ELECTROLYTE METABOLISM DURING THE OESTROUS CYCLE IN THE RAT

BY

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CONTENTS

SUMMARY

In the present study renal electrolyte function and adrenal cortical activity were determined during the rat oestrous cycle, and in ovariectomised animals given oestradiol and progesterone therapy.

Initial studies showed a variation in the sodium content of uterus, kidney, liver, plasma, heart and skeletal muscle during the oestrous cycle, with higher levels of sodium at oestrus than at dioestrus. The water and potassium content of the tissues examined showed a less marked variation during oestrous cycle.

The variation in tissue electrolytes during the oestrous cycle was paralleled by a variation in renal electrolyte function. Urine flow, urinary sodium concentration and excretion rate were significantly lower at oestrus, when ovarian hormones are raised, than at dioestrus. Urinary potassium concentration and excretion rates were significantly higher at oestrus than at dioestrus. No significant variation in glomerular filtration rate was observed during the oestrous cycle.

Spironolactone blocked the increased sodium and water retention and potassium excretion observed at oestrus, but had little effect on renal electrolyte function in dioestrous or ovariectomised animals. Spironolactone has been shown to inhibit the action of mineralocorticoids on the distal tubules of the kidney. This, therefore, suggested that the adrenal cortex was involved in the variation in renal electrolyte function observed during

the oestrous cycle, with an increase in adrenal activity at oestrus when ovarian hormones are also raised.

In order to investigate further the role of ovarian hormones in the observed variation in renal electrolyte function during the oestrous cycle, oestradiol and progesterone were administered to ovariectomised rats.

Prolonged administration of oestradiol, progesterone and oestradiol + progesterone all significantly reduced urine flow, urinary sodium concentration and excretion rate and increased potassium excretion, but had no effect on glomerular filtration rate in ovariectomised animals. Spironolactone administration blocked the action of ovarian hormone administration on renal electrolyte function, again implicating adrenal cortical action. A single, physiological dose of oestradiol resulted in a progressive increase in sodium and water retention and potassium excretion 5 to 8 hours after hormone administration. Spironolactone was found to block the action of oestradiol on renal function.

Twelve hours after oestradiol administration renal electrolyte function was not significantly different to that observed in control ovariectomised animals.

In contrast to the effect of oestradiol administration on renal electrolyte function, it was found that administration of physiological doses of progesterone had little effect on renal electrolyte function compared with control ovariectomised rats. The evidence obtained from investigation of renal electrolyte function during the oestrous cycle suggested a variation in adrenal activity at this time. A significant

variation in aldosterone and corticosterone secretion rates were found during the rat oestrous cycle, the highest secretion rates being observed at pro-oestrus when oestrogen levels were also raised.

It is suggested, therefore, that the action of ovarian hormones on renal electrolyte function is a result of an initial stimulation of the adrenal cortex by the ovarian hormones, primarily oestrogens.

INTRODUCTION

The endocrine glands, and the hormones which they secrete, form a complex system for the regulation and integration of certain physiological and metabolic processes. Therefore, the endocrine glands must be considered as a system, since they rarely act independently from each other. A hormone may influence the activity of other endocrine glands in two ways: by a direct action on the endocrine gland, or by an indirect action via an alteration in the internal environment. Thus the variations in levels of gonadotrophins (Armstrong, 1970; Bell & Christie, 1970; Fukushima, Stevens & Gantt, 1964; Wallach, 1970; Deane & Parlow, 1971 and Harrington & Bex, 1970), oestrogens (Lloyd, Lobotsky, Baird, McCraken & Weisz, 1971; Barlow & Logan, 1966 and Yoshinaga, Hawkins & Stocker, 1969) and progesterone (Woolever, 1963 and Feder, Brown-Grant & Corker, 1971), observed during the femal sex cycle, are thought to be associated with the reported cyclic variations in the activity of other endocrine glands. Money, Kirschner, Kraintz, Merrill & Rawson (1951) suggested that the high levels of progesterone at oestrus depressed thyroid function and the high levels of oestrogen at pro-oestrus stimulated thyroidal iodine uptake. Bailey & Matty (1971) have postulated that the increased levels of plasma insulin, which they observed at pro-oestrus, and oestrus compared with dioestrus, are a result of the increased levels of sex hormones at this time. There is a great deal of evidence to suggest that there is a variation in the activity of the adrenal gland during the female sex cycle. A number of workers have shown that there is an increase in the weight of the adrenal gland at oestrus compared with dioestrus,

and that this variation is absent in ovariectomised animals (Tepperman, Engel & Long, 1943 , Chester & Jones, 1957).

It is known that the adrenal cortical hormones induce sodium and chloride retention, with increased renal excretion of potassium. Thorn, Nelson & Thorn (1938) have also reported sodium and chloride retention at the time of ovulation in women and Pye & Matty (1968) have demonstrated that there is a reduction in the sodium-potassium ratio at oestrus compared with dioestrus in the rat. The work of Thorn & Emerson (1940), Thorn & Harrap (1937), and Thorn & Engel (1938) suggests that oestrogen and progesterone, the plasma levels of which are increased at the time of ovulation and during the luteal phase of the cycle, cause sodium retention and chloride retention.

This early work indicates, therefore, that there is an important variation in salt and water balance during the female sex cycle, associated with the variation in sex hormones, which could be mediated through the adrenal cortex.

The present studies were carried out in order to examine further the variation in electrolyte function, in relation to changes in adrenal cortical function, during the oestrous cycle, and the role played by the ovarian hormones in this variation.

LITERATURE REVIEW

Introduction

This literature survey is concerned with the action of the ovarian hormones, oestrogens and progesterone, on renal electrolyte function and the role played by the adrenal cortical hormones in these changes. It is therefore necessary to include evidence of changes in renal electrolyte function and adrenal cortical activity in conditions where ovarian hormone secretion alters, as well as after oestrogen and progesterone therapy.

During the menstrual cycle oestrogen levels are low throughout the early part of the follicular phase, followed by a mid-cycle peak, and a second rise during the luteal phase of the cycle (Barlow & Logan, 1966 and Bell & Christie, 1970). A number of workers (Woolever, 1963; Johansson, 1969 and Bell & Christie, 1970) have reported very low or undetectable levels of progesterone during the follicular phase of the menstrual cycle in women but a marked rise after the formation of the corpus luteum. The level remains high for a few days, and then declines.

In human pregnancy oestrogen and progesterone levels were found to be significantly increased from week seven and rose to very high levels prior to parturition (Brown, 1956 and Greig, Coyle, Cooper & Walker, 1962).

Yoshinaga, Hawker & Stocker (1969) have also demonstrated a significant increase in oestrogen secretion in rats on day four of pregnancy and reported that levels rose gradually from days thirteen to twenty and thereafter rose sharply to

parturition. Progesterone levels showed a similar pattern to those of oestrogen during gestation in the rat (Eto, Masuda, Suzuk & Hosi, 1962).

It has been demonstrated that during the oestrous cycle in the rat the oestrogen levels are highest at pro-oestrus (Yoshinaga et al., 1969), whilst progesterone levels reach a peak late on the day of pro-oestrus, which continues to early oestrus (Feder et al., 1971).

The variations in ovarian hormones during the mammalian sex cycle are therefore well established. The work presented in this thesis is concerned with the effect on the adrenal cortex of the observed variations in ovarian hormones during the oestrous cycle. However, evidence of alterations in adrenal cortical activity associated with the variation in ovarian hormones during the menstrual cycle, pregancy and during oestrogen and progesterone therapy, has a direct bearing on this work and must, therefore, be considered.

SODIUM AND POTASSIUM LEVELS IN TISSUES AND PLASMA

Introduction

Sodium homeostasis is considered to be largely controlled by the action of aldosterone. The main site of action of aldosterone is the kidney where aldosterone induces an increase in sodium retention and an increase in potassium excretion in the distal convoluted tubule (Hierholzer & Stolte (1969). This action of aldosterone on renal tubule leads to increased potassium excretion in the urine with eventual cellular potassium deficiency accompanied by sodium retention.

Although there is evidence to suggest that the ovarian hormones influence aldosterone levels (which will be discussed later), there are surprisingly few reports on the action of ovarian hormones on plasma and tissue electrolytes.

Variation in Plasma Electrolytes During the Human Menstrual Cycle

Danforth et al. (1946) were unable to detect any significant variation in plasma sodium and potassium during the human menstrual cycle and this was confirmed by the work of Gray, Straussfeld, Watanabe, Sims & Krantz (1968). However, the more recent work of Michelakis, Stant, & Brill (1971) (1971) has shown that serum sodium levels are higher during the luteal phase of the menstrual cycle, when oestrogens and progesterone are increased, than during the following phase of the cycle.

Variations in Plasma Electrolytes During the Rat Oestrous Cycle

No evidence has been presented to suggest that there is a variation in plasma electrolytes during the oestrous cycle and no significant difference could be demonstrated between the plasma sodium levels of pregnant rats compared with non-pregnant animals (Pike & Yao, 1971).

The Effect of Ovarian Hormone Administration on Plasma Electrolytes

Pharmacological doses of oestrogens, administered for long periods of time to women, induced no significant change in plasma electrolytes (Knowlton, Kenyon, Sandiford, Lotwin & Fricker, 1942; Zelewski, 1965; Richardson & Houck, 1951).

Similarly, Jacobs (1969) could not detect any alteration in serum sodium chloride in women given 16 α-progesterone for twelve days.

Dance, Lloyd & Pickford (1959) found that single injections of oestrogen did not induce any change in serum electrolytes in dogs.

From these results it is apparent that little or no change in plasma electrolytes occurs with variations in the levels of ovarian hormones.

The Action of Ovarian Hormones on Tissue Electrolytes

Evidence for the action of oestrogen and progesterone on tissue electrolytes has mainly been concerned with changes which take place in the uterus. Hawk, Bitman, Cecil, Wiltbank, Bond & Sykes (1961) demonstrated that the sodium and water content of the cow

uterus is increased at oestrus. Talbot, Lowry & Astwood (1940) showed that during oestrogen administration to rats the uterus gains water and extra-cellular electrolytes. More recently Clementson, Mallikarjunewara, Mashteghi, Carr & Wilds, (1970) have shown a decrease in the sodium to potassium ratio in the uterus with progesterone administration. However, little information is available on the variation in electrolyte content of other tissues during sex hormone administration, or when plasma ovarian hormone levels are altered.

Therefore there is little available evidence to suggest that variations in ovarian hormones affect electrolyte homeostasis sufficiently to induce changes in plasma or tissue electrolytes.

KIDNEY FUNCTION

Introduction

The volume and composition of the extracellular fluid, and, indirectly, the intracellular fluid, is primarily regulated by the kidneys. Hence the kidneys are vitally important for the maintenance of homeostasis in the animal.

Effect of Ovarian Hormones on Kidney Function

1. Oestrogens

Previous reports have failed to reveal an effect of oestrogens on glomerular filtration rates in man (Dean, Ables & Tayler, 1945, Chesley & Tepper, 1967 and Dignam, Voskain & Assali, (1956).

Glomerular filtration rate was not found to be altered in dogs (Richardson & Houck, 1951, White, Henbecker & Rolt, 1947) in rats (Nocenti & Cizek, 1964). However, Dance et al. (1959) and Selkurt, Talbot & Houck (1944) have reported increased glomerular filtration rates following oestrogen administration in dogs.

2. Progesterone

Landau & Lugibihl (1961) found that progesterone had no effect on glomerular filtration rates in human beings. However, Chesley & Tepper (1967) found that large doses of progesterone (300 mg for 4 days) increased inulin and para-amino hippurate (P.A.H.) clearance.

3. Variations during the Menstrual Cycle

From the evidence presented above the lack of a significant variation in glomerular filtration rate (G.F.R.)

during the menstrual cycle is not unexpected (Gray, Straussfeld, Watanabe, Sims & Solomon, 1969; Sims & Krantz, 1958).

4. Pregnancy

Observations on glomerular filtration rates during pregnancy have often been contradictory and have indicated that there is a species difference. In 1963 Lichton reported that, in rats, inulin clearance was slightly elevated on day 13 of gestation but was depressed on day 20; however, these results were not significantly different from non-pregnant levels. However, in a later paper (1968) the same worker found that glomerular filtration rates were unchanged or slightly increased in late pregnancy in the rat. It would appear, therefore, that there is little variation in glomerular filtration rates during pregnancy in the rat.

In the dog glomerular filtration rates are significantly elevated throughout pregnancy, but a significant decrease, from the high level in mid-pregnancy, occurred in the last half of pregnanct (Robb, Davis, Johnson, Blaine, Schneider & Baumber, 1970

De Alvarez (1958) found that glomerular filtration rates and renal plasma flow are elevated in the first trimester of human pregnancy and then fall steadily until they are 8% below non-pregnant levels by the 38th week. However, this work has been contradicted by that of Sims & Krantz (1958). They clearly demonstrated that this is a progressive increase in para-amino hippurate and inulin clearance from the 15th to the 38th week of pregnancy. Glomerular filtration rates are increased by 50% and renal plasma flow by 25%, but in late pregnancy the elevated glomerular

filtration rates are maintained in the face of declining renal plasma flow. There is no evidence to suggest that the increase in glomerular filtration rates is due to renal hypertrophy.

Berlin, Goetsch, Hude & Parsons (1953) have shown that plasma volume reaches 125% of normal by the 16th week of gestation and increases to 155% by the 36th week. It can be seen, therefore, that renal plasma flow has declined to non-pregnant levels at the time of maximum plasma volume. Therefore it seems unlikely that the increase in renal function observed by Sims & Krantz is a reflection of hypervolaemia.

It is well established that the secretion of a number of hormones is markedly increased during pregnancy. There is evidence to suggest that thyroid hormones (Heinbecker, Rolt & White, 1943), adrenal cortical hormones (Garrod, Davis & Cahill, 1955) and somatotropic hormones (White et al., 1947) all have the capacity to increase glomerular filtration rates and may contribute to the increase in glomerular filtration rates during pregnancy. In view of this the observations of Lichton on glomerular filtration rates in pregnant rats are surprising.

ELECTROLYTE EXCRETION BY THE KIDNEY

Introduction

Aldosterone increases sodium reabsorption and potassium excretion by acting on the distal tubules of the kidney.

1. Variation in Renal Electrolyte Function During the Menstrual Cycle

Thorn & Emerson (1940) demonstrated that there is a variation in electrolyte excretion during the menstrual cycle. They reported a slight decrease in electrolyte excretion and urinary volume at the time cf ovulation, with a more pronounced effect during the luteal phase of the cycle, thus confirming the earlier work of Thorn, Nelson and Thorn (1938). This cyclic variation was observed in women who had been hysterectomised but not in women who had been ovariectomised, thus establishing a close association between the variation in electrolyte excretion and urinary volume with the cyclic activity of the ovaries.

The recent work of Michelakis et al. (1971) has reaffirmed these early findings demonstrating that sodium and potassium excretion was highest during the luteal phase of the cycle and that the sodium to potassium ratio is lowest during menstruation.

However not all workers agree with these findings. Gray et al. (1968) and Reich (1962) could detect no fluctuation in the sodium to potassium ratio during the menstrual cycle. It would appear that this may be a result of their careful selection of subjects to exclude women

with a history of premenstrual oedema and therefore probably excludes a variant of the normal.

These observations on variations in electrolyte function may be involved in the generalised oedema described by a number of workers (Thomas, 1933; Sweeney, 1934 and Molnar & Gruber, 1934) associated with menstruation. Thorn, Nelson & Thorn (1938) showed that 76% of women gained weight at the time of ovulation and 48% premenstrually, but this weight gain was rapidly lost at the onset of menstruation. This variation in body weight during the menstrual cycle was confirmed by Danforth, Boyer & Grott (1946) and Chesley & Hellman (1957).

Thorn & Emerson (1940) noted that female patients suffering from disorders which predispose them to water retention, accumulated excessive quantities of extracellular fluid in the premenstrual period and that ovariectomy abolished significant premenstrual oedema. It is, therefore, a well established fact that there is a variation in fluid retention during the menstrual cycle.

Although there may be an association between the cyclic oedema and variation in electrolyte function during the menstrual cycle, other mechanisms may be involved. Alteration in capillary permeability would provide a simple explanation for the observed fluid retention. Jones, Fox, Vero and Asscher (1966) presented some interesting evidence suggestin that there is an increase in capillary permeability during the luteal phase of the menstrual cycle. Since it has been shown that renin can increase vascular permeability to

proteins (Asscher & Anson, 1963) and it is known that plasma renin is increased in the luteal phase of the cycle (Brown Davies, Doak, Lever & Robertson, 1964) It is possible that change in vascular permeability are a result of variations in plasma renin during the menstrual cycle.

2. Variations in Electrolyte Excretion During the Oestrus Cycle

There have been surprisingly few observations on water and electrolyte excretion during the oestrous cycle. However, Pye & Matty (1967) have shown that there is a reduction in the sodium to potassium ratio at oestrus compared with dioestrus in the rat.

3. Electrolyte Excretion During Pregnancy

A number of workers have shown that sodium and water are retained during pregnancy in human beings (Rinsler & Rigby, 1957; Gray et al., 1964; MacGillivray & Buchanan, 1958), in dogs (Robb et al., 1970) and in rats (Lichton, 1963). Lichton, Rosa & Hugh (1968) demonstrated that the anti-diuresis observed in late pregnancy cannot be accounted for by the action of vasopressin. The failure to retain administered sodium after injection of spironalactone suggested that mineralocorticoids are important in the sodium conservation observed in late pregnancy. However, Robb et al. (1970) have shown that DOCA administration to dogs, late in pregnancy, initially induced sodium retention but this was followed by 'escape' from the sodium retaining action of the steroid. They also found that a normal rate of aldosterone secretion occurred during pregnancy in dogs. This suggested that some mechanism other than high

mineralocorticoid levels caused sodium retention during pregnancy in dogs.

The problem of the mechanism of sodium retention is complicated further by the fact that there is a progressive increase in uptake of sodium by the foetus and placenta. It is possible that this process leads to a depletion in maternal extracellular fluids, thus resulting in increased reabsorption of sodium and potassium by the kidney.

From the preceding observations, and those presented in the previous section concerning changes in glomerular filtration rates, it is difficult to directly relate the sodium and water retention observed during pregnancy with variations in the levels of oestrogen and progesterone which occur at this time.

EFFECTS OF OESTROGENS AND PROGESTERONE TREATMENT ON ELECTROLYTE EXCRETION

Introduction

A number of workers have attempted to further elaborate the action of ovarian hormones on renal electrolyte function by the administration of oestrogens and progesterone separately and in combination.

1. Oestrogen

Several investigations have shown that oestrogens decrease the renal excretion of sodium. Thorn & Engel (1938) reported that a single 5 mg injection of oestradiol to dogs induced a marked decrease in renal sodium and water excretion for up to 3 days; 15 mg of oestrone produced a decrease in sodium and water excretion for 24 hours. The synthetic oestrogen, stilboestrol, has been reported to depress urinary sodium and water excretion, without any consistent alteration in potassium excretion, in the dog (Dance et al., 1959). However, Richardson & Houck (1951) have previously shown that administration of 2.6-6.0 mg of oestradiol benzoate for 17 days produced a decrease in potassium reabsorption, but sodium reabsorption was only decreased when glomerular filtration rates were also decreased.

