major site, if not the only one, of potassium secretion in the kidney is the distal tubule (102, 103). In this region there is a linked sodium,

potassium and hydrogen pump whereby sodium is removed from the tubule lumen in exchange for either potassium or hydrogen ions by anactive process; and chloride ions follow sodium by a passive diffusion (102,103). If potassium and iodide secretion are closely linked it would mean that both ions use this distal tubule pump.

The method by which the adrenal steroids, particularly aldosterone, increase sodium and chloride retention is thought to be by stimulating the distal tubule sodium pump (102, 103). It is possible, therefore, to ascribe all the effects of the adrenal steroids on iodide excretion to alterations in potassium secretion: A high circulating level of adrenal steroids increases potassium secretion and increases iodide clearance, while in the adrenalectomised animal potassium secretion is reduced and iodide excretion is also reduced. Thus, there are three theories to explain urinary iodide excretion all of which involve the distal tubule sodium, potassium pump. (see Fig.A.)

There is evidence, from data on the method by which the thyroid traps and accumulates iodide, that iodide transport is dependent on a sodium pump (104-108). One of these authors states that -"Iodide transport is parasitic upon the energy supply of the sodium, potassium pump" (108). It would seem possible therefore that iodide transport in the kidney, as well as in the thyroid, involves either directly or indirectly the sodium, potassium exchange mechanism.

Four possible mechanisms for the control of iodide excretion have been mentioned, perhaps all of them are involved in the renal handling of iodide.

The importance of chloride in modifying iodide excretion has been known for a number of years from the fact that chloride administration increases the renal excretion of iodide. This has been demonstrated in the rat (24, 96, 119-111), the dog (112), the mouse (113), and in calves (114). A number of explanations can be given for this effect of chloride. Firstly that by increasing body chloride, iodide is displaced from the tissues and enters the blood, thus increasing iodide filtered by the kidneys. Secondly, the additional chloride raises the renal tubular concentration of this ion so that the passive tubular reabsorbtion of anion which accompanies sodium will be predominantly chloride, with the result that less iodide is reabsorbed. Thirdly, if there is a preferential reabsorbtion of chloride in the kidney, increasing tubular chloride concentration will lead to a higher proportion of chloride being reabsorbed, to the exclusion of iodide.

The literature concerning the way iodide is excreted by the kidney extends mainly over the last fifteen years or so, however, the literature on bromide excretion has a longer history. This is due to the fact that bromide compounds were widely used, particularly in the nineteenth and beginning of this century, in the treatment of various mental disorders. Consequently, the medical profession were increasingly confronted with cases of bromide intoxication and the need

to clear this accumulated bromide from the body. Methods of clearing bromide were obviosuly centred on increasing urinary excretion of this ion and this led to the comparison of the behaviour of bromide and chloride ions in the kidney. The literature on bromide handling by the kidney is well revued (115-117), and while it is not the intention of this author to confuse the question - how is iodide handled by the kidney? It would seem relevant to mention some of the work carried out on bromide excretion.

Like iodide, urinary bromide excretion is increased by the administration of chloride, this has been demonstrated in man (115, 118-122), and dog (116, 123, 124); and also like iodide, adrenal steroid administration increases bromide excretion in man (115, 122). One important difference between bromide and iodide however, is that mercurial diuretics increase bromide excretion in man (120, 121) whereas this diuretic does not increase iodide excretion (30).

There is evidence throughout the literature on bromide and iodide excretion that halogens are not necessarily treated by the kidney in the same way. This has been attributed to the difference in size of each ion, which will affect the rate at which these ions cross biological membranes. Moreover, considerable differences have been noted between species in the way one particular halogen is excreted by the kidney.

The work to be described here is an attempt to study aspects of urinary iodide excretion in one species, the rat, and in particular to study the relationships between iodide excretion and the excretion of other major urinary electrolytes.

It is hoped that by such a study the mechanism by which iodide is handled by the rat kidney can be determined.

It was decided to examine electrolyte excretion under various conditions of saline and water diuresis and to further manipulate electrolyte excretion by means of diuretics. The diuretics chosen for this purpose were a thiazide and, considering the possible importance of the distal tubule sodium pump in iodide excretion, an aldosterone-antagonist.

METHODS AND MATERIALS

Female rats of approximately 250. g. body weight were used in this work, and all experiments were started at 9.30.a.m. to standardise procedures as much as possible. Animals were deprived of solid food the night before they were used for an experiment. Diuretics were administered under two separate experimental conditions, in animals "loaded" with isotonic saline and animals "loaded" with water. Two diuretics were used chlorothiazide and an aldosterone-antagonist SC-14266.

Sodium chloride loading:

The experiments in this group consisted of administering two saline-loads or a single saline-load.

(a) Double saline-loading

Each rat was injected subcutaneously with approximately 3μc of carrier free radioactive iodide (NaI^{131}) in an accurately measured volume of isotonic saline (0.1 ml); immediately following this procedure the rat was given 5 ml. of 0.9% Na Cl by stomach tube. The

rats were then placed in cages, with absorbent paper bedding, so that any radioactive urine was soaked up and to prevent the animals collecting sawdust etc. on themselves, which they would do if returned to their normal cages (This avoided contamination of the urine collected later). Two hours later a 0.1. ml. whole blood sample was obtained from each rat by slightly cutting the tip of the tail with a sharp scalpel. The rats usually urinated during this blood sampling, but to ensure that the bladder was emptied a slight pressure was applied to the pubic region. This was followed by another oral load(5 ml.) of isotonic saline after which each animal was placed in a small metabolism cage. Urine was collected over a 4-hour period at the end of which they were required to breath ether in order to stimulate bladder emptying. A second tail blood sample was then taken (0.1 ml.), the animals killed and the thyroids dissected out and homogenised in 10% Na OH. Urine volume was measured. Throughout the 6-hour experiment the animals had no access to food or water. The metabolism cages were washed with 25 ml of KI (10% solution) and this wash retained.

Blood samples, Aliquots of urine, cage wash, and thyroid homogenate from each rat, together with an aliquot of the injected radiodide were counted for I^{131}. The percentage of the injected dose of I^{131} excreted during the collection period was calculated, together with renal iodide clearance values, after correcting for the I^{131} in the cage wash. The urine was analysed for sodium, potassium, and chloride and the amount of each of these ions in the collected urine calculated. It was possible to calculate the quantities of sodium,

chloride and potassium in the cage wash from working out what percentage the I^{131} in the cage washing was, of the I^{131} in the urine collected. Thus the total quantities of sodium, chloride and potassium excreted in the 4-hour period could be determined, and these values were expressed as μEq/100 g. body wt/4 hrs. One major error in this procedure is that an accurate measure of urine volume cannot be obtained because of urine which adheres to the sides of the metabolism cage. However, it could be assumed that this error existed, more or less, to the same extent throughout all the experiments.

Chlorothiazide was given dissolved in the first saline-load, but because of the low solubility of this thiazide in water a small quantity of 0.1. N NH_4OH was added to the saline to dissolve the drug. SC-14266, which is readily soluble in water, was given in the first saline-load; and when given in combination with chlorothiazide, it was again in the first load. The pH of the first saline-load was adjusted in all experiments to pH 8.4 and a control experiment performed with the first load at pH 8.4. In one experiment, which did not have a control, chlorothiazide was given in the second saline-load again at pH 8.4.

(b) <u>One saline-load</u>

The procedures in this series were the same as in (a) except that the first saline-load was not given, the route of SC-14266 administration was different, and no combined diuretic experiments were done. Chlorothiazide was given in the saline-load at pH 8.4 and a control experiment performed. SC-14266 was given in the saline-load at pH 8.4, or intraperitoneally in o.5 ml. of water at the time of I^{131} injection and the saline-load was given at pH 8.4.

Water-Loading

The experiments in this group consisted of double water and single water-loading. The procedures in these water-loading experiments were the same as in (a) and (b) except for the dosage and route of administration of drugs.

(c) Double water-loading

Chlorothiazide or SC-14266 or both combined were given in the first water-load at pH 8.4 with a control water-loading experiment at the same pH. In one experiment without a control, chlorothiazide was given in the second load at pH 8.4.

(d) One water-load

Chlorothiazide was given in the water-load at pH8.4., and a control loading experiment done at the same pH. SC-14266 was given intraperitoneally in o.5.ml. of water at the time of I^{131} injection and the waterload adjusted to pH8.4.

RESULTS

Double saline-loading

The results of this series of experiments are shown in Table 1. Iodide excretion is not significantly altered by treatment with diuretics except that in the combined drug group there is a significant reduction from control values in the percentage of iodide excreted.

Sodium chloride elimination was increased above control levels by the 20 and 30 mg. doses of chlorothiazide but not by the lower dose of this drug or by SC-14266. Even when SC-14266 is combined with chlorothiazide id did not significantly alter sodium chloride excretion from conttrol values; this is seen when the 20 mg. chlorothiazide group is statistically compared with 20 mg. chlorothiazide + SC-14266 group ($P > 0.05$)

The 5 and 20 mg. doses of chlorothiazide slightly, but significantly, reduced potassium excretion. On the other hand there was a marked reduction in the excretion of this cation by Sc-14266 treatment, by itself or in combination with chlorothiazide.

The increased Na:K ratio seen with the higher doses of chlorothiazide was due mainly to an increase in urinary sodium; but the high ratio found with SC-14266 was due to a reduction in potassium excretion.

Urine volume was significantly increased in all experimental groups except the lowest chlorothiazide dose and SC-14266 by itself.

Thyroidal iodide uptake differed from control values only in the aldosterone-antagonist-treated rats; and in these groups thyroid iodide levels were three to four times higher than in the control group.

In Figs. 1 - 4 iodide clearance has been plotted against chloride, sodium, potassium and urine volume and the data for the construction of these graphs was derived from individual rat experiments from Table 1. Iodide clearance is nor correlated with any of these other values.

One saline-load

The results of the single saline series of loading experiments are shown in Table 2.

All doses of the two diuretics used significantly increased the excretion of chloride, sodium, iodide and water and raised the urinary Na:K ratio and thyroidal iodide uptake. There was no significant change from control values in potassium excretion in drug-treated groups.

A significant difference found between chlorothiazide and SC-14266 was that chlorothiazide induced a greater increase in chloride and sodium excretion than did SC-14266; conversely SC-14266 increased iodide excretion to a significantly greater extent than did chlorothiazide.

Figs. 5-7 show the relationship between iodide clearance and chloride, sodium, potassium and urine volume; the data being derived from individual rat experiments from Table 2. Figs. 5 and 6 demonstrate more clearly the difference between the two diuretics as regards their ioduretic and chloruretic-naturetic properties.

There is a significant correlation between iodide clearance and chloride (Fig. 5), sodium (Fig. 6) and water (Fig. 8) excretion; but no such correlation exists between iodide clearance and potassium excretion (Fig. 7).

Double water-loading.

Results of this series of experiments are shown in Table 3.

In all chlorothiazide-treated groups sodium chloride excretion and the urinary Na:K ratio were elevated above control values; and similarly this drug increased iodide excretion in all groups except that the percentage iodide excretion in one group was not significantly different from control. Potassium excretion, urine volume and thyroidal iodide uptake were either unchanged or increased by chlorothiazide.

SC-14266 by itself significantly increased sodium urine Na:K ratio and thyroidal uptake and significantly reduced potassium excretion. All other values were not altered by treatment with this drug.

When the 20 mg. chlorothiazide group and the 20 mg. chlorothiazide + SC-14266 group are statistically compared the only differences that SC-14266 cause are a reduced potassium excretion ($P < 0.001$), a higher urine Na:K ratio ($P < 0.001$) and a higher thyroidal iodide uptake ($P < 0.001$)

Figs. 9-12 show the correlation between iodide clearance and chloride, sodium, potassium and urine volumes. Iodide excretion is significantly correlated with chloride, sodium, and potassium but not with urine volume.

One water-load

Data for this series of experiments are shown in Table 4.

Both doses of chlorothiazide significantly increased chloride, sodium, the urinary NaK ratio and both parameters of iodide excretion above control levels. This drug either increased or did not alter the potassium and urine excretion; and either reduced thyroidal iodide uptake slightly, but significantly, or did not alter this value from control uptake levels.

SC-14266 in the group data in Table 4 did not significantly alter chloride excretion but sodium excretion was significantly increased by all doses of this drug. Potassium excretion was either reduced or unchanged; the urine Na:K ratio significantly increased; urine volume not altered; iodide excretion unchanged or increased by SC-14266. Thyroidal iodide uptake was either significantly reduced or increased above control levels.

Figs. 13-16 show the correlation between iodide clearance and chloride, sodium, potassium and urine volume, Iodide clearance is correlated with all these values except urine volume. Figs. 13 and 14 exemplify the greater chlouretic action of chlorothiazide, there is also an indication in these Figs. that SC-14266 can produce a more marked ioduretic action than chlorothiazide.

Discussion

In three types of experiment in this work, double water, single water and single saline-loading the renal iodide clearance had been shown to be correlated either with sodium, chloride, potassium, or urine volume. The correlation, however, between iodide clearance and sodium chloride excretion is common to all three types of experiment. This suggests that iodide clearance is primarily linked to either sodium or chloride excretion.

It is possible, therefore, that when iodide clearance is correlated to urine volume it is not due to any direct link between these two values, but to the fact that urine volume is determined to a large extent by the renal tubular reabsorbtion of sodium chloride.

The hypothesis (d) that iodide is secreted by the cells of the distal tubule in a similar manner to potassium i.e. using the aldosterone - controlled Na $\rightleftharpoons$ K pump mechanism is disproved by these results. For the aldosterone-antagonist SC-14266, which reduces potassium secretion by the distal tubule pump, does not in these experiments reduce iodide clearance. This is seen in the double saline (Table 1), double water (Table 3) and single water (Table 4) loading experiments for instance, where a 10 mg. dose of SC-14266 reduces potassium excretion by fifty per cent yet iodide clearance is not altered.

The very close correlation between iodide clearance and chloride excretion found, clearly does not support the hypothesis (c), that chloride is preferentially reabsorbed to iodide by the renal tubules. This indicates, in fact, that the hypothesis (b) that iodide and chloride are handled in a similar manner by the kidney is correct. The fact that sodium as well as chloride is correlated with iodide excretion is further evidence that iodide and chloride ions are reabsorbed, impartially, by the renal tubule. This is because active reabsorbtion of sodium is accompanied by reabsorbtion, by a passive process, of anion which is usually chloride as this is the predominant anion in the glomerular filtrate. Thus as iodide excretion is correlated to sodium and chloride it suggests that iodide as well as chloride is reabsorbed passively secondarily to an active sodium reabsorbtion.

While this work was in progress McCARTHY, FREGLY and NECHAY (125) published data on the effect of diuretics on iodide excretion in the

rat and the dog. They also found that iodide and chloride excretion are closely correlated, and, therefore, concluded that these two ions are reabsorbed by the renal tubules by a passive process. Thus the work by this author using similar experimental procedures confirms the results of McCARTHY et al.

The results of the single water and saline-loading experiments demonstrated that SC-14266 is a greater ioduretic than chlorothiazide and that this latter drug is greater chloruretic than SC-14266 (Figs. 5 and 13). These differences can be attributed to the fact that these two diuretics have a different site and mechanism of action in the renal tubules.

SC-14266 acts primarily on the aldosterone-controlled Na⇌K exchange pump of the distal tubule, by antagonising the action of aldosterone; thus increasing, sodium, chloride and water, and reducing potassium excretion (126, 127). The mechanism of actionof thiazides and chlorothiazide in particular is to reduce sodium, chloride and water reabsorbtion by the renal tubule but the site of this action is uncertain. Early work on this drug placed the site of action in the proximal tubule (23, 128, 129, 130) but in more recent work authors suggest that the site of action is not the proximal tubule but more distally such as the ascending loop of Henlé (131) or just proximal to the distal tubule Na⇌K pump (132-134)

As approximately 85 per cent of sodium chloride reabsorbtion in the renal tubule occurs in the regions proximal to the distal tubule Na⇌K exchange (102) it would explain why chlorothiazide is a greater chloururetic than the aldosterone- antagonist SC-14266.

One would expect, therefore, if chloride and iodide are reabsorbed in a similar manner, that the principal site of iodide reabsorbtion would occur proximal to the Na⇌K exchange. This is not so, however, for SC-14266 increased iodide excretion more than did chlorothiazide. It is suggested therefore that iodide is preferentially reabsorbed in the distal tubule and is linked to the Na⇌K pump.

In a study of the effects of two diuretics on bromide excretion in the rat KAGAWA and VAN ARMAN (117) found that Amisometradine and chlorothiazide both increased bromide and chloride excretion but that the former diuretic was a greater bromuretic than chlorothiazide. They concluded that Amisometradine inhibits more strongly a renal mechanism for bromide reabsorbtion than for chloride - a conclusion that also applies to the action seen in this work, of SC-14266 on iodide reabsorbtion.

IVANCEVIC and TABORSKY (135)also found in the rat that both a mercurial diuretic and hydrochlorothiazide increased chloride excretion but that only hydrochlorothiazide increased bromide excretion. As mercurial diuretics exert their chloruretic effect on the proximal tubule (23, 132, 136) it could be postulated from these results that bromide reabsorbtion occurs in a distal part of the nephron, where hydrochlorothiazide exerts its action.

Whatever the precise explanation for these results on bromide excretion it would appear that this ion as well as iodide is reabsorbed more strongly in a specific region of the renal tubule.

It was possible in the rats given a single saline-load to increase iodide excretion to a greater extent with SC-14266 than in animals which received a single water-load and this drug (Tables 2 and 4). An explanation for this difference, is afforded by the work of KOVACS, DAVID and LASZLO (137) who found that an aldosterone-antagonist was more effective in increasing sodium chloride excretion in saline than in water-loaded rats. This is because more salt reaches the distal tubule for Na⇌K exchange in the saline-treated rats and, therefore, the aldosterone-antagonist will produce a greater increase in the excretion of sodium chloride.

In the saline treated rats of these experiments, therefore, more sodium, chloride and iodide is reabsorbed using the distal tubule Na⇌K pump than in water-loaded rats. Blocking the pump with SC-14266 will therefore result in a greater iodide excretion in the saline group of rats.

The absence of any correlation between iodide clearance and sodium chloride excretion in the double saline-loaded rats cannot be explained by the author. A number of factors, not involved in the other experiments, may be responsible for this lack of correlation. For instance an intravenous saline infusion in the rat is known to raise the glomerular filtration rate and reduce proximal and distal tubular reabsorbtion of sodium chloride (138). The double oral saline-loading in these experiments may produce effects similar to those seen in saline infusions and iodide clearance may be influenced by the increased filtration rate.

The observation by other authors that chloride increases iodide excretion is confirmed in these experiments. If the iodide clearance measurements of the control groups of rats in Tables 1 - 4 are compared there is seen an increase in this value - 2.07, 2.80, 5.91, 10.56 for the double water, single water, single saline and double saline-loaded groups respectively. A possible explanation for this chloride effect is that as the concentration of chloride in the glomerular filtrate increases there will be more chance that chloride ions will be reabsorbed by the tubule cells with the result that iodide excretion is increased. There will in effect be a preferential chloride to iodide reabsorbtion due, not to any "selection" on the part of the tubule cell between the two ions, but to the fact that more chloride ions are available for reabsorbtion.

In conclusion this work has demonstrated that under conditions where renal tubular reabsorbtion has been blocked by diuretics the renal excretion of chloride and iodide are closely correlated; and that no preferential tubular chloride to iodide reabsorbtion occurs. Also these experiments have shown that the distal tubule Na⇌K pump is an important mechanism in the control of renal iodide excretion. This latter observation in fact agrees with those authors who found that mineralocorticoids, which act primarily on the distal tubule Na⇌K pump, have a marked influence on iodide excretion. These authors, however, found that stimulating this pump by means of adrenal steroids increased iodide excretion, which is evidence, albeit indirect, that chloride is preferentially reabsorbed to iodide.

The work described here, because it has only dealt with blocking the distal pump, does not, therefore, exclude the possibility that in fact stimulating this distal pump mechanism causes a preferential chloride reabsorbtion.

TABLE 1: The effect of diuretics on thyroidal iodide uptake, water, and electrolyte excretion in double saline-loaded rats.

Treatment	No. of Rats	Body wt. g.	Cl^-	Na^+	K^+	$\frac{Na}{K}$	Urine ml.	RCI^- cc/min x100	I^- % inject	Thyroid I^- % inject x10
			μEq							
Control	15	255±4	335±18	274±16	89±5	3.2±0.2	1.9±0.1	10.56±0.51	13.47±0.98	3.09±0.85
5 mg CZ	14	245±5	300±15	279±16	73±4*	3.9±0.2 *	2.2±0.2	10.00±0.54	14.15±1.20	1.71±0.33
20 mg CZ	17	248±4	401±18 *	368±16*	72±6 *	5.6±0.4*	2.7±0.1*	10.60±0.38	12.13±0.71	3.78±0.81
20 mg CZ ***	8	262±5	503±20*	464±17*	89±19	5.3±0.3*	3.3±0.2 *	10.10±0.45	12.49±0.58	1.22±0.25
30 mg CZ	8	250±3	439±25*	449±23*	91±9	5.4±0.7*	3.3±0.2*	11.26±0.27	11.43±0.61	5.20±0.33
10 mg SC	18	245±4	339±19	333±20	46±5*	9.3±1.5*	1.9±0.1	11.79±0.70	12.52±0.59	10.33±1.49 *
20 mg CZ + 10 mg SC	12	269±5*	403±12*	420±15*	44±4*	10.4±1.0*	2.6±0.1*	10.93±0.36	10.11±0.37*	13.33±2.43*

± I standard error of mean CZ Chlorothiazide SC SC-14266 *** Drug given in second saline-load.

* Significantly different from control ($P. < 0.05$)

Table 2. The effect of diuretics on thyroidal iodide uptake, water, and alectrolyte excretion in single saline-loaded rats.

Treat-Ment	No. of rats	Body wt. g.	Cl^-	Na^+	K^+	Na/K	Urine ml.	RCI cc/min x100	I^- %inject	Thyroid I^- % inject x 10.
				μEq.						
Control	24	269±4	96±7	79±5	63±4	1.3±0.1	0.8±0.1	5.91±0.40	8.17±0.43	1.61±0.16
10 mg CZ	8	264±3	337±22*	301±23*	74±10	4.3±0.4*	1.8±0.2*	7.45±0.56*	11.08±0.58*	5.66±1.13*
20 mg CZ	8	274±6	318±11*	295±9*	78±8	4.0±0.4*	2.0±0.1*	7.56±0.40*	10.48±0.68*	4.21±1.02*
5 mg SC i.p	8	241±3*	174±19*	157±12*	48±8	4.1±0.8*	1.6±0.2*	10.08±0.42*	14.52±1.57*	2.36±0.41*
10 mg SC i.p.	8	241±5*	209±15*	210±18*	54±5	4.0±0.4*	1.9±0.2*	13.00±0.48*	14.27±0.77*	7.00±0.79*
20 mg SC	8	244±4*	189±10*	163±11*	67±4	2.5±0.2*	2.0±0.2*	9.53±0,57*	11.85±0,62*	16.91±1.35*

± I standard error of mean SC Chlorothiazide SC Sc-14266 *Significantly different from control. ($P. < 0.05$)

i.p. Intraperitoneally.

Table 3. The effect of diuretics on thyroidal iodide uptake, water, and electrolyte excretion in double water-loaded rats.

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	Na/K	Urine ml.	RCI cc/min X 100	I^- % inject	Thyroid I^- % inject X10
				µ Eq						
Control	16	242 ± 3	16±3	10±2	49±4	0.21±0.03	2.4±0.1	2.07±0.27	3.92±0.48	0.47± 0.08
10 mg CZ	16	257±3*	66±7*	69 ±7*	40±3	1.8±0.2*	2.6±0.1	3.42±0.26*	4.32±0.35	2.51±0.32*
20 mg CZ	15	250±3	158±10*	162±10*	68±5*	2.4±0.2*	3.0±0.1*	6.64±0.24*	7.58±0.44*	0.48± 0.06
20 mg CZ ***	8	290±7*	118±19*	139±16*	56±5	2.8±0.6*	2.7±0.1*	6.66±0.56*	7.65±0.61*	15.84±1.88*
30 mg CZ	16	245±3	106±11*	110±9*	51±3	2.3±0.2*	3.1±0.1*	5.73±0.33*	5.98±0.33*	7.03±1.13*
10 mg SC	11	248±5	22±3	42±4*	22±3*	2.9±0.9*	2.3±0.1	2.51±0.25	4.06±0.45	1.90±0.68*
20 mg CZ + 10 mg SC	8	237±4	182±11*	187±12*	38±4	5.5±0.8*	3.1±0.1*	6.30±0.24*	8.53±0.50	7.17±0.81*

± I standard error of mean CZ Chlorothiazide SC SC-14266 *** Drug given in second water-load

* Significantly different from control ($P < 0.05$)

Table 4. The effect of duiretics on thyroidal iodide uptake, water, and electrolyte excretion in single water-loaded rats.

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	$\frac{Na}{K}$	Urine ml.	RCI^- cc/min X 100	I^- % inject	Thyroid I^- % inject X10
			μ Eq							
Control	7	281±6	37±10	21±4	45±9	0.5±0.1	2.3±0.1	2.80±0.77	3.51±0.86	11.30±2.32
10 mg CZ	8	260±5*	170±22*	141±15*	59±8	2.5±0.3*	2.2±0.2	5.37±0.41*	7.35±0.56*	6.10±0.80*
20 mg CZ	16	269±5*	169±9*	157±11*	69±4*	2.4±0.2*	2.8±0.1*	5.74±0.22*	7.82±0.35*	8.25±0.92
10 mg SC	8	228±6	41±9	60±12*	24±5*	2.6±0.3*	2.6±0.2	3.42±0.64	6.40±0.92*	2.54±0.39*
15 mg SC	8	261±3	69±13	58±10*	51±7	1.2±0.1*	1.9±0.2	7.13±0.84*	7.74±0.77*	18.33±1.04*
20 mg SC	4	243±7	67±22	83±31*	22±4	3.5±0.8*	2.0±0.4	6.14±2.20	7.49±2.16	2.31±0.60*

± I standard error of mean CZ Chlorothiazide SC SC-14266 * Significantly different from control ($P < 0.05$)

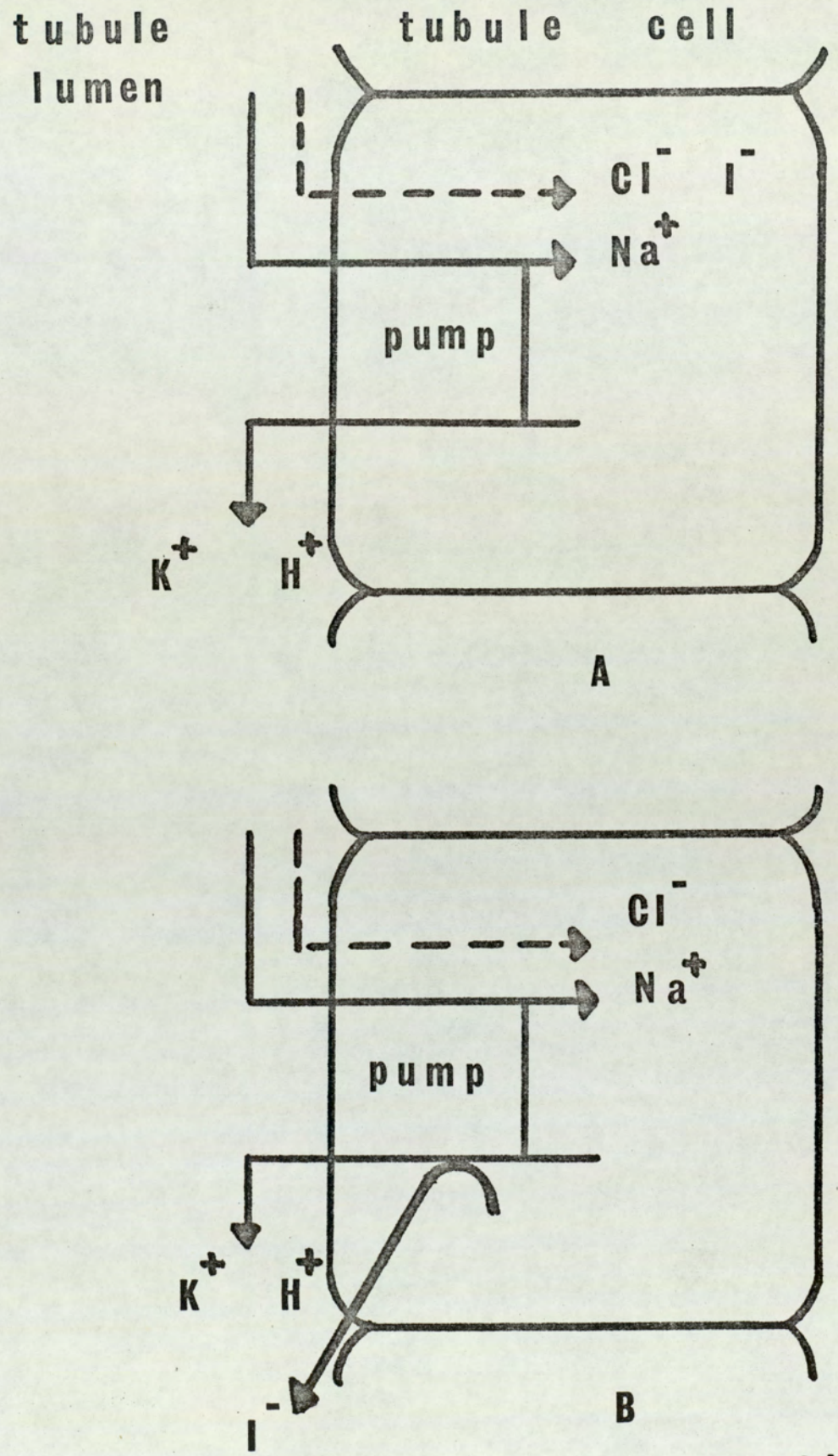
tubule lumen
tubule cell
Cl⁻ I⁻
Na⁺
pump
K⁺ H⁺
A
Cl⁻
Na⁺
pump
K⁺ H⁺
I⁻
B
active transport
passive transport

Fig. A

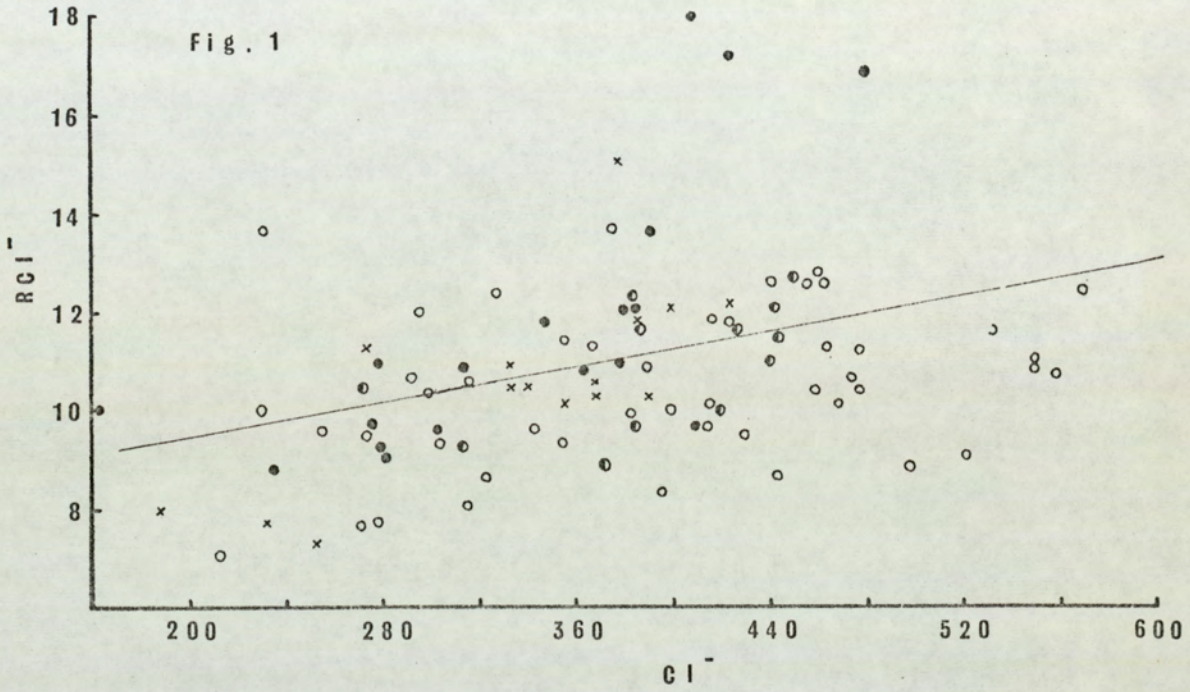
Fig. 1
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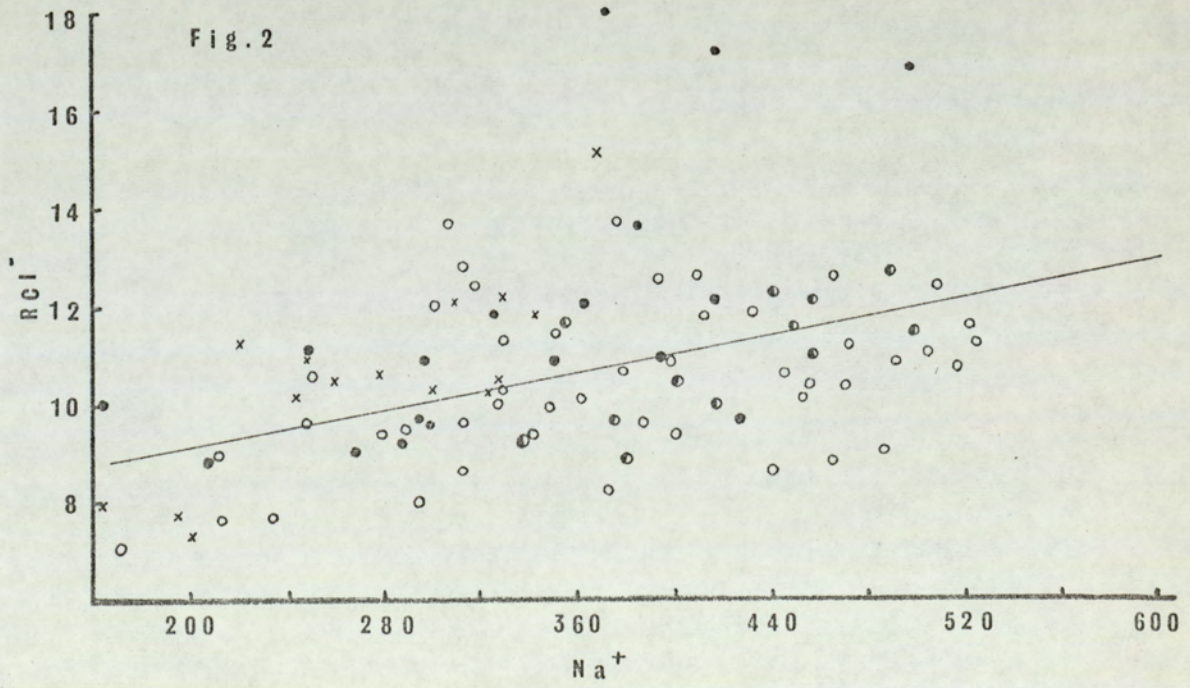
Cl⁻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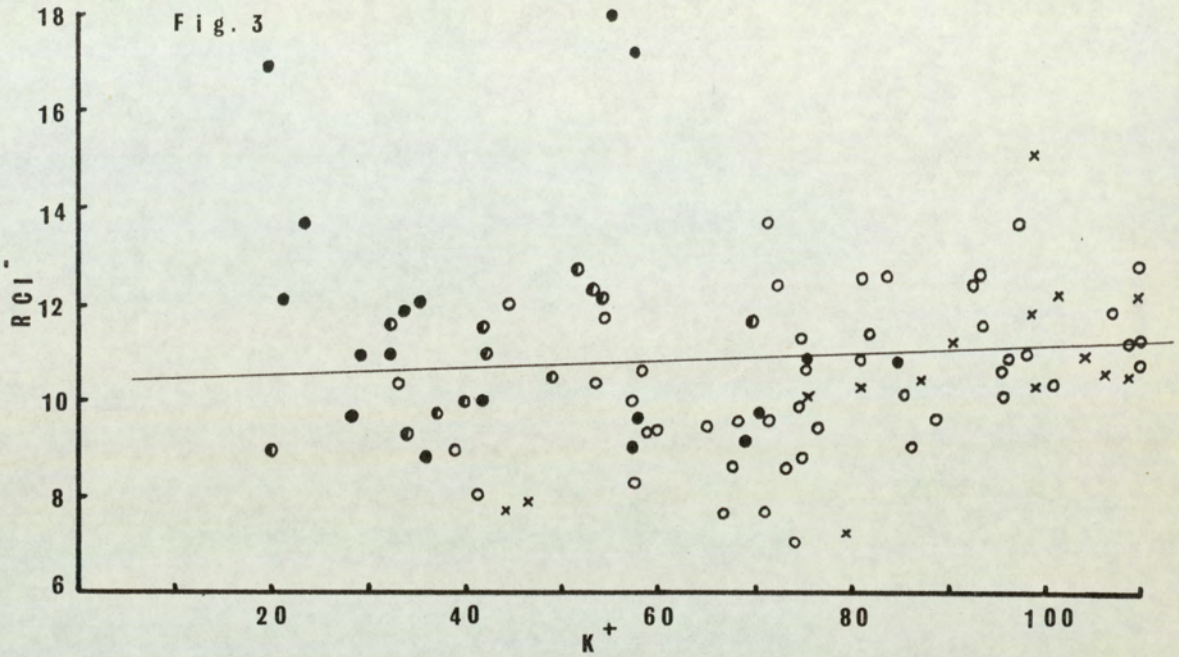
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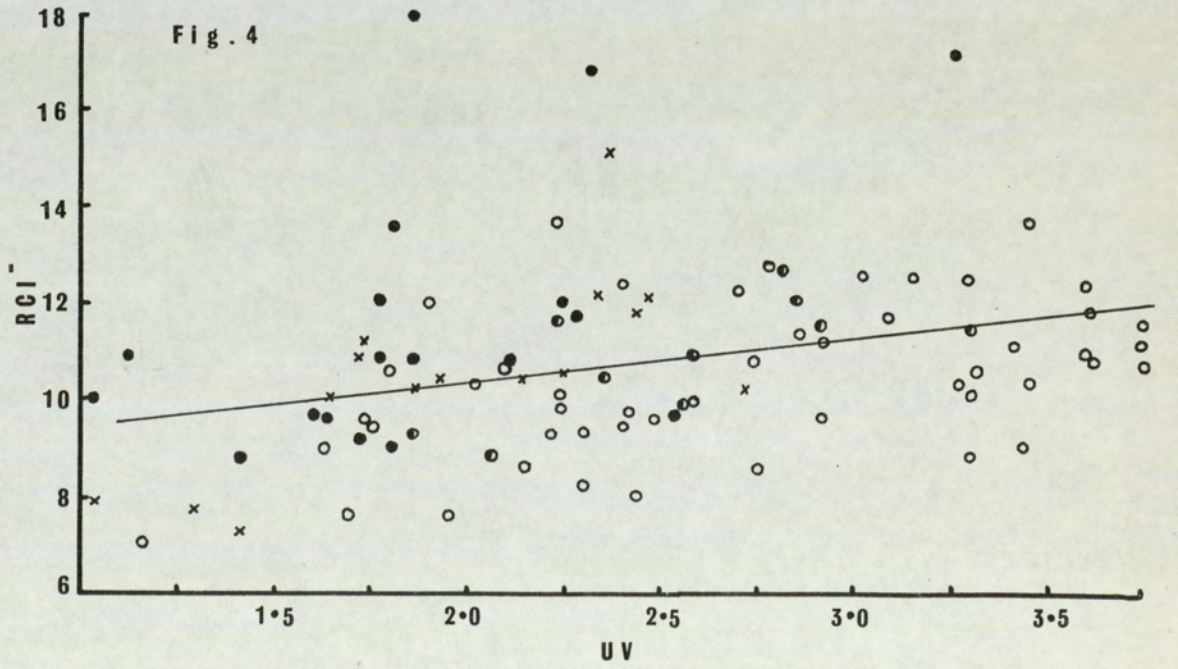
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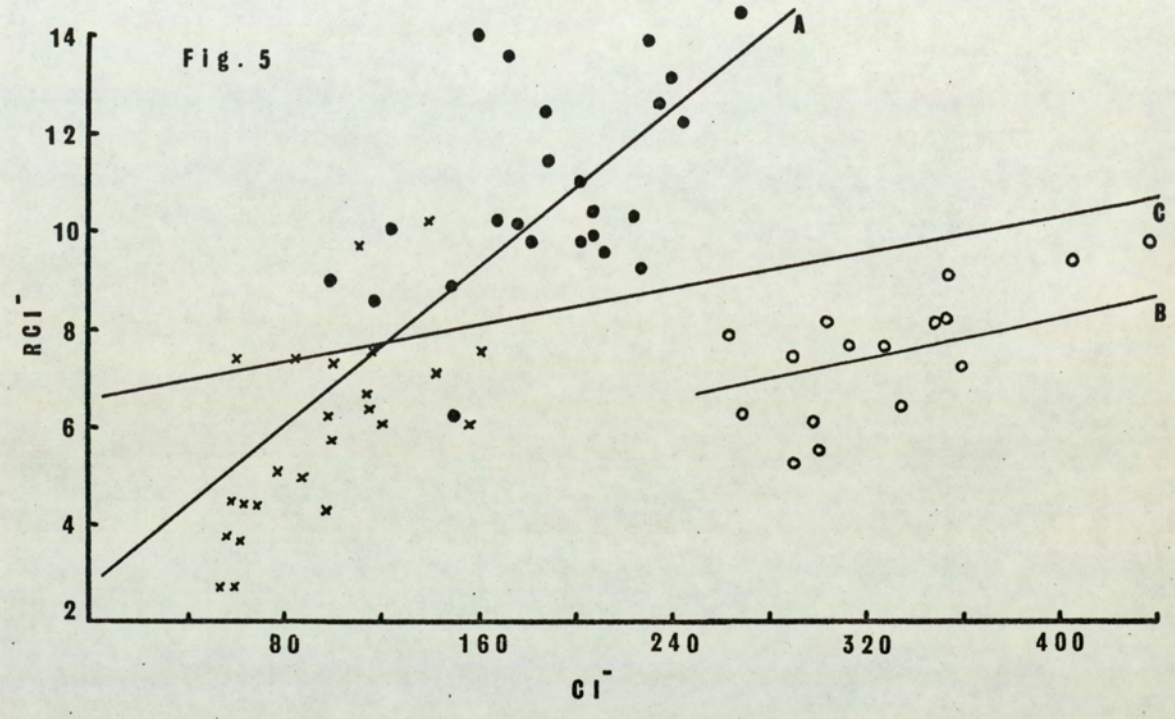
3•5
UV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Cl⁻
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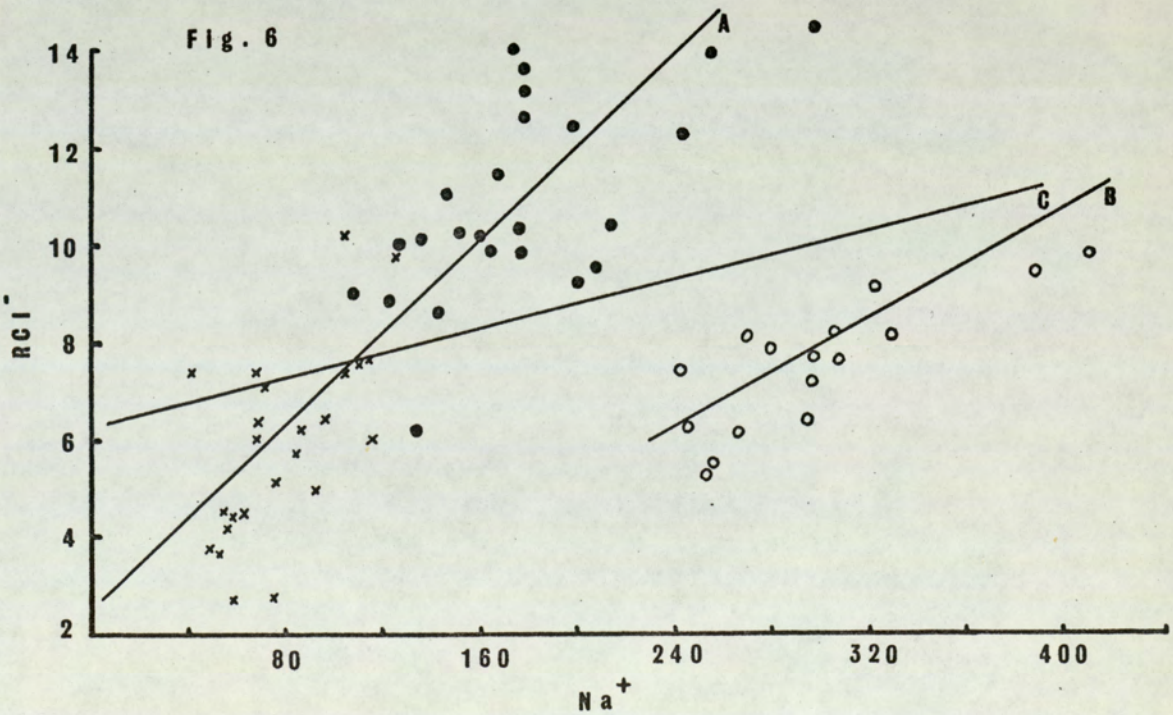
Fig. 6
RCl⁻
Na⁺
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Fig. 7

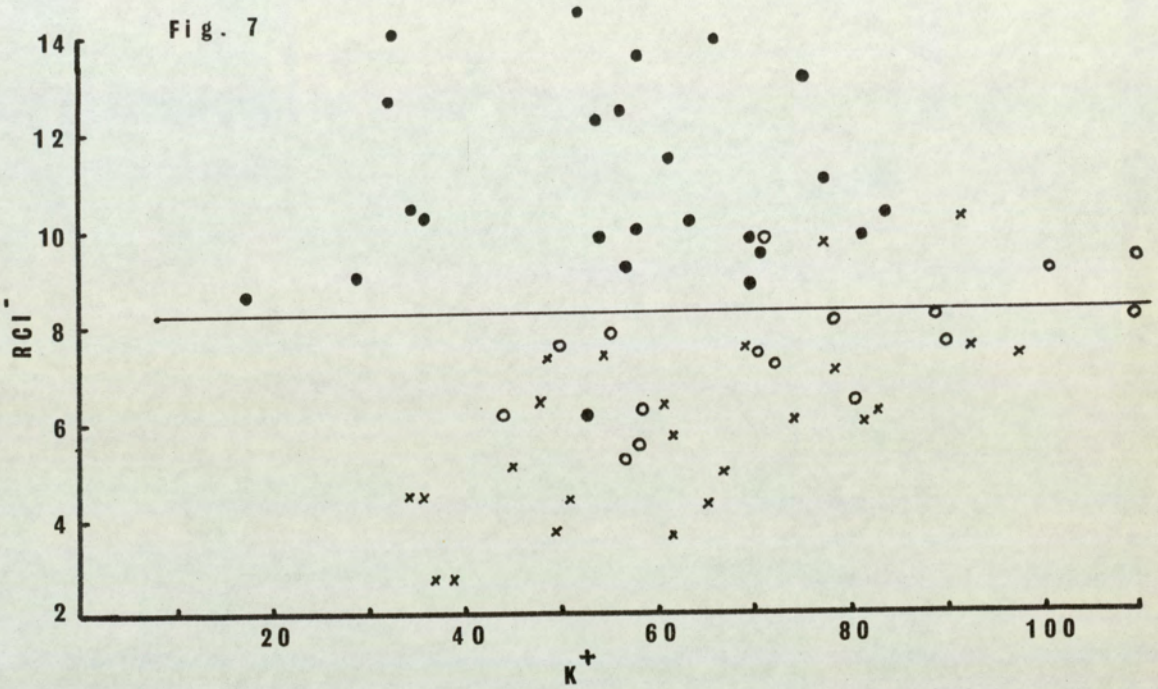
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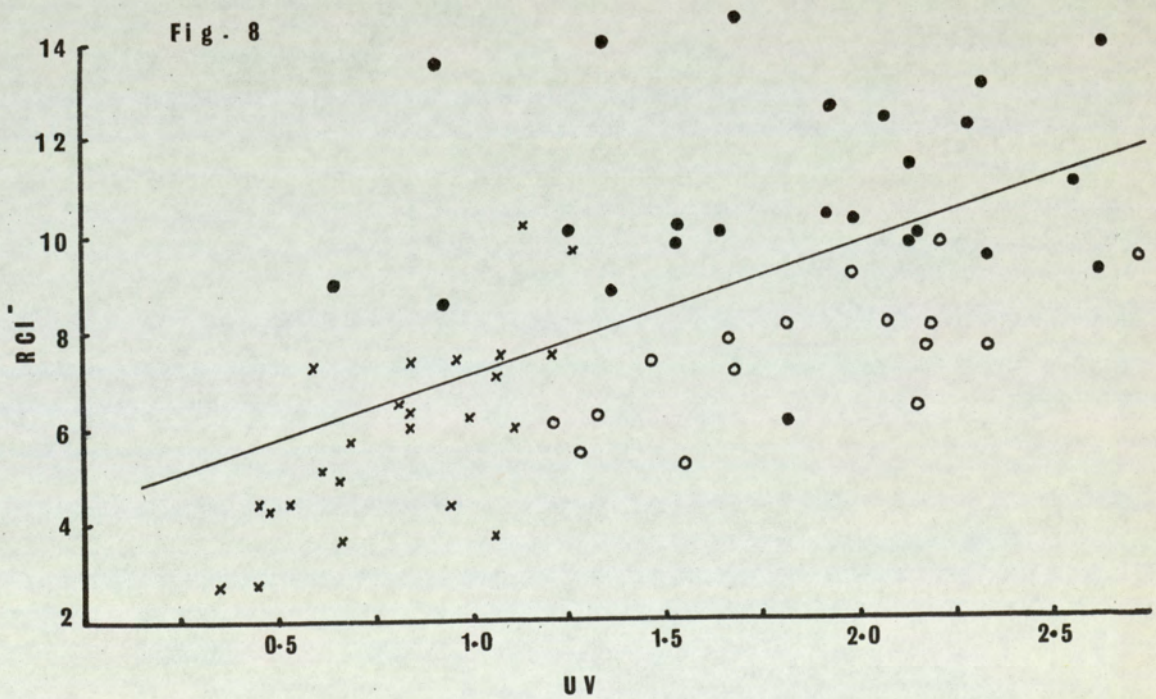
Fig. 8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RCl⁻
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2·0
2·5
UV