However, Thorn & Harrap (1937) have shown that continued injection of oestrogen to dogs for more than 5 days does not prevent the return of sodium and water excretion to normal. Since Richardson & Houck administered oestradiol benzoate for 6-17 days they have probably observed the return of sodium and water excretion to normal.

A similar response to oestrogen administration was found in rats. Zelewski (1965) administered 26.6 μ moles of oestradiol-17β and found a decrease in urinary citrate, sodium and chloride excretion. This decrease was not observed in adrenalectomised rats, which suggested that oestrogen acts via adrenal function. Nocenti & Cizek (1964) administered diethyl stilboestrol (D.E.S.) to rats. They found that diethyl stilboestrol produced a decrease in urine volume, sodium, potassium and chloride excretion and the sodium to potassium ratio. In 1970 the same workers showed that oestradiol completely inhibited the polyuria-polydipsia-natriuretic syndrome which develops in rabbits deprived of food.

In normal human subjects Preedy & Aitkin (1956) found that daily intramuscular injections of 10 mg of oestradiol resulted in significant reductions in the urinary excretion of sodium, chloride and water from the second to the sixth days of steroid administration. With a smaller dose of oestradiol-17β, Dignam et al. (1956) demonstrated a significant fall in daily excretion of sodium and chloride. The decline in urine excretion observed by these workers reaffirmed the earlier observations of Shapiro (1938) which Sharper-Schafer (1939) had been unable to confirm.

With large doses of oestradiol (20 mg/day) Katz & Kappas (1967) found an initial one to two day diuresis which was followed by a pronounced decrease in urinary sodium excretion for the duration of the ten day period of oestradiol administration. Landau et al. (1957) observed

sodium retention in human subjects receiving 0.5 mg of oestradiol benzoate daily.

Although these studies demonstrate that oestrogen decreases sodium and water excretion all the preceding workers with the exception of Landau, Bergenstal, Lugibihl, Dimick & Rashide,(1957) administered large doses of oestrogen, often for long periods.

The more recent work of Johnson Davis, Baumber & Schneider (1970) administering 1 mg, 250 µg and 100 µg/day to dogs, is of particular interest. They found that doses of 1 mg and 250 µg/day induce the same moderate sodium retention, whereas 100 µg/day produced only slight sodium retention.

The mechanisms through which oestrogens produce a decrease in sodium and water excretion are uncertain. Pfeiffer (1940) suggested that oestradiol benzoate produced renal hypertrophy in rats. However, this cannot contribute significantly to the observed changes in renal function during oestrogen treatment since there appears to be no significant change in glomerular filtration rates. The lack of effect of oestrogens on glomerular filtration rates reported in the previous section suggests that the decrease in sodium and water excretion does not take place by this mechanism. Other possibilities include: an increase in mineralocorticoid levels with oestrogen, a direct action of oestrogen on the kidney tubules to retain sodium or the action of oestrogens on some other endocrine factor which may affect renal electrolyte excretion.

2. Progesterone

Previous reports on the action of progesterone on electrolyte excretion have demonstrated both a natriuretic

and an antinatriuretic action.

Studies on the action of progesterone in man have repeatedly shown a natriuretic action for progesterone. It has been demonstrated that 50 mg of progesterone cause sodium diuresis (Landau et al., 1957; Landau & Lugibihl, 1958) and even with doses as high as 250 mg per day, natriuresis is still observed (Jacobs, 1969). However, Hempel-Jørgensen & Eilersen (1960) found that progesterone was ineffective as a diuretic in cases of hepatic cerrhosis and ascites if administered for long periods. They suggested that the decreased effectiveness after 17 days was due to an increase in aldosterone secretion. Landau & Lugibihl (1961) found that progesterone had no effect on sodium excretion in the absence of aldosterone. This suggests that progesterone acts by inhibiting the action of aldosterone on renal tubular receptor sites.

In contrast O'Connell & Welsh(1969) and Johnson et al. (1970) could detect no effect of progesterone on sodium excretion in the dog. Thorn & Engel (1938) also demonstrated that 1-5 mg of progesterone had no influence on electrolyte excretion in dogs. They did show however, that an injection of 20 mg of progesterone delayed sodium diuresis in adrenalectomised animals, thus supporting the observations of Thorn & Harrap (1937) that large doses of progesterone produce sodium retention in dogs.

Reports of experiments concerned with progesterone administration to rats vary considerably. The early work of Gaun Nelson & Loomis (1940) found that administration of 1-2 mg

of progesterone per day prolonged the life-span of adrenalectomised rats; this work was confirmed by that of Emery & Greco (1940). These results suggested that progesterone had a mineralocorticoid-like activity in adrenalectomised rats. However, Rosemberg & Engel (1961) presented evidence to suggest that the same dose of progesterone (2 mg) had no effect on adrenalectomised rats but that 2 mg of progesterone blocked the sodium retaining activity of 6 µg of DOC in rats. These findings confirmed the earlier findings of Kagawa (1958) who also demonstrated a blocking of D.C.A. activity by progesterone. These results are obviously contradictory and further evidence for the action of progesterone on renal electrolyte excretion in rats is required.

RENIN-ANGIOTENSIN SYSTEM

Introduction

The renin-angiotensin system may affect salt and water balance in several ways. It has a direct effect on the kidney tubule and an indirect effect via its influence on aldosterone production. Angiotensin also increases blood pressure which may change blood flow through the kidney.

The enzyme renin is produced by the juxtaglomerular apparatus of the nephron, and is released into the plasma via the efferent arteriole. In the plasma renin reacts with angiotensinogen to form Angiotensin I which undergoes enzymatic conversion to Angiotensin II:-

$$\text{Renin} + \underset{(\alpha\text{-2-globulin})}{\text{Angiotensinogen}} \longrightarrow \underset{\text{(decapeptide)}}{\text{Angiotensin I}} \xrightarrow[\text{conversion}]{\text{enzymatic}} \underset{\text{(octapeptide)}}{\text{Angiotensin II}}$$

The Effects of Ovarian Hormones on the Renin-Angiotensin System

1. Oestrogens

A great deal of evidence has been presented to suggest that oestrogens increase angiotensinogen concentration in the plasma (Crane & Harris, 1969; Menard, Malmejac & Milliez, 1970 Nasjletti, Matsunaga & Masson, 1970, 1971a). The work of Nasjlett (1971b) on the action of stilboestrol on angiotensinogen in adrenalectomised rats suggests that the observed effect of oestrogens is not mediated via the adrenal glands.

2. Progesterone

Nasjletti et al,(1971a) could detect no effect of progesterone on renal renin. However, Chesley & Tepper (1967) have

shown that progesterone reduced the response of the glomerular filtration rates and water and electrolyte excretion rates to Angiotensin II.

3. Variations in the Renin-Angiotensin System During the Human Menstrual Cycle

A number of workers have shown that there is an increase in plasma renin concentration (Brown et al., 1964; Skinner, Lumbers & Symonds, 1969) and plasma angiotensin II (Sundsfjord & Aavaag, 1970) during the luteal phase of the human menstrual cycle. Most authors favour the theory that the high levels of progesterone during the luteal phase of the cycle induces a natriuretic respons which, in turn, stimulates the secretion of renin.

4. Variation in the Renin-Angiotensin System During the Oestrous Cycle

Nasjletti et al. (1971a) has shown that angiotensinogen was increased at oestrus and that this increase was suppressed by ovariectomy. These data therefore implicate oestrogens in the variation in activity of the renin-angiotensin system during the oestrous cycle.

5. The Renin-Angiotensin System During Pregnancy

Renin concentration is increased during human pregnancy; this rise is very marked during the first trimester (Brown, Davies, Doak, Lever & Robertson, 1966; Helmer & Judson, 1967; Weir, Paintin, Fraser, Robertson, Tree & Young, 1970).

Consistent increases in angiotensinogen have also been found during pregnancy (Helmer & Judson, 1967; Gould, Skeggs & Kahn, 1966; Weir et al., 1970).

Robertson et al. (1971) found a 300% elevation in angiotensin II during the first trimester of pregnancy. The increase in plasma levels of oestrogen and progesterone undoubtedly play some part in the observed changes in the renin-angiotensin system in pregnancy. It is possible that the natriuretic action of progesterone stimulates the renin-angiotensin system. The previously described stimulation of angiotensinogen by oestrogens may also be involved. However, the increase in glomerular filtration rate early in pregnancy, leading to increased sodium excretion, and the possibility that maternal sodium depletion may occur in the first weeks of pregnancy, probably contribute to the increased activity of the renin-angiotensin system during pregnancy.

ADRENAL CORTICAL HORMONES

Introduction

The mineralocorticoids, principally aldosterone, are the most important hormones in the control of renal electrolyte function, although the other corticosteroids do have sodium retaining ability. However, the mineralocorticoids affect only 2% of the filtered sodium, but the control of the reabsorption of the electrolytes in this small amount is vital in maintaining sodium balance. Although the major sites of action of the mineralocorticoids are the distal tubules, Hierholzer (1966) has suggested that sodium reabsorption and potassium excretion in the proximal tubule may also be affected.

1. The Action of Ovarian Hormones on the Adrenal Cortex

A relationship between the adrenal cortex and the gonads has been recognised for a number of years. Early workers noted a variation in the size of the adrenal gland during the oestrous cycle, the maximum weight being achieved at oestrus (Anderson & Kennedy, 1932; Parkes, 1945). This variation was found to be abolished in ovariectomised animals (Tepperman, Engel & Long (1943). The first evidence that ovarian hormones might have some adrenal-like activities was derived from the observation that the sexual condition of the animal had some influence on survival time after adrenalectomy (Swingle, Parkin s, Taylor & Morrell, 1936; Snyder & Wyman, 1951). Since the presentation of these early findings aa considerable amount of evidence has been published on the

relationship between the ovarian hormones and the adrenal cortex.

2. Progesterone

Laidlaw, Ruse & Gornall (1962) showed that progesterone administration induced an increase in aldosterone excretion. These findings were confirmed by Layne, Meyer, Vaishwanar & Pincu (1962

Singer, Losito & Salmon (1963) found that progesterone administration in rats induced an increase in aldosterone. secretion rates. Most workers have postulated that progesterone causes a natriuresis which induces an increase in renin secretion, resulting in increased levels of aldosterone.

3. Oestrogens

The evidence presented by Layne et al. (1962), Laragh et al. (1967), Crane & Harris (1969) and Katz & Kappas (1967) suggests that oestrogens induced a rise in aldosterone excretion rates. From the evidence presented in the preceding section it is possible that the observed increase in aldosterone excretion rates were stimulated via the action of oestrogens on the renin-angiotensin system.

A number of workers have shown that oestrogens increased plasma levels of corticosteroids (Layne et al.,1962; Laidlaw et al., 1962; Peterson, Nokes, Chen & Black (1960) but depressed their secretion rates (Layne, et al., 1962; McKerns, Coulomb, Kaleita & De Ronzo 1958; Holzbauer, 1957 & Vogt, 1955).

It has been demonstrated that oestrogens diminish the conversion of corticosteroids to their metabolites (Robertson, 1959; Layne et al., 1962 and McKerns, 1958). It has been suggested, therefore, that the elevated levels of

corticosteroids observed with oestrogen treatment were a result of increased protein-binding of the corticosteroids which led to a diminished conversion of their metabolites and retention in the intravascular compartment. This theory is supported by the work of Bradley & Waterhouse (1966) who have demonstrated a marked increase in intravascular cortisol, without any effect on extravascular cortisol, during oestrogen treatment in women.

4. Variations in Adrenal Cortical Activity During the Menstrual Cycle

A number of workers have shown that aldosterone excretion (Reich, 1962; Sundsfjord & Aakvaag, 1970 and Nowaczynski, Koiw, Biron, Chretein & Genest (1962) and secretion rates (Gray et al. 1968) were significantly increased during the luteal phase of th menstrual cycle.

The evidence presented in the preceding section suggests that the activity of the renin-angiotensin system was increased at this time. This has led most workers to postulate that the high levels of progesterone, observed during the luteal phase of the cycle, induced natriuresis stimulated renin release, thus leading to increased secretion of aldosterone.

5. Variations in Adrenal Cortical Activity During the Oestrous Cycle

Variations in aldosterone levels during the oestrous cycle have not been reported. However, Dean, Cole & Chester Jones (1959) showed that plasma corticosterone levels were highest at pro-oestrus and oestrus, and lowest at dioestrus during the rat oestrous cycle. Telegday (1957) also

demonstrated an increase in corticosteroid levels at oestrus, but these levels were not significantly different from those observed at dioestrus. The results presented by Raps Barthe & Desaulles (1971) only partially agree with these findings. They demonstrated that the highest levels of corticosteroids occur at pro-oestrus, with maximal levels between 20.00 h and 24.00 h, but that the levels at oestrus were comparable with, or lower than, those at dicestrus. Zondek & Burnstein (1952) found that there is an 80% increase in corticosterone levels at oestrus in the guinea-pig.

It is apparent, therefore, that there is some discrepancy in the time when the peak in corticosterone levels has been observed during the oestrous cycle. Even so, these findings demonstrate a relationship between the cyclic activity of the ovaries and the adrenal cortex. The observed increases in corticosterone levels may be mediated through an increase in protein binding of corticosterone induced by increased levels of oestrogens.

6. Adrenal Cortical Activity During Pregnancy

The increase in aldosterone secretion and excretion rates and plasma concentration during human pregnancy are well documented. The results presented by Rinsler & Rigby (1957) Martin & Mills,(1956), Venning, Primrose,Caligaris & Ryrenfurth (1957), Kumar (1959) and more recently Kuryanova, (1970) demonstrated that there was a progessive increase in the excretion of aldosterone metabolites in women. It has been shown by other workers however, (Jones et al., 1959, Tate & Little, 1968 and Kuryanova (1970) that there

was an alteration in the pattern of aldosterone metabolism during human pregnancy. The formation of the 3 oxo-4 ene conjugate of aldosterone was found to be increased but conversion to metabolit conjugated with glucoronic acid was decreased. Therefore the assumption that increased excretion of aldosterone metabolites indicates an increase in aldosterone secretion is not necessarily correct.

A number of workers have presented results which show very considerable increases in aldosterone secretion rates during pregnancy. Watanabe, Meeker, Gray, Sims & Solomon (1963) found aldosterone secretion rates of up to 15 times greater than non-pregnant levels. Jones, Lloyd-Jones, Riondel, Tait, Tait, Bulbrook & Greenwood (1959) and Wiele, Gurpide, Kelly, Laragh & Lieberman (1960) have demonstrated increases of 300% in aldosterone secretion rates during pregnancy.

Significant increases in plasma aldosterone (Weir et al., 1970 and cortisol (Doe, Dickinson, Zinnerman & Seale, 1969; O'Connell & Welsh, 1969) have also been demonstrated during pregnancy in women. In rats, plasma corticosterone levels were found to be increased in pregnancy (Vogt, 1969).

The increased activity of the renin-angiotensin system during pregnancy would appear to significantly contribute to the stimulus for increased aldosterone levels during pregnancy. However, Melby, Dale, Wilson & Nicholas (1966) have shown that intravenous administration of human placental somatotropin (H.P.S.) stimulates aldosterone secretion. This would indicate that H.P.S. may also stimulate aldosterone levels during pregnancy. It is suggested that the increased cortisol levels observed are a result of increased plasma binding.

GENERAL MATERIALS AND METHODS

Animals

250-280 g female and male Wistar rats were used throughout the study and were housed for one week preceding use. During experimental studies female rats were kept in a room separated from the main animal house to which only the operator had access. The room was maintained at approximately 23°C with controlled lighting (Takahashi & Suzuki, 1969).

The rats were kept in cages containing 4 animals and were supplied with tap water and a standard 41B pellet diet ad libitum (Cooper & Haynes, 1969).

Vaginal Smears

The various stages of the oestrous cycle were determined by taking vaginal smears at approximately 8.30 a.m. each day. The stages of the oestrous cycle were designated as follows:-

Dioestrus - the day on which the smear consisted mainly of leukocytes.

Pro-oestrus - the day on which the smear consisted mainly of nucleated epithelial cells.

Oestrus - the day on which large numbers of squamous epithelial cells were present in the smear.

Metoestrus - the day on which the smear contained many leukocytes with a few remaining cornified epithelial cells.

Statistical Evaluation of Results

The mean and standard error for each set of results was calculated from the following formulae:-

$$\text{Mean} = \frac{Ex}{n}$$

where 'x' is the variable and 'n' the number of variables.

$$s = \sqrt{\frac{Ex^2}{n-1} - \frac{(Ex)^2}{n(n-1)}}$$

where 's' is the standard deviation.

$$\text{Standard error} = \frac{s}{\sqrt{n}}$$

Students' 't' test was applied to compare observations from different populations.

For samples of equal numbers of observations:

$$t = \frac{Ex_1 - Ex_2}{\sqrt{(SE_1^2 + SE_2^2)}}$$

For samples of unequal observations:

$$t = \frac{x_1 - x_2}{Q\sqrt{1/n_1 + 1/n_2}}$$

where $Q = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{(n_1 + n_2 - 2)}}$

The value of probability, p, was obtained from the appropriate tables.

SECTION I

Variation in Water and Electrolytes in Tissues and Plasma during the Oestrous Cycle

Materials and Methods

Anaesthesia

The animals were anaesthetised with an intraperitoneal injection of 60 mg/kg sodium pentobarbitone (Nembutal, Abbott Laboratories Ltd.).

Blood Samples

All blood samples for plasma electrolyte determinations were obtained from the right carotid artery. This was cannulated using heparinised Portex No: 25 polythene tubing. The blood samples were centrifuged in order to obtain the plasma.

Tissue Samples

The whole liver, right kidney, heart and left gastrocnemius muscle were removed, cleared of extraneous tissue, blotted and weighed.

Preparation of Tissue Samples for Analysis

The fresh tissues were dried to a constant weight at 105^{o}C and then ground in a mortar with three changes of a mixture of equal volumes of diethyl ether and light petroleum ether (b.p. 40-60^{o}C) in order to remove any fat.

The resulting powder was dried at 105^{o}C for 12 hours and stored in a dessicater over silica gel. Duplicate 20 mg samples of the dry, fat-free powder were weighed into nickle crucibles and ashed at 500^{o}C for 18 hours in a Muffle furnace.

After cooling to room temperature in a dessicator, the ash was dissolved in 4 ml of 1.16 N HCl for flame photometry.

Sodium and Potassium Estimations

Sodium and potassium levels in the plasma and fat-free dry tissue were determined using an EEL flame photometer.

The standard sodium solutions contained 6.34 g of dry 'Analar' sodium chloride per litre of distilled-deionised water. The standard potassium solution contained 4.77 g of dry 'Analar' potassium chloride per litre of distilled-deionised water. Sodium was included in the potassium standard in order to compensate for spectral interference caused by high concentrations of sodium in the sample solutions. The standard solutions were diluted 50 times and the plasma samples were diluted 1,000 times before the analyses were carried out. After initial calibration of the flame photometer with standard solutions, deionised water and standard solution were sprayed after each sample. The standards for the tissue samples were made up in 1.16 N HCl instead of distilled-deionised water. Distilled-deionised water was sprayed after each sample.