Fig . 9

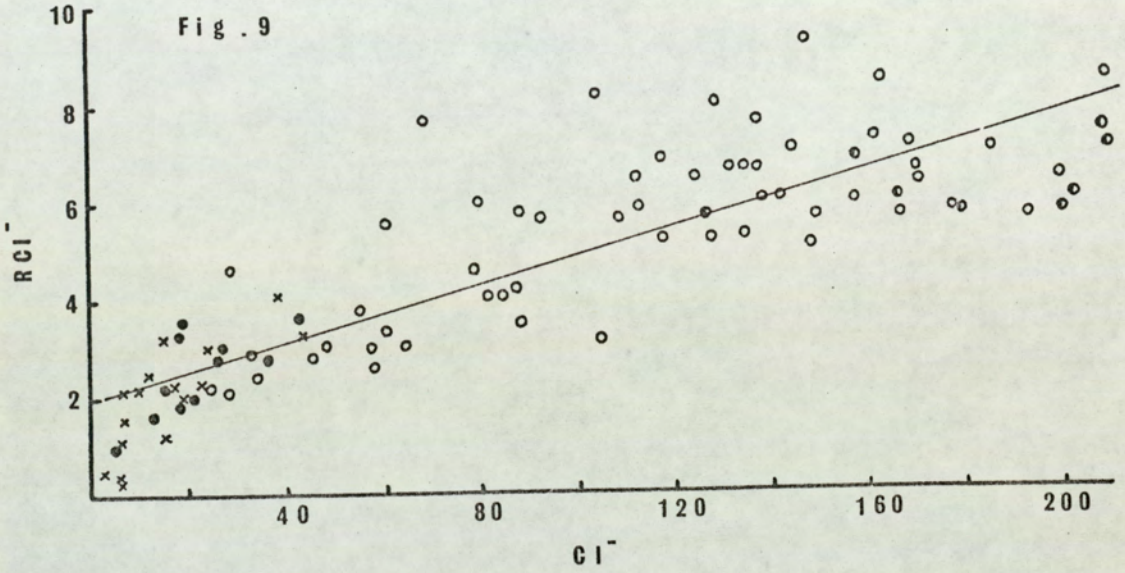

Fig. 10

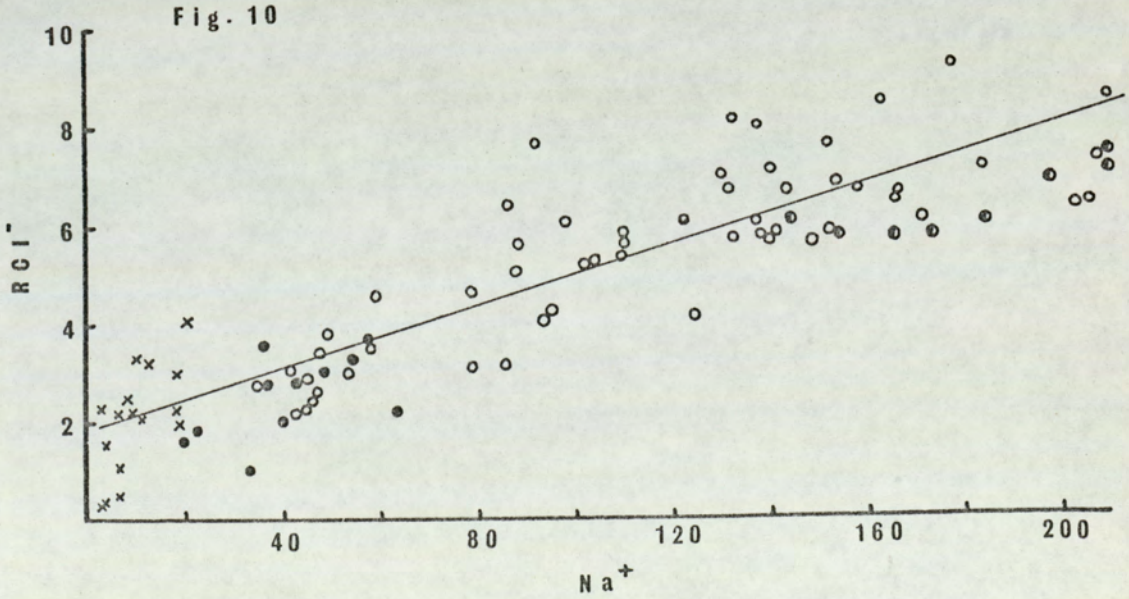

Fig.11

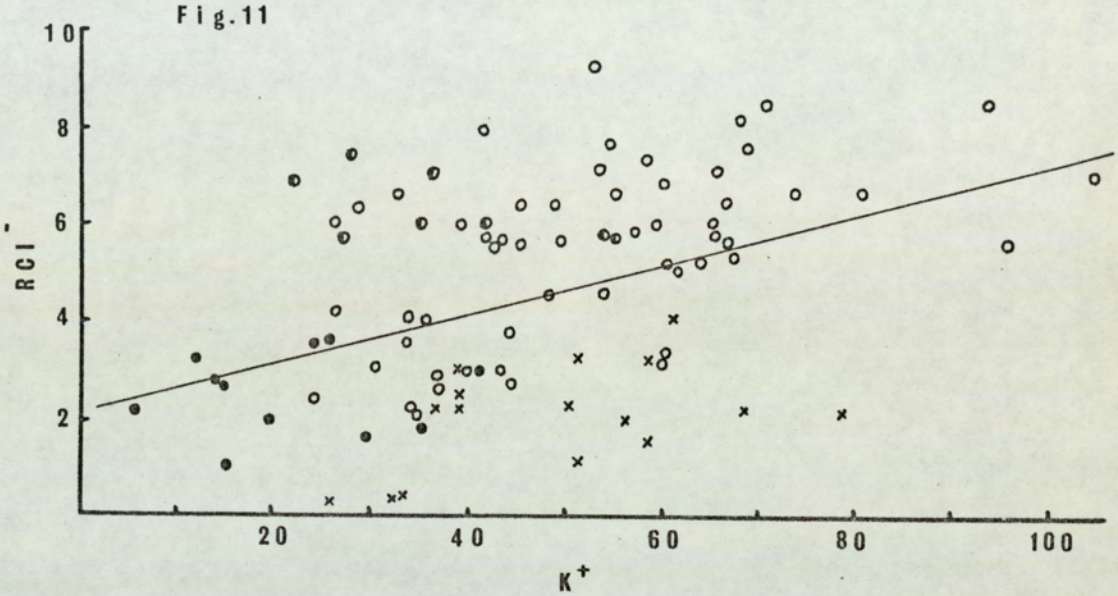

Fig.12

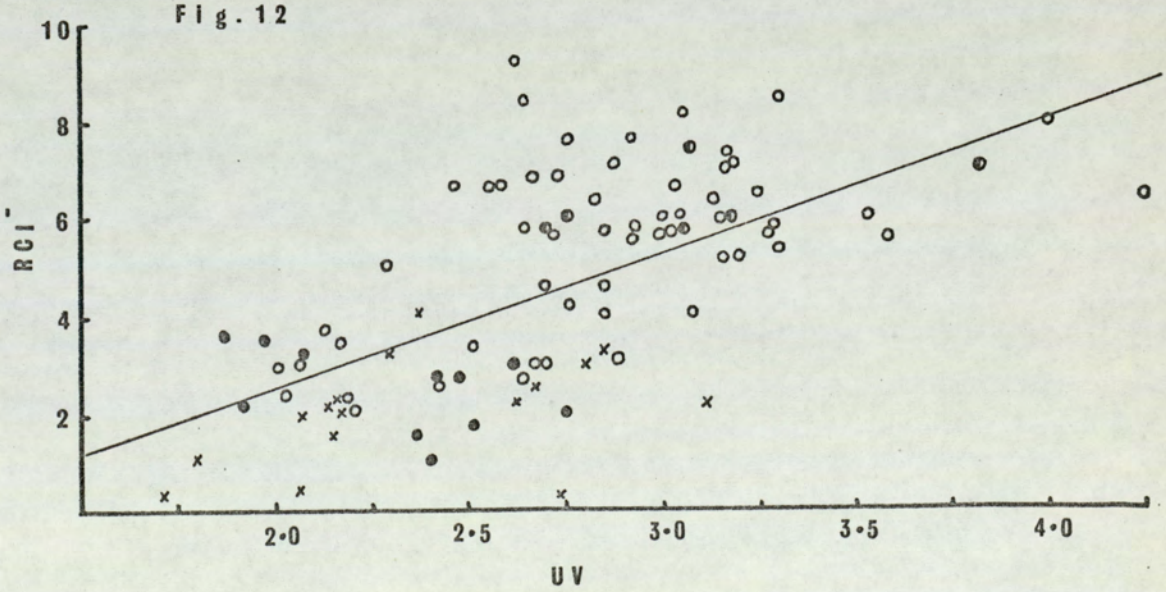

Fig. 13

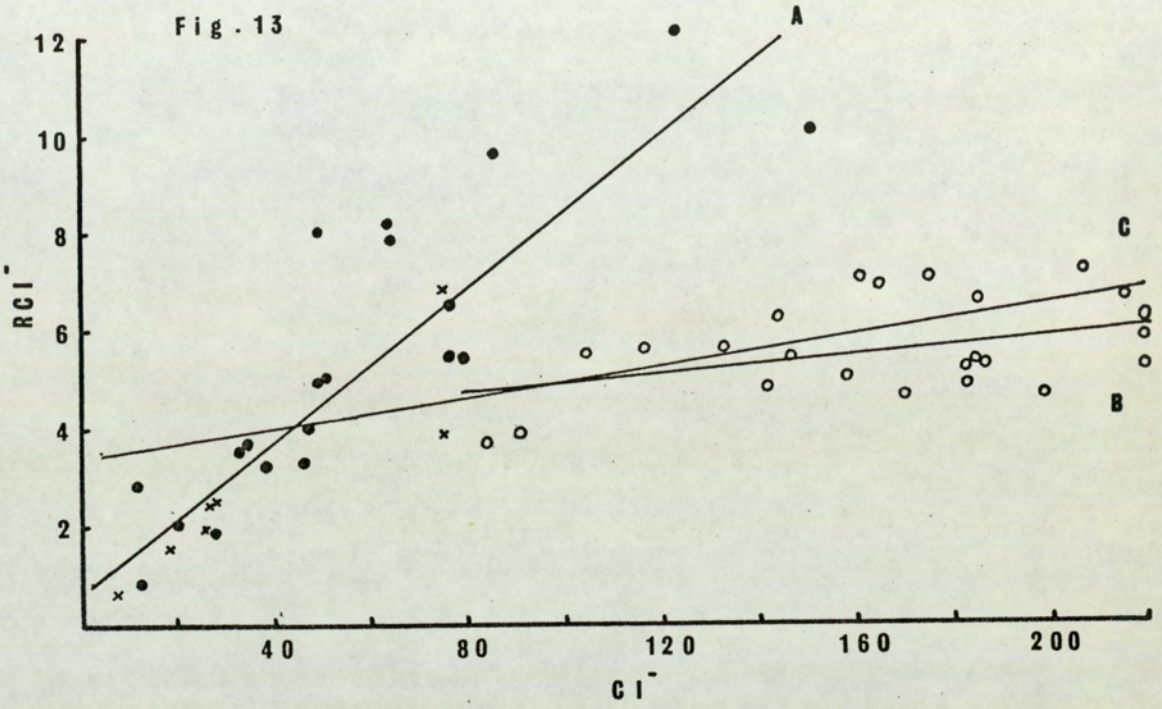
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Fig. 14

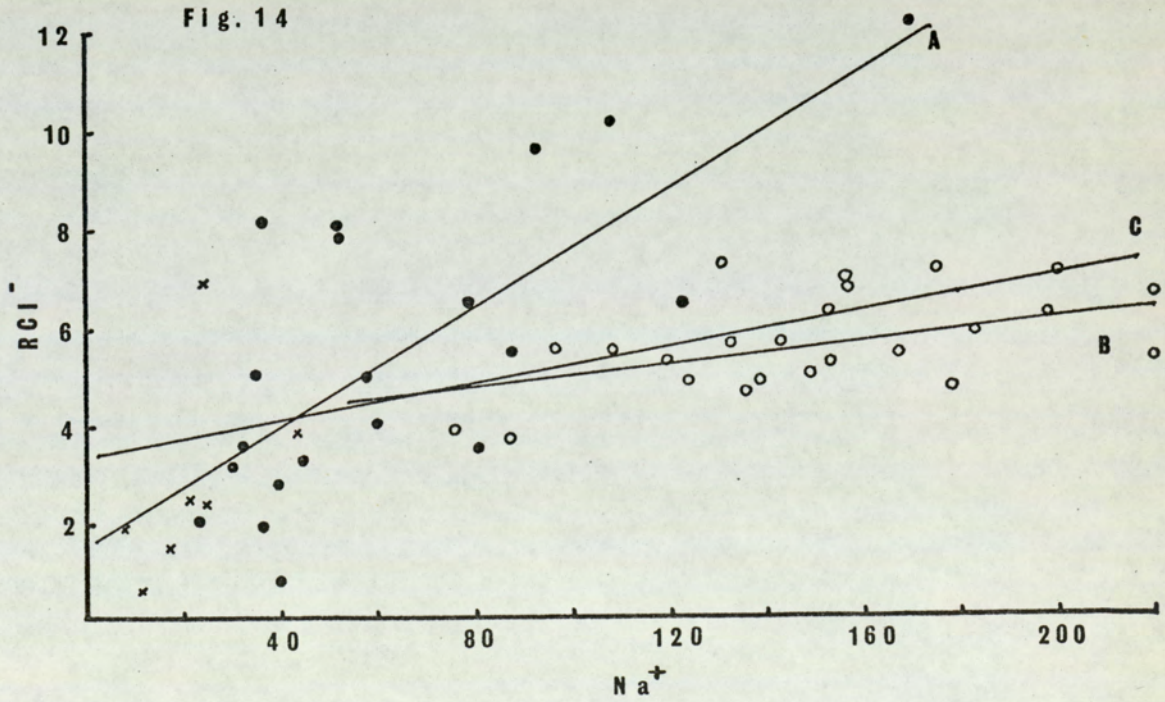
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Fig.15

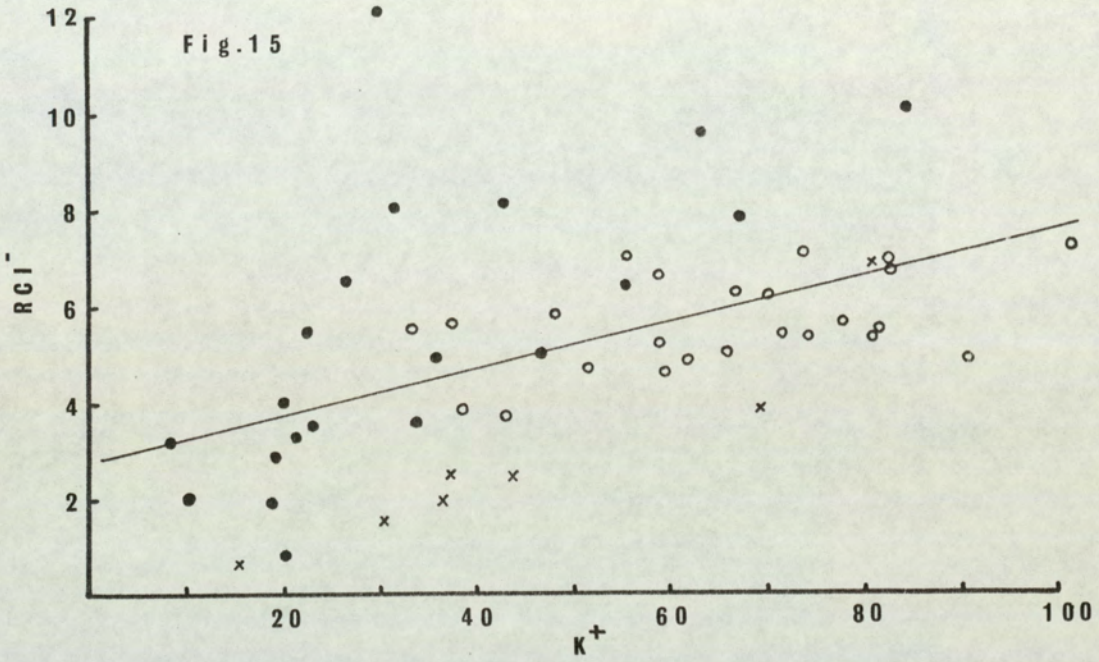
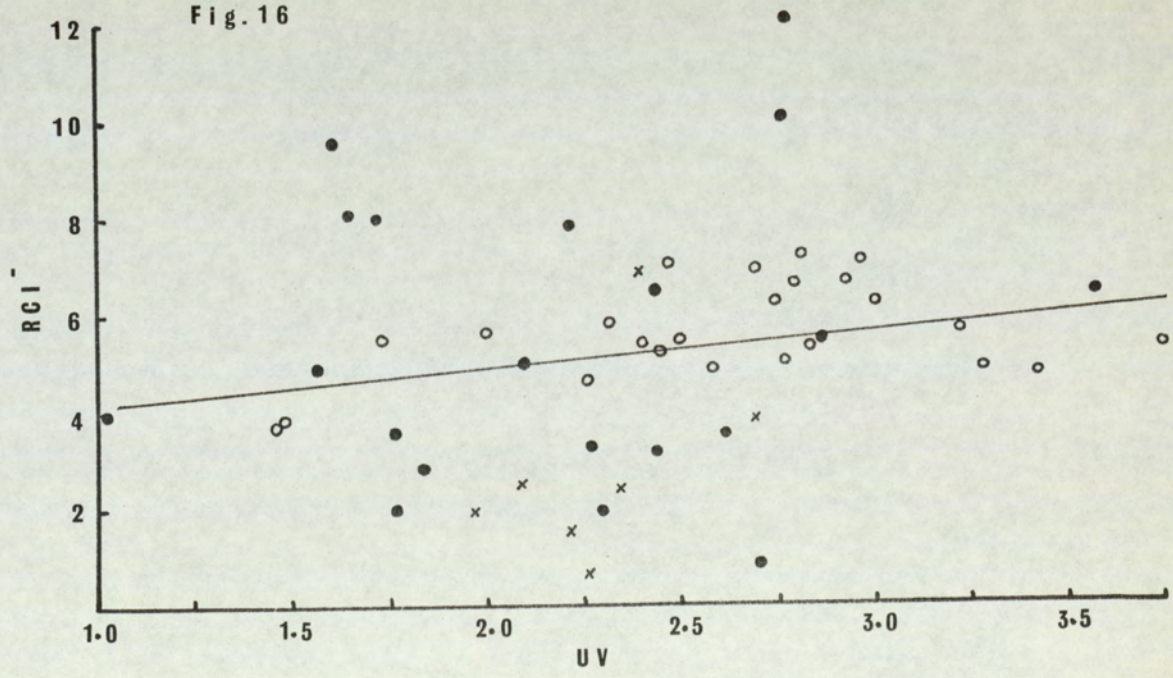


Fig.16



IODIDE EXCRETION: II

THE EFFECT OF PROPYLTHIOURACIL ON IODIDE EXCRETION

Introduction:

Propylthiouracil (P T U) has been used for a number of years as a goitrogenic agent for experimental purposes, and the treatment of hyperthyroidism (70). It is known that this drug induces goitre by preventing iodide, which enters the thyroid gland, from being organically bound, thus reducing the manufacture of thyroid hormone (139). While P T U per se does not prevent iodide accumulating in the gland, after 2 - 8 days of drug administration iodide uptake by the thyroid is reduced. This is because further uptake is inhibited by the high levels of accumulated unbound iodide in the gland (140,141).

Besides the direct action of P T U on the thyroid gland this drug is also known to show a number of peripheral metabolic effects, in particular its effect on deiodination: in vivo it reduces the deiodination of thyroxine and triiodothyronine in the rat (85, 142-146); in vitro the drug reduces the deiodination of thyroxine in rat kidney slices (147) and P T U inhibits the spontaneous deiodination of thyroxine which occurs when this hormone is applied to filter paper (148). Another extra-thyroidal effect of P T U is that it increases the renal clearance of iodide, which BROWN (109) first demonstrated. He found that acute doses of the drug increased the renal clearance of injected I^{131} in the thyroxine-maintained, thyroidectomised rat; an affect

which he attributed to inhibition of an active tubular transport mechanism for kidney iodide. An increased urinary iodide excretion after PTU treatment has also been demonstrated in the intact rat (149). It is pertinent to note that two years after the work of BROWN (109), HALMI et al (24) in their studies of iodide excretion in the rat, used PTU to block organic binding of iodide by the thyroid; they were unaware that by doing this they would in fact increase iodide clearance values.

Since iodide excretion has been shown in the previous section to be closely correlated to chloride excretion, it was of interest to the author to see whether or not PTU produced a chloruretic effect as well as an ioduresis.

Methods and materials:

The methods used were essentially the same as those used in the previous section for single water-loading (Page 24) Intact female rats were used and a single water-load a pH 8.4 containing the PTU was given, and four dose levels of the drug tested. Urinary electrolytes were determined as before. An additional experiment was done where 20 µg of thyroxine in 0.1.ml. distilled water was given at the time of water-loading.

Results:

The results of the effect of PTU on electrolyte excretion are shown in Table 5. Sodium, chloride, potassium, the Na:K ratio and both parameters for iodide excretion were significantly increased above control levels by all doses of the drug ($P < 0.0001$). There was,

however, no significant difference ($P > 0.05$) in chloride excretion between any of the PTU-treated groups. There was no significant difference between any group as regards thyroidal iodide uptake ($P > 0.05$), and the only significant difference in urine output was in the 30 mg. PTU group which was lower than the control group ($P < 0.01$)

The only difference seen between the thyroxine treated groups (Table 6) and their corresponding non-thyroxine treated groups (Table 5) were: potassium excretion was higher in thyroxine treatment than the control group of Table 5 ($P < 0.01$) and thyroidal iodide uptakes of both thyroxine treated groups were higher ($P < 0.01$). Individual rat data from Table 5 was used to plot iodide clearance against chloride and sodium, the regression lines so obtained are shown in Figs. 17 and 18. The correlation coefficients for both slopes are highly significant ($P < 0.001$).

Discussion:

It was thought possible that by using intact animals for this work the administration of PTU might reduce the circulating blood levels of thyroxine in the experimental period. The purpose of the thyroxine series of experiments (Table 6) was to check that the PTU effects seen in (Table 5) were not due to any reuction in blood thyroid hormone. Apart from the differences in iodide uptake by the thyroid and potassium excretion thyroxine did not alter electrolytes which agrees with the finding that in man thyroxine does not affect electrolyte excretion up to 8 hours post-injection (159). It is concluded that the increase in electrolyte excretion seen

with PTU treatment is due to the drug per se and not to any reduction of circulating thyroxine.

These experiments confirm that acute doses of propylthiouracil increase iodide clearance (109) by a mechanism which is independent of its action on the thyroid gland and, furthermore, this work demonstrates that this drug is also a chloruretic agent.

An inverse relationship is said to exist between iodide excretion and thyroidal iodide uptake, but although iodide excretion is increased (Table 5) there was no reduction in the amount of iodide in the thyroid gland. This may be accounted for by the fact that the PTU prevents organic building of the iodide, and therefore prevents loss of iodide by the thyroid.

How or where in the kidney PTU exerts its chloruretic ioduretic action cannot be determined from the work presented here. However, the regression lines obtained when iodide clearance and chloride excretion are plotted, are similar in the case of SC-14266 (Fig. 13 slope A) and PTU treatment (Fig. 17). On the other hand the slope of the line in the case of chlorothiazide treatment (Fig. 13 B) is quite different from that obtained by PTU. It might be possible therefore that PTU acts on the kidney in a similar manner to SC-14266, by an aldosterone-antagonist effect exerted at the distal tubule. Additional evidence from the data in Table 5 to support this hypothesis is that the urinary Na:K ratio is increased and this also occurs with SC-14266 (Table 4) or indeed any aldosterone-antagonist.

More indirect evidence suggesting an aldosterone-antagonist action of PTU is afforded by the work of DEAN and GREEP (150) who found that the zona glomerulosa - the principal site in the adrenal gland for aldosterone production - is temporarily exhausted by PTU treatment but not by surgical thyroidectomy. This exhaustion then could be due to the adrenal gland producing more aldosterone in an attempt to maintain sodium balance while unable to do so because of the blocking action of PTU on aldosterone at the kidney tubule.

It is known that rats chronically treated with PTU show a negative sodium balance and elevated urinary NA:K ratio (151); and that such rats, when given a water or saline load excrete more sodium, chloride, potassium and water than do untreated animals (152-155). These effects of the PTU on the mechanism for renal tubular reabsorbtion of these ions do not appear to be exerted by virtue of any extrathyroidal action. This is illustrated by the fact that physiological doses of thyroxine administered daily prevent this increase in electrolytes excretion in PTU treated rats (155). The chronic treatment of rats with this goitrogen induces degeneration of the kidneys (156), reduces the sensitivity of the kidneys to aldosterone to a tenth of the euthyroid state (154), and reduces the aldosterone secretion rate of the adrenal glands (157). These effects are not seen if thyroxine is given with the PTU.

There is thus irrefutable evidence that chronic, (four

weeks or more), treatment of rats with PTU increases electrolyte excretion - an effect which is due to the hypothyroid state of these animals. In the acute experiments described here the increase in electrolyte excretion is due to an extrathyroidal action of PTU. These two effects of PTU are not irreconcilable and a three stage effect of this drug can be postulated: a) an increase in salt excretion due to an extrathyroidal action on the kidney; b) a tendency for alectrolyte excretion to return to normal by means of increased mineral ocorticoid secretion by the adrenals; and c) an increase in electrolyte excretion due to diminishing levels of circulating thyroxine. It has indeed been shown that the elevated sodium and chloride excretion in chronic PTU-fed rats does return to normal two weeks after commencing drug treatment (158). What is needed to definitely establish that this three stage effect exists is a continuous, daily study of electrolyte excretion in PTU-treated animals. Moreover to establish whether or not PTU in acute doses has an aldosterone-antagonist effect in the kidney requires further experimentation.

Whatever the mechanism might be for the chloruretic, ioduretic action of propylthiouracil on the kidney this work confirms that the excretion of these two halogens is closely correlated.

Table 5. The effect of propylthiouracil (PTU) on thyroidal iodide uptake, water, and electrolyte excretion in water - loaded rats.

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	Na/K	Urine ml.	RCI^- cc/min X 100	I^- % inject	Thyroid I^- % inject X 10
				μ Eq						
Control	12	242±5	27±3	10±1	42±4	0.3±0.03	2.2±0.1	2.53±0.32	3.62±0.50	2.99±0.94
10 mg PTU	8	240±7	76±14*	61±10*	81±4*	0.8±0.1*	2.2±0.2	5.41±0.89*	7.74±1.27*	1.75±0.18
15 mg PTU	8	258±5*	94±12*	75±14*	80±5*	0.9±0.2*	2.3±0.1	7.57±0.91*	10.40±0.93*	2.27±0.34
20 mg PTU	8	241±5	130±19*	120±15*	80±8*	1.5±0.1*	2.4±0.1	10.04±1.14*	12.97±1.51*	1.13±0.15
30 mg PTU	7	251±5	97±19*	103±26*	78±15*	1.3±0.2*	1.5±0.2*	10.33±1.94*	12.39±1.51*	4.27±0.67

Table 6. The effect of PTU and Thyroxine on thyroidal iodide uptake, water, and electrolyte excretion in water - loaded rats.

Thyroxine 20μg	8	239±5	31±2	10±2	57±3	0.2±0.03	2.3±0.1	2.73±0.48	4.05±0.58	17.88±1.82*
Thyroxine 20μg + 15 mg PTU	8	258±4	97±17*	67±13*	80±7*	0.8±0.1*	1.9±0.2	6.96±1.04*	9.02±0.92*	5.80±0.73

± I standard error of mean * Significantly different from control ($P < 0.05$).

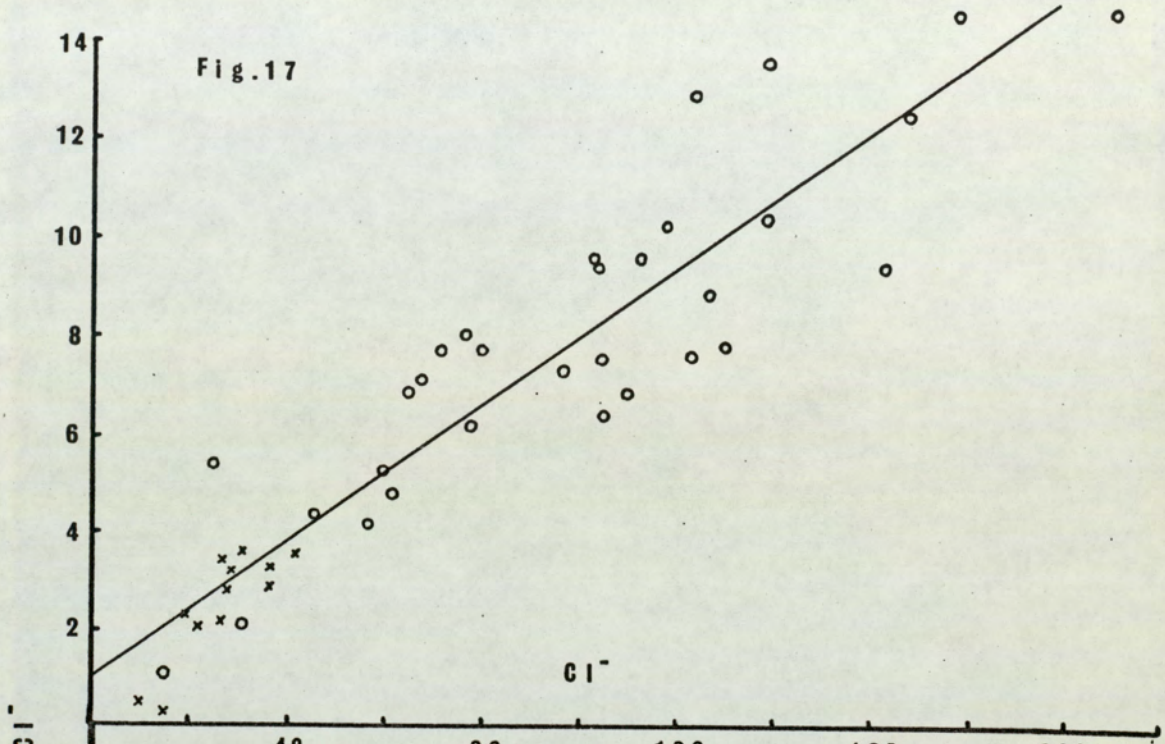


Fig.17
14
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RCl⁻
Cl⁻
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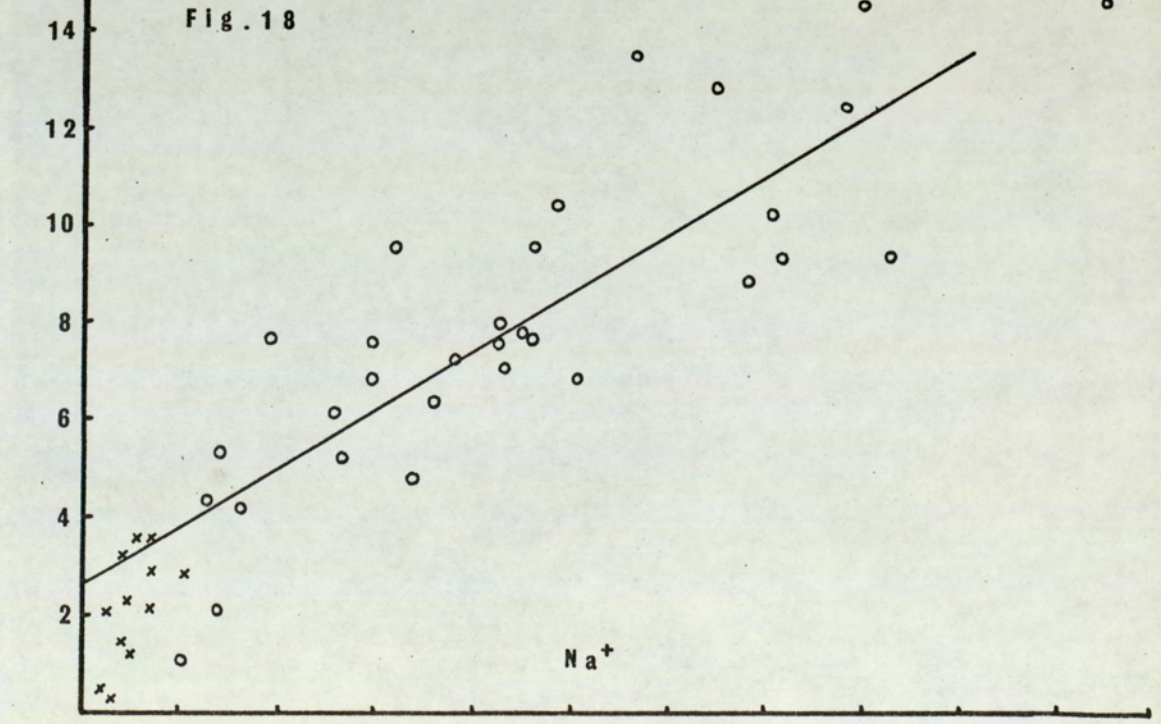
Fig.18
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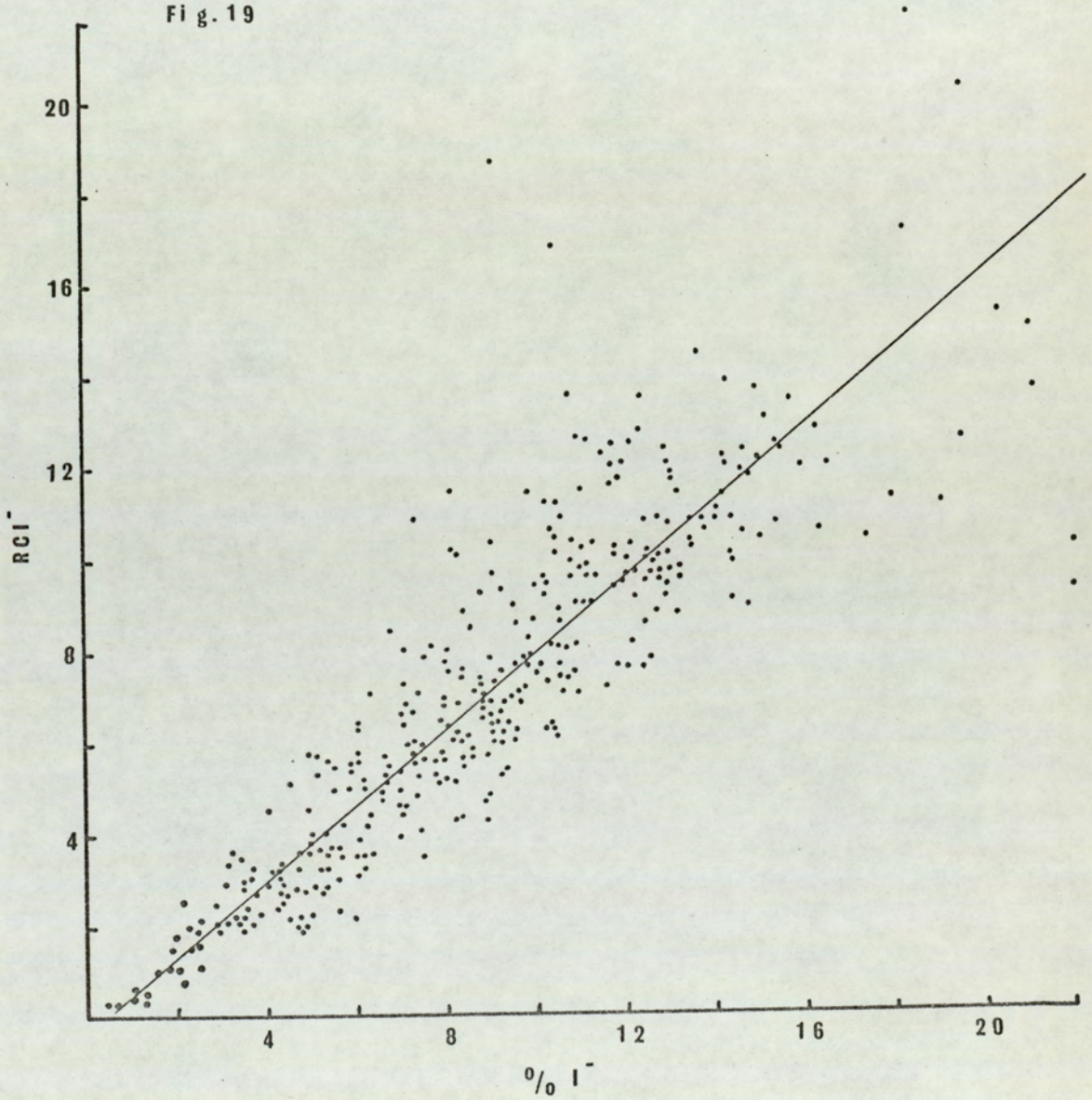
Fig. 19



IODIDE EXCRETION III

THE EFFECT OF OESTROGEN ON IODIDE EXCRETION

Introduction:

The previous sections of this work have shown that blocking renal tubular reabsorbtion of chloride ions also prevents the reabsorbtion of iodide. No evidence was seen for the proposition that chloride is preferentially reabsorbed to iodide by the kidney. Nevertheless, increasing renal tubular chloride reabsorbtion has been shown to increase iodide excretion (27, 29, 94, 95) which in fact supports the concept that chloride is reabsorbed in preference to iodide. In this section the excretion of iodide and chloride will be examined under conditions where renal tubular reabsorbtion of chloride has been enhanced by oestrogen treatment.

It is unequivocal that oestrogens increase the renal tubular reabsorbtion of sodium, chloride and water; this has been demonstrated in a number of species. In the dog oestrogens decrease the excretion of sodium, chloride and urine volume (35, 36, 37, 41). Similar reffects have been demonstrated in man (34, 39, 40, 42, 160 - 163), though one group of workers were unable to show that oestrogen treatment reduced urine volume in men and women (164). The reat is also known to respond to oestrogen administration by reducing sodium and chloride excretion (38, 167).

Compared to the work done on the effect of oestrogens on thyroidal iodide uptake very lettle has been done on the effect of

these hormones on iodide excretion. FELDMAN, however, has studied both these aspects of iodide metabolism after oestrogen treatment in the rat. He showed that short-term treatment with oestrogen did not affect the urinary excretion of an injected amount of I^{131} (43, 65, 67) but a more prolonged treatment reduced I^{131} excretion (44, 45). He did, however, find in another series of experiments that oestrogen increased I^{131} excretion following administration of radiotriiodothyronine (67) but this may have been due to an increased deiodination of this compound for oestrogens have been shown in the rat to increase the deiodination of thyroxine (66). The increase in urinary iodide excretion at puberty in women (5, 28) may, for the same reason, be due to an increased peripheral deiodination of thyroid hormones.

No simultaneous measurement of both iodide and chloride excretion have been made after oestrogen treatment. It is, therefore, the intention of this work to examine the urinary excretion of both these anions after a single injection of oestrogen in both normal male and female rats.

Methods

The techaniques for this work are essentially similar to those used in the single saline and water-loading experiments in the previous sections on diuretics and P T U. In Fig.19 the two parameters of iodide excretion measurement for every rat used in these previous sections have been compared. The correlation between the renal iodide clearance measurement and the percentage of injected

iodide excreted measurement is highly significant ($P < 0.001$). In this section, therefore, only one measurement of iodide excretion has been used - the percentage of injected iodide that is excreted in the urinary collection period.

Female rats of approximately 250g. body weight were divided into two large groups. One group was injected subcutaneously with 400 µg. of oestradiol benzoate dissolved in 0.1 ml. of arachis oil at 9.30 a.m., and batches of these rats taken, 1, 2, 4 and 6 days post - oestrogen treatment for the urinary electrolyte experiments. The night preceeding such an experiment each batch of rats was deprived of solid food and at 9.30 a.m. on the experimental day each rat was injected subcutaneously with approximately 3 µc. NaI^{131} in 0.1 ml. of distilled water and an isotonic saline load (5 ml.) given by stomach tube. Each rat was then placed in a metabolism cage for urine collection for a four-hour period. At the end of this time the rats were killed and urinary electrolytes and thyroidal I^{131} uptake were determined as in previous experiments of this type.

This technique was repeated with another group of oestrogen-treated female rats, but which instead of receiving an isotonic saline-load were given 5 ml. of distilled water in the urinary electrolyte experiments. Control experiments were performed, for both water and saline - loading procedures; rats were given just 0.1 ml. arachis oil subcutaneously and the loading experiments done the next day. Vaginal smears were taken from each rat every day after either oestrogen treatment or arachis oil injection.

Similarly two groups of male rats were given 400 µg. of oestradiol benzoate in oil and the experimental precedures repeated as above.

Results:

Female rats.

The results of the saline-loading series of experiments are shown in Table 7.

One day post-oestrogen treatment iodide excretion is reduced but one day later it had increased above the control level and by the fourth day it had returned to normal. A further increase above normal levels occured on the sixth day post-oestrogen injection. Chloride excretion in the group data did not always follow the same pattern of excretion as iodide. For instance chloride increased above control levels on the second day, but it was not a significant increase, also whereas iodide excretion had returned to normal by the fourth day, chloride excretion was significantly lower than the normal; yet again chloride excretion on the sixth day was not significantly different from the control value but iodide excretion was significantly higher than control excretion. The excretion of sodium in this saline-loading series followed the same pattern as that of chloride. Potassium excretion was only different from the control level on day four and this reduction was responsible for the high Na:K ratio seen at this time. No statistically significant alteration in urine volume occured in the oestrogen-treated groups. The thyroidal iodide uptake was, however, higher than normal on each experimental

day with the greatest uptake on day two.

While in the group data iodide did not always follow the same pattern of excretion as chloride, in the individual rat data from this Table (Table 7) the excretion of iodide is significantly correlated ($P < 0.001$) with the excretion of chloride. This is shown in slope B Fig.21.

The vaginal smears showed that one day after oestrogen injection rats were either in proestrous or oestrous, while all the rats at days two and four had oestrous smears. In the sixth day batch of rats six were in metoestrous and two still in oestrous. It was possible to separate two large groups of rats oestrous and dioestrous - from the control batch and the electrolyte excretion of these two groups are shwon in Table 8. There were no significant differences in any of the values between these two groups ($P > 0.05$).

The results of the water-loading experiments on female rats are shown in Table 9.

The excretion of iodide followed exactly the same pattern as in the saline-loaded group of rats. Chloride, once again, did not always follow iodide excretion in the group data and for instance fell below normal on the fourth day and returned to normal on the sixth, whilst iodide excretion was correspondingly normal and elevated on these days.

The excretion of sodium did not always follow that of chloride, but it did on day one when the Na:K ratio fell below normal due to a reduced sodium excretion. Potassium excretion

tended to remain unchanged except that on day four there was a significant reduction in the excretion of this ion. The only difference in urine volume seen after oestrogen treatment is that it was slightly, but significantly, reduced on day four. Thyroidal iodide uptake, unlike the saline series of experiments was only increased on one day but this did happen to coincide with the greatest increase seen in the saline group - on day two.

As with the saline experiments, the correlation between iodide and chloride excretion in individual rats was highly significant ($P < 0.001$) in these water-loading tests, this correlation is shown in Fig.20 slope B.

The vaginal smears taken in this series were slightly different from those seen in the saline groups for on the sixth day all rats showed a dioestrous smear. It was not possible to separate large oestrous and dioestrous groups from the control rats for statistical comparison because there were only three oestrous and four dioestrous rats, the rest being either in proestrous or metoestrous.

Male rats.

The group data results for the saline series of experiments are shown in Table 10.

Iodide excretion was reduced on all days after oestrogen treatment except that the reduction on the first day was not significant. Sodium and chloride excetion and urine volume followed the same pattern as that of iodide. Thyroidal iodide uptake

remained unchanged or increased by oestrogen treatment.

Individual rat data for chloride and iodide excretion from this Table are shown in Fig.21 slope A. Once again the correlation between these halogens is highly significant ($P < 0.001$).

Table 11 shows the results of the water-loaded groups of male rats treated with oestrogen.

Iodide excretion is reduced on all the days the loading experiments were done, but only on day one is the reduction statistically significant. Sodium and chloride excretion tended to follow the same reduced pattern as seen with iodide excretion. Urine volume only differed from control levels on the first day where it was slightly but significantly reduced.

Once again the correlation between iodide and chloride excretion in individual rats is significant ($P < 0.001$) and this can be seen in Fig.20 slope A.

Discussion

The excretion of iodide in these experiments on female rats follows the same pattern whether under conditions of saline or water induced diuresis. This fluctuation in iodide excretion bears some resimblance to the fluctuating T:S iodide ratio that was found by BOCABELLA and ALGER (47) after a single dose of oestrogen given to ovariectomised rats.

These workers found that after a single 50µg. dose of oestradiol benzoate given subcutaneously the T:S iodide ratio was reduced one day after injection, it then rose above nromal to reach

a peak about two days post-oestrogen treatment and then the ratio gradually fell and returned to normal five days later. The iodide excretion pattern in this work does differ from the T:S iodide movements in that iodide excretion returns to normal after four days and rises again. Nevertheless there is such a similarity between these two measurements of iodide metabolism that a possible link between the two suggests itself.

For instance, this work confirms that exogenous oestrogen increases the thyroidal iodide uptake (44, 49, 54) which is a major factor in raising the T:S iodide ratio - an effect of exogenous oestrogen seen by a number of people (44, 45, 47, 49). But also, if there is a simultaneous increase in iodide excretion, as found in the female rats of these experiments on days two and six, this would tend to reduce serum iodide levels and thus contribute to the raising of the T:S iodide ratio.

The changes in T:S ratio that are seen after oestrogen treatment may well be due, therefore, not only to alternations in iodide content of the thyroid, but also to changes in serum iodide levels as a consequence of increasing or decreasing the urinary excretion of iodide.

There is a tendency in the female experiments for sodium and chloride excretion (but not urine), to move in the same direction as that of iodide excretion in the group data. It is interesting to note, therefore, that ZUCKERMAN et al (165) found that a single

injection of oestradiol in immature rats caused a cycle in water uptake by skin, uterus and vagina. These movements of water most probably occur secondary to changes in sodium and chloride uptake (166). They found that water uptake was reduced one day after oestrogen injection, rose to a maximum one day later and returned to normal by three days post-oestrogen treatment. This cycle is similar, but the inverse, to that seen in urinary excretion of sodium, chloride and iodide of this work.

The pattern of iodide excretion in the male rat series of experiments differs from that of the females in that excretion tends to be reudced all the time atter oestrogen treatment. BOCABELLA and ALGERA (47) also found a sex difference in the T:S iodide ratio. They found that in the spayed male rat a single 50 µg. dose of oestradiol benzoate caused a rise in this ratio to reach a maximum two days after oestrogen injection and a gradual return to normal levels five days later. The pattern of iodide excretion in the male series of experiments reported here shows an inverse relationship to the T:S iodide ratio values found by BOCABELLA and ALGER. These workers do not account for the six difference in T:S iodide ratio in response to oestrogen and this author cannot give any concrete explanation for the sex differences in iodide, sodium and chloride excretion seen in this work. There appears to be in the male rats a continuous stimulation of renal tubular reabsorbtion of these electrolytes whilst in the female rats increased tubular reabsorbtion alternates with an increased excretion of these ions.

This work confirms the results of FELDMAN (44, 45) that oestrogens can reduce the excretion of iodide, but a strict comparison of these results and his cannot be made because of the different experimental procedures used. Feldman, for instance administered estrone to castrate male rats in one piece of work in doses of 1000 or 100 µg. over a four day period followed by a tracer dose of I^{131} and urine collection for a twenty four hour period.

Although in the group data of these experiments chloride excretion does not always follow that of iodide excretion, there is a very close correlation in individual rats between the excretion of these two anions (Fig.20, 21). There is no evidence that increasing renal tubular chloride reabsorbtion leads to an increased excretion of iodide. Indeed the close correlation in the excretion of these two ions confirms the results of the duiretic and P T U work that iodide and chloride are handled in a similar manner by the kidney.

It is possible that the way in which oestrogens exert their effect on electrolyte excretion is mediated through the adrenal glands. ZELEWSKI (38) found that in contrast to the normal rat, the adrenalectomised rat did not show a reduced sodium and chloride excretion following oestradiol treatment. It has been shown, however, that the adrenalectomised dog still reduces sodium and chloride excretion after oestrogen treatment, and although an adrenal mediated effect of oestrogen in this species is not entirely excluded it was concluded that oestrogens have a direct action on the kidney in the

dog (35).

The findings that oestrogens do not alter the glomerular filtration rate in man (163), dog (41) and the rat (167) would exclude glomerular filtration as a factor in the sodium - retaining effect of oestrogens on the kidney.

The most consistent finding after oestrogen treatment as regards electrolyte excretion is that sodium and chloride excretion is reduced but that potassium excretion is either unaltered (160, 163, 38, 39, 40) or increased slightly (162, 35, 41). There is thus a reduced urinary Na : K ratio (167) which is an indirect indication of an increase in aldosterone activity.

The finding that oestrogen had no effect on electrolyte excretion in the adrenalectomised rat (38) supports the proposal that in the rat at least, aldosterone may be involved in the oestrogen-induced sodium and chloride retention - a possibility that was voiced by NOCENTI and CIZEK (167).

Table 7. The effect of oestradiol benzoate on thyroidal iodide uptake, water, and electrolyte excretion in saline-loaded female rats.

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	$\frac{Na}{I}$	Urine ml.	I^- % inject	Thyroid I^- % inject X10
			μ Eq						
Control	20	274±4	109±8	81±5	56±4	1.6±0.1	0.8±0.1	7.33±0.35	2.48±0.47
1 Day	12	270±3	75±10*	61±8*	55±4	1.1±0.1*	1.0±0.1	4.44±0.66*	4.37±0.59*
2 Days	8	270±4	140±17	105±12*	56±9	2.1±0.3	0.7±0.1	9.38±1.12*	20.31±2.68*
4 Days	8	252±6*	52±6*	62±6*	25±3*	2.8±0.5*	0.6±0.1	7.58±0.88	7.97±2.20*
6 Days	8	262±5	87±17	87±11	49±5	1.9±0.3	0.8±0.1	8.96±0.65*	12.63±2.00*

± I standard error of mean * Significantly different from control ($P < 0.05$)

Table 8. Thyroidal iodide uptake, water, and electrolyte excretion in oestrous and dioistrous rats given a single saline-load. (Rats are from the control group in Table 7.).

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	Na/K	Urine ml	I^- % inject	Thyroid I^- % inject X 10●
			μ Eq						
Oestrous	8	268±7	120.1±14.1	84.6±10.5	52.5±6.0	1.68±0.23	0.85±0.14	7.18±0.57	3.14±1.06
Dioestrous	10	281±6	101.5±9.6	76.3±6.7	57.6±4.6	1.40±0.17	0.77±0.14	7.22±0.48	2.18±0.37

± I standard error of mean.

There is nosignificant difference between any of the above values ($P > 0.05$) when the two groups are compared.

Table 9. The effect of oestradiol benzoate on thyroidal iodide uptake, water, and electrolyte excretion in water-loaded female rats.

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	$\frac{Na}{K}$	Urine ml	I^- % inject	Thyroid I^- % inject X10
				μ Eq					
Control	11	264±3	23±2	16±2	30±5	0.6±0.1	1.7±0.1	1.35±0.20	4.98±0.72
1 Day	12	283±10	14±1*	9±2*	32±5	0.2±0.03*	1.6±0.2	0.65±0.21*	4.87±1.32
2 Days	16	275±5	34±4*	12±2	30±4	0.5±0.1	1.5±0.1	2.80±0.41*	11.81±2.02*
4 Days	8	259±5	12±2*	13±4	9±3*	1.9±0.8	1.3±0.1*	1.26±0.36	6.35±2.84
6 Days	6	269±6	17±3	28±6*	23±5	1.4±0.4*	1.6±0.1	2.64±0.31*	5.72±1.18

± I standard error of mean * Significantly different from control ($P < 0.05$)

Table 10. The effect of oestradiol benzoate on thyroidal iodide uptake, water, and electrolyte excretion in saline - loaded male rate.

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	$\frac{Na}{K}$	Urine ml.	I^- % inject	Thyroid I^- % inject X10
			μ Eq						
Control	12	316±9	143±20	84±16	55±7	1.4±0.2	1.8±0.2	6.27±0.60	3.02±0.20
1 Day	11	333±6	101±14	63±8	53±5	1.2±0.1	1.4±0.1	4.86±0.75	6.51±0.54*
2 Days	7	402±8*	41±6*	23±5*	36±6	0.7±0.1*	0.7±0.1*	2.67±0.46*	3.66±0.96
4 Days	8	426±7*	28±8*	18±4*	21±6*	0.9±0.2	0.5±0.1*	2.80±0.53*	3.50±0.94
6 Days	8	407±10*	38±11*	24±7*	24±6*	1.2±0.3	0.5±0.1*	2.76±0.71*	7.18±0.89*

± I standard error of mean * Significantly different from control ($P < 0.05$)

Table 11. The effect of oestradiol benzoate on thyroidal iodide uptake, water, and electrolyte excretion in water - loaded male rats.

Treatment	No. of Rats	Body Wt. g.	Cl^-	Na^+	K^+	$\frac{Na}{K}$	Urine ml.	I^- % inject	Thyroid I^- % inject X 10
				µ Eq					
Control	8	384±6	20±3	10±2	28±5	0.4±0.1	1.4±0.1	1.17±0.29	5.40±0.74
1 Day	8	376±7	11±2*	6±1	29±4	0.3±0.1	0.9±0.2*	0.51±0.13*	9.03±1.52
2 Days	8	410±8*	21±3	6±1	18±4	0.4±0.1	1.3±0.2	0.80±0.19	7.68±1.06
4 Days	8	429±12*	13±3	9±3	7±2	1.5±0.4*	1.9±0.1	0.93±0.25	9.26±0.42*
6 Days	8	419±8*	9±2*	5±1*	15±3	0.5±0.1	1.0±0.1	0.81±0.21	6.32±1.20

± I standard error of mean * Significantly different from control ($P < 0.05$)

Fig. 20

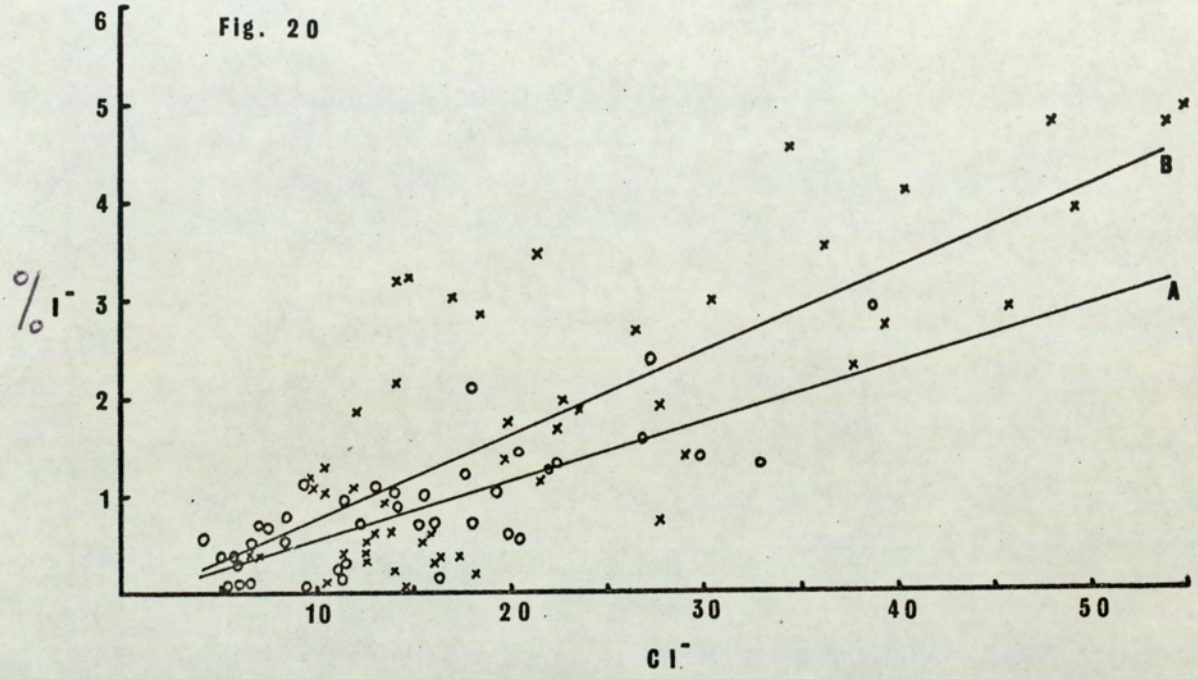

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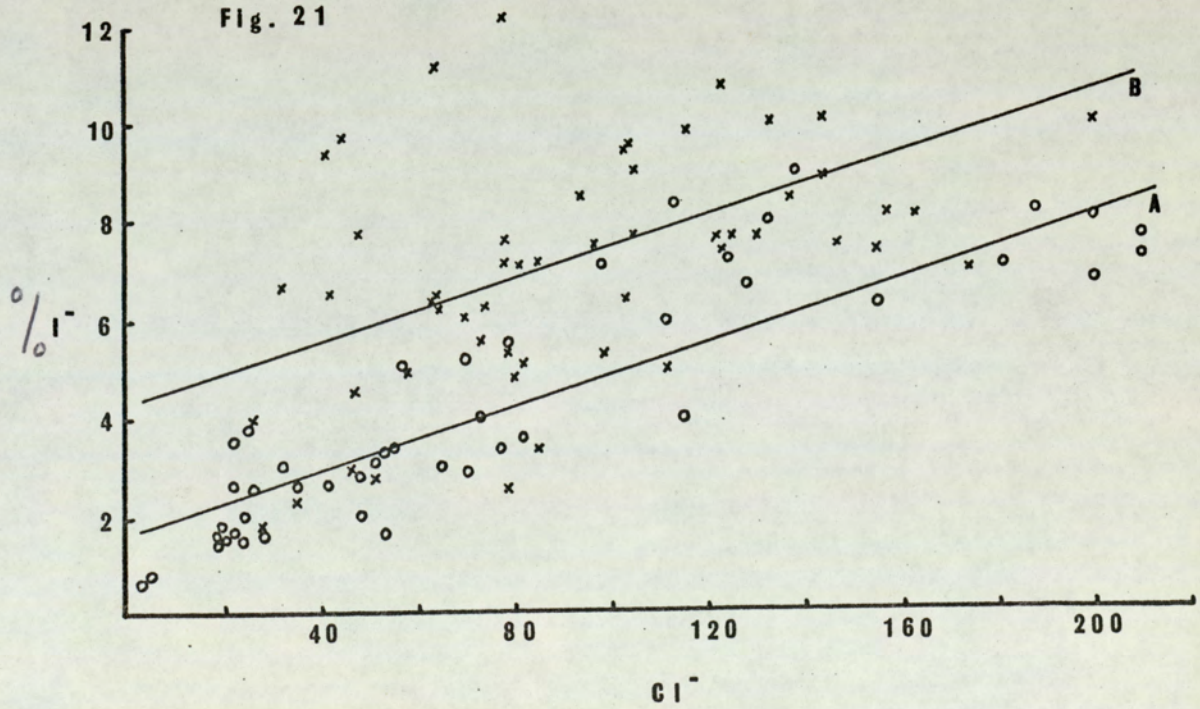
Fig. 21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URINARY ELECTROLYTE EXCRETION IN THE RAT OESTROUS CYCLE

Introduction:

Exogenously administered oestrogen increases iodide uptake by the thyroid gland in the mouse and rat, and increases the T:S iodide ratio in these two species (43, 44, 45, 47, 49, 54, 55, 56, 59, 60, 61, 63). It has been shown moreover that these two values show a cyclic pattern associated with the oestrous cycle in the mouse and rat. In the rat a high thyroidal iodide uptake and T:S iodide ratio are found at oestrous, and low values found at dioestrous (46, 49, 50, 52, 53, 54) when blood oestrogens levels are correspondingly high and low (168, 169). Thus the work on the effect of exogenous oestrogen on thyroidal iodide uptake and T:S iodide ratio fits well with the work on the observed values occuring in the oestrous cycle.

While it is known that exogenously administered oestrogens cause an increased renal retention of sodium and chloride in the rat (38, 167), and other species (34-37, 39-42) no systematic measurement of sodium and chloride excretion in the rat oestrous cycle has been reported in the literature. Would there be a connection, as in the case of the iodide experiments above, between the endogenous cycle of oestrogen levels of the oestrous cycle and the results of exogenous oestrogen treatment as regards sodium and chloride excretion?

As it has been shown that exogenously administered oestrogens in the rat can reduce urinary iodide excretion and that in this work a close correlation has been shown to exist between the excretion of iodide and chloride it was of interest to examine the above question

in the hope that it would indicate the excretary pattern of iodide in the oestrous cycle.

Methods and Materials:

Nine normal female rats of between 240-280g. body weight were placed individually in HOWELLS WRIGHT and HARRISON metabolism cages and daily urine collected for a number of weeks. In three rats a 41 B diet was fed and in the other six a Remington (170) "low iodine" test diet No.347 was given; both diets were given in paste form (1 g food/ 1cc. distilled water) to prevent spillage. Moreover each rat was given a constant amount of the diet daily and the amount restricted so as to ensure that all the diet was eaten on every day. Distilled water, in non-drip bottles was freely available to every animal.