Cleaning of Glassware

All glassware was rinsed thoroughly in tap water and immersed in a strong solution of Pyroneg (Diversy, Ltd.) for at least 24 hours. The glassware was then brushed and rinsed thoroughly in tap water. This was followed by several rinses in distilled water.

After washing, the nickle crucibles were placed in a Muffle furnace for 12 hours at 500^{o}C before use.

SECTION II

The Action of Ovarian Hormones on Renal Electrolyte Function

Materials and Methods

Anaesthesia

For non-recovery experiments anaesthesia was induced by an initial intraperitoneal injection of 45 mg/kg of sodium pentobarbitone (Nembutal, Abbott Laboratories Ltd.) and maintained with small supplementary doses where necessary. The animals were tracheotomised with Portex No. 5 polythene tubing in order to ensure a free air passage.

For the recovery experiments anaesthesia was induced by inhalation of a mixture of 80% nitrous oxide and 20% oxygen containing 3.5% halothane (Fluothane, I.C.I. Ltd.) and maintained by halothane at 1-1.5% in the same gaseous mixture.

Measurement of Body Temperature

Rectal temperature was taken as an indication of the maintenance of deep body temperature during the 8 hour infusion experiments. The temperature was determined using a standard 6 inch mercury thermometer inserted 2 inches into the rectum.

Ovariectomies

Ovariectomies were carried out under sterile conditions in an operating theatre.

The animals were anaesthetised using halothane with an oxygen-nitrous oxide gas mixture. All instruments were sterilised in a mixture of 5% Hibitane (I.C.I. Ltd.) and

70% methyl alcohol, 2:5 v/v, and rinsed in distilled water before contact with the animals tissues.

The mid-lateral area was shaved and washed with 0.5% aqueous Hibitane and the abdomen opened on each side by two small incisions. The Fallopian tubes and associated blood vessels were ligated and the ovary removed. The muscle wall was sutured using plaited silk thread (size 0) and a curved triangular needle (size 14). The skin was closed in the same way. The area was washed with 0.5% aqueous Hibitane (I.C.I. Ltd.) covered with sterile sulphathiazole with penicillin (5,000 i.μ./g., Macarthys) and sprayed with Aeroplast dressing (Parke-Davis).

The animals were housed individually in clean cages 12 hours post-operatively and then housed four animals per cage under conditions previously described.

Successful removal of the ovaries was verified by taking vaginal smears from day 4 to day 7 after the operation. At least 14 days elapsed after the ovariectomy before the animal was used.

Cannulations

All cannulations for the 8 hour renal function experiments were carried out under sodium pentobarbitone anaesthesia.

a) Vein Cannulations

The left jugular vein was cannulated, using Portex No. 30 polythene tubing for saline infusions.

b) Arterial Cannulations

All blood samples were taken from the right carotid

artery. This was cannulated using heparinised Portex No. 25 polythene tubing. Blood samples were centrifuged to obtain the plasma.

c) Bladder Cannulations

The bladder was catheterised using Portex No. 5 polythene which was kept in place with a ligature thread. Urine was allowed to collect into a graduated centrifuge tube. A short mid-line incision was made in the lower abdomen and the bladder emptied at the end of each collection period by manual compression.

Saline Infusion

All solutions were infused via the jugular vein using a Palmer Injection Apparatus. The pump was set to deliver a small constant infusion of 3mls/hour. The saline solution used throughout the experiments was Tyrodes without glucose and had the following composition:-

Compound	Grams/100mls water
NaCl	0.80
KCl	0.02
$CaCl_2$	0.02
$MgCl_2$	0.01
$NaHCO_3$	0.10
NaH_2PO_4	0.005

(Biochemist's Handbook).

All solutions were filtered through Whatman No. 1 filter paper before use.

Determination of Glomerular Filtration Rate

Inulin Infusion

A primer dose of 1ml of 5% inulin in saline was given, followed by a sustaining infusion of 1% in saline (3 ml/hour). All solutions were filtered through Whatman No.1 filter paper before infusion (Johns, 1968).

Inulin Estimations

The inulin concentration in urine and plasma were determined by the method of Schreiner (1951):-
5 mls of 30% HCl and 2 mls of 0.1% resorcinol in 95% alcohol were added to 2 ml of the inulin sample or standard. The mixture was incubated at 80°C for 25 mins. and cooled to room temperature. The optical density was read at 490 mu against a reagent blank and standards.

Glomerular filtration rate was calculated from the following formulae:-

$$c = \frac{u \times v}{b}$$

where 'c' = clearance

'u' = urinary inulin concentration

'v' = urinary volume

'b' = plasma inulin concentration

Flame Photometry

The sodium and potassium concentrations of the urine were determined using an EEL flame photometer.
Urine samples were diluted 1:000 with distilled-deionised water and the sodium and potassium concentrations determined by the procedure described for plasma in section I.

Commercial Hormone Preparations

Spironolactone

Spironolactone was obtained as Aldactone® from Searle and Co., in tablet form. Each tablet contained 25 mg of spironolactone. The dose to be administered was prepared by grinding the tablets in a mortar and suspending the required quantity of fine powder in 0.2 ml of distilled water for oral injection.

Oestradiol

Oestradiol - 17β benzoate was supplied in powder form by the Sigma Chemical Co. The required dose was dissolved in 0.1 ml Arachis Oil (British Drug Houses, Ltd.) by gently heating the oil for intramuscular injection.

Progesterone

Progesterone was supplied by the Sigma Chemical Co. This hormone was dissolved in 0.1 ml Arachis Oil for intramuscular injection. For direct infusion experiments the hormone was dissolved in a small volume of absolute alcohol and diluted to the required concentration with saline. The solution was filtered through a Whatman No.1 filter paper prior to use.

Cleaning glassware

All glassware was thoroughly rinsed in tap water and immersed in a strong solution of Pyroneg for at least 24 hours, brushed and thoroughly rinsed in tap water. This was followed by several rinses in distilled water.

All glassware which had contained steroids was rinsed in acetone prior to immersion in Pyroneg.

SECTION III

Determination of Aldosterone and Corticosterone Secretion Rates During the Oestrous Cycle

Materials and Methods

Anaesthesia

Anaesthesia was induced by an intraperitoneal injection of sodium pentobarbitone (60 mg/kg).

Renal Vein Cannulation

The left kidney was exposed by a mid-ventral laporotomy. The renal and adrenal veins and the renal artery were carefully cleared and small blood vessels associated with the adrenal gland and vein were cauterized. The renal vein, and ovarian vein in the female rats, were ligatured and an artery clamp applied to the renal vein close to the inferior vena cava.

Heparinised Portex No. 30 polythene tubing was inserted into the renal vein so that blood entering the renal vein from the adrenal vein flowed into the cannula.

The artery clamp was removed and 200 i.u. of heparin in saline (Boot's Pure Drug Co. Ltd.) infused into the cannula. A ligature was applied close to the inferior vena cava.

Blood was collected into an ice-cooled vessel for exactly 15 mins. The volume in this time was determined and aldosterone 4-C^{14} (2,000 c.p.m.) and corticosterone 4-C^{14} (2,500 c.p.m.) added together with an equal volume of distilled water. The mixture was stored at -20^{o}C.

Double Isotope Derivative Assay for Aldosterone and Corticosterone

Aldosterone and corticosterone secretion rates were determined by a modification (Kinson, 1967) of the double isotope derivative assay of Kliman & Peterson (1960).

Whole blood was extracted rather than plasma since Holzbauer & Vogt (1961) demonstrated that adrenocorticoids are bound to red blood cells in a number of animals.

In the method of Kliman & Peterson C^{14} - steroids, as an indicator of steroid losses, were not added until after the acetylation stage. Therefore losses prior to this step were undetected. In the modified method, C^{14} - marker steroids were added at the beginning of the procedure. The paper chromatography systems of Kliman & Peterson involved at least 16 hours running time and therefore thin-layer chromatography systems were substituted. The solvent systems described by Benraad & Kloppenburg (1964) were found to be suitable for the separation of aldosterone diacetate ahead of corticosterone acetate on thin-layer.

The chromic oxide step was retained in order to increase the specificity of the assay through the formation of new products with different chromatographic characteristics.

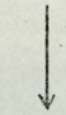
Solvents

All solvents were of the 'Analar' grade (British Drug Houses Ltd.) and were used throughout the assay without further purification.

Reagents

Analar pyridine was refluxed over barium oxide for 4-6 hours and distilled in a fractionating column. The

FIG. 1a



MODIFIED DOUBLE-ISOTOPE DERIVATIVE ASSAY FOR ALDOSTERONE AND CORTICOSTERONE

EXTRACTION OF WHOLE BLOOD + C^{14} LABEL

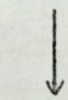
ACETYLATION
(0.03 ml H^3 acetic anhydride + 0.025 ml pyridine at 38°C for 24 hrs)

T.L.C. I
($CHCl_3$ for 3 hrs)

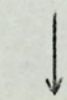
T.L.C. II
($CHCl_3$:MeOH:H_2O 94:6:0.5)

T.L.C. III
(Ethyl Acetate:Dichloroethane:H_2O 90:10:1)

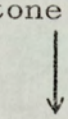
OXIDATION
(0.1 ml of 0.5% chromium trioxide in acetic acid)



PAPER CHROMATOGRAPHY
(Cyclohexane:Dioxane:MeOH:H_2O 40:40:20:10)



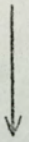
T.L.C. IV
(Benzene:Acetone:H_2O 75:50:0.2)



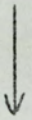
T.L.C. III

COUNTING

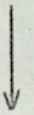
FIG. 1b



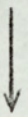
DOUBLE-ISOTOPE DERIVATIVE ASSAY OF KLIMAN & PETERSON (1960)



EXTRACTION OF PLASMA



ACETYLATION
(0.03 ml H^3 acetic anhydride + 0.025 ml pyridine
at 38°C for 24 hours)



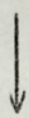
ADDITION OF C^{14} - INTERNAL STANDARD

PAPER CHROMATOGRAPHY I
(Cyclohexane:benzene:methanol:water 4:2:4:1)
18 hours

PAPER CHROMATOGRAPHY II
(Cyclohexane:dioxane:methanol:water 4:4:2:1)
20 hours

CHROMIUM TRIOXIDE OXIDATION

PAPER CHROMATOGRAPHY III
(Cyclohexane:benzene:methanol:water 4:3:4:1)
18 hours



COUNTING

middle fraction, boiling at 115°C, was collected and stored over calcium chloride in a dessicator. Glacial acetic acid was refluxed over an excess of chromium trioxide for 6 hours. The chromate was decanted off and the acetic acid distilled over fresh chromium trioxide. The fraction boiling at 118°C was collected.

Chromium trioxide reagent was freshly prepared for each oxidation. Liquid scintillation fluid was prepared by dissolving 4 g of 2,5-diphenylxyloxazole and 0.4 g of 1,4,di2(5-phenyloxazole) in 1 L of toluene. 1% alcohol was added to this mixture in order to increase the efficiency of counting by decreasing the absorption of steroids onto the surface of the glass vials. Nitrogen was bubbled into the mixture for 15 mins. in order to displace oxygen from the solution and therefore reduce the quenching.

All labelled compounds were supplied by the Radiochemical Centre, Amersham. Aldosterone 4-C^{14} (50mCi/mMol in benzene/ethanol) and corticosterone 4-C^{14} (50mCi/mMol in benzene/ethanol solution) were redissolved in methanol and stored at -29°C.

Acetic anhydride-H^3 (100uCi/mMol in benzene solution) was stored at -20°C in a dessicator.

The radioactive standards used were n-hexadecane-1, 2-H^3(1.98uCi/g) and n-hexadecane-1-C^{14} (1.01uCi/g).

Aldosterone-18, 21-diacetate and corticosterone-21-monoacetate (Sigma Chemical Co.) were used as unlabelled markers for the visual detection of the steroids on the chromatograms.

Determination of the Specific Activity of Acetic Anhydride

0.5 ml of cortisol (Sigma Chemical Co.) was dissolved in a mixture of 0.03 ml acetic anhydride-H^3 and 0.025 ml pyridine in a tightly stoppered conical tipped test tube. After incubation for 18 hours at 25°C, 0.5 ml of 25% ethanol was added and the cortisol acetate extracted with 5 volumes of dichloromethane. The aqueous phase was removed by aspiration and the dichloromethane washed twice with 0.5 ml of distilled water. The dichloromethane was evaporated off under a stream of nitrogen at 40°C.

The cortisol acetate was purified by thin-layer chromatography systems II and III (Fig. 1). The resultant compound was dissolved in methanol and precise aliquots analysed for tritium activity on a Packard 3003 liquid scintillation counter.

The concentration of cortisol acetate was determined by measuring the ultraviolet absorbance at 240 mu on a Beckman DB spectrophotometer.

Procedure

Extraction of Blood Samples

It was found that ethyl acetate did not have the tendency shown by other solvents to form an emulsion with the blood samples. (Kinson, 1967). Therefore, ethyl acetate was used for all blood extraction procedures.

The blood sample was mixed thoroughly with C14-marker steroids and an equal volume of distilled water and extracted in a separating funnel with 5 volumes, followed by 2.5 volumes of ethyl acetate. The combined extracts were

washed twice with 1/40 volume 0.1N sodium hydroxide, followed by several washings with distilled water.to neutrality. The organic phase was transferred to a round bottomed flask and the solvent evaporated off at 40°C using a rotary film evaporator. The residue was transferred to a conical tipped test tube in acetone, which was subsequently evaporated at 40°C under nitrogen.

Acetylation

The extracts were acetylated to form the products shown in Fig 2. The extracts were dessicated under reduced pressure for 12 hours and 0.03 ml of H^3-acetic anhydride and 0.025 ml of pyridine were added using a Hamilton syringe.

The tubes were tightly stoppered and the solutions thoroughly mixed with the extracts in the tip of the tube. The mixture was incubated at 38°C for 24 hours. 1 ml of distilled water was added, followed by 10 ml of chloroform. The phases were thoroughly mixed on a Whirlimixer (Fisons Scientific App. Ltd.)

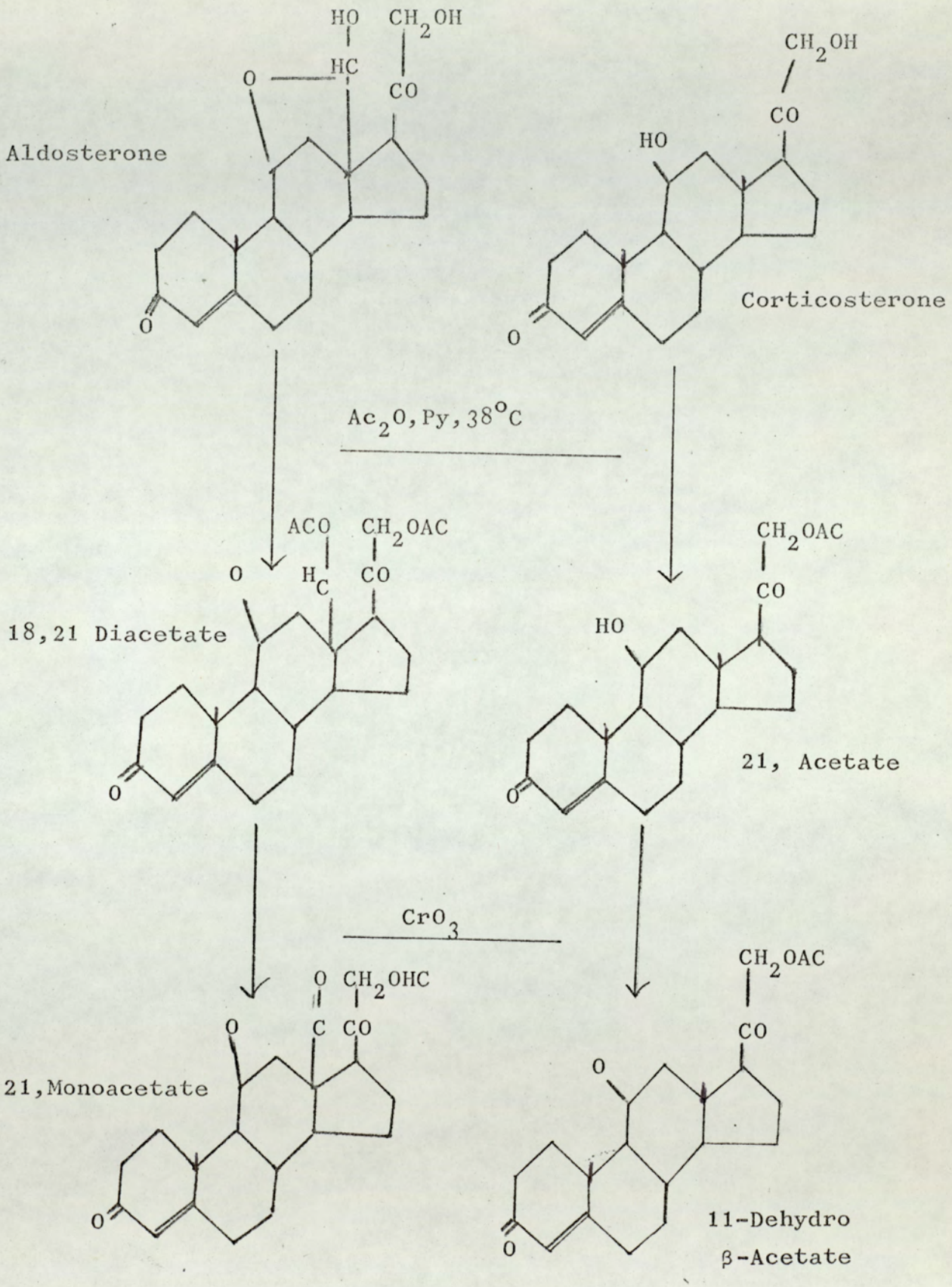
The aqueous phase was removed by aspiration and the organic phase washed twice with 1 ml of distilled water. The solvent was evaporated to dryness under a stream of nitrogen.

Thin Layer Chromatography

Details of the chromatography systems used are presented in Fig.2. Thin layer chromatography was carried out on 10 x 20 cm glass plates, coated with 0.25 mm layers of MH-Kieselgel G/UV_{254} (Macherey, Nagel & Co.). The material was taken up in 0.05 ml of solvent and applied as

FIG. 2.

THE ACETYLATION AND OXIDATION OF ALDOSTERONE AND CORTICOSTERONE



a strip 3 cm from the lower edge of the plate. The run was completed 15 cm from the origin.

The tanks (28 x 22 x 13 cm) were lined with filter paper and saturated for one hour before use with 150 ml of solvent. The tanks were freshly prepared for each run.