Animals were transferred to clean cages between 5.30 - 7.0 p.m. every day, and at this time urine output was measured and collected, fresh food given, water consumption measured and vaginal smears taken. Daily urine samples were analysed for sodium, potassium and chloride and the water balance (water intake - urine volume) calculated.

Three male rats of 220-230 g. body weight, and three female rats of 230-260 g. body weight, ovariectomsed three months previously were similarly placed in metabolism cages and daily electrolyte and water excretion determined. With these six rats the diet given was the Remington No.347.

Electrolyte excretion was expressed as mEq/Day. Only proestrous (P) or oestrous smears (E) are shown in these results,

smears on any other day are either met or dioestrous.

Results:

The daily electrolyte excretion in three female rats fed on 20 g. of 41 B diet a day are shown in Figs. 22, 23, 24.

The rat in Fig 22 showed a 4 day cycle in its vaginal smear pattern. Associated with this smear cycle was an indication of a cycle of similar length in urinary Na:K ratio - five such cycles in this ratio were clearly shown. A reduced ratio occured at proestrous and sometimes at oestrous, and a high ratio was found at met and dioestrous.

The excretion of sodium and chloride followed each other closely, and whilst a cycle in the excretion of these two ions was not as clear as in the Na:K ratio, from day fifteen three cycles were observed. In these three cycles sodium and chloride excretion was reduced at proestrous or oestrous and increased at met or dioestrous. Potassium excretion in the three cycles after day fifteen increased at proestrous and decreased at met or dioestrous. Thus the cycle in sodium and potassium excretion resulted in the observed cycles of Na:K ration.

Electrolyte excretion in the second female rat maintained on a 41 B diet is shown in Fig. 23.

In this rat there was also a 4-day oestrous cycle as shown by the vaginal smears. There was a tendency for the Na:K ratio in this rat to be reduced at proestrous and higher at other stages of the cycle, but cycles in this ratio were not as clear as in the previous rat.

Sodium and chloride excretion again followed each other, but a correlation between the vaginal smear cycle was not very apparent, but low levels of excretion of both these electrolytes occured consistently when the rat was in oestrous. Potassium excretion showed a cycle with highest excretion occuring at proestrous.

Fig. 24 shows the daily electrolyte excretion of the third rat on the 41 B diet.

This animal also had a 4-day oestrous cycle as demonstrated by the vaginal smears. The Na:K ratio did not show a clear cycle, but low ratio values were found at proestrous. Sodium and chloride excretion moved together, but no real cycle in the excretion of these ions, correlated with the oestrous cycle, was apparent, and this also applied to the excretion of potassium.

In all three rats fed this diet no obvious cycle in water balance was observed which could be connected with the vaginal smear pattern.

The daily electrdyte excretion in six female rats fed a "low iodine" Remington diet are shown in Figs. 25 - 28. In three rats (Figs. 25, 26, 27) vaginal smears were only taken from day 20-49 to avoid permanent cornification of the vagina which is known to occur with excessive smearing (171). In two of these rats (Fig. 25, 26) 10 g. of diet were given and in the rest (Figs. 28 and 27) 15 g. of diet were given daily. In view of the fact that sodium and chloride excretion moved together, chloride excretion has only been shown graphically in three rats (Fig. 28 a,b,c.)

In the rat in Fig. 25, there was 4-day oestrous cycle and throughout the experiment there was seen a very marked cycle in the Na:K ratio. Lowest values in this ratio were seen at proestrous or oestrous, and highest levels occur at met or dioestrous. The main factor contributing to the cycle in this Na:K ratio was the excretion of potassium which increased at proestrous or oestrous and was reduced at met or dioestrous. These chages in potassium excretion were very marked indeed, and there is often a hundred per cent difference between the excretion of this ion at proestrous or oestrous and that of met or dioestrous.

Sodium and chloride excretion in contrast, did not fluctuate as much in the oestrous cycle, but there was all the same a pattern of excretion associated with the oestrous cycle. The excretion of these two ions tended to move in an opposite direction to that of potassium i.e. A reduced excretion occuring at proestrous or oestrous and an increased excretion occuring at met or dioestrous.

The rat in Fig. 26 showed a similar cycle in Na:K ratio, potassium, sodium and chloride excretion in the oestrous cycle as in the previous rat. There was, however, a more marked cycle in the sodium and chloride excretion than in the rat before. Similarly, the rat in Fig. 27 showed the reduced Na:K ratio and sodium chloride excretion at proestrous or oestrous and elevated potassium excretion at this time.

The three female rats (A,B,C) of Fig. 28, tended to show the changes in urinary electrolyte excretion seen in the previous three rats, but the cycle length varied from four-five days.

These graphs demonstrate the close correlation between the excretion of sodium and chloride. Moreover, in the first two cycles of rat (A) and rat (C) the excretion of these two electrolytes in an opposite direction to the excretion of potassium was seen quite well.

In all the female rats fed the Remington diet, there was a cycle in water balance, an increased water retention occuring at proestrous or oestrous.

The results of the electrolyte excretion in three male rats of 220-240 g. body weight, fed the Remington diet are shown in Figs. 29, 30 and 31. Two days of urine collection were missed in these experiments, and in addition, changes in the quantity of diet fed were made. Each male rat was started on 10g. of diet and were given on the fifteenth day 15g/day and at day thirty were returned to 10g/day (see arrows in Figs.)

No cycle in Na:K ratio, potassium, sodium chloride excretion or water balance, comparable to the cycles seen in the female rats on this diet were seen.

Increasing the quantity of diet given to these rats caused a marked increase in the excretion of sodium chloride in all rats, but an increased excretion of potassium was not so large; indeed in one rat (Fig. 31) potassium excretion remained practically unaltered. This demonstrates the importance of maintaining a constant daily dietry intake in this sort of experiment.

As with the male rats three ovariectomised rats of 240-260 g. weight showed no consistent cyle in electrolyte excretion or water balance, and the vaginal smears taken throughout these experiments

showed no proestrous or oestrous appearance.

Discussion:

In all the intact female rats of these experiments, with the exception of one rat, there was an indication of a cycle in the urinary sodium, chloride, potassium and Na:K ratio associated with the oestrous cycle. In the intact female rats fed on a Remington diet the cycles are more pronounced, particularly the cycle in Na:K ratio and potassium excretion. The reasons why rats on this diet exhibit such a marked cycle in these two values compared to animals fed the standard 41B diet, is most probably twofold. Firstly, the potassium content of the Remington diet is $^{1}/_{7}$th that of a 41B diet (see page 10) and this most probably results in changes in the renal handling of this electrolyte in the oestrous cycle being more emphasised and magnified. Secondly, measurements of the faecal weight of rats fed the same quantity of 41B diet or Remington diet show that in the former fed rats 72.5% of the daily food intake is reabsorbed by the gut whilst 97% reabsorbtion occurs in Remington diet fed rats. Thus variability in reabsorbtion of electrolytes from the gut would conceivably be less in rats fed the Remington diet and so contribute to the consistant cyclic electrolyte excretion pattern shown by these animals.

Rats fed on both diets, however, particularly the Remington, demonstrated that the Na:K ratio is reduced at proestrous or oestrous, and that potassium excretion is elevated at this time.

An obvious difference in the urinary Na:K ratio of rats fed on the 41B diet and those fed the Remington, is that in the Remington

series of experiments, the ratio is very high indeed. This again is due to the low level of potassium in the Remington diet. The Na:K ratio of the 41B diet is 0.68 whilst the Remington diet has a ratio of 3.25 (see page 10) and this difference in dietary Na:K ratio is reflected in the urinary Na:K ratio.

The use of the Remington diet in these experiments and the finding that pronounced changes in electrolyte excretion could be seen in the oestrous cycle was fortuitous. It was originally intended to use this diet to measure stable electrolyte excretion together with tracer I^{131} because of the "low iodine" content.

The cycle in urinary sodium and chloride excretion was seen in most of the female rats whatever diet they were fed. An increased renal retention of these ions occuring at proestrous or oestrous, and an increase occuring at met or dioestrous. The cycle in the excretion of these ions in rats fed the Remington diet was not, however, as pronounced as that seen with potassium, probably because the sodium content of the diet was not reduced like potassium. The cycle in water balance seen with the females on the Remington diet was most probably the result of the cycle in sodium chloride i,e. increasing renal sodium chloride reabsorbtion increased renal water reabsorbtion.

The observations that no cycles of water and electrolyte excretion occured in male and ovariectomised female rats demonstrates

that the cyles observed in normal female rats were due to the oestrous cycle; and also that the "low iodine" content of the diet was not a factor in inducing such cycles in water and electrolyte excretion.

Summarising these results:- In the intact female rat there is a retention of sodium, chloride and water, an increased potassium excretion and a reduced urinary Na:K ratio at proestrous or oestrous, and at dioestrous and metoestrous there is an increased excretion of sodium, chloride and water, a reduced excretion of potassium and an increase in the Na:K ratio.

The changes in water and electrolyte excretion occuring at proestrous or oestrous in these rats are similar to those which occur one day after exogenous oestrogen treatment in female rats, but the marked increase in potassium excretion seen in the oestrous cycle is more than is seen after an oestrogen injection.

In a personal communication GRANT and JENNER (172) have confirmed some of these finding from data in some of their rat experiments. They also found that there is a renal retention of sodium chloride, and increased excretion of potassium, and a reduced Na:K ratio at proestrous or oestrous and an increase in the excretion of sodium chloride, reduction in potassium and a raised Na:K ratio at met or dioestrous. They did not, however, conduct any water balance studies in their experiments.

Sodium and water retention by the kidney is known to occur in the human menstrual cycle at the time of ovulation, and premenstrually, periods when oestrogen levels are highest (173, 174). Water retention in the sexual skin of the pig-tailed macaque (MACACA NEMETRINA) has been similarly demonstrated to occur at the time of greatest

oestrogenic stimulation in the menstrual cycle - at ovulation and premenstrually (175, 176). It is likely, therefore, that the sodium chloride and water retention which occurs in proestrous or oestrous in the female rat is due to the action of oestrogens, particularly as the blood oestrogen levels are highest at the proestrous stage of the cycle (168, 169). Other hormonal influences cannot, however, be excluded as the cause for these electrolyte changes in the rat oestrous cycle. Progesterone for instance is known to show a cyclic secretory pattern throughout the rat oestrous cycle (177, 178) and this hormone is known to influence electrolyte excretion (179 - 184).

The evidence that oestrogens are responsible for sodium and chloride retention in the rat oestrous cycle is further strengthened by the fact that these hormones given exogenously, produce similar effects on electrolyte excretion. If oestrogens are responsible for salt and water retention in the cycle, how exactly do they do this?

In the previous section, indirect evidence was put forward for the possibility that oestrogens exert their action on electrolyte excretion indirectly by means of stimulating the adrenal, and particularly aldosterone secretion. There is in fact much more evidence to suggest that oestrogens do indeed stimulate the adrenal glands, and this additional evidence will be considered here.

In rats, exogenously administered oestrogens cause an increase in adrenal weight (185 - 189) and it has been shown that ACTH, which stimulates the adrenal, is increased in the peripheral blood of rats given oestrogen treatment (190). Also the adrenal glands in the rat oestrous cycle increase in weight at oestrous (191) or proestrous (192)

and reduce weight at dioestrous (191, 192).

Measurements of steroid output by the adrenal gland of the rat have shown that injections of oestrogens stimulate adrenal corticoid secretion in vivo (190, 193) and in vitro (194). Studies of steroid output by the rat adrenal gland during the oestrous cycle have shown that corticosterone in the adrenal venous blood is highest at proestrous and lowest at metoestrous and dioestrous (195). A similar pattern of urinary corticoid excretion has been seen in the guinea pig, where corticoid excretion reaches a peak at the time of ovulation (196). The pituitary gland of rats also shows changes in weight throughout the oestrous cycle, increasing in weight at oestrous and reducing in weight at dieoestrous (197).

There is evidence then that exogenous oestrogens and the increased blood oestrogens, at proestrous and oestrous in the rat oestrous cycle, stimulate the adrenal directly or indirectly via the pituitary (190), resulting in increased adrenal corticosterone secretion. The reduced urinary Na:K ratio found in the oestrous cycle of the rat is indicative of an increase in mineralo- corticoid activity. Although corticosterone has a mineralocorticoid action in that it does reduce urinary sodium chloride and increases potassium excretion, it is not very potent in this action compared to aldosterone (198). However, no published data on the blood levels of aldosterone in the rat oestrous cycle exists.

However, a number of reports, some conflicting, do occur in the literature concerning the effect of administered oestrogen on aldosterone levels. LLAURADO et al (199) have found a 70% increase in faceal

aldosterone in the oestrogen-treated male rat, together with an increase in blood corticosterone levels forty times that of control rats. As corticosterone is a precursor of aldosterone, it reinforces the findingsof the increase in aldosterone excretion. An increased aldosterone secretion rate has been seen in a man treated with stilbestrol phosphate (200); and an increase in the urinary aldosterone excretion has been observed in another male subject receiving oestradiol (201). Other studies on human sujects have shown no effect of oestrogens on aldosterone levels (201, 202).

Some indirect evidence that aldosterone is increased at proestrous or oestrous in the rat oestrous cycle is afforded by the evidence that blood levels of ACTH in the rat increase after oestrogen tratement (190) and is highest at the proestrous stage of the oestrous cycle (203); and it is known that ACTH can stimulate aldosterone secretion by the adrenal gland (204, 205). Also, the zona glomerulosa of the adrenal gland, the main site of aldosterone production, is narrower at oestrous than at dioestrous (191), which may indicate a cycle in aldosterone secretion associated with the oestrous cycle.

Unpublished data by MATTY, HINSUL and CROCKER (206) perhaps resolves the question - is aldosterone increased in the proestrous and oestrous phase of the rat oestrous cycle? Their preliminary data shown below indicates that aldosterone secretion rate does in fact increase at these times:-

No. of rats	stage of cycle	aldosterone µg./ml/hr/ad/Kg.Bwt
5	DIOESTROUS	0.6267 .. ..
5	PROESTROUS	1.3006 .. ..
6	OESTROUS	1.0147 .. ..
4	METOESTROUS	0.8464 .. ..

Progesterone which increases at dioestrous in the rat (177, 178) is known to antagonise the effects of aldosterone and other mineralocorticoids at the kidney tubule. Thus it increases the excretion of sodium, chloride, raises the urinary Na:K ratio, and reduces the excretion of potassium (179, 181, 183). The changes in electrolyte excretion in the rat oestrous cycle could, therefore, be explained solely by the cycle in blood progesterone levels.

REINEKE and SOLIMAN (54) demonstrated that exogenous oestrogen increased and progesterone depressed iodide uptake by the tyroid gland of the rat and, furthermore, showed that in the oestrous cycle thyroidal iodide uptake increased at oestrous - the time when oestrogen levels are elevated - and decreased at dioestrous when progesterone levels are highest. These workers suggested, therefore, that these changes in thyroid function during the oestrous cycle are mediated by changes and proportions of oestrogen and progesterone.

The changes in electrolyte excretion seen in the oestrous cycle of the rats in these experiments could similarly be due to both oestrogens and progesterone activity throughout the cycle.

Whatever the exact cause, there is a renal retention of chloride

at proestrous and oestrous in the rat. If iodide follows the same excretary pattern as chloride, as shown in previous sections of this work then iodide retention also occurs at these times. The iodide retention would thus contribute to the increased thyroidal iodide uptake which is known to occur at oestrous in the rat (46, 49, 50, 52, 53, 54).

Fig. 22

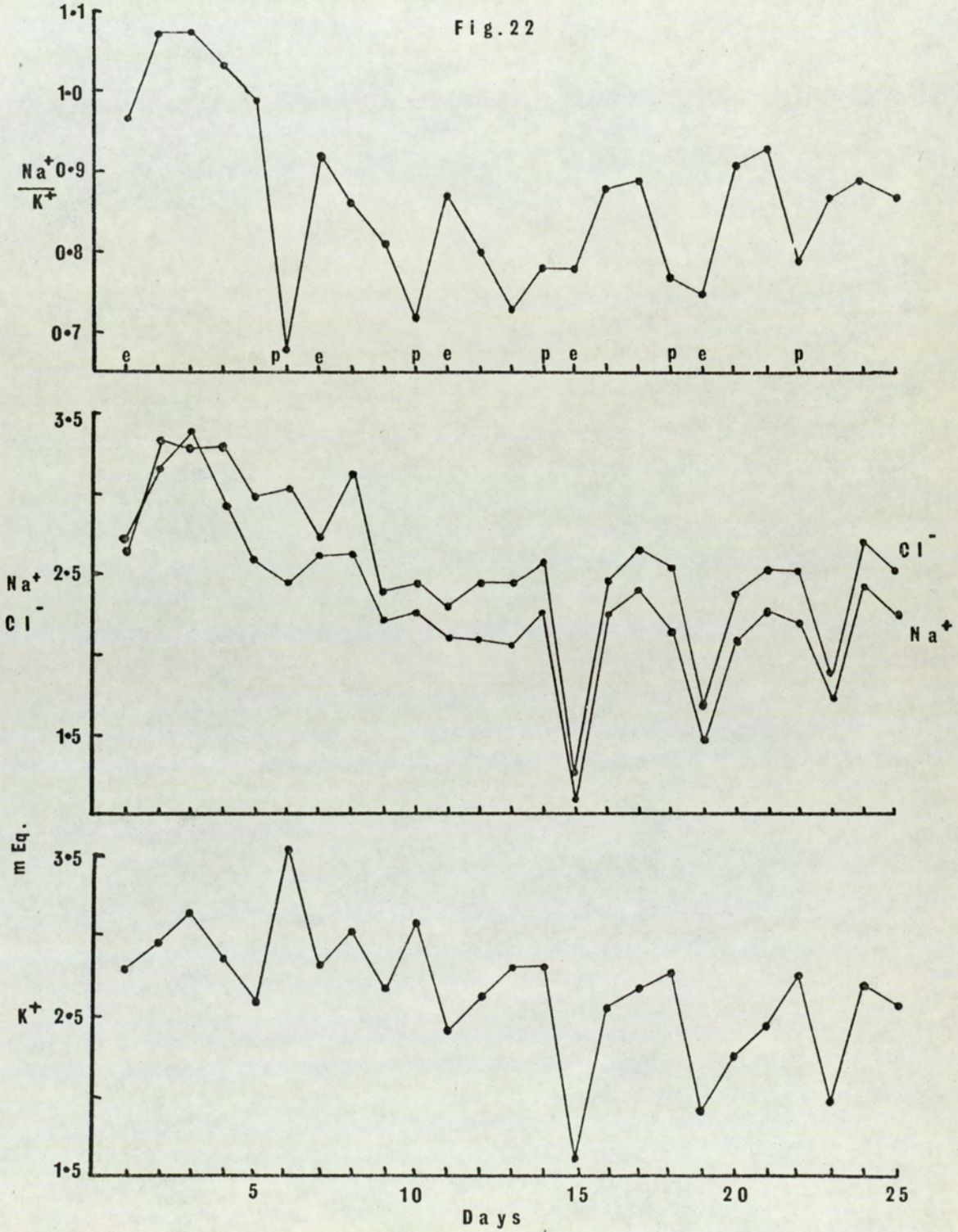
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Fig. 23

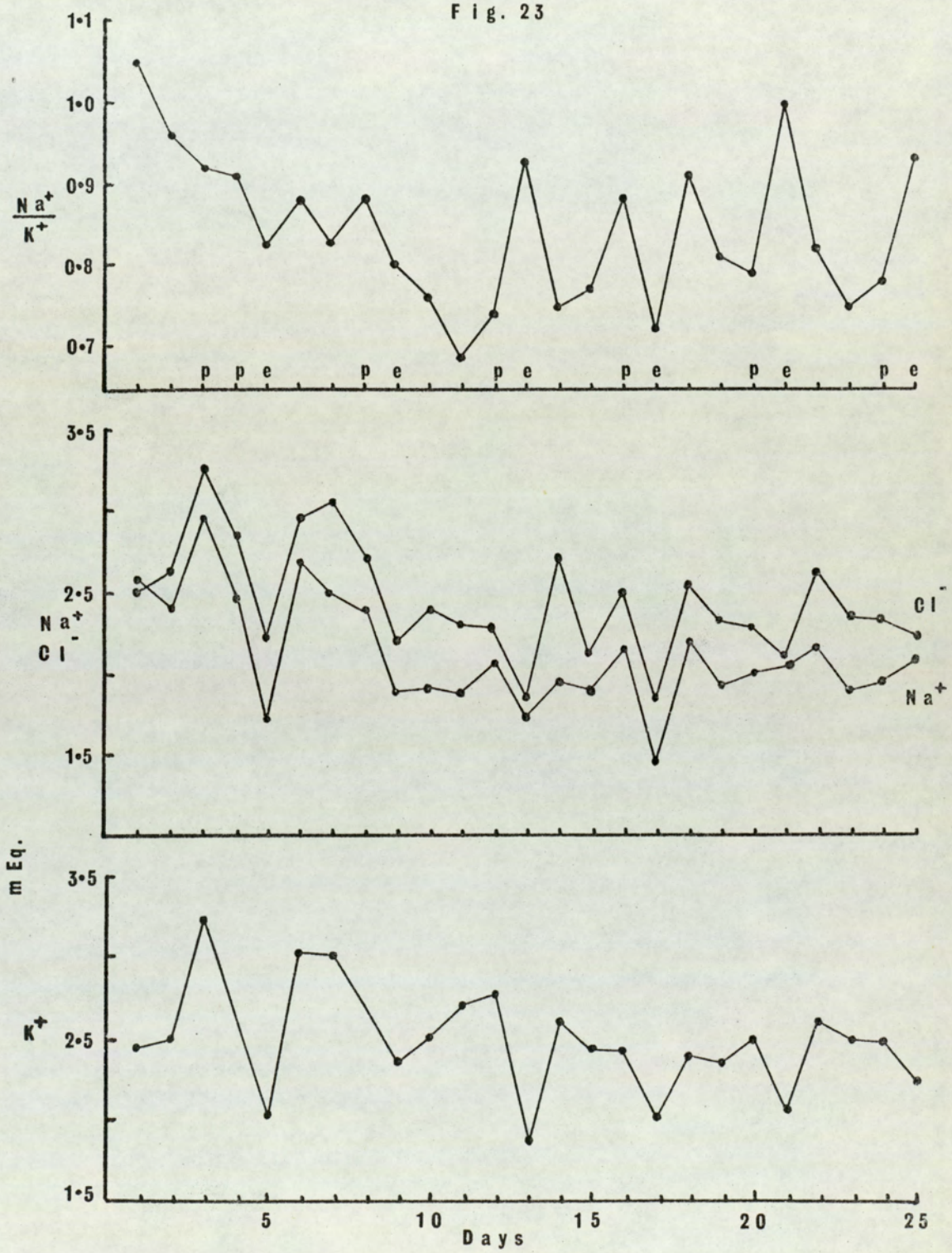
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Fig. 24

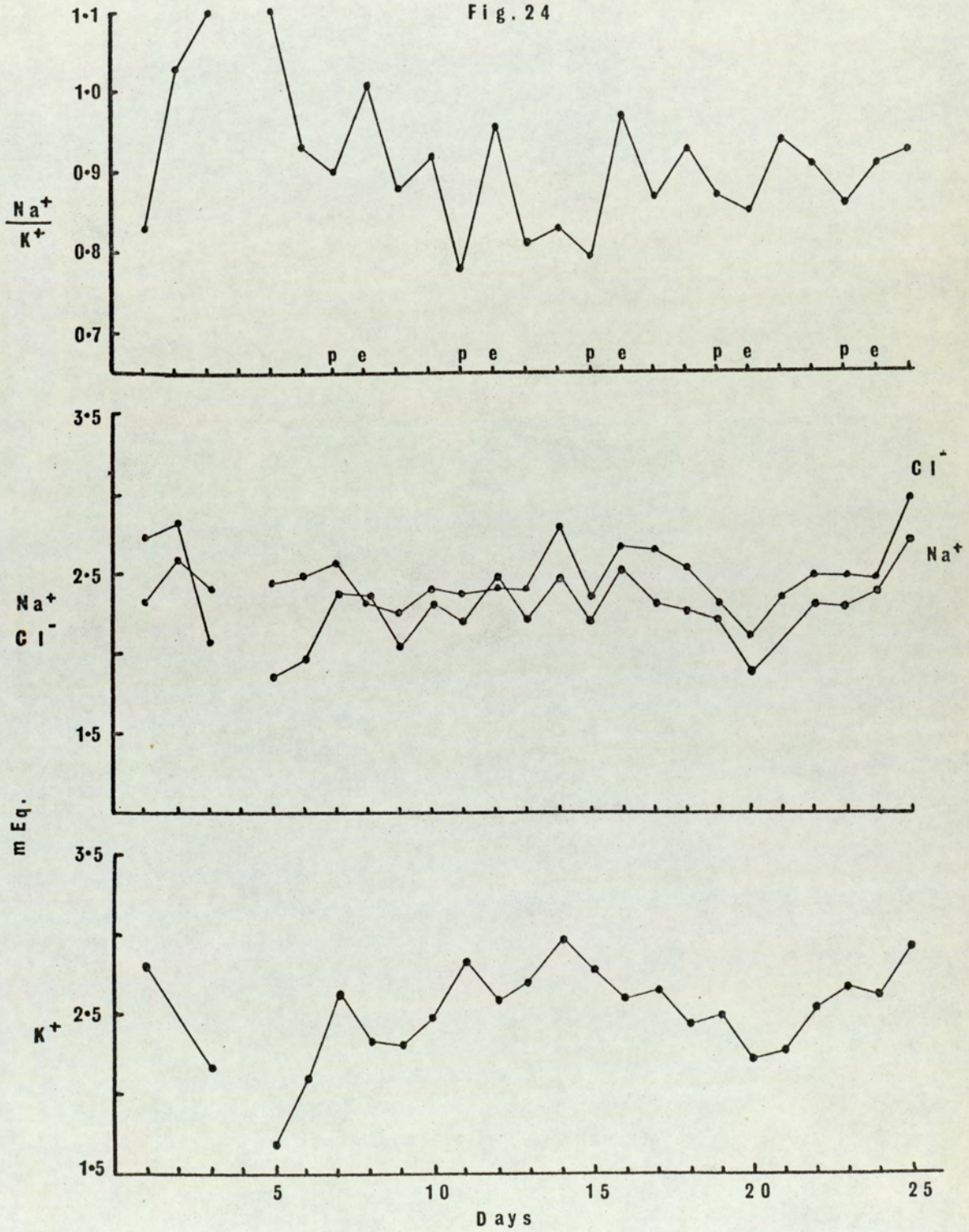
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Fig. 25