Elution from Thin Layer

The steroid zones were located by UV light and transferred to conical tipped test tubes. 5 ml of a 3:1 mixture of ethyl acetate:methanol with 0.5% distilled water was added to the tube and the contents thoroughly mixed. This was followed by centrifugation at 40,000 r.p.m. for 5 min. and the solution decanted into a second tube. The procedure was repeated twice with 2 ml of solvent. The solvent was evaporated under nitrogen and transferred to the two further thin layer systems.

Oxidation

0.1 ml of 0.5% chromium trioxide in glacial acetic acid was added to each sample in a conical tipped test tube. The reaction was allowed to proceed at room temperature for exactly 15 mins., when it was terminated by the addition of 1 ml of 25% aqueous ethanol. The steroids were extracted with 10 ml of chloroform and the extract washed twice with 1 ml of distilled water. The solvent was evaporated off at 40^{o}C under a stream of nitrogen.

Paper Chromatography

The products of oxidation were run through the paper chromatography system. Paper chromatograph was carried out in a tank (56 x 28 x 19 cm) lined with filter paper and

saturated with 600 ml of solvent. The tank was saturated for 48 hours prior to a run and the solvent renewed every 4 weeks.

The material was taken up in 0.05 ml of solvent and applied 9 cm from the top of Whatman No. 20 strips. The chromatograph paper was allowed to equilibrate in a saturated tank for 6 hours before introduction of the running solvent mixture. The paper chromatrograms were run for 16 hours.

Elution from Paper

Steroid zones were visualised with a UV light and the areas cut out and transferred to conical tipped test tubes. The steroids were eluted by incubation in 10 ml of ethyl acetate:methanol (3:1) for 30 mins. at 40°C.

The products were run through two further thin layer systems.

Liquid Scintillation Counting

The samples were taken up in 10 ml of liquid scintillator and counted in low potassium vials on a Packard 3003 counter for 15 mins. each.

Calculation of Results

Adrenal secretion rate - µg/Kg/adrenal/hr.

$$= (c/c \times H/SA) - m \times 60/T \times 1000/BW.$$

When C = cpm C^{14}-internal standard added to sample,

c = c = cpm H^3 counted

H = cpm H^3 counted

SA = specific activity of acetic anhyride in cpm H^3/µg steroid

m = µg of internal standard added to sample

T = blood collection period in minutes

BW = bodyweight of animal in grams.

RESULTS

SECTION I.

Variations in Tissue and Plasma Electrolytes During the Oestrous Cycle in the Rat

Introduction

The evidence cited in the review of the current literature suggests that there is a cyclic variation in electrolyte metabolism during the human menstrual cycle. In the present study initial investigations were carried out to determine the electrolyte content of tissues and plasma during the oestrous cycle in the rat.

The electrolyte and water content of tissues were determined at the various stages of the oestrous cycle in the rat. Samples of uterus, kidney, liver, plasma, heart and skeletal muscle were taken from female rats at the various stages of the oestrous cycle. All tissues were prepared for analysis by the method of MacIntyre & Davidsson (1958) and the sodium content of tissue and plasma samples determined by flame photometry.

The results presented in Fig. 3a show that there was a significant increase in the sodium content of heart and skeletal muscle at oestrus compared with dioestrus, and plasma sodium levels were higher at oestrus than dioestrus. The sodium content of the uterus was significantly higher at pro-oestrus than at met- or dioestrus.

The water and potassium content of the uterus showed a similar variation to that of sodium during the oestrous cycle. However, the variation in water and potassium content of the other tissues studied was less marked (Fig 3B and 3C).

LEGENDS

'' Compared with dioestrus

' " " metoestrus

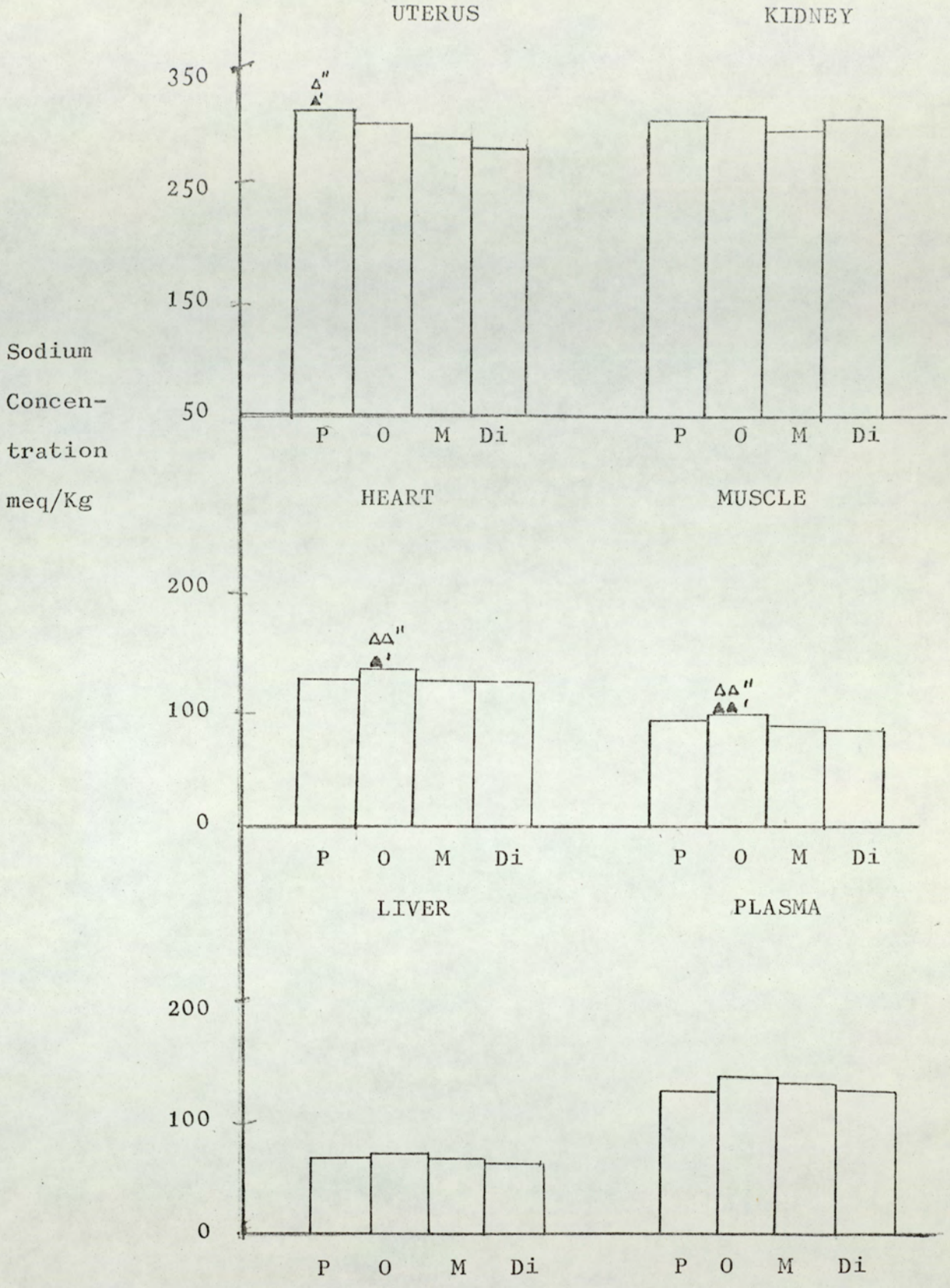
△ $p < 0.05$

△△ $p < 0.02$

▲ $p < 0.01$

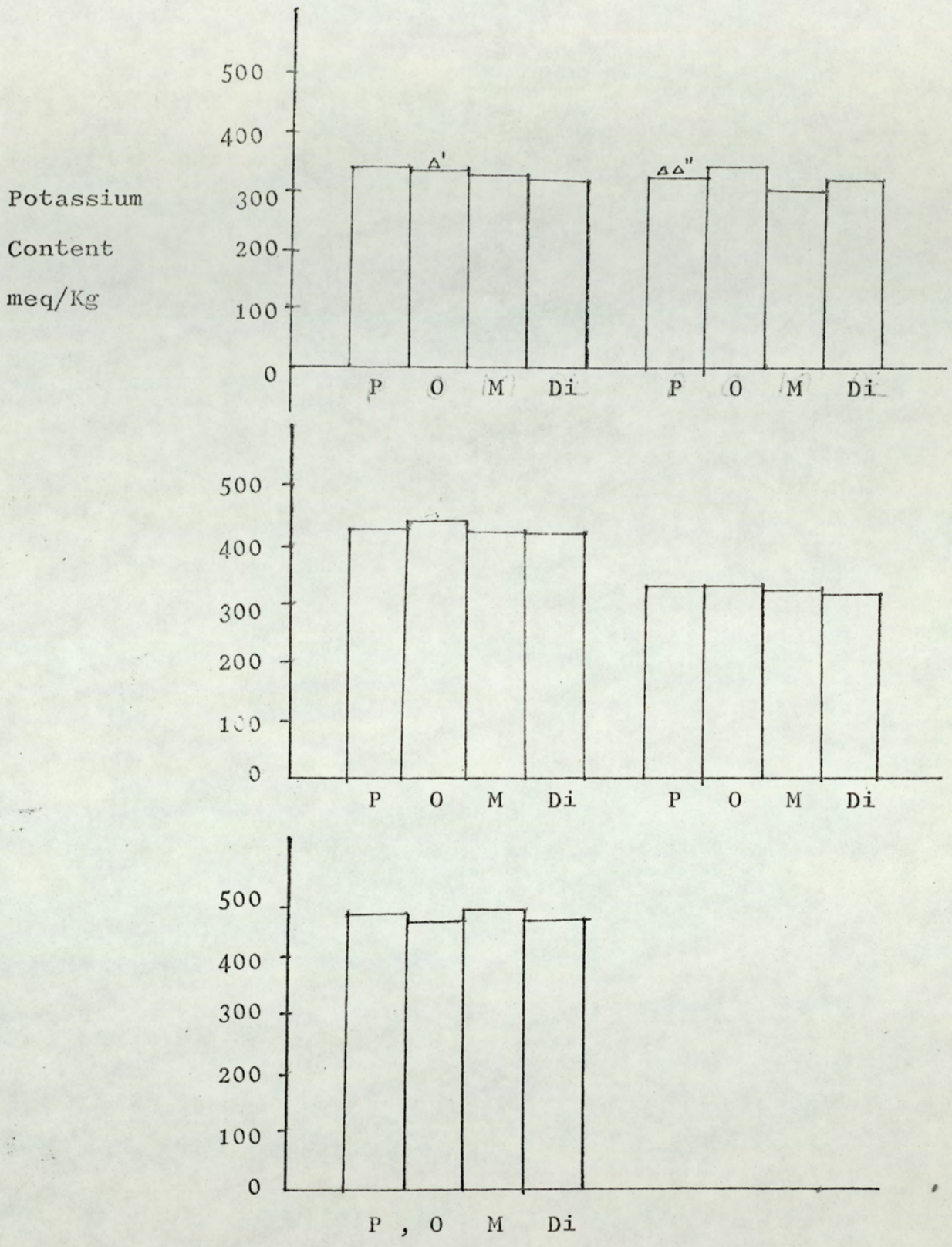
▲▲ $p < 0.001$

FIG. 3a



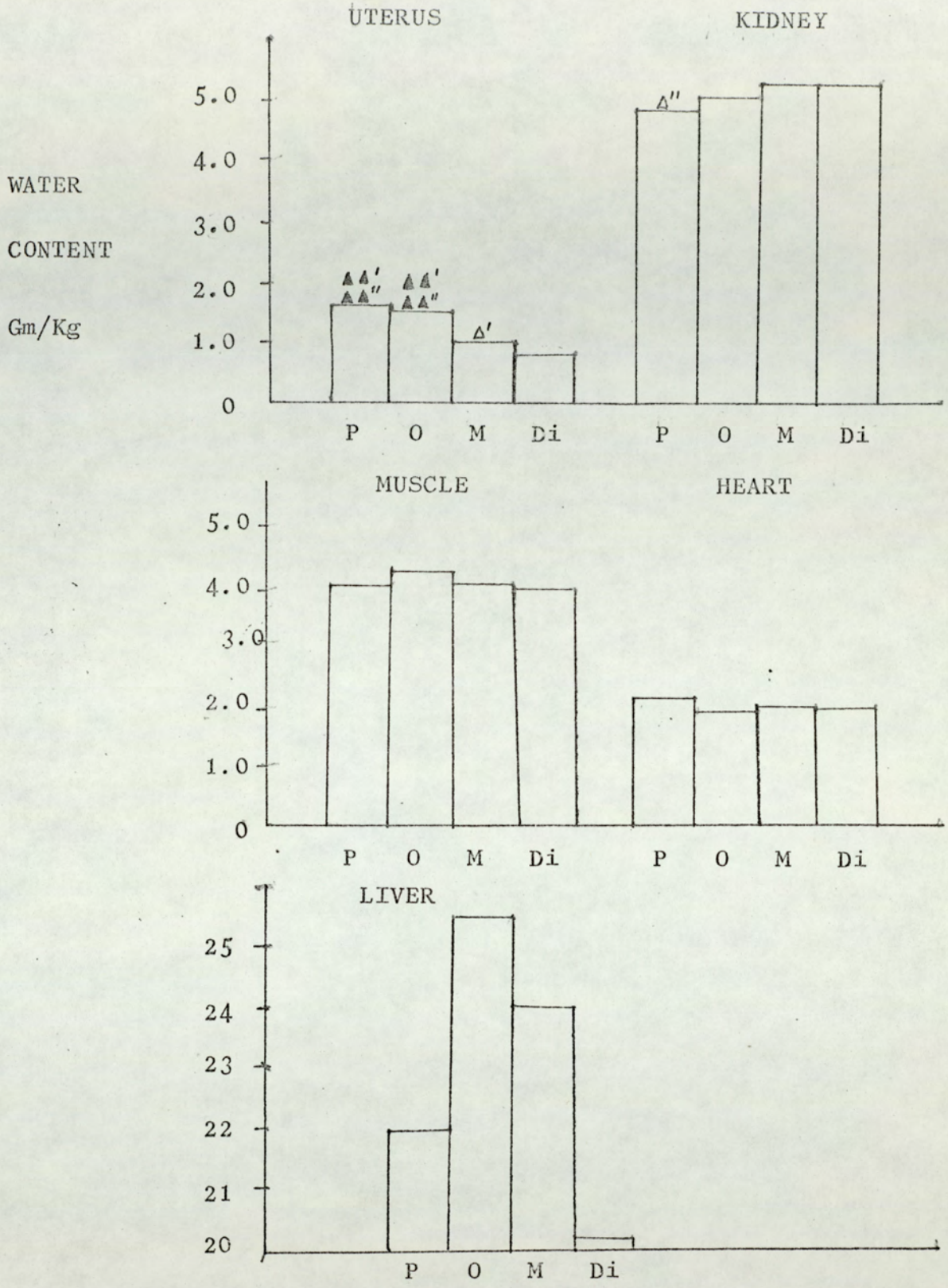
The Sodium Content of Tissues During the Oestrous Cycle

FIG. 3b



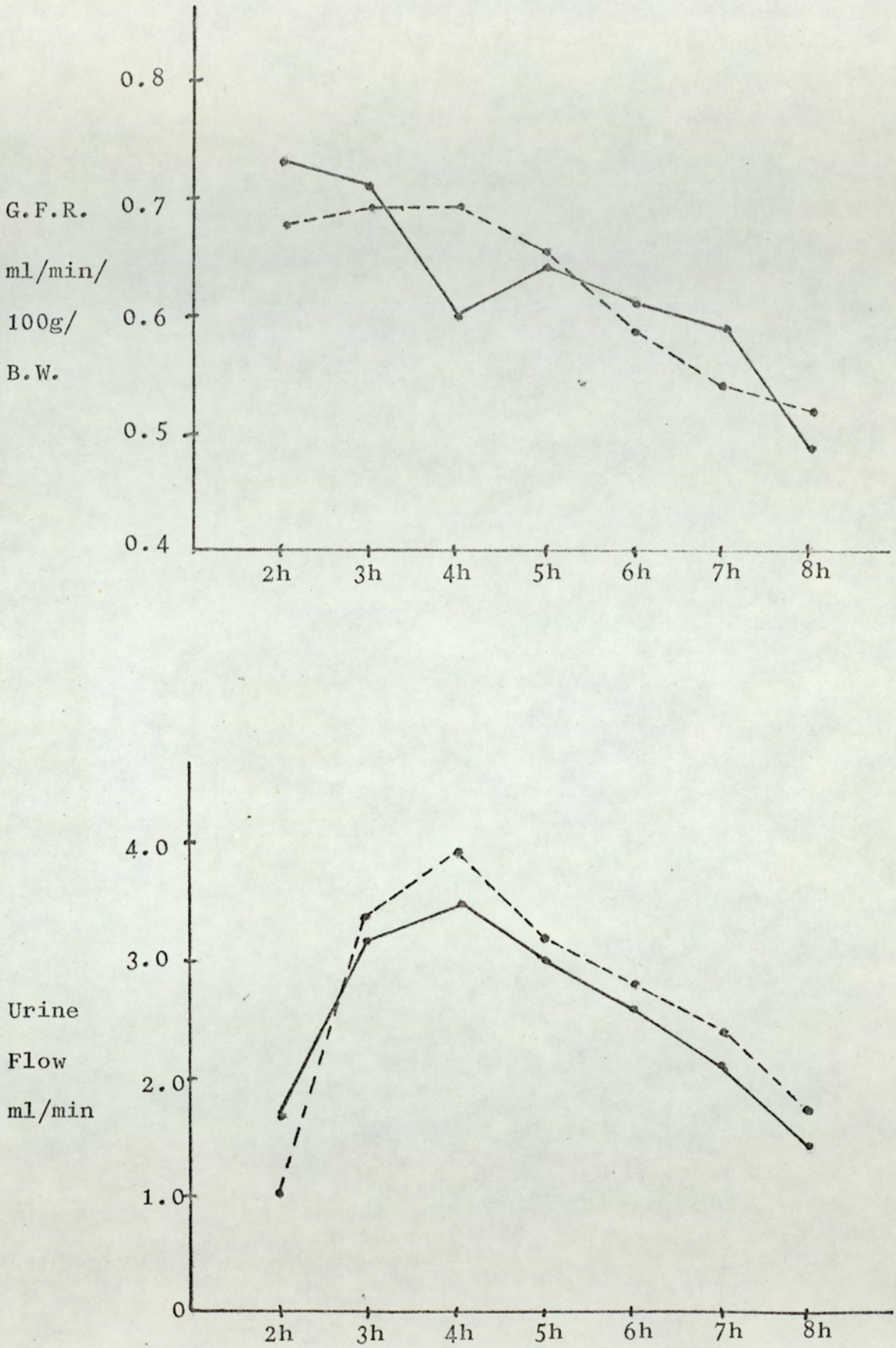
The Potassium Content of Tissues During the Rat Oestrous Cycle

FIG . 3c



The Water Content of Tissues During the Rat Oestrous Cycle

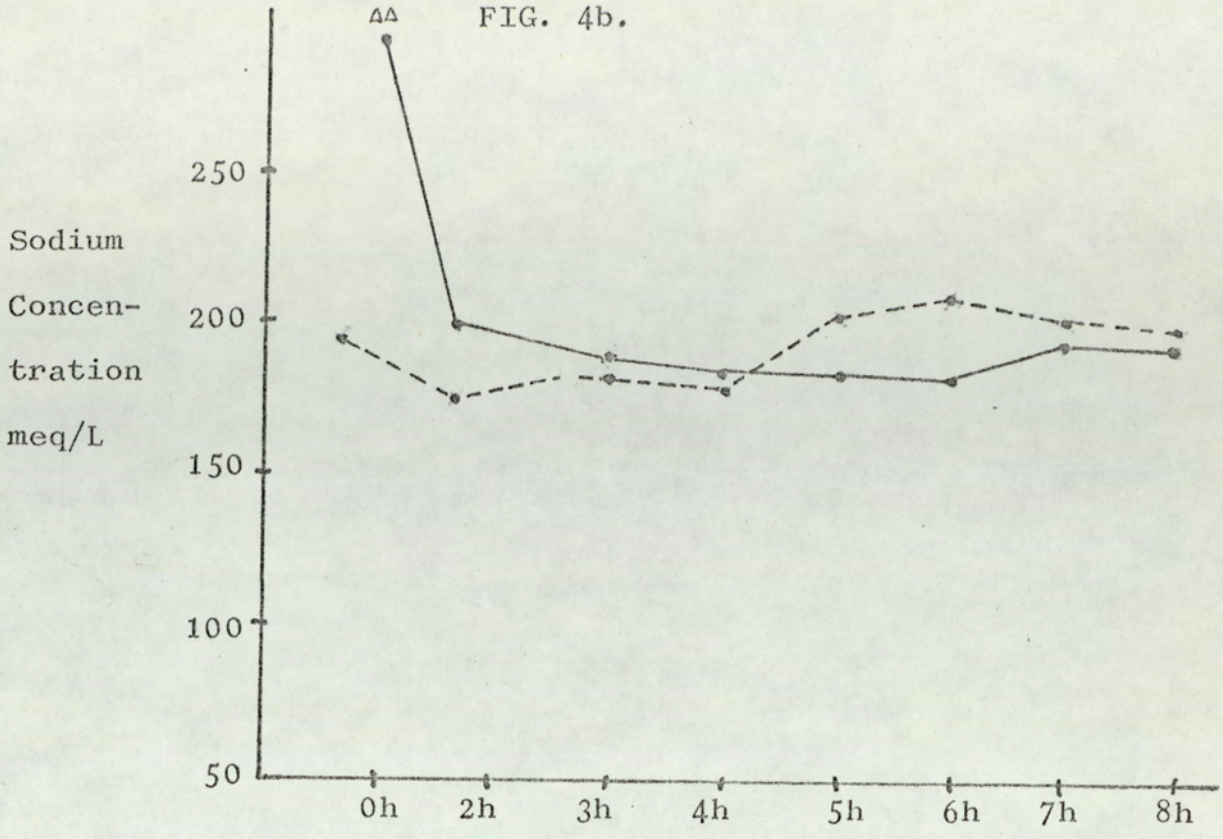
FIG. 4a

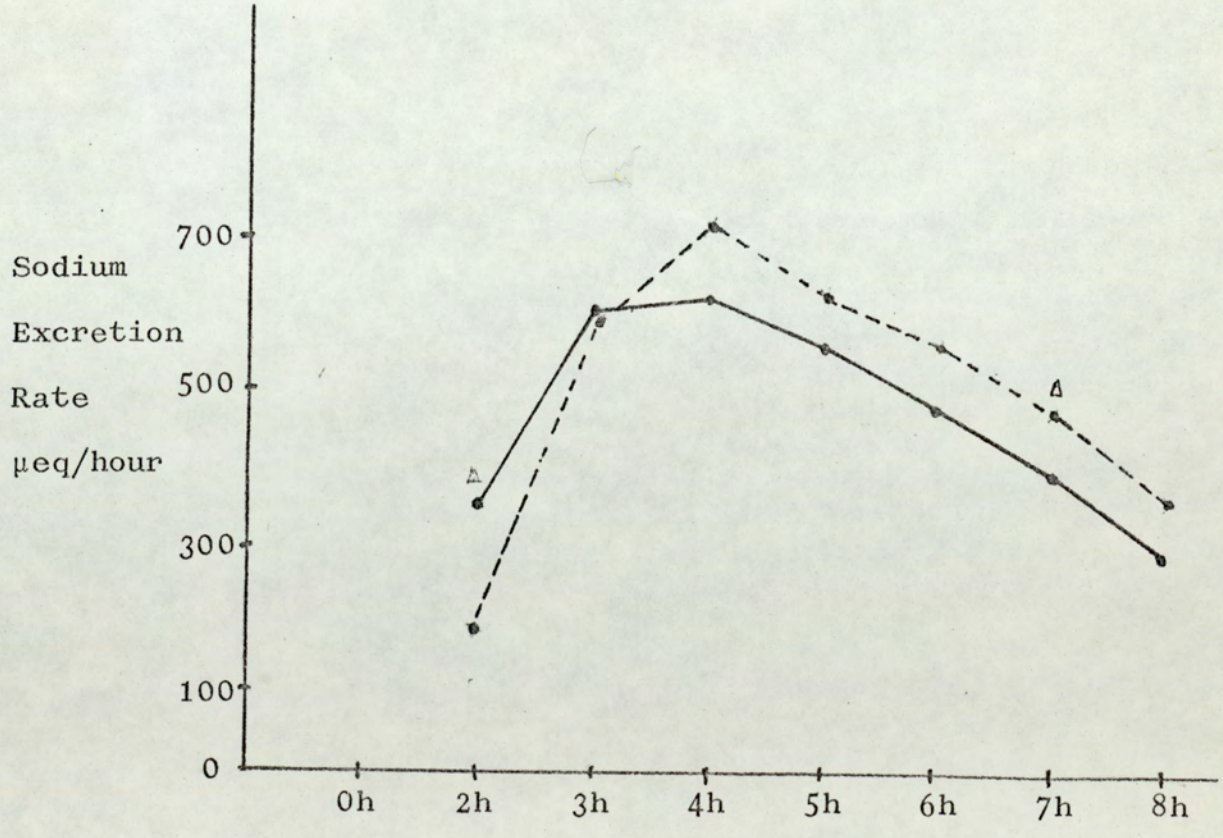


The Action of Saline Infusion on Urine Flow and G.F.R. in ovariectomised Rats.

Expt. 1. ——— Expt. 2 - - - -

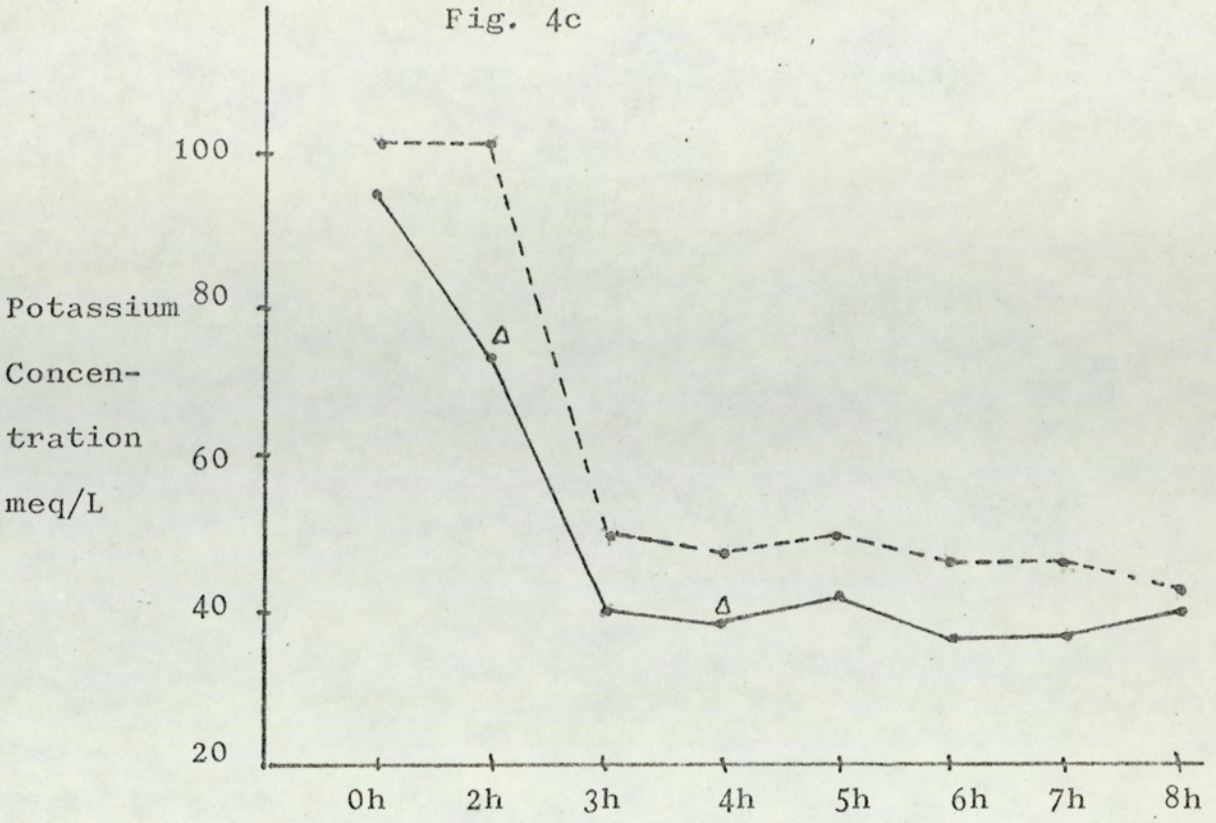
FIG. 4b.

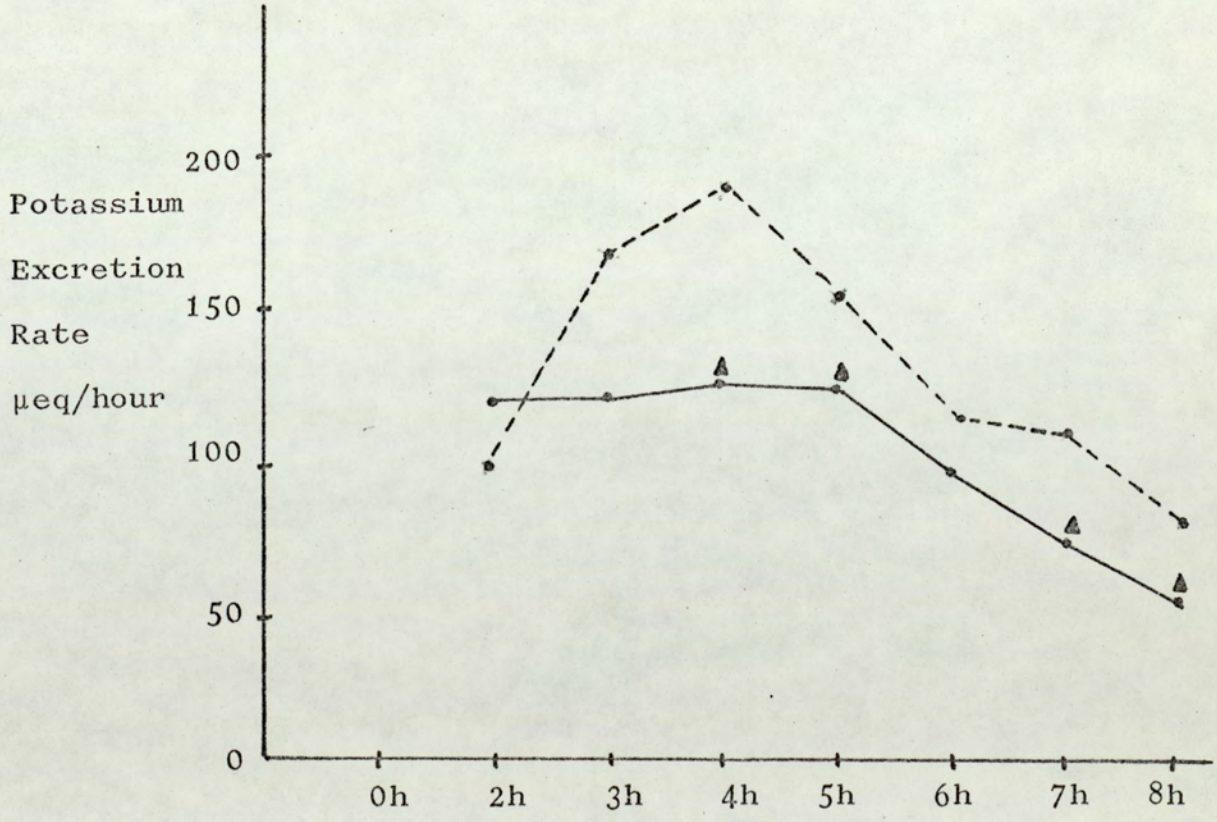




The Action of Saline Infusion on Urinary Sodium Concentration and Excretion Rate in Ovariectomised Rats

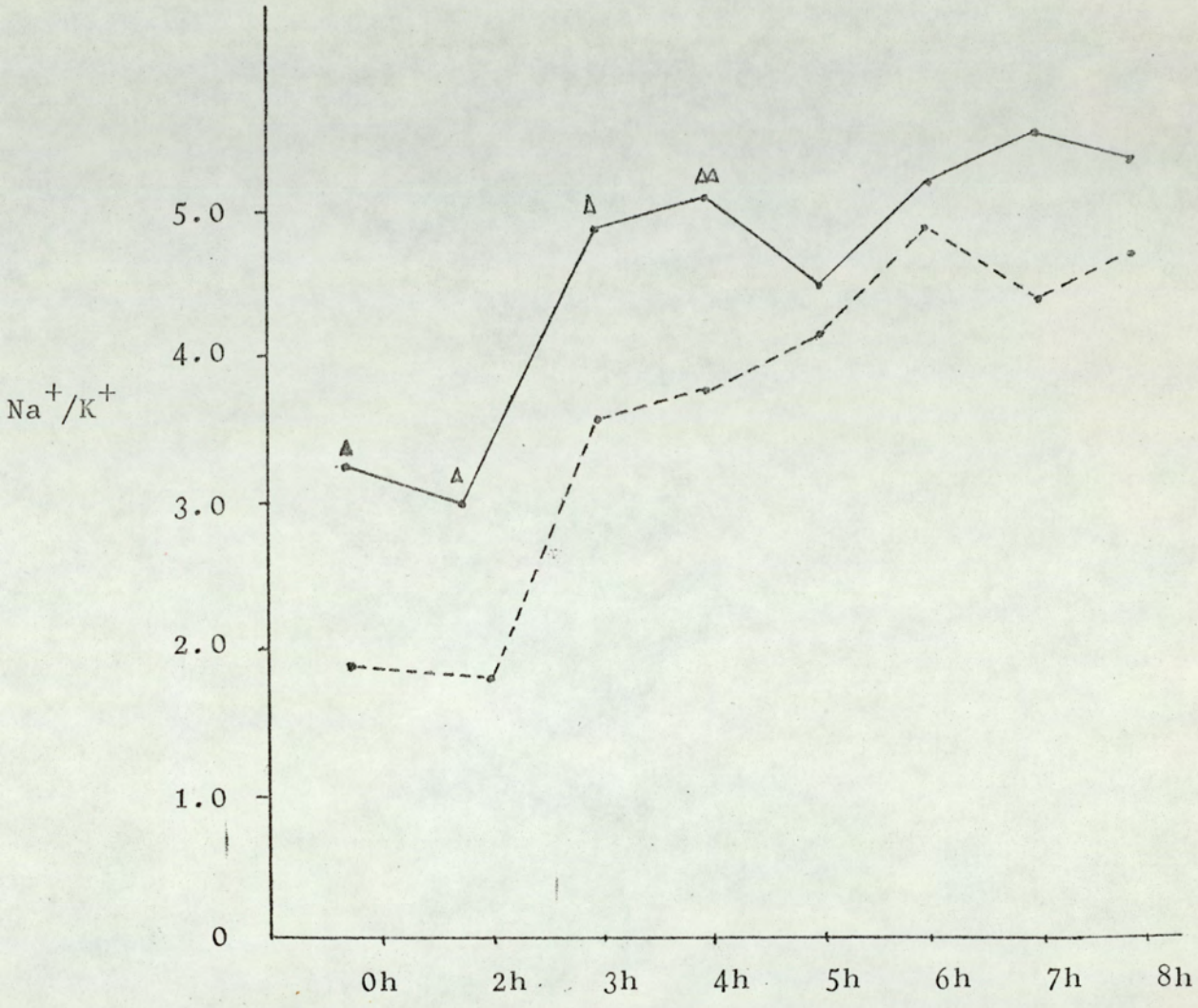
Fig. 4c





The Action of Saline Infusion on Urinary Potassium Concentration and Excretion Rate in Ovariectomised Rats

FIG. 4d



The Action of Saline Infusion on theSodium Potassium Ratio in Ovariectomised Rats

These results would suggest, therefore, that there is a variation in electrolyte metabolism during the rat oestrous cycle, with an increase in sodium content at oestrus compared with dioestrus.

SECTION II

The Action of Ovarian Hormones on Renal Electrolyte Function

Introduction

The results presented in Section I suggest that there may be a variation in electrolyte balance during the oestrous cycle in the rat. This was further investigated by determining the action of ovarian hormones on renal electrolyte function.

Urine flow, glomerular filtration rate (G.F.R.), urine sodium and potassium concentration and excretion rates were determined. The sodium to potassium ratio of the urine was taken as an indication of mineralocorticoid activity (Hetzel, McSwiney, Mills & Prunty, (1956). Glomerular filtration rate was determined by inulin clearance. A primer dose of inulin was given at 0 hours and sustained by administration of 1% inulin incorporated in saline solution which was infused at the rate of 3 ml per hour into the external jugular vein. The saline solution contained the components necessary to keep the animal in salt and water balance throughout the experimental period. Urine and blood samples were taken at 60 min intervals, following a 2 hour equilibration period.

Renal Function in Ovariectomised Rats

It is known that intravenous saline infusion in rats is followed by a marked increase in tubular rejection fractions for sodium and water without any significant alteration in inulin clearance (Keller & Schneiden, 1958). It was therefore essential to determine the renal response to saline in the strain of rat used throughout this investigation. Therefore the variation in renal electrolyte function under saline infusion was determined in ovariectomised rats over an 8 hour period, beginning at 9 a.m. (experiment 1).

The experiment was repeated, commencing at 3 p.m. (experiment 2), to determine if a possible diurnal variation affected renal function during the 8 hour experimental period.

Urine Flow

The results presented in Fig.4 show that there was a 100% increase in urine flow from 2 hours to 4 hours after the start of the saline infusion period. This was followed by a progressive decline in flow, with a marked fall in urine flow 6 hours after commencing infusion. The variation in urine flow was found to be similar in both experimental groups and was not accompanied by parallel changes in G.F.R. There was a decrease in G.F.R. at 7 hours and 8 hours after the start of the infusion. No significant diurnal variation in G.F.R. was observed.