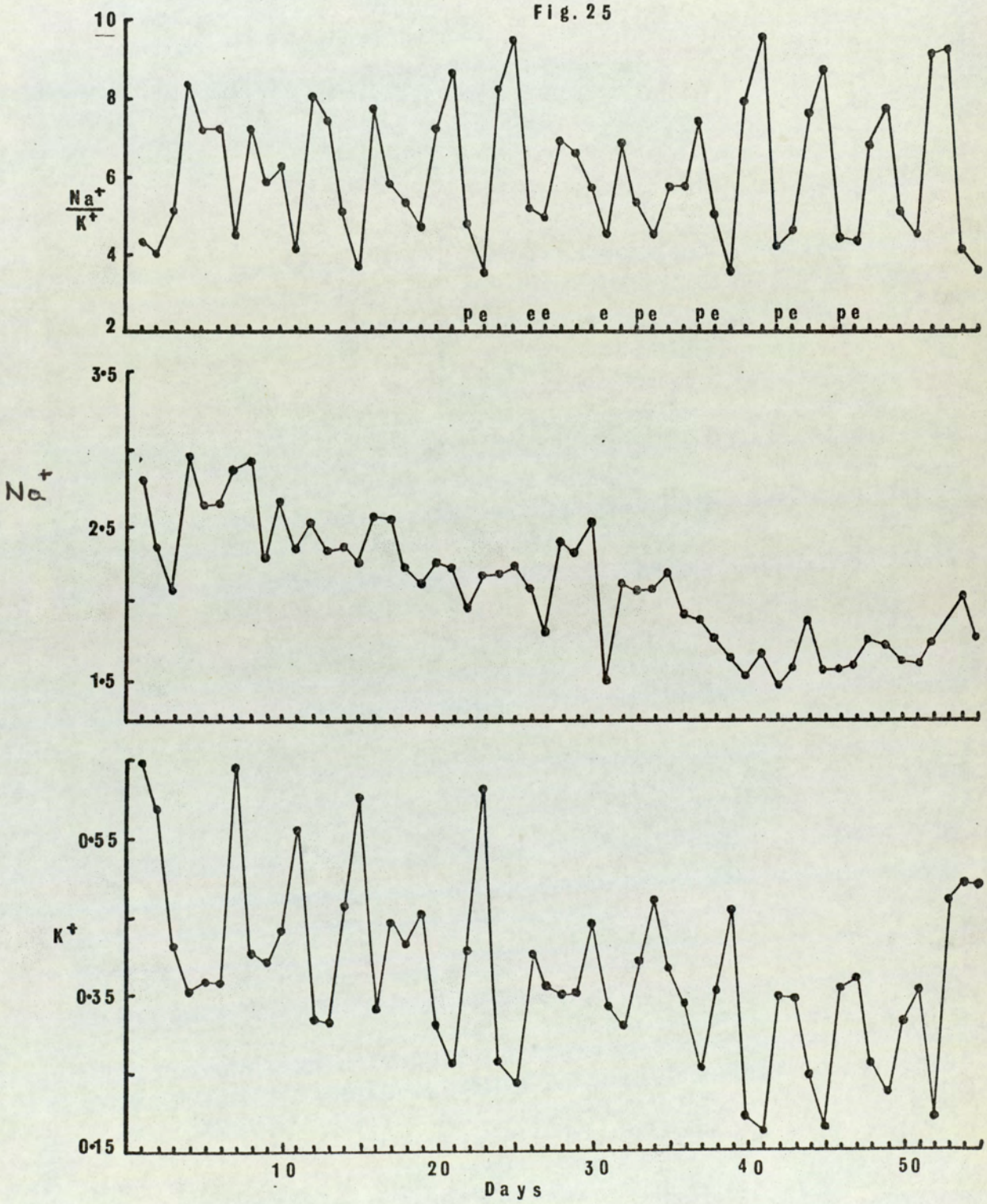
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Fig.26

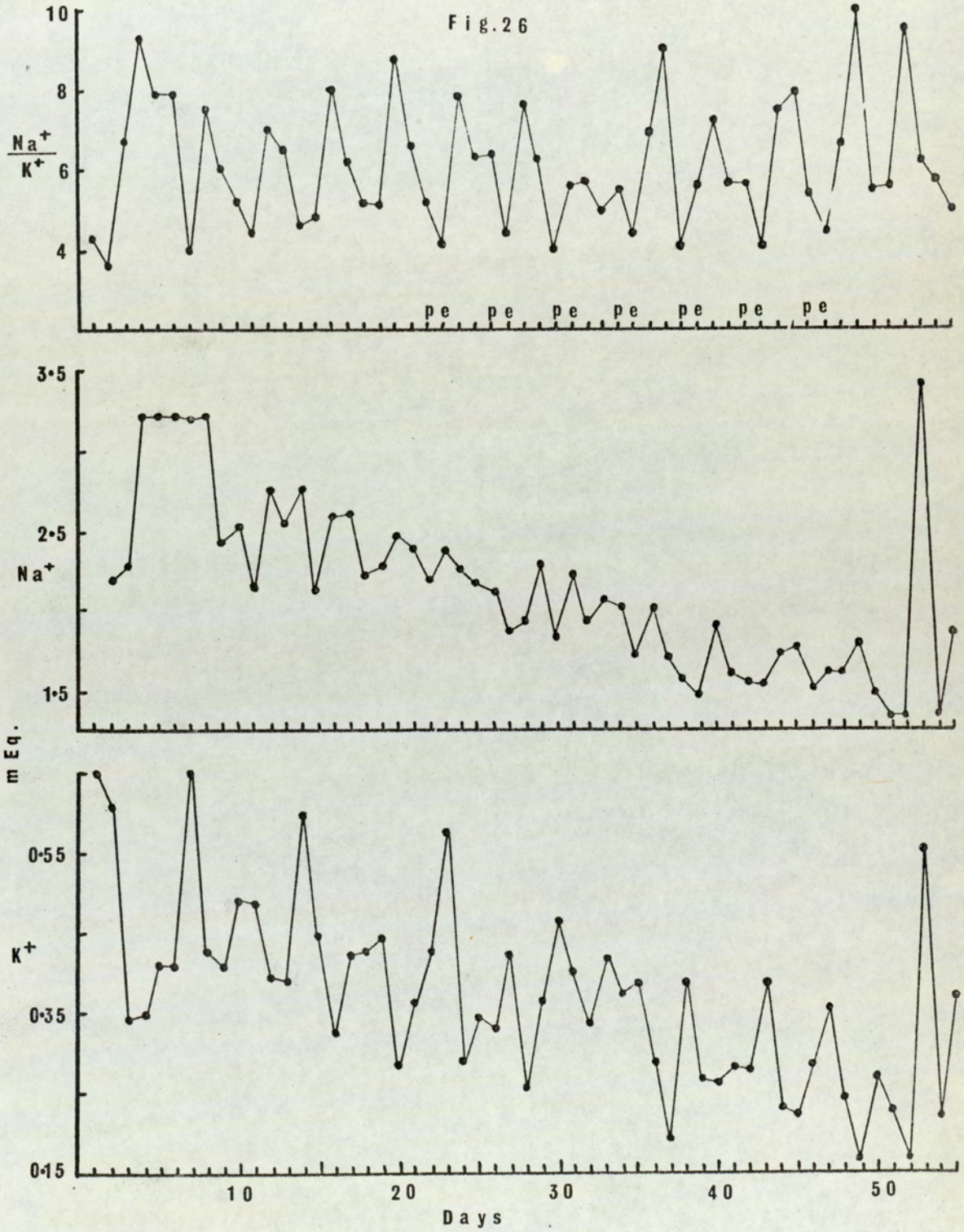
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Fig. 27

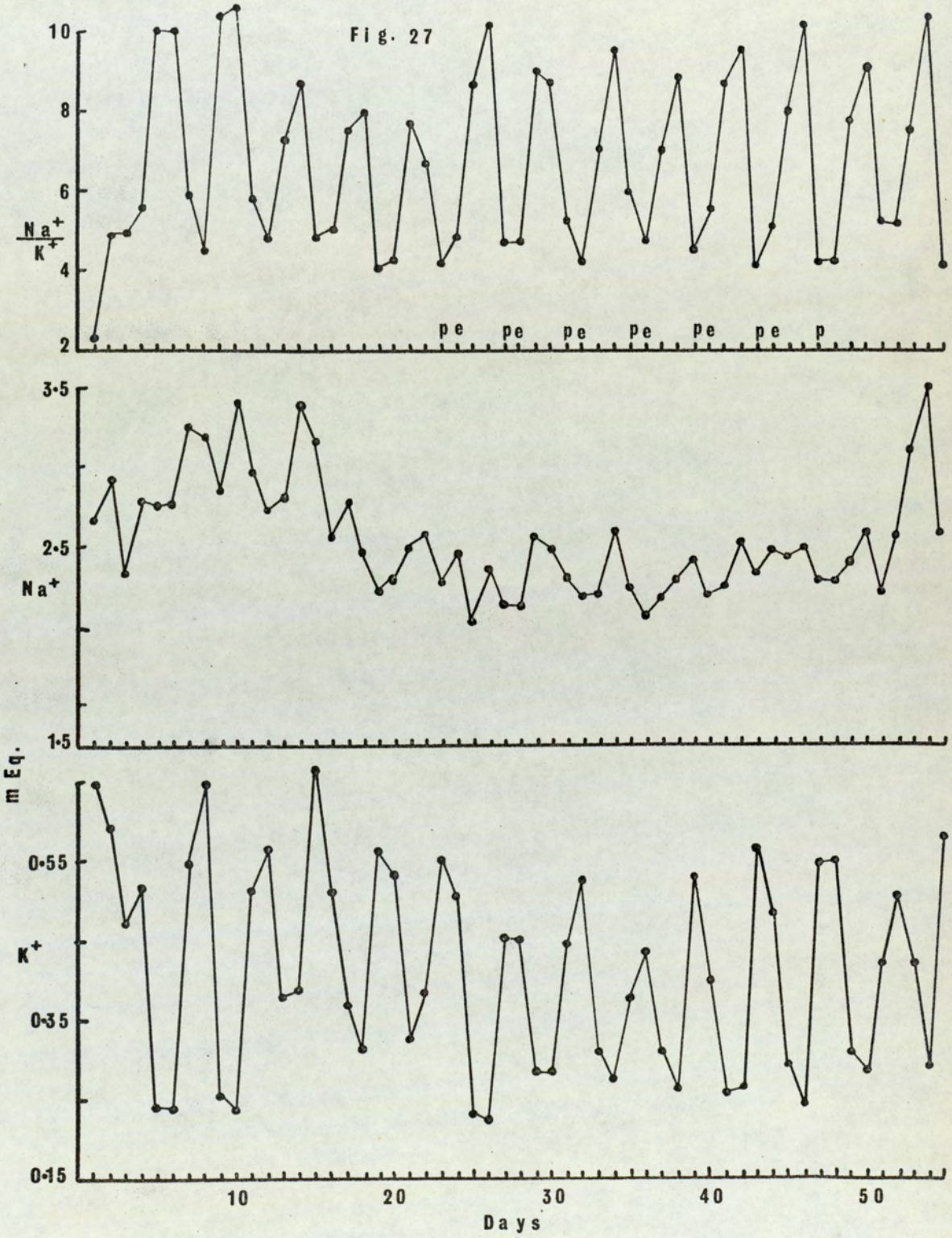
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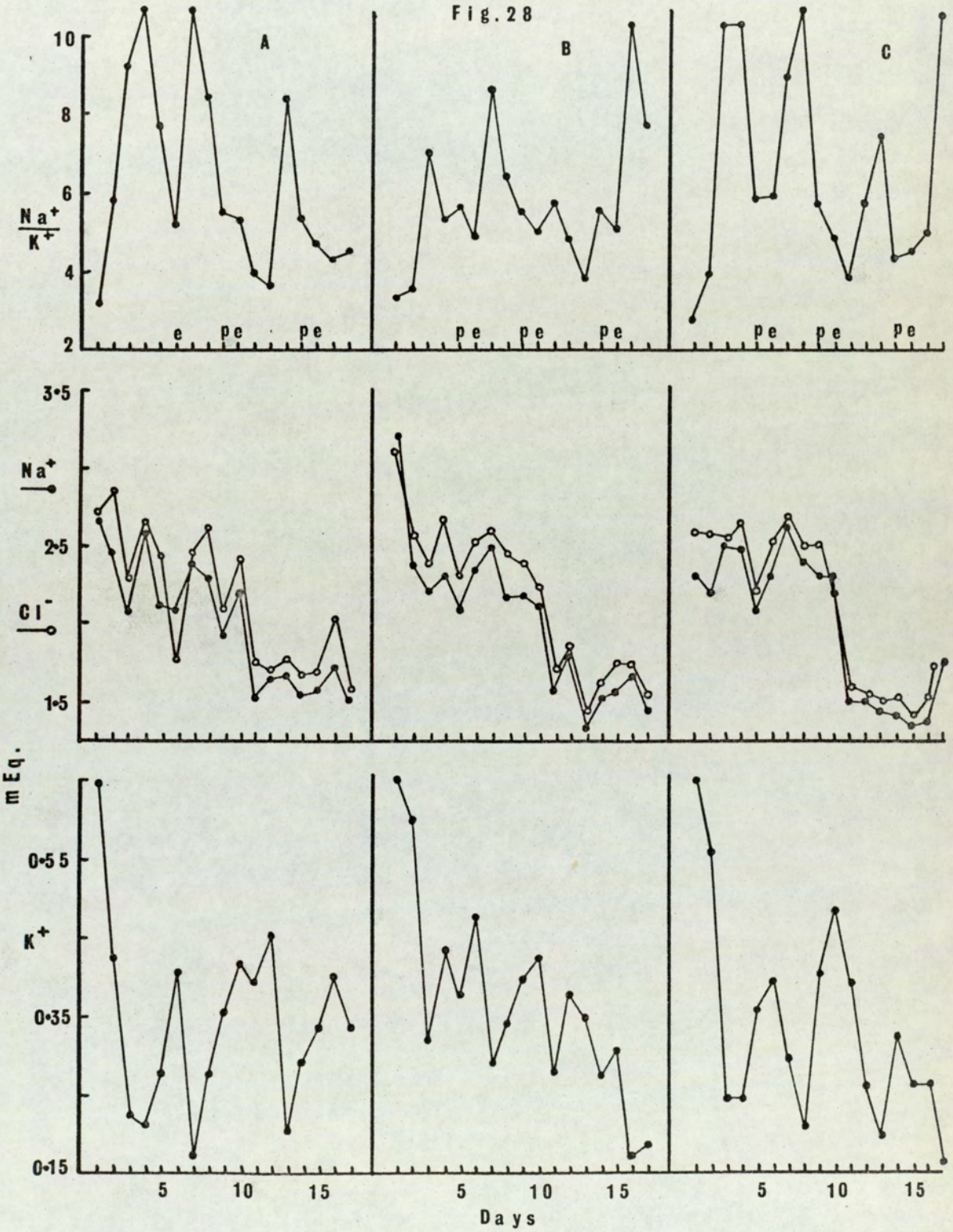
Fig. 28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Days