Sodium Excretion

After an initial fall in urine sodium concentration, the levels did not significantly alter in either group of experimental animals throughout the remainder of the 8 hour experimental period.

Sodium excretion rates showed a similar pattern to that of urine flow in both experimental groups but was significantly lower at 0 hours and 8 hours after starting saline infusion in experiment 2.

Sodium:Potassium Ratio

Although the sodium to potassium ratio was found to be significantly lower in experiment 2 than in experiment 1 from 0 hours to 4 hours after starting saline infusion, a progressive rise in the sodium:potassium ratio was still observed.

Potassium Excretion

It was found that potassium concentration fell by 100% 3 hours after beginning saline infusion. After this time the concentration did not vary significantly.

Potassium excretion rates reached a peak 4 hours after starting saline infusion after which they progressively fell. Potassium excretion rates were significantly lower in experiment 2 than in experiment 1 at 4 hours, 7 hours and 8 hours after starting saline infusion.

The results presented above are in accordance with those of Keller & Schreiden (1958) demonstrating a rise in the rate

of sodium excretion and urine volume 2 hours after commencing a saline infusion. It has been suggested that changes in plasma volume may stimulate volume receptors which play a part in the control of body water. The increase in extracellular volume may also lead to a decrease in aldosterone secretion.

The fall in G.F.R. and urine volume observed after 6 hours of saline infusion suggested that renal activity was failing. Therefore renal function was determined over a 6 hour period in the following experiments. It was considered important to continue experiments over this length of time since other workers had failed to do this (see literature review) and it is possible that their observations did not take into account the effect of saline infusion on renal function. This might have resulted in a failure to detect any alteration in renal electrolyte function produced by sex hormones because of the delay in the action of these hormones on the kidney.

Summary

After intravenous saline infusion in ovariectomised rats:

1. Urine volume increased by 100% from 2 to 4 hours after starting saline infusion.
2. G.F.R. remained constant throughout the experimental period.
3. Urinary sodium concentration decreased during the initial 2 hours of saline infusion, but remained constant for the remainder of the experimental period.

4. Sodium excretion rate showed a similar pattern to that of urine flow.
5. The sodium:potassium ratio was not significantly affected by saline infusion.
6. Urinary potassium concentration decreased during the initial 3 hours of saline infusion, after which the concentration did not vary significantly.
7. Potassium excretion rate increased (expt. 2) or remained constant (expt. 1) for 4 hours after starting saline infusion after which they fell.
8. The sodium:potassium ratio was significantly higher in expt. 1, compared with expt. 2, for 4 hours after starting saline infusion.

Variations in Renal Electrolyte Function During the Oestrous Cycle

Urine Flow

The results presented in Fig.5Ashow that urine flow was significantly decreased at oestrus compared with dioestrus. Urine flow per hour at dioestrus was 2.7 ml, 4 hours after starting saline infusion, compared with 1.3 ml at oestrus ($p<0.01$).

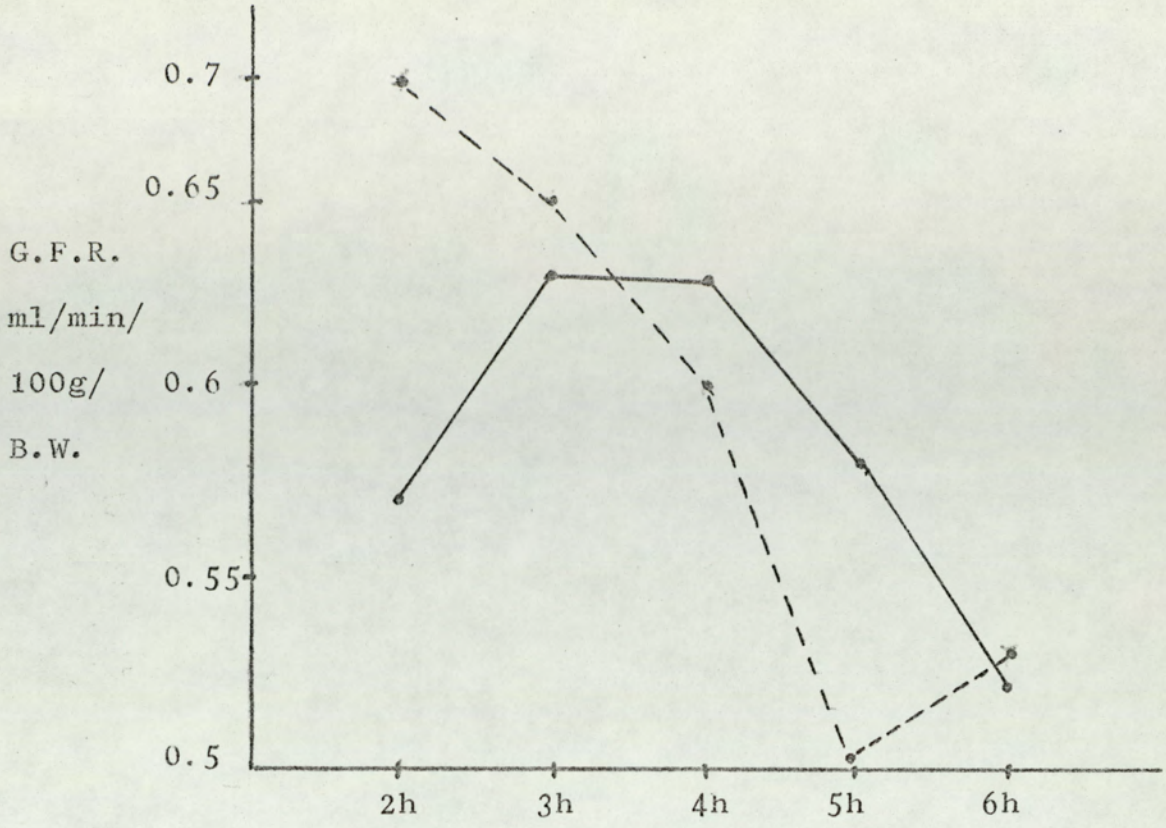
The decrease in urine flow observed at oestrus was not associated with an alteration in glomerular filtration rate, which was not significantly different from the glomerular filtration rate at dioestrus.

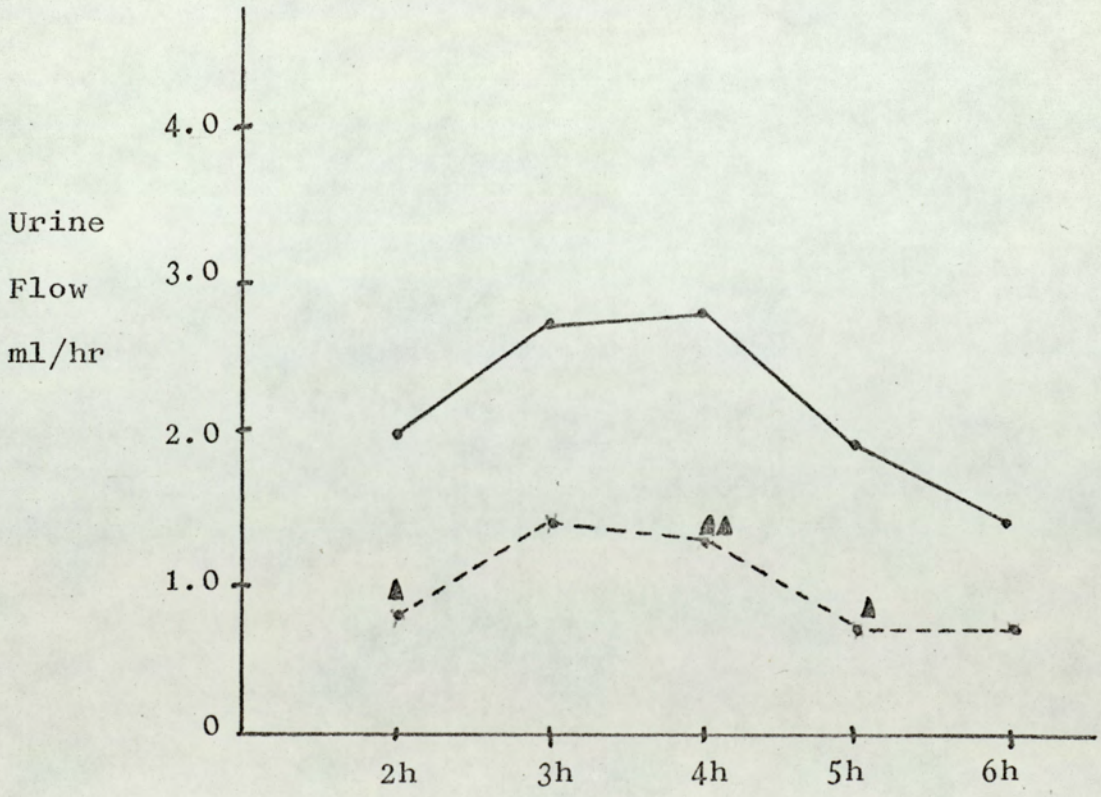
Sodium Excretion

Urinary sodium concentration was high at dioestrus, 175.1 $\pm$ 19.0 meq/L 4 hours after starting saline infusion, and fell significantly ($p<0.02$) at oestrus, 117.7 $\pm$ 5.4 meq/L at four hours.

Sodium excretion rates were significantly lower at oestrus compared with dioestrus. Four hours after starting infusion the sodium excretion rate at oestrus was 156.9 $\pm$ 28.4 µeq/hour, whereas in dioestrus animals the sodium excretion rate was 520.3 $\pm$ 69.7 µeq/hour at this time ($p<0.01$).

FIG. 5a

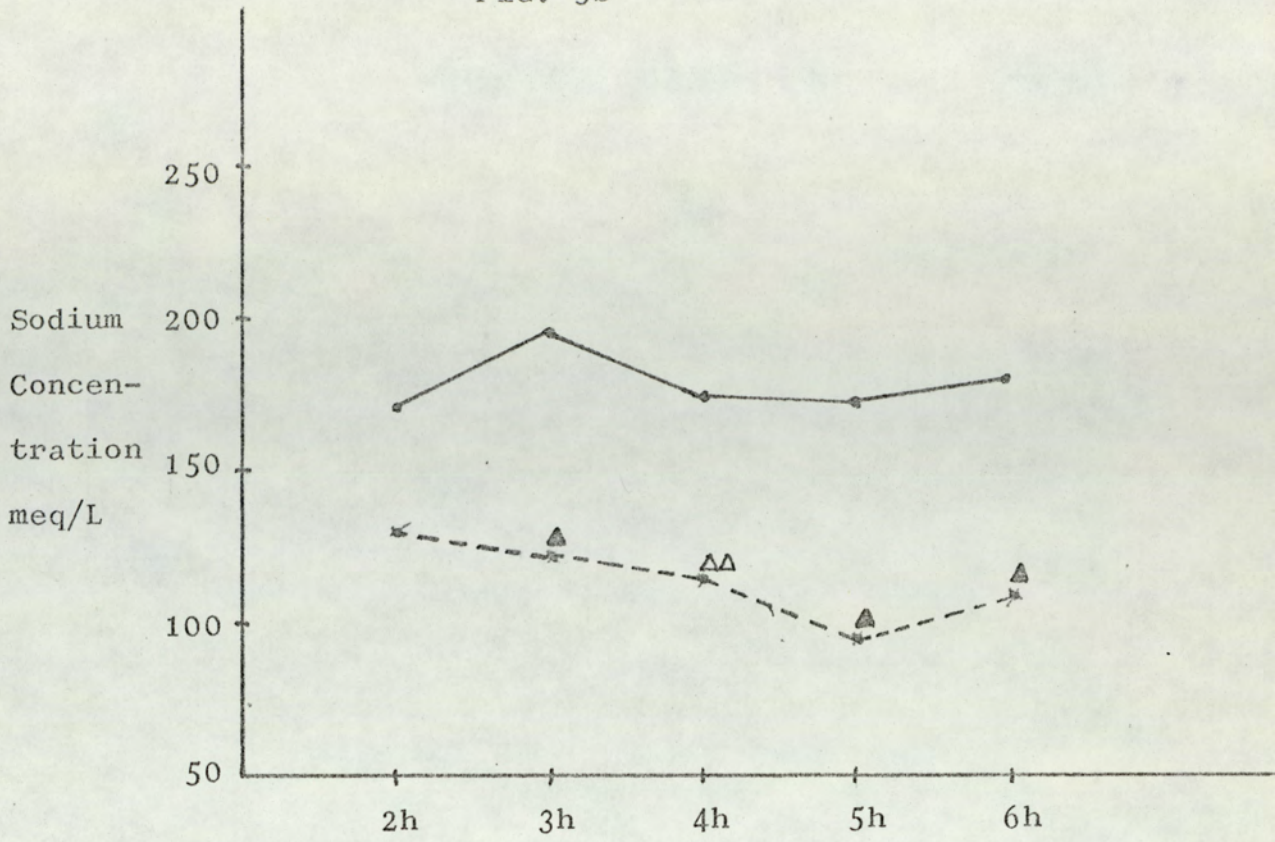


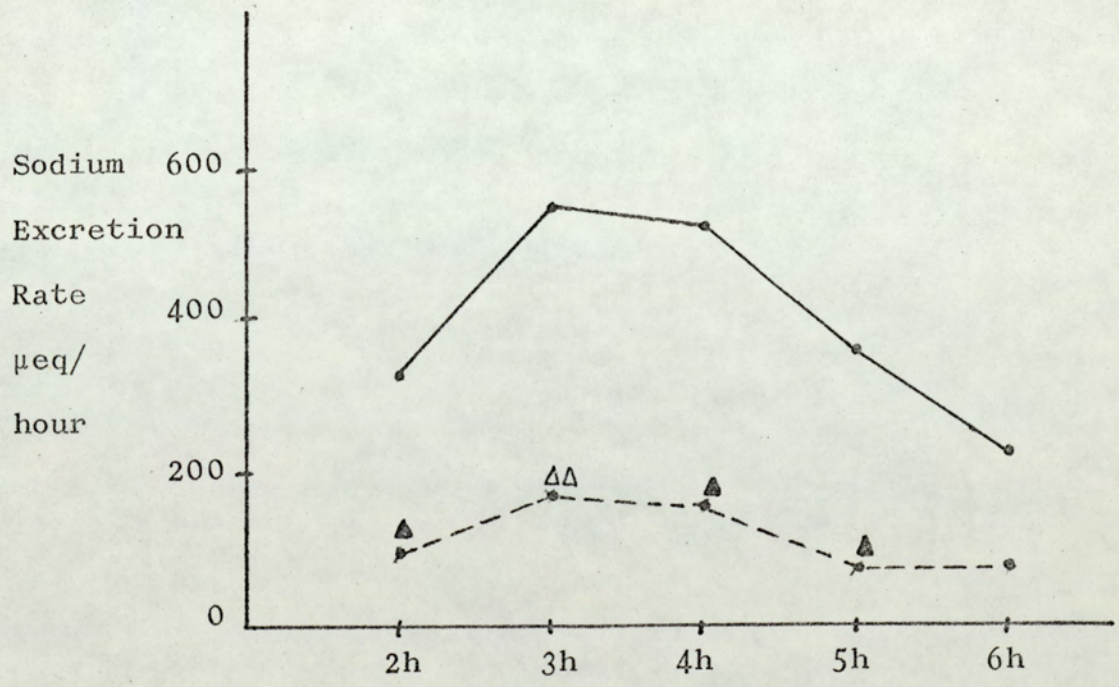


G.F.R. and Urine Flow during the Oestrous Cycle

Dioestrus ——— Oestrus - - - - -

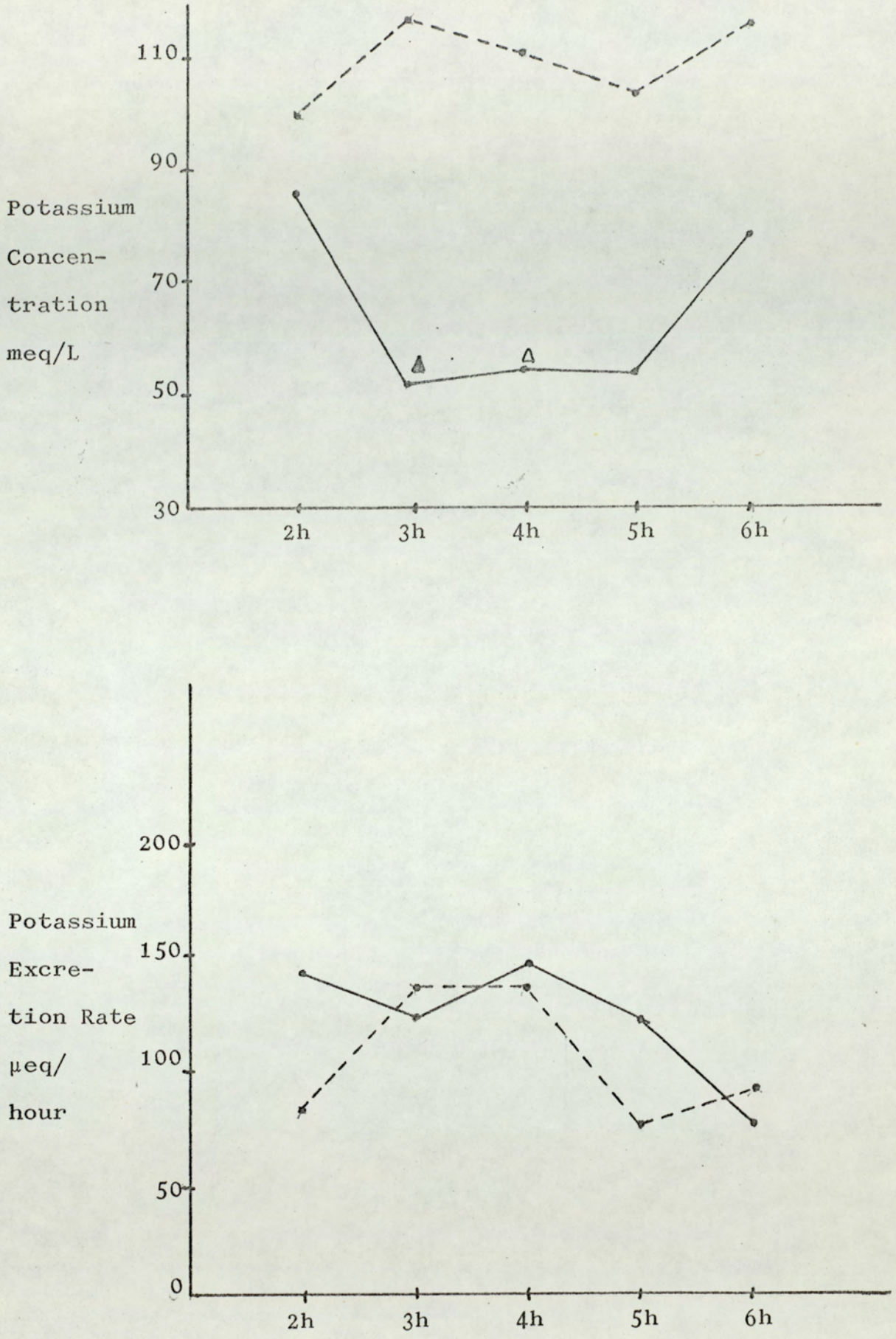
FIG. 5b





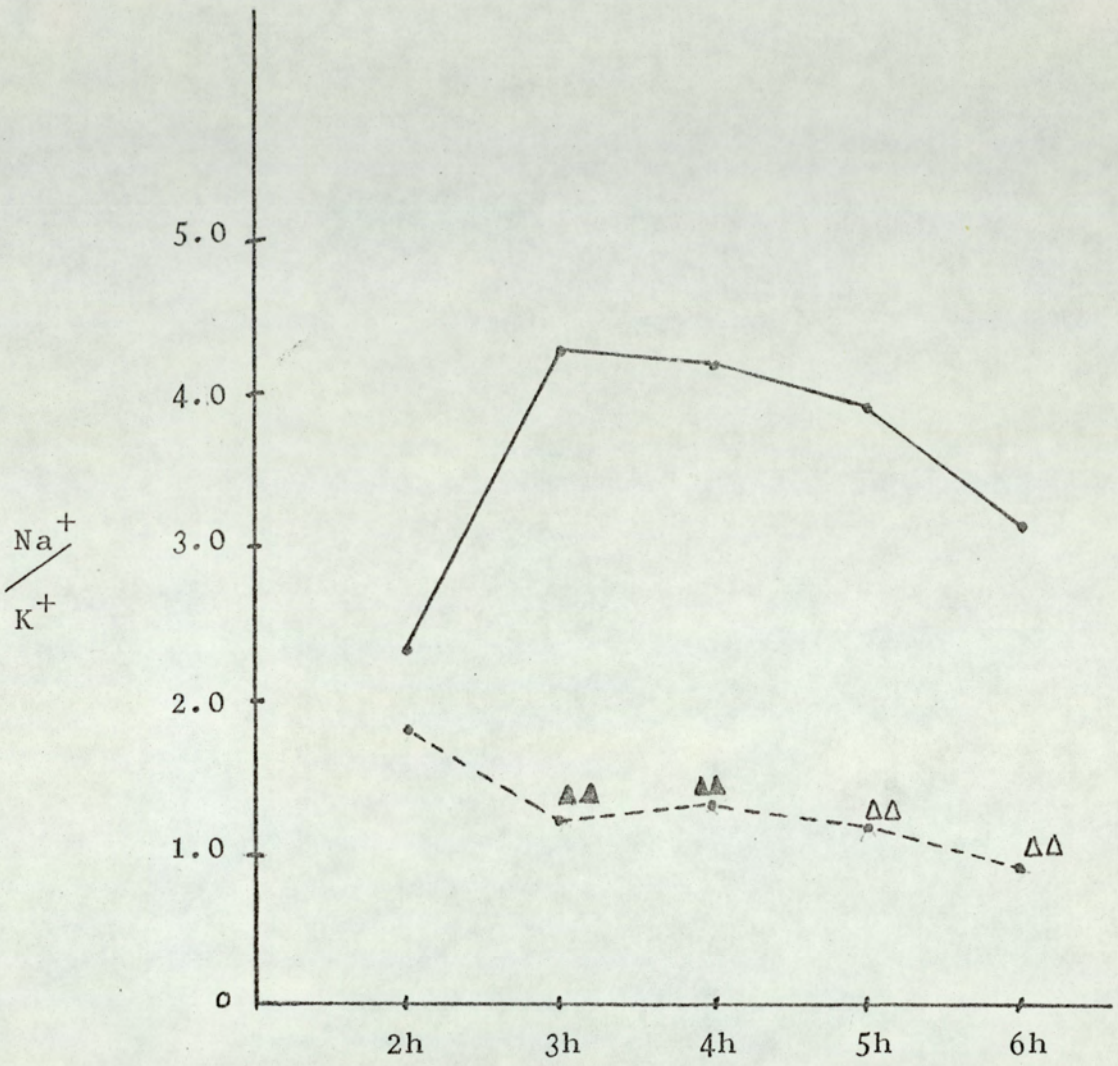
Urinary Sodium Concentration and Excretion Rates During the Oestrous Cycle

FIG. 5c



Urinary Potassium Concentration and Excretion Rate During the Oestrous Cycle

FIG. 5d



Sodium:Potassium Ratio During the Oestrous Cycle

Sodium:Potassium Ratio

A lower sodium:potassium ratio was observed at oestrus, 1.3 $\pm$ 0.3 than at dioestrus, 4.2 $\pm$ 0.8. These values were obtained 4 hours after starting infusion and were significantly different ($p<0.001$). The low ratio at oestrus suggested an increase in mineralocorticoid activity (Fig. 5D).

Potassium Excretion

No statistical difference was found in urinary potassium concentration at oestrus compared with dioestrus. However, the mean values for potassium concentration were at least 50% higher at oestrus than at dioestrus, suggesting an increased movement of potassium into the tubule lumen (Fig. 5C).

Potassium excretion rates were significantly lower at dioestrus than oestrus 3 hours after starting saline infusion ($p<0.02$), but were higher 4 hours after starting saline infusion.

These results suggest that there is a variation in renal electrolyte function during the rat oestrous cycle. The fall in urinary sodium concentration at oestrus appears to be a consequence of the kidneys' increased ability to conserve sodium, and this is associated with increased water retention. Thus sodium excretion rates are also decreased. Potassium concentration in the urine is increased at oestrus but decreased urine flow caused little significant change in potassium excretion rates. From these initial results experiments were designed to investigate further the cause of the observed changes in renal electrolyte function during the oestrous cycle.

Summary

1. There is a variation in renal electrolyte excretion during the rat oestrous cycle.
2. Urine flow was found to be significantly lower at oestrus that at dioestrus.
3. There was no significant difference in G.F.R. at oestrus compared with dioestrus.
4. Sodium concentration and excretion rates were significantly lower at oestrus than at dioestrus.
5. Potassium concentrations and excretion rates were higher at oestrus than at diestrus.

The Action of Spironolactone on Renal Electrolyte Function During the Oestrous Cycle

Introduction

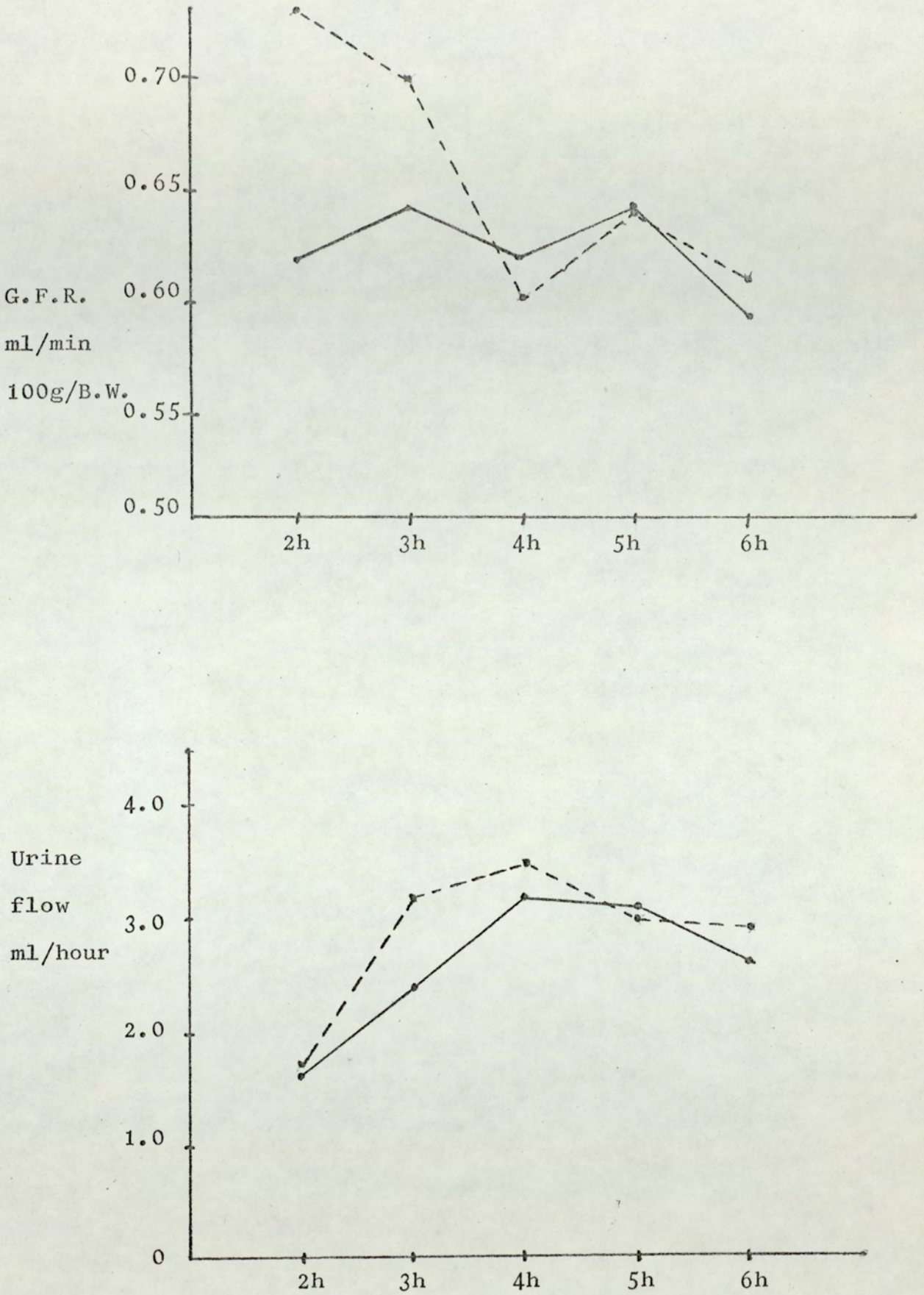
The results presented in the previous section indicated that a variation in mineralocorticoid activity may contribute to the observed variation in renal water and electrolyte excretion during the oestrous cycle. The synthetic steroid spironolactone is believed to act by antagonizing the physiological effects of endogenous aldosterone and related mineralocorticoids (Kagawa et al., 1957). Spironolactone was therefore used in the present experiments as a tool to study the role of mineralocorticoids in the sodium and water retention observed at oestrus.

Spironolactone was administered as Aldactone® (Searle & Co.), in an aqueous suspension, by stomach tube. Edmunds & Marriot (1968) have shown that 5 mg of spironolactone, administered in two doses 24 hours and 12 hours prior to experimentation, had no effect on normal sodium handling by the colon but blocked the effect of increased aldosterone induced by sodium depletion in rats. So, 2.5 mg of Aldactone® in 0.2 ml of water were given 24 hours, 12 hours and 2 hours prior to experimentation. The additional injection of spironolactone 2 hours prior to experimentation was administered because of the prolonged nature of the experiment.

Ovariectomised Animals

The effect of spironolactone on renal electrolyte function in ovariectomised rats is shown in Figs 6. It can