Fig. 29

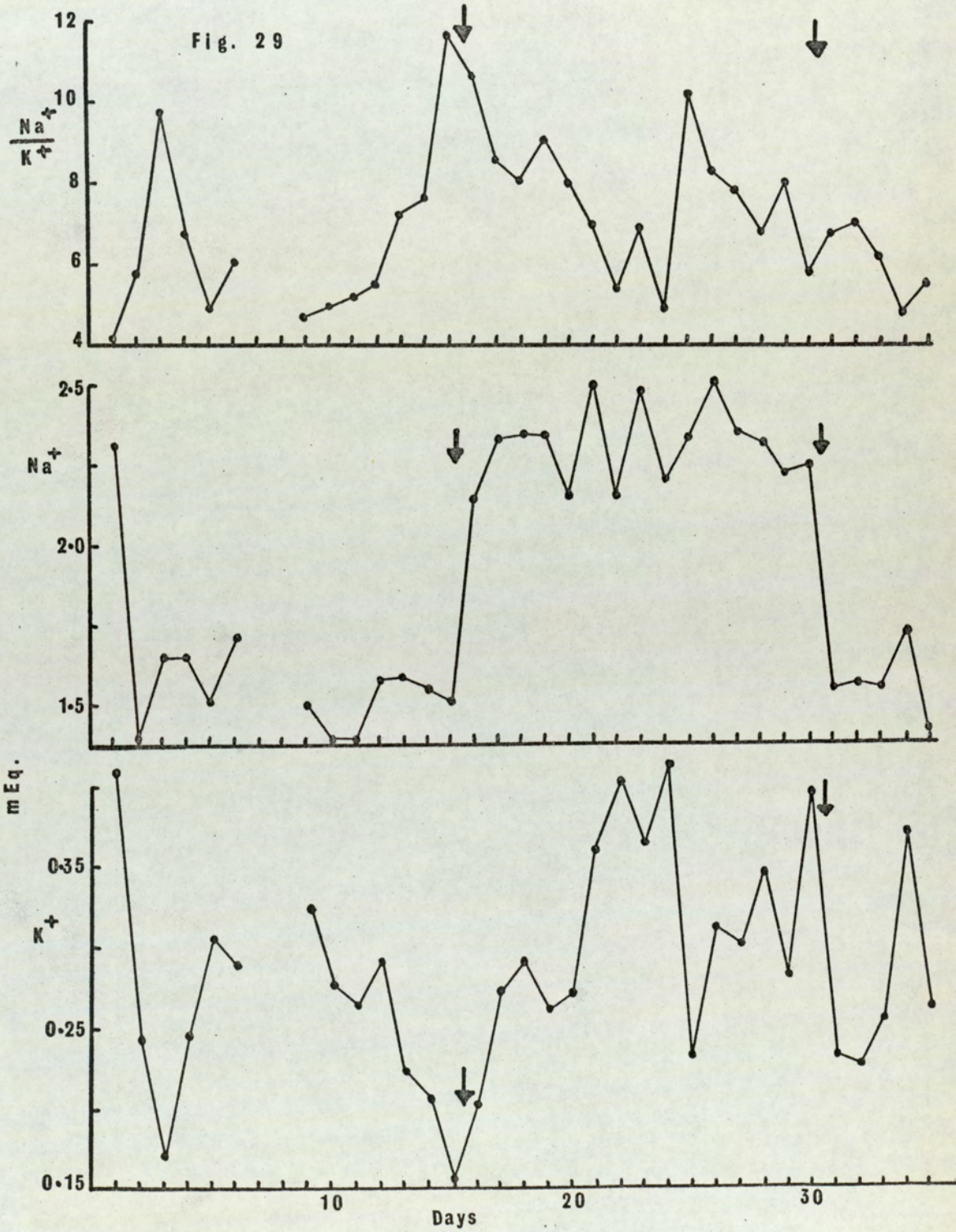


Fig. 30

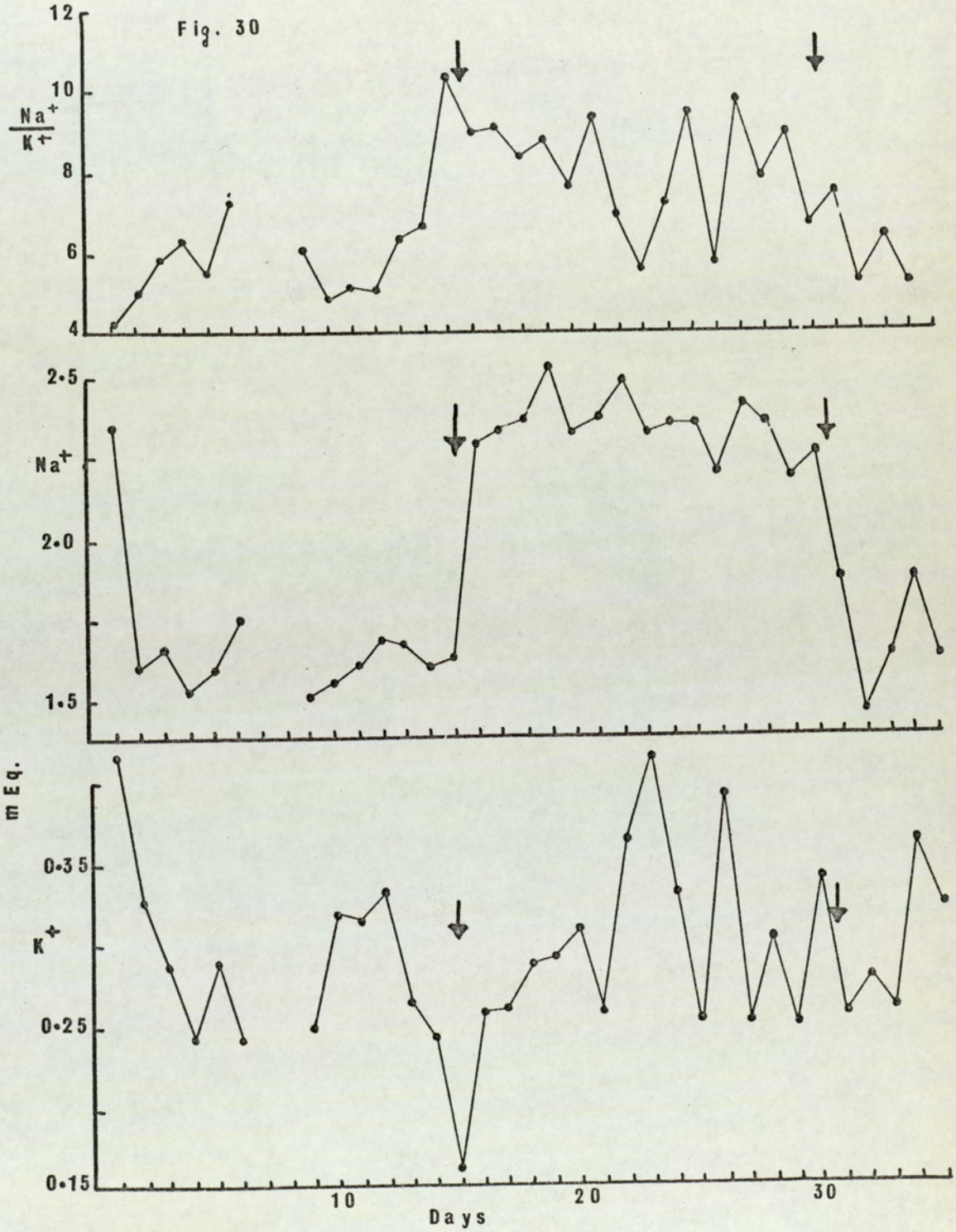


Fig. 31

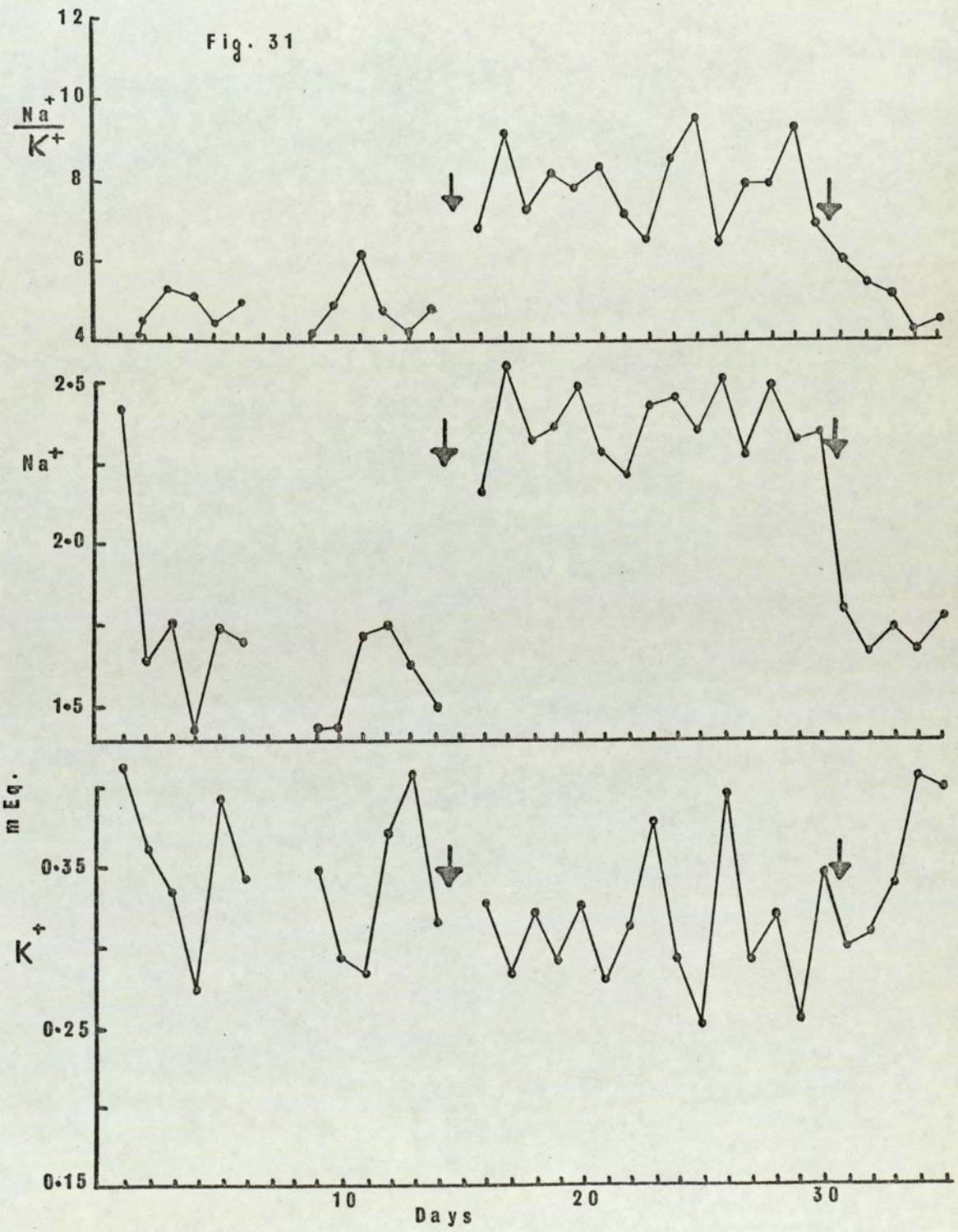


Fig. 32

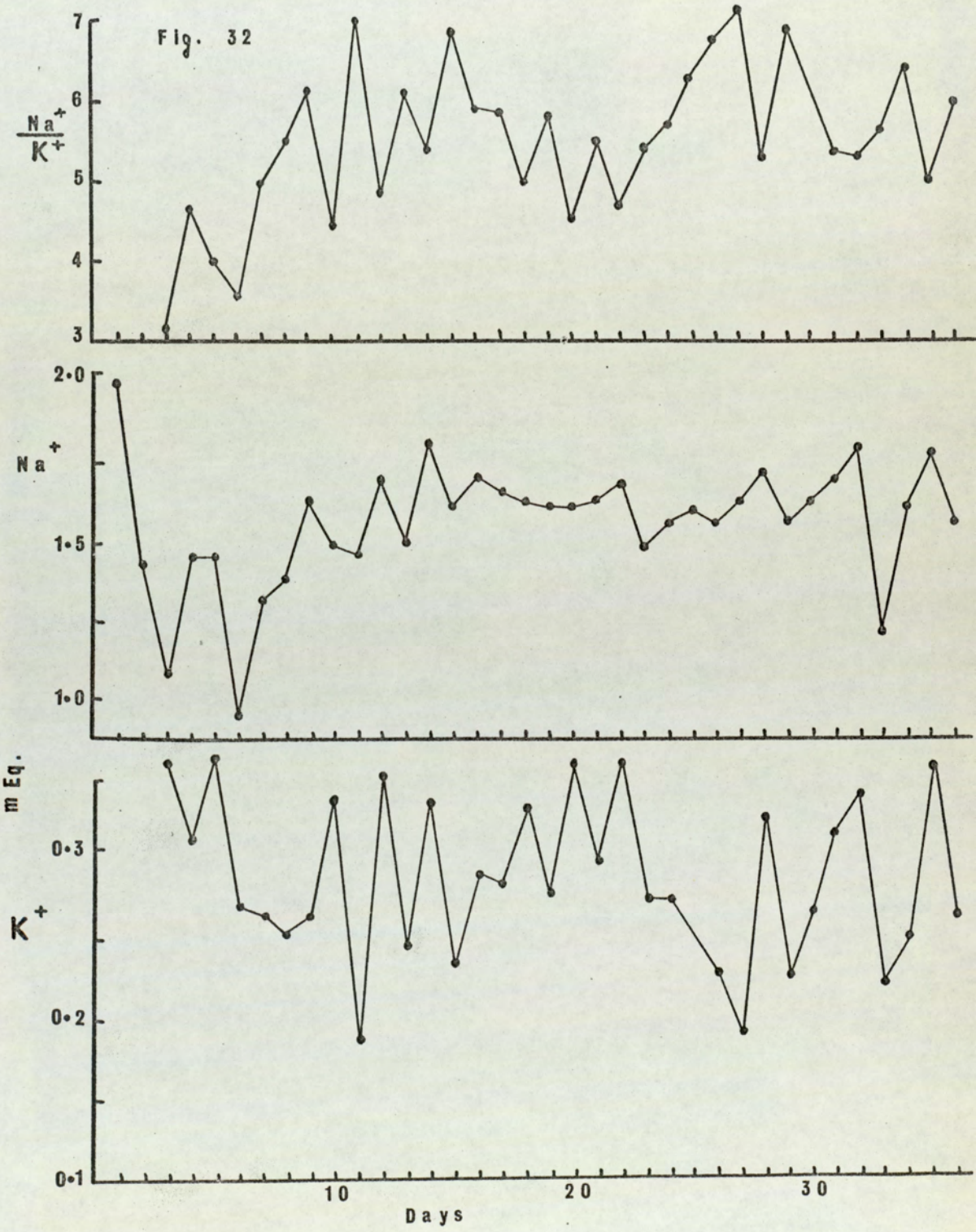


Fig. 33

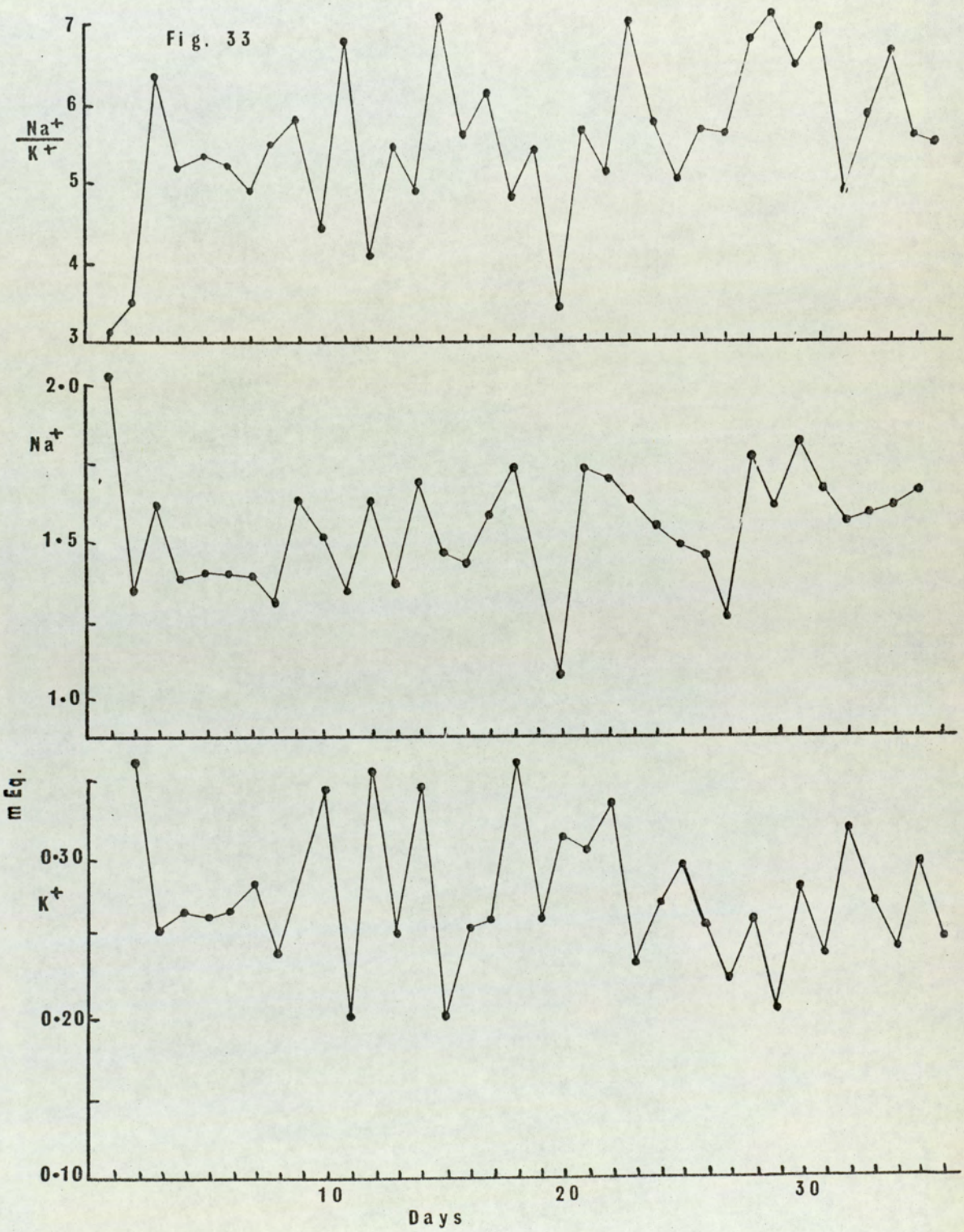
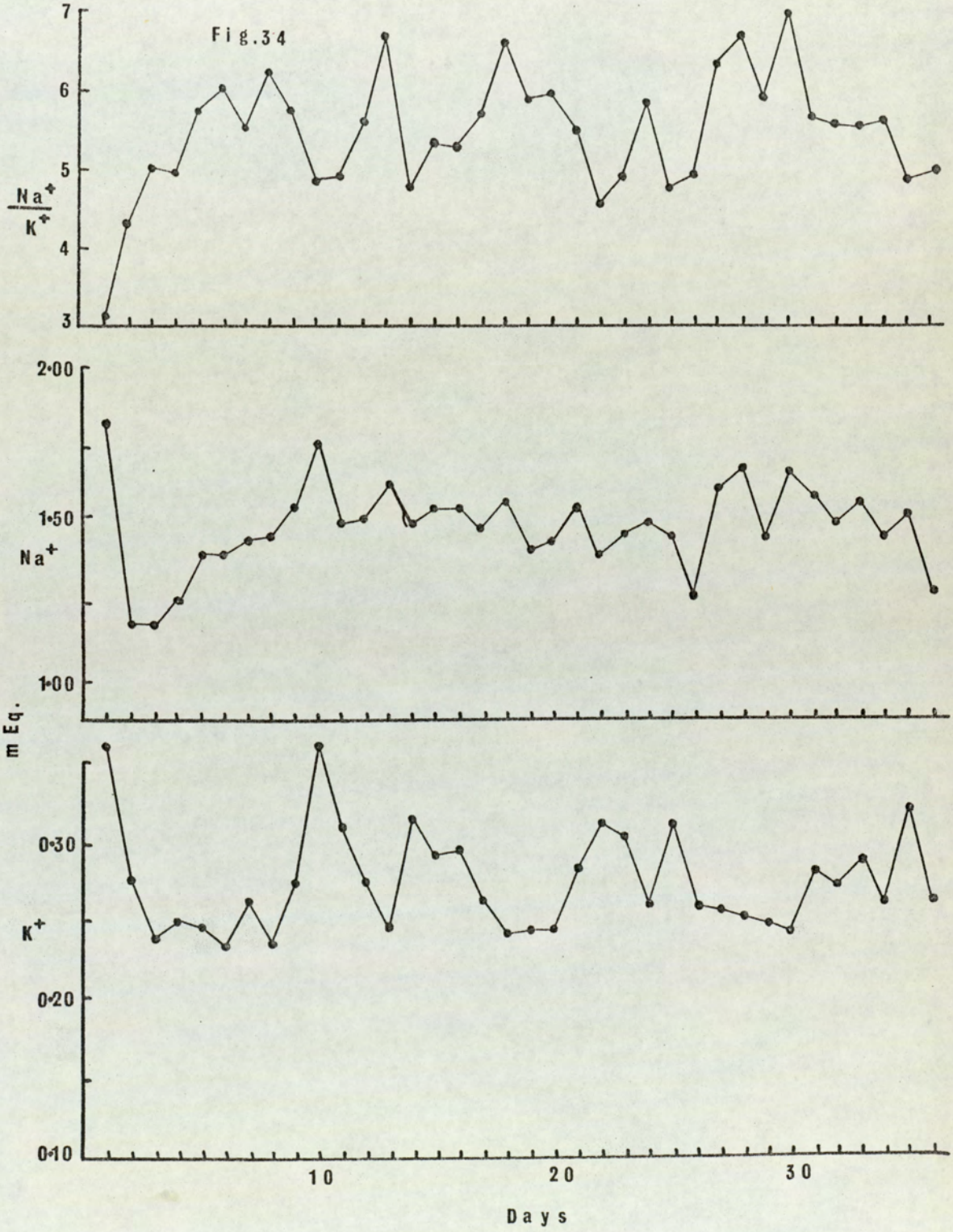


Fig.34



DAILY URINARY ELECTROLYTE EXCRETION - INCLUDING IODIDE - IN OVARIECTOMISED AND NORMAL FEMALE RATS FED A "LOW IODINE" REMINGTON DIET

Introduction:

The conclusions from the measurements of iodide excretion using tracer I^{131} in the previous experiments were that urinary excretion of iodide and chloride closely follow each other, and that exogenous oestrogen given in both male and female rats could reduce iodide and chloride excretion. These experiments, however, were performed in drug-treated water and saline-loaded animals and the duration of such experiments only four hours. In addition it was shown in the previous section that a reduction in chloride excretion occured at the proestrous or oestrous stage of the rat oestrous cycle, and it was postulated that a reduced iodide excretion would also occur at this time.

It was decided therefore to measure daily urinary iodide excretion together with chloride, sodium and potassium in female rats which were not subjected to acute experimental conditions, and which enjoyed as much as possible a normal existance. By performing such experiments, urinary iodide excretion could be determined throughout the oestrous cycle; and also the conclusions derived from the acute experimental procedures could be confirmed in rats under near natural environmental conditions.

The technique of VAN MIDDLESWORTH (207, 209, 211) and VAN MIDDLESWORTH and INTOCCIA (149, 208) provide a method of measuring

urinary and faecal iodine excretion using tracer I^{131} in rats maintained in metabolism cages. The basic method of these workers is employed in the work to be reported here.

Methods and materials.

Rats were housed individually in HOWELLS, WRIGHT and HARRISON metabolism cages and fed a "low iodine" Remington diet (No. 347) in paste form (I g. diet /1 ml. distilled water). A constant amount of diet was given to each animal daily and the amount restricted slightly to ensure that all of it was eaten each day. Distilled water was available to each animal ad libitum. A stock solution of Na I^{131} in distilled water was prepared containing 0.2 µc. Na I^{131} per ml; and I ml. of this stock solution mixed with the pasted diet of every rat each day. Daily urine and faeces were collected in every rat.

Each animal was transferred to a clean cage each day between 5.30 - 7.00. p.m. and at this time urine was measured and collected, water intake measured, fresh diet with the 1^{131} tag given and vaginal smears taken. Every cage was washed with 25 ml. of 10% KI, and this wash retained for analysis.

Daily urine samples were analysed for sodium, potassium and chloride and the daily excretion of these ions expressed as m Eq./Day. An aliquot of urine, cage wash, and stock solution of Na 1^{131} was counted for 1^{131} and the total daily urinary radioactivity calculated and expressed as percentage of the daily dietary dose of 1^{131}.

The total weekly faeces of every rat was counted for I^{131} together with an aliquot of stock Na I^{131} solution and the weekly faecal radioactivity expressed as percentage of the daily dose of I^{131}.

Decontamination of the metabolism cages was carried out in the following manner. The glass urine faeces seperator units were soaked in DECON 75 for one day, rinsed in running water for another day, and then washed in distilled water before being used on the cages again. As there were three sets of glassware for every cage, complete decontamination of the glass was possible. The metal parts of the cages were only duplicated once, and the corrosive nature of the DECON 75 and the size of the metal units made it impossible to decontaminate with Decon; and were therefore washed thoroughly in running hot water, and rinsed with distilled water before use. Checks for complete decontamination of metabolism cages carried out periodically showed that these washing procedures left no residual I^{131}.

This basic procedure was used in three groups of rats which had been previously maintained on a Remington diet ad libitum for some weeks before the metabolism cage experiments were performed and I^{131} tagging began.

GROUP 1 3 Ovariectomised rats fed the Remington diet 4 weeks before I^{131} tag.

These rats had been ovariectomised three months before being placed on the Remington diet and were between 220-250 g. body weight when placed in metabolism cages for daily I^{131} tagging. All rats in this group were fed 10 g. of Remington diet each day from the begining of the experiment.

After changing cages on day 9, all animals were given additional potassium in the diet. Potassium carbonate was added to the Remington diet to raise the K content three times to 0.353%. (a)

After five days on this modified diet a further addition of potassium carbonate was made to bring the K content up to 1.294% (b) i.e. a K content eleven times that of the original Remington diet. On the last day of the experiment, the original diet was given to all animals.

Faeces were only collected for the first two weeks of these studies and counted for I^{131}.

GROUP 2 3 Ovariectomised rats fed the Remington diet 14 months before I^{131} tag.

The rats used in this group were the same animals that were used in the previous group. Throughout the 14 months on the "low iodine" diet these rats received a vitamin supplement (see page 9) in their drinking water once a week.

When they started on the I^{131} tag, they weighed between 220-270 g. Each animal received 10g./day of diet. 6p.m. on day 11, all animals were given 300 µg. of oestradiol benzoate subcutaneously in 0.1.ml. arachis oil; similarly on day 18 at 6 p.m. 400 µg. of this oestrogen was given to each rat subcutaneously.

GROUP 3 2 intact female rats fed the Remington diet 14 months before I^{131} tag.

Three intact female rats were fed the Remington diet for 14 months, vitamin solution was given in the drinking water once a week throughout this time. One rat had to be destroyed because a tumor

developed in the animal, but the other two rats were in good condition at the time of beginning the I^{131} experiments.

One rat weighed 300 g. and the other 250 g. at the beginning of these experiments. The heavier rat was fed 18 g. diet and the smaller rat 15 g. of diet each day. Daily sodium, chloride and potassium excretion was measured from day 19 and on day 24 fresh stock solution (0.2μc/ml) of I^{131} was given to each animal.

12. a.m. on day 41 both rats were given 500 μg. of oestradiol benzoate subcutaneously in 0.1 ml. arachis oil, and one rat received an additional injection of this oestrogen at the same dose level on day 44.

Faeces were collected weekly from days 24-51 of these experiments and counted for I^{131}.

RESULTS

The excretion of chloride and sodium follow each other closely, and because of this only the daily chloride values have been shown graphically in these results.

GROUP 1

Daily chloride, potassium, I^{131} (% I^-) and Na:K ratios for the rats in this group are shown in figs, 35-37.

In the rat of fig. 35, chloride and iodide excretion tended to move in the same direction within two or three days after starting I^{131} tagging of the diet.

The predominant pathway by which the I^{131} tag was eliminated from the rat was via the urine, faecal elimination was very small. In the first and second weeks faecal I^{131} accounted for 21.75% and

and 18.5 % daily 1^{131} intake, which meant that only approximately 3% of the daily 1^{131} intake was excreted by the faecal route. Moreover this small amount of faecal iodine may well have been due to incomplete reabsorbtion of the tracer from the diet, and also perhaps to contamination of the faeces with 1^{131} from the urine.

Raising the daily dietary K intake by three times (a) raised the daily excretion of this ion by almost the same amount and reduced the urinary Na:K ratio to a third of the original value. Increasing K intake still further (b) increased potassium excretion still further and reduced the Na:K ratio. The addition of potassium to the diet did not alter the excretary pattern of 1^{131}.

Electrolyte excretion in the rat of Fig. 36 is similar to the previous rat except that on some days chloride and iodide excretion did not move in the same direction.

The faceal elimination of iodine was also low in this animal being 19.95% and 23.25% daily 1^{131} intake for week one and two respectively.

The other rat in this group (Fig. 37) showed the same electrolyte excretion characteristics of the other two rats; with high urinary iodide excretion and low faecal radioactivity - First week 16.61% second week 18.70% daily 1^{131} intake.

GROUP 2.

The daily excretion of chloride, potassium, iodide (1^{131}) and the Na:K ratio are shown for the rats in this group in Figs. 38-40.

The excretion of chloride and iodide tended to move in the same direction in all these animals, and as was seen in Group 1 the urinary excretion of iodide was still very high and the faecal

elimination of the tracer I^{131} low (Table 12).

TABLE 12. The weekly faecal weight and faecal I^{131} in ovariectomised rats fed a Remington diet tagged with I^{131}

RAT	WEEKS	FAECAL WT. g.	I^{131} (% of daily I^{131})
Fig. 38.	1	2.4	2.26
	2	2.7	4.59
	3	2.0	2.65
Fig 39.	1	2.9	5.72
	2	2.4	6.98
	3	2.1	6.03
Fig. 40.	1	2.6	5.22
	2	2.7	11.30
	3	1.8	6.48

In the rat of Fig. 38, the vagina was completely closed up, and the vaginal smears taken the first eleven days in the other two rats showed no cornification whatsoever.

The first injection of oestradiol benzoate (arrows in Figs.) resulted one day later in a decrease in the Na:K ratio, a decrease in chloride and an increase in potassium excretion. A marked reduction in iodide excretion was only seen in one rat (Fig. 40) where there was the greatest reduction in chloride excretion. In the two rats with open vaginae, the vaginal smears showed cornified cells for five days after this oestrogen injection.

A second injection of oestradiol benzoate on day 18 in the rats of Figs. 38, 39 reduced the Na:K ratio, chloride and iodide excretion and either did not alter or increase potassium excretion. In the rat of Fig.40 which gave a large reduction in iodide and chloride excretion after earlier oestrogen treatment, no reduction in chloride or Na:K ratio occured after a second dose of oestradiol benzoate, even though the vagina became cornified and remained so until day 22.

GROUP 3

The daily electrolyte excretion of the rats in this group are shown in figs 41, 42.

The pattern of iodide excretion in the two rats was similar to the rats in the previous two groups i.e. very high urinary iodide excretion and low faecal I^{131}. Table 13 shows the weekly faecal weights and I^{131} content of faeces in the rats of this group.

After day 24, when fresh stock I^{131} solution was given, the excretion of iodide and chloride closely paralleled each other in both animals. Unfortunately both rats had a very irregular vaginal smear pattern, both having a dioestrous smear most of the time. Similarly there was no cyclic pattern in the excretion of the electrolytes.

TABLE 13. The weekly faecal weight and faecal I^{131} in normal rats fed a Remington Diet tagged with I^{131}

RATS	WEEKS	FAECAL WEIGHT g.	I^{131} % of daily I^{131}
Fig. 41	1	3.1	10.54
	2	4.0	12.36
	3	4.3	13.07
	4	2.7	10.18
Fig. 42	1	3.4	10.85
	2	3.3	8.63
	3	4.0	11.50
	4	4.0	7.78

The injection of oestradiol benzoate (arrows) in the rat of Fig. 42 produced a marked reduction in chloride and iodide excretion, increased potassium excretion and therefore reduced an urinary Na:K ratio. This rat showed an oestrous smear for two days after the oestrogen injection.

The first injection of oestradiol benzoate in the rat of Fig. 41 increased chloride, iodide, and potassium excretion and reduced the urinary Na:K ratio; while the second injection of oestrogen given shortly afterwards reduced iodide and chloride excretion. The urinary elimination of these two ions remained reduced

for four days after this second dose of oestrogen.

In both animals the injection of oestrogen caused a reduction in the voluntary food intake which lasted three or four days. Consequently, the calculation of I^{131} excretion on these days of reduced food intake had to take into account a reduced I^{131} intake.

Discussion

The pattern of I^{131} excretion found in the rats of this work does not conform to that found by VAN MIDDLESWORTH (209-211), and VAN MIDDLESWORTH and INTOCCIA (149, 208) who used similar I^{131} tagging experiments in rats. These workers found that most of the tracer appeared in the faeces, and typical values found by them were 60 and 30% of daily I^{131} intake for faeces and urine respectively. They consider that the faecal radioactivity of their experiments was organically bound I^{131} and the urinary I^{131} as being iodide.

The very high urinary and low faecal I^{131} excretary pattern found in this work, therefore, indicates that very little organic I^{131} is being excreted by the faecal route. Two factors could account for this state of affairs, notably that circulating organic I^{131} is very rapidly deiodinated and or the I^{131} tag is not entering the thyroid gland. The fact that a high urinary I^{131} excretion is seen soon after adding the tag to the diet would indicate that the latter explanation is more likely.

In iodine balance experiments of this type, the technique depends on depleting the body of stable iodine by maintaining animals on an iodine defficient diet. Thus when the I^{131} tag is given the tracer is rapidly trapped by a much enlarged thyroid gland and therefore

follows the same metabolic pathway as stable iodine. The fact that in these rats even after fourteen months on the Remington diet, the tag wa still excreted predominantly in the urine indicates that body stable iodine was not depleted prior to I^{131} tagging. In effect the Remington diet was not sufficiently low in iodine content to produce a thyroid goitre and the iodine requirement of the thyroid was fully satisfied; with the result that the I^{131} tracer was not trapped by the thyroid and was eliminated from the body directly into the urine.

High urinary and low faecal I^{131} excretion has been observed in dietary I^{131} tagging experiments by VAN MIDDLESWORTH (207) when the low iodine diet used has not been sufficiently low in iodine to produce an iodine-deficiency state (and therefore goitre). Indeed this author in another study (212) found that the Remington "low iodine" diet produced goitre in rats very slowly.

It was suspected that the low potassium content of the Remington diet could be responsible for the rapid urinary elimination of the tracer, but raising dietary potassium intake (Fig.35-37) did not, however, reduce urinary iodide excretion.

It is concluded therefore that the pattern of excretion of the tracer I^{131} seen in this work is due entirely to the Remington "low iodine" diet being sufficiently high in iodine content to satisfy the iodine requirements of the rats. The iodine balance studies reported here then show only the urinary excretory fate of iodide, iodide derived directly from dietary sources and not from the deiodination of thyroid hormone.

The close correlation seen in these experiments between the

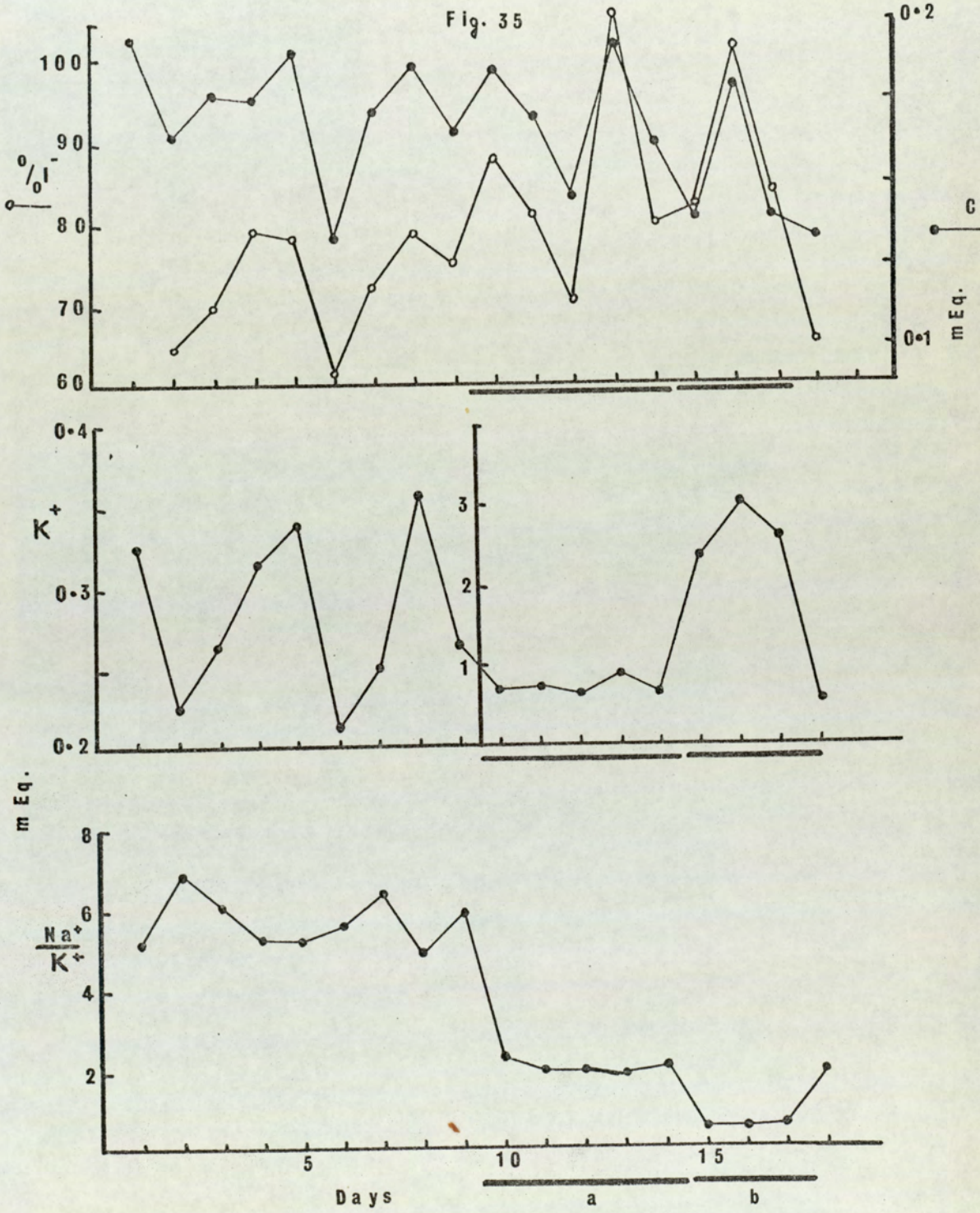
urinary excretion of chloride and iodide confirms the results of the short-term "loading" work.

Oestrogen treatment induces, with some exceptions, a renal retention of sodium, chloride and iodide, and an increase in potassium excretion; stimulating renal chloride retention causes a renal retention of iodide. It is, however, difficult, from five experimental rats, to compare the results of oestrogen treatment in this work with the effect of oestrogen treatment in the water and saline-loading series of experiments.

Unfortunately the intact female rats in these experiments did not show an oestrous cycle or cycle in urinary electrolyte excretion. It is not possible, therefore, to state that a cycle in urinary iodide excretion occurs, linked with the oestrous cycle. It has been reported that no cyclic pattern in urinary iodide excretion occurs in the human menstrual cycle (7). On the other hand some workers found that a premenstrual and menstrual increase in renal iodide excretion occured in the human (213).

Circumstantial evidence certainly indicates that there is a reduction in iodide excretion associated with a reduced chloride excretion at proestrous or oestrous in the rat. Further experiments are obviously required to substantiate this indirect evidence.

Fig. 35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mEq.
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Na⁺/K⁺
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10
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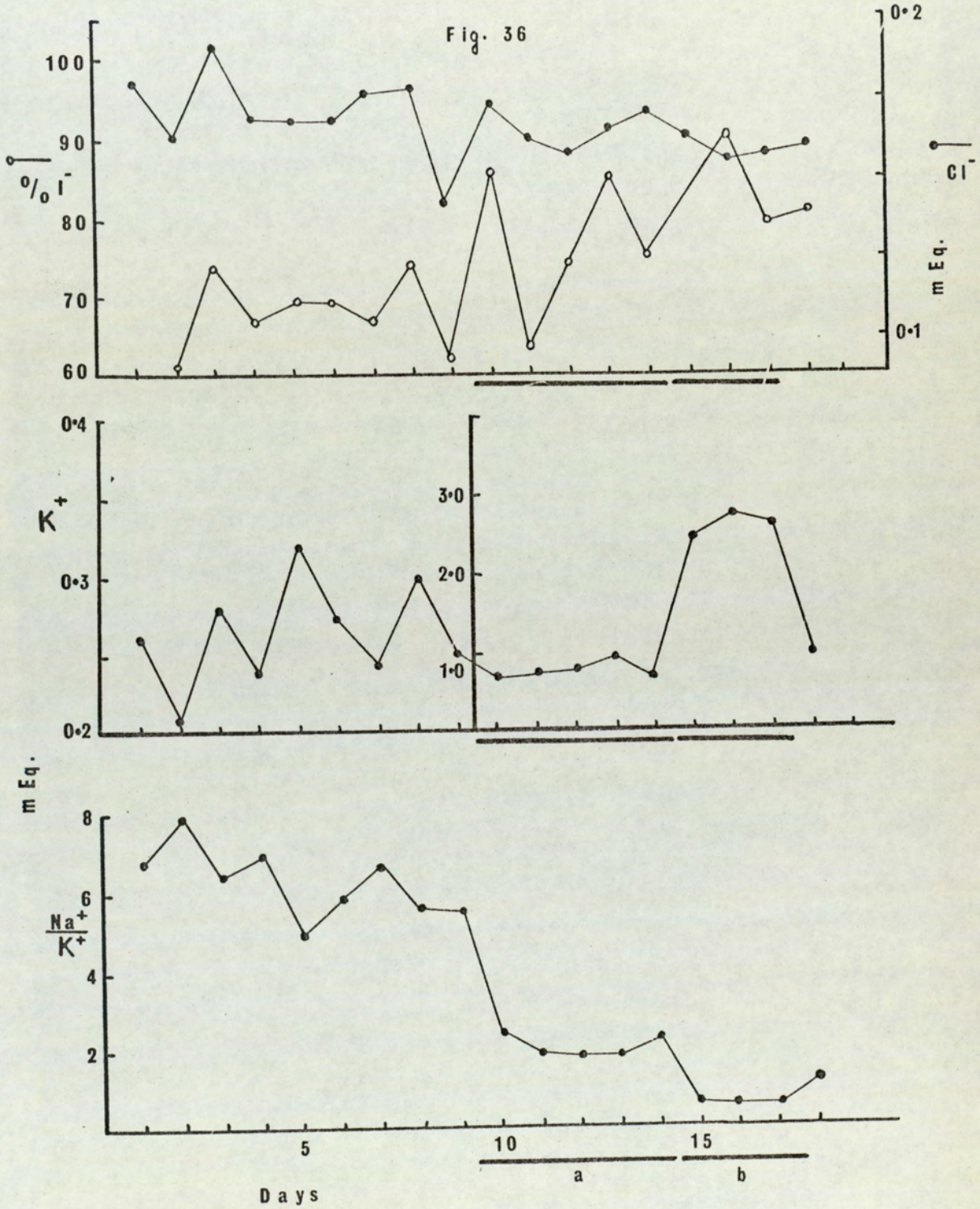
Days
a
b

Fig. 36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Na⁺/K⁺
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a
b
Days

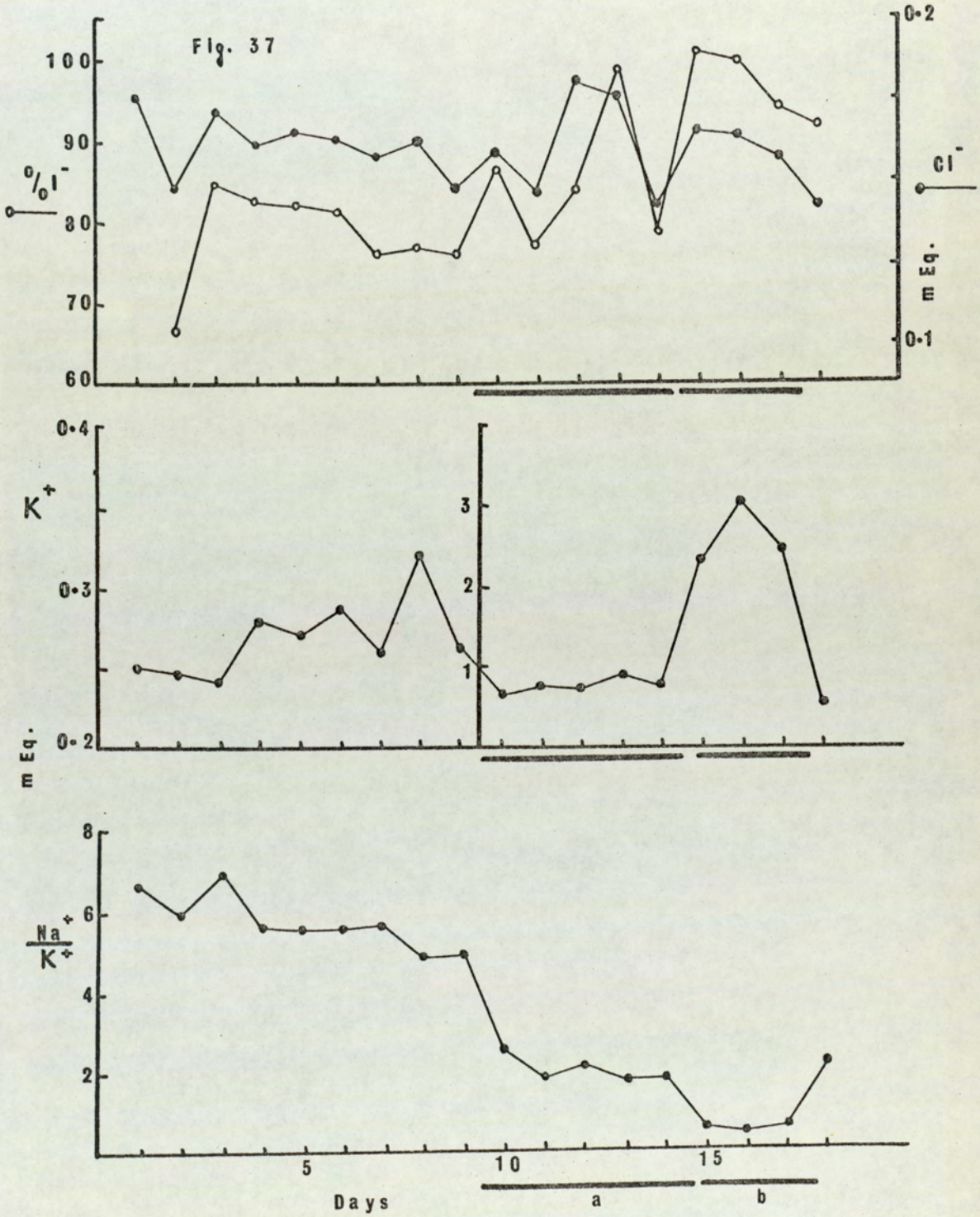
Fig. 37
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Days
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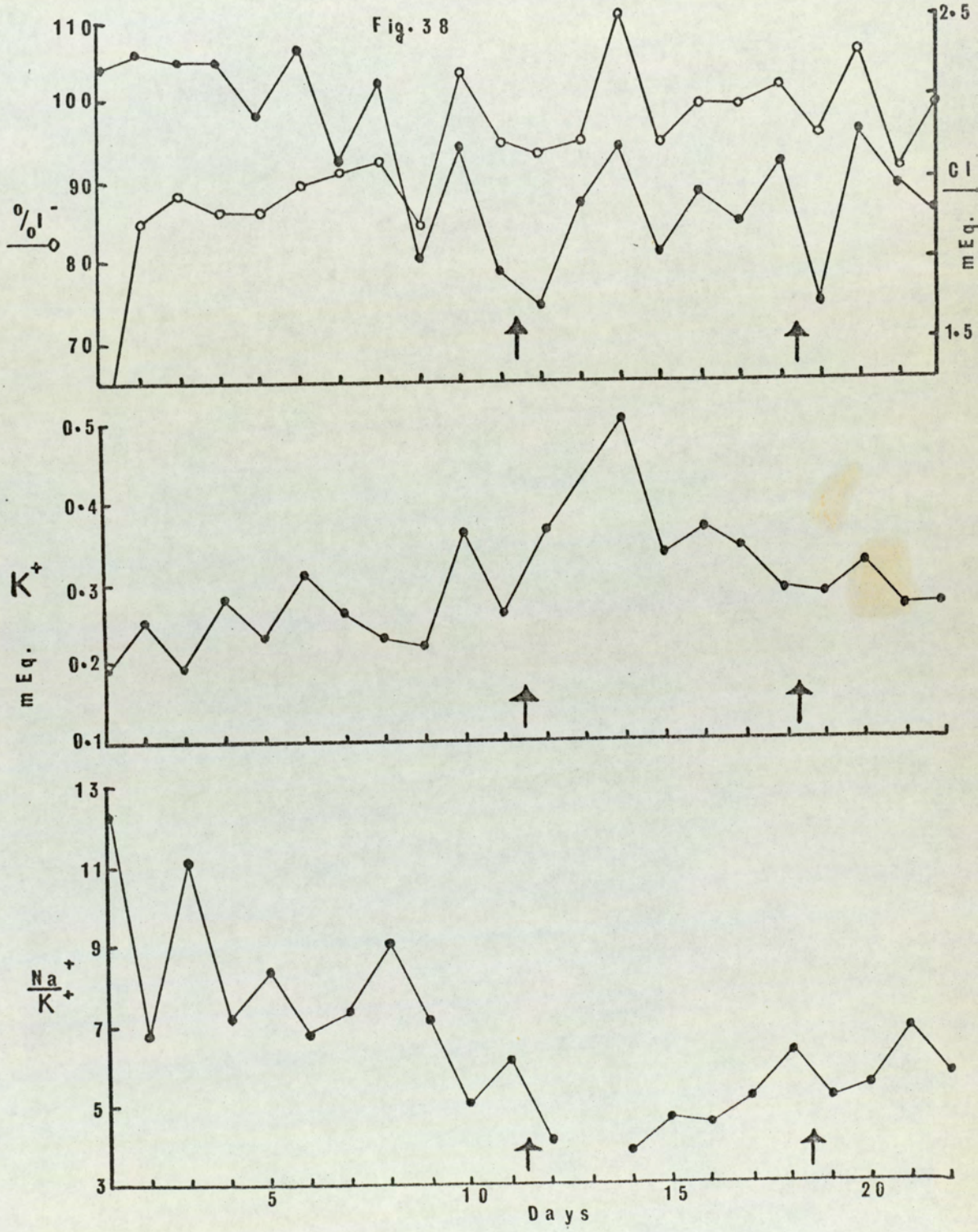
Fig. 38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Na⁺/K⁺
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Days

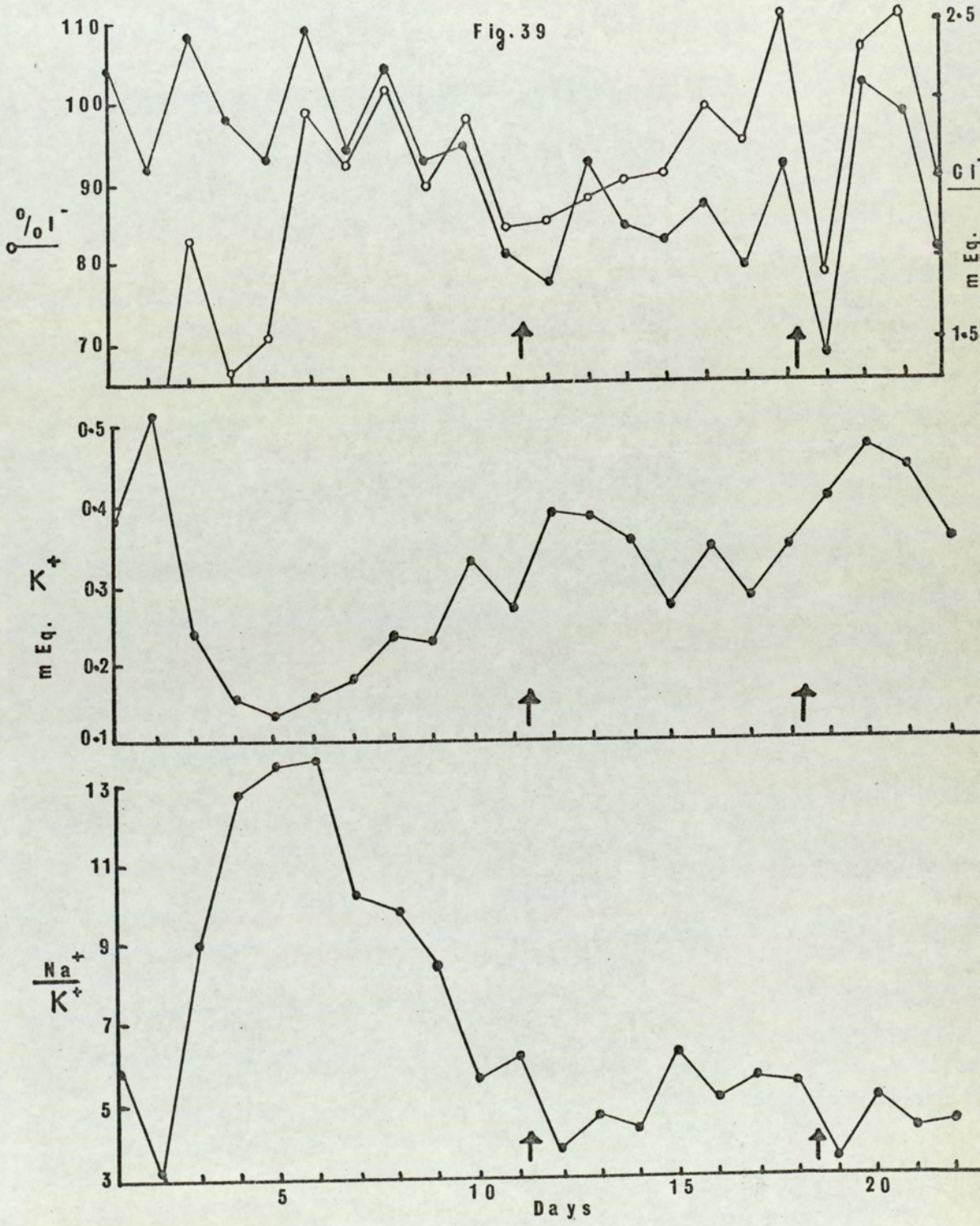
Fig. 39
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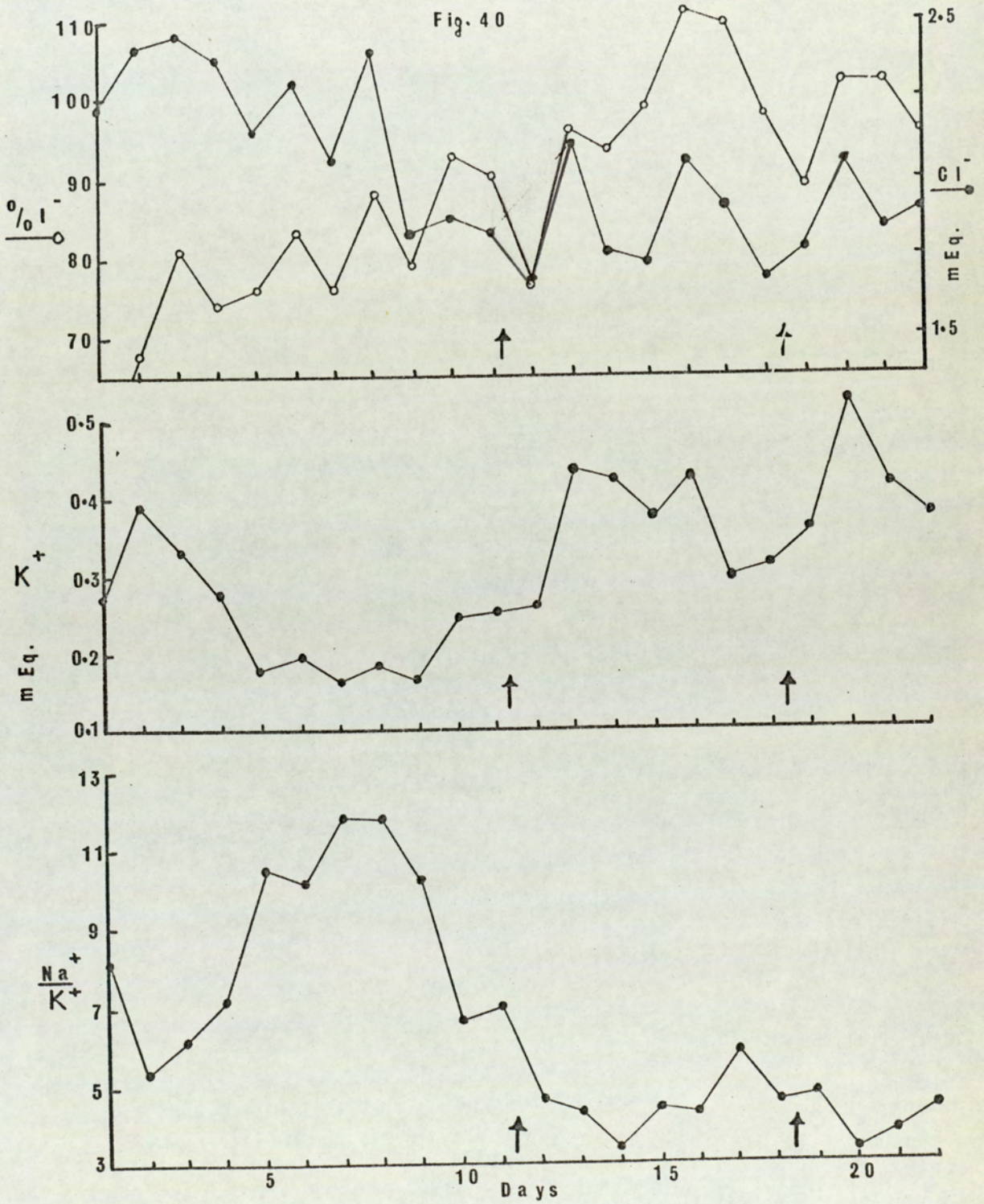
Days

Fig. 40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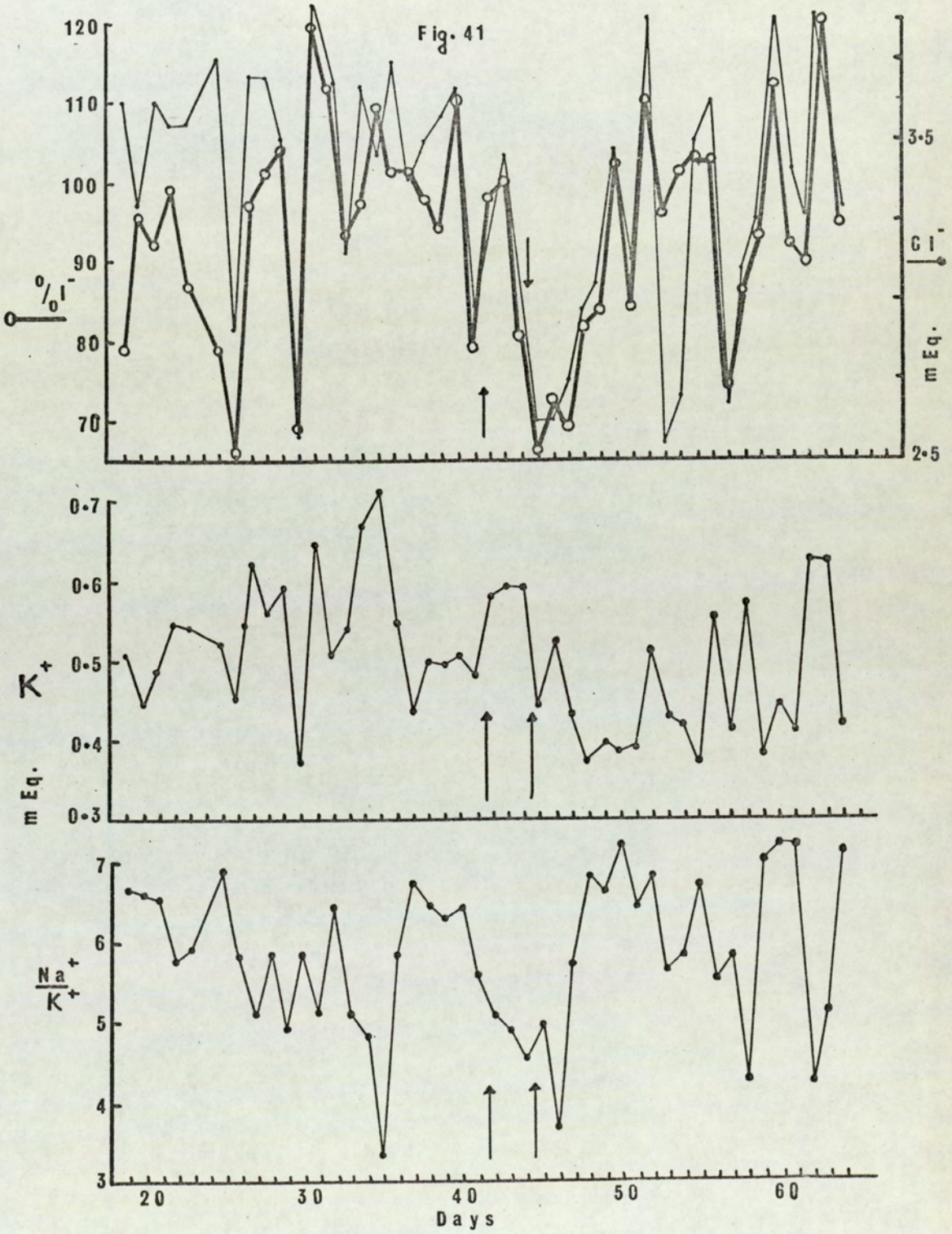
Days

Fig. 41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30
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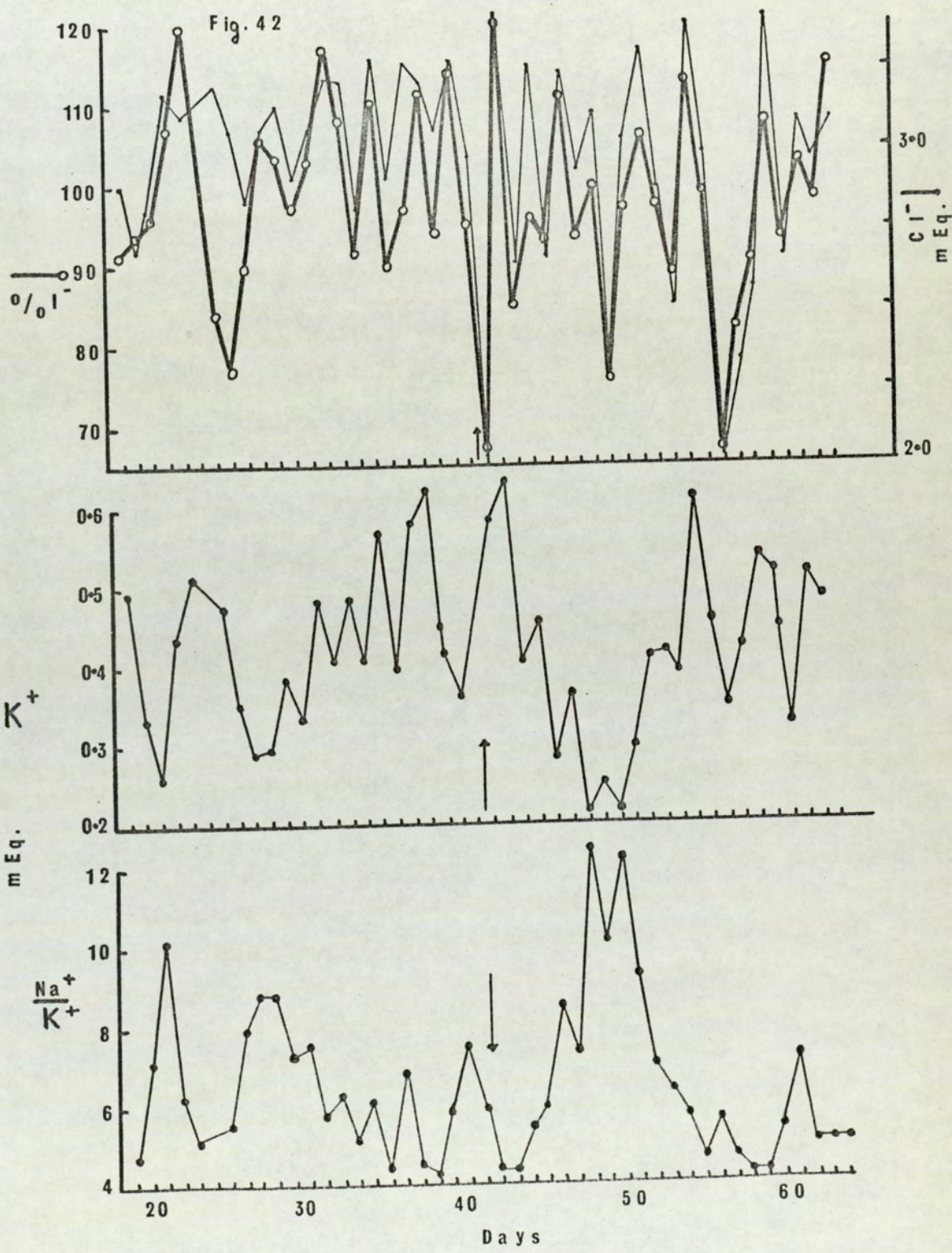
Days

Fig. 42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Na⁺/K⁺
20
30
40
50
60
Days

CONCLUDING REMARKS.

The original stimulus for the work reported here stemmed from the observation that an increase in urinary iodide excretion occured in pregnancy. It was proposed that the basic reason for this increase was due to a preferential reabsorbtion of chloride to iodide in the renal tubules.

It is apparent from the data presented here that, in the rat at least, there is a close correlation between the urinary excretion of iodide and chloride; when chloride excretion is reduced, so is iodide and vice versa. This was established in short-term experiments under conditions where renal tubular reabsorbtion was inhibited by the use of diuretics and where reabsorbtion was stimulated by oestradiol benzoate, and confirmed in the dietary I^{131} tagging experiments. Thus if the kidney of the pregnant rat handles iodide and chloride in a similar manner to the kidney of the non-pregnant rat another explanation for the increase in iodide excretion found in rat pregnancy is required.

GALTON and GALTON (9) have shown that the increased iodide excretion found in the pregnant Syrian hamster is due to the ability of the foetus, especially the foetal liver, to deiodinate maternal thyroxine. They also indicate that this is the reason for the increased urinary iodide excretion which occurs in the pregnant rat. Possibly this explanation, coupled with the fact that the glomerular filtration rate increases, are the reasons for the ioduresis of human pregnancy.

It was concluded from the diuretic experiments of this thesis that iodide reabsorbtion in the renal tubule occured to a greater extent in the distal segment and is linked to the aldosterone-controlled Na⇌K pump of this region. (Fig. A). This fits in well with observations that iodide transport in the thyroid is linked to a Na⇌K pump mechanism (105-108). Is iodide transport through the intestinal wall also dependant on this mechanism?

The numerous reports in the literature that adrenal steroids increase iodide excretion suggests evidence to indicate a preferential chloride to iodide renal tubular reabsorbtion mechanism. However, as no evidence for this preferential handling of these halogens was seen in this work, the ioduretic properties of these steroids remains an enigma. It is possible that the adrenal steroids exert their action on iodide excretion by reason of their effect on kidney glomerular filtration rate.

The cyclic nature of electrolyte excretion in the female rats demonstrated in this work, and the low urinary Na:K ratio found at the time of greatest blood oestrogen level (proestrous) indicated increased aldosterone secretion by the adrenals at this time. Direct evidence has since shown that this is indeed the case.

It is tempting, therefore, to ascribe the effects of exogenous oestrogen on sodium chloride and iodide excretion found in the male and female rats of this work to adrenal stimulation, particularly to an increased aldosterone secretion. The sex difference in electrolyte excretion in response to oestradiol benzoate can be explained in terms of adrenal stimulation for it has been shown that adrenal stimulation

in the rat produces a greater secretion of steroids in the female than in the male (193). Thus in the male rats oestradiol benzoate could stimulate the adrenal slowly without exhausting the steriod content of the gland, and a steady action on the kidney and reduction in electrolyte excretion would result (Tables 10,11). In the female rat, however, oestrogen injection may result initially in a large increase in steroid output followed by exhaustion, recovery, and exhaustion again of the adrenal gland and so on until the oestrogen stimulation ceases. The result of such a cycle in adrenal steroid output will be reflected in the kidney by a cycle in electrolyte excretion (Tables 7, 9).

No difference in electrolyte excretion was seen between dioestrous and oestrous rats which had been given an oral saline-load (Table 8). However, on the other hand striking differences were obtained when animals were maintained, and daily urine obtained, under near normal conditions. Data obtained from acute experimental procedures may therefore be misleading. It is noteworthy, therefore, that the I^{131} tagging experiments carried out under non-stressed conditions confirmed that a close correlation exists between iodide and chloride excretion.

Although female rats on the Remington diet show a cycle in sodium and chloride excretion it is not as clear as the cycle shown by potassium. Furthermore, the I^{131} tagging experiments failed to demonstrate a cycle in urinary iodide excretion in female rats (because they were not cycling). A Remington diet with a reduced sodium chloride content has therefore been obtained from the manufacturer and it is intended to repeat these I^{131} tag experiments

on young cycling female rats. In this way it is expected that large fluctuations in sodium, chloride and iodide excretion (as found with potassium) will be found to occur throughout the oestrous cycle, and so establish for certain whether a cycle in urinary iodide excretion occurs.

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