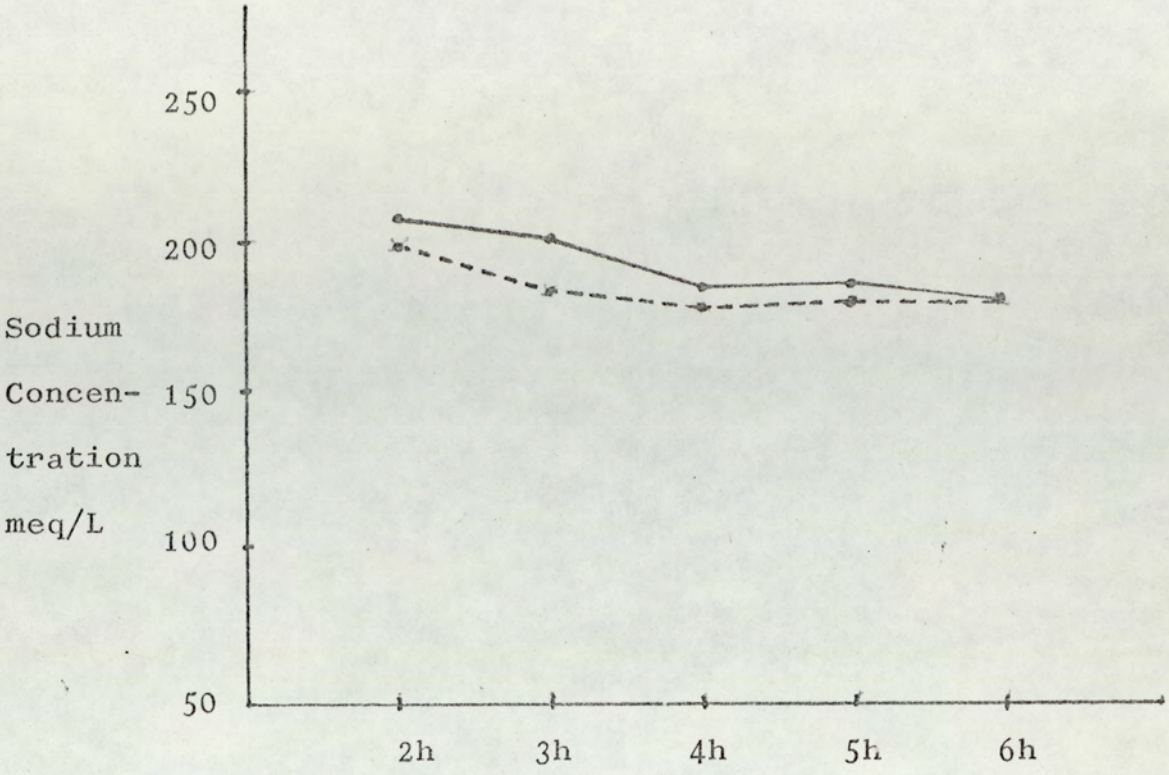
FIG. 6a

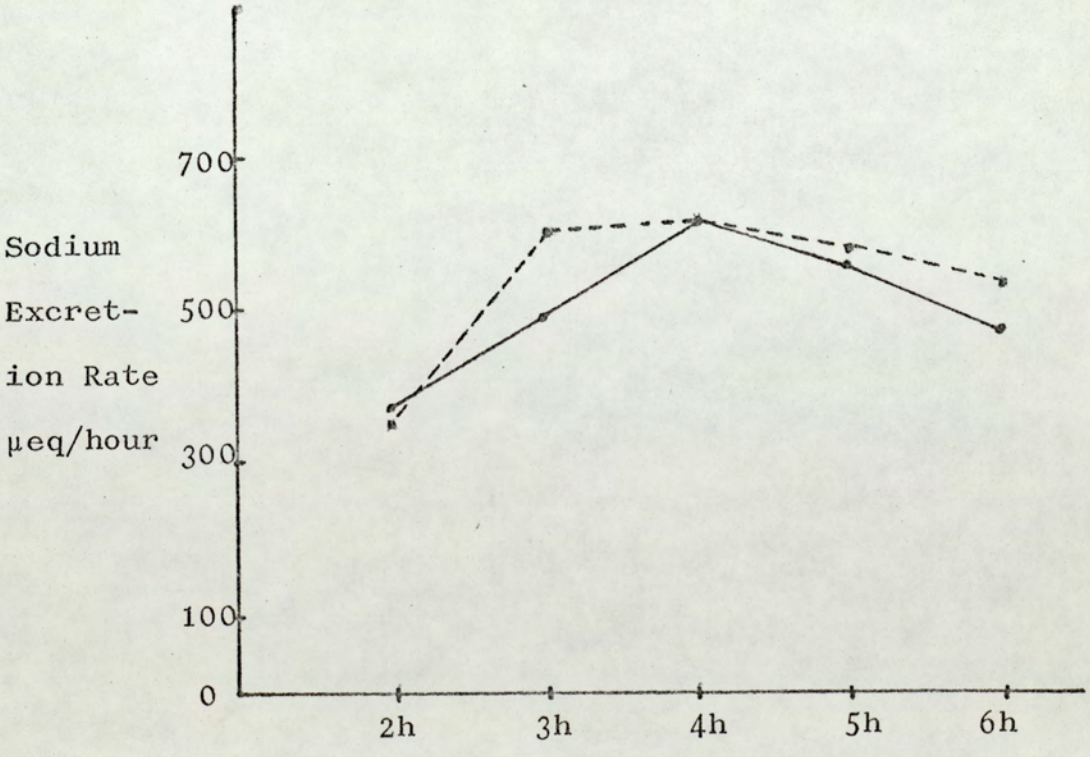


The Action of Spironolactone on G.F.R. and Urine Flow in Ovariectomised Rats

Spironolactone ——— Ovx. Controls - - - -

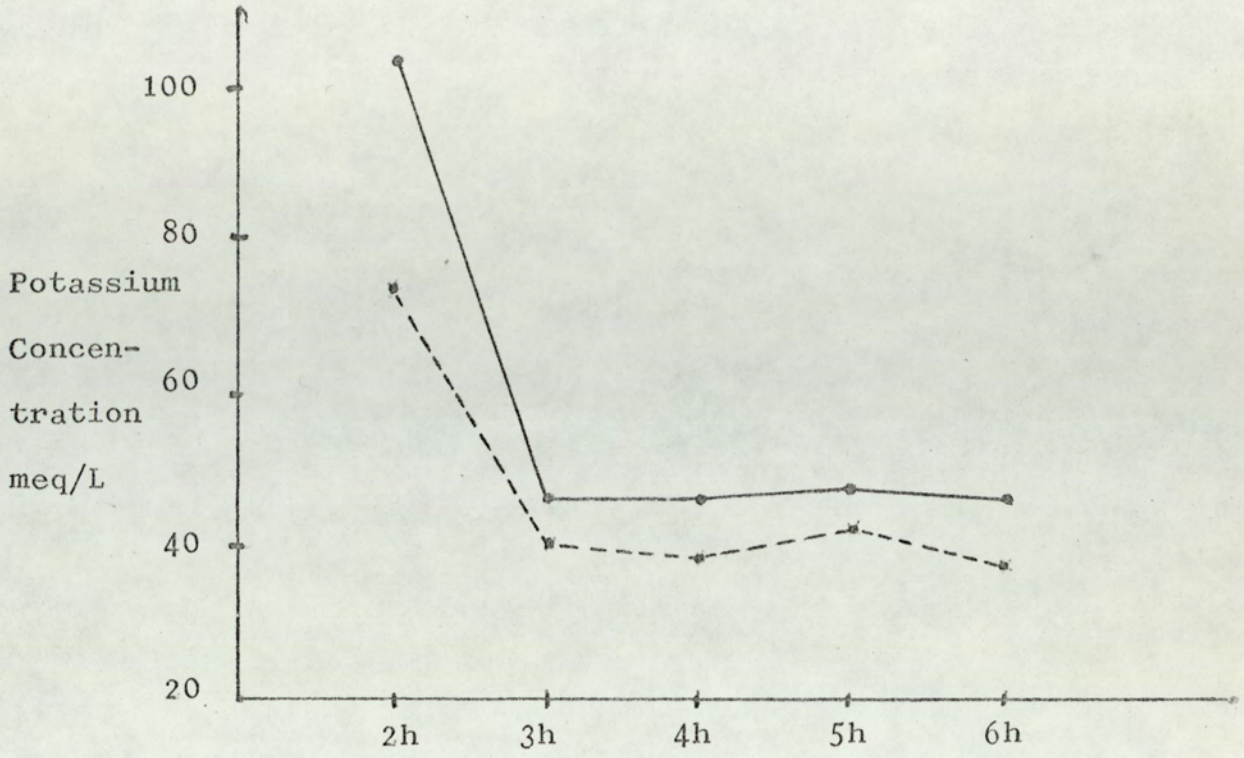
FIG. 6b

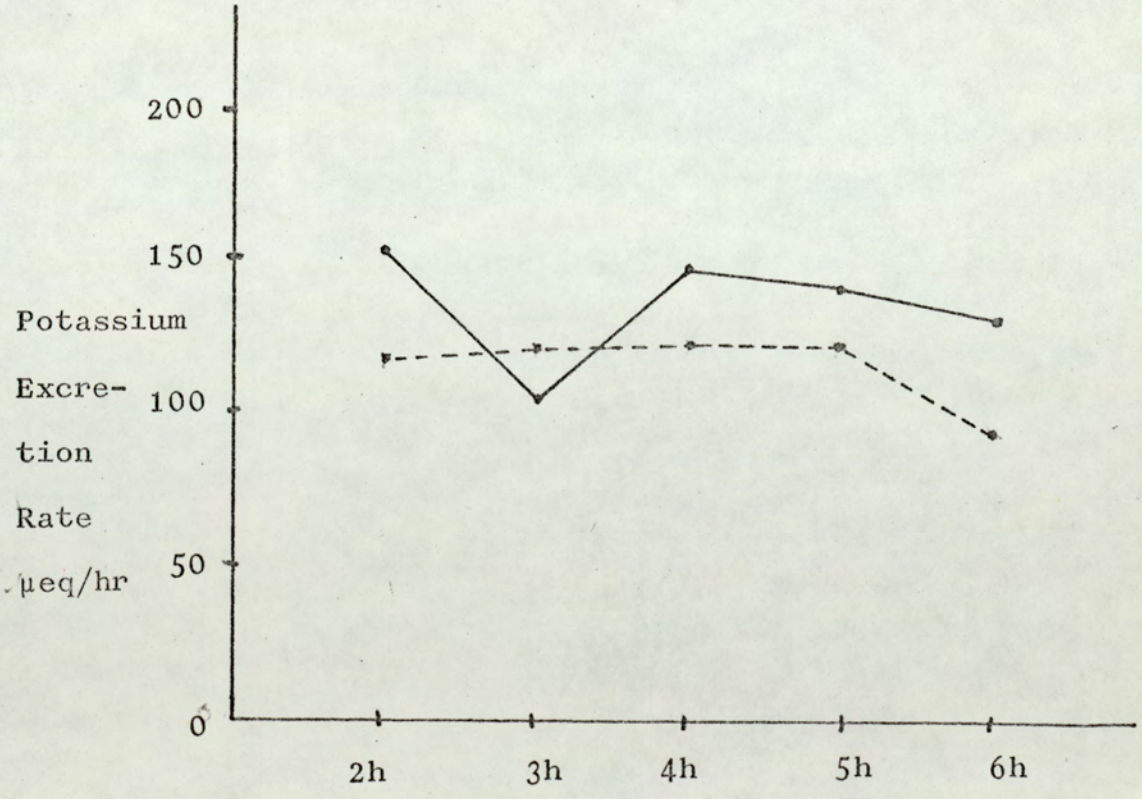




The Action of Spironolactone on Urinary Sodium Concentration and Excretion Rates in Ovariectomised Rats.

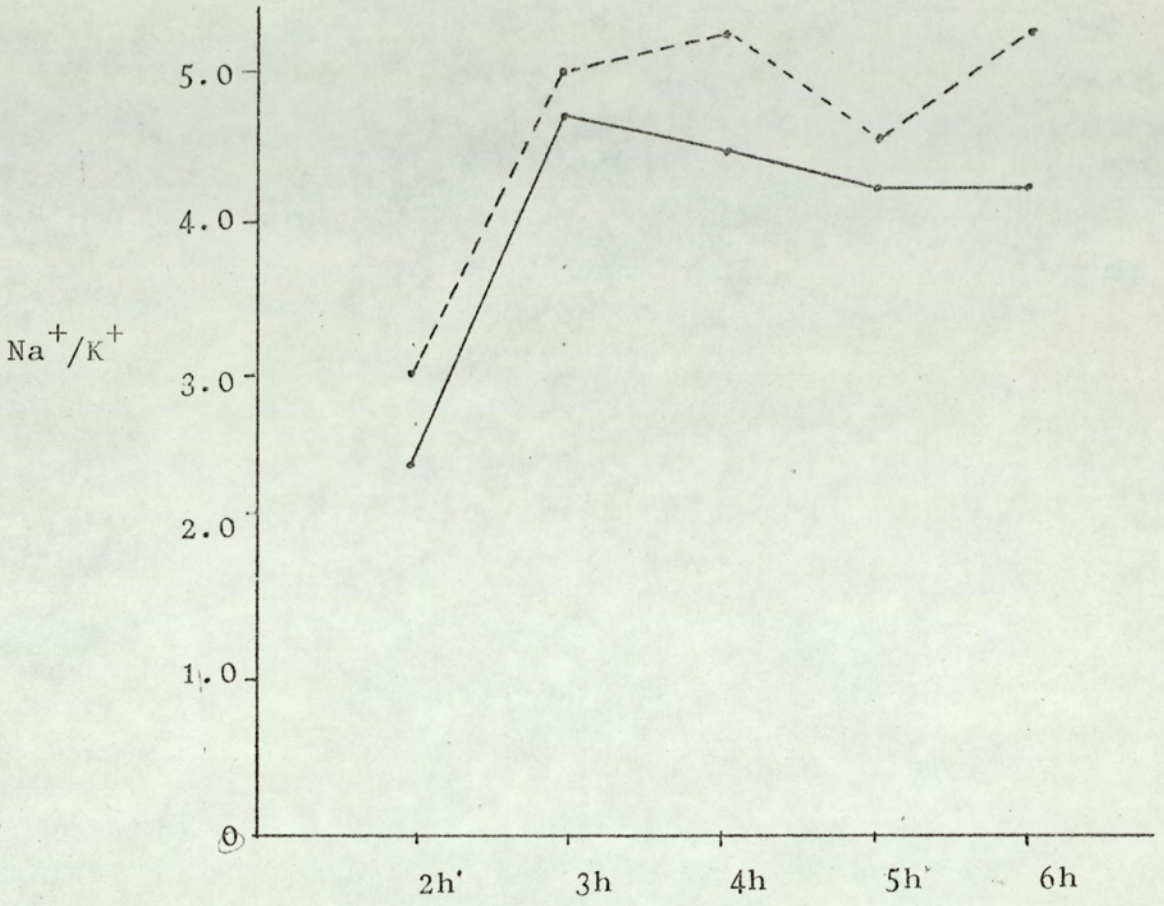
FIG. 6c





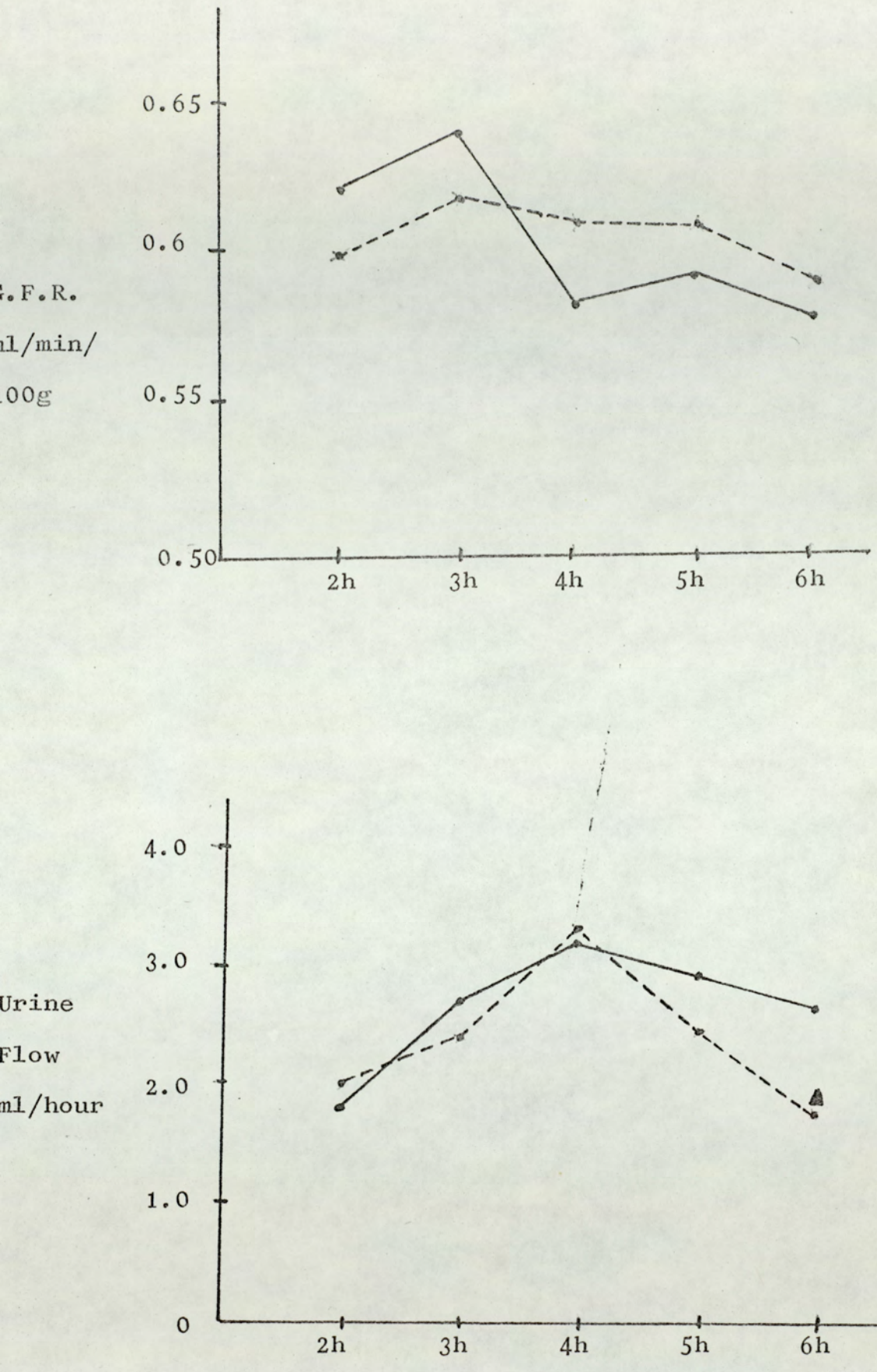
The Action of Spironolactone on Urine Potassium Concentration and Excretion Rates in Ovariectomised Rats

FIG. 6d



The Action of Spironolactone on the Sodium: Potassium Ratio in Ovariectomised Rats

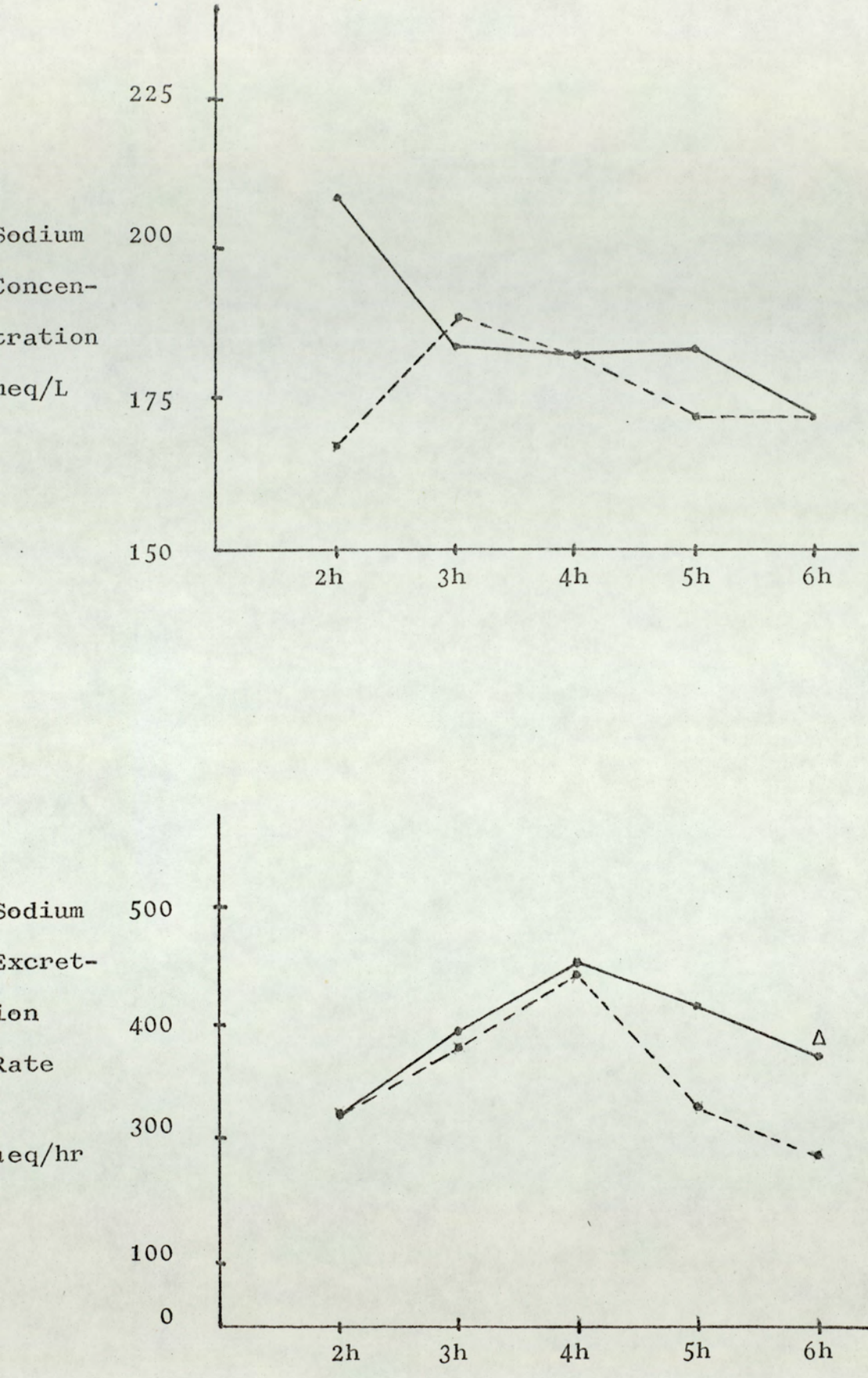
FIG. 7a



The Action of Spironolactone G.F.R and Urine Flow in Dioestrous Rats.

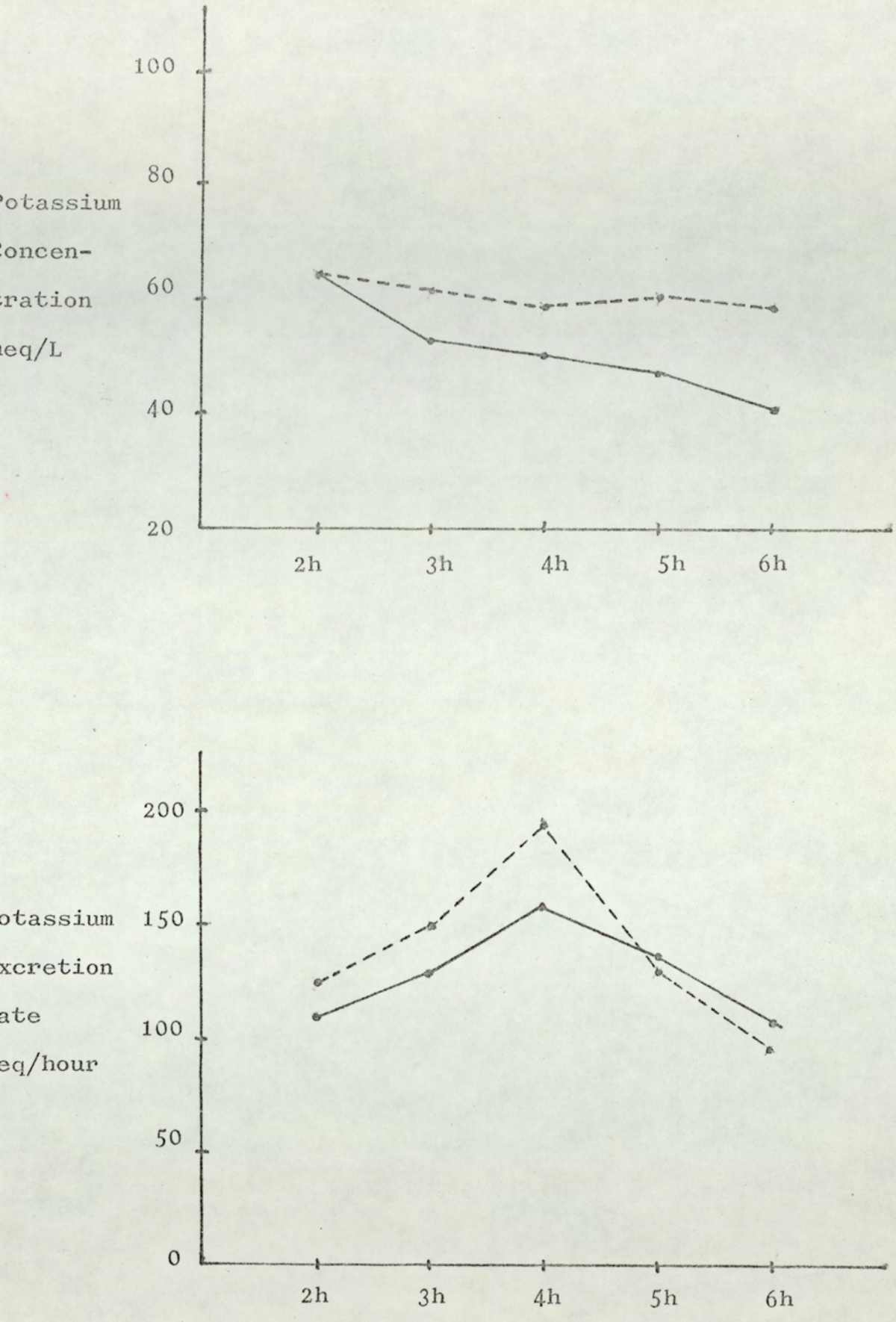
Spironolactone ——— Dioestrous Control - - - - -

FIG. 7b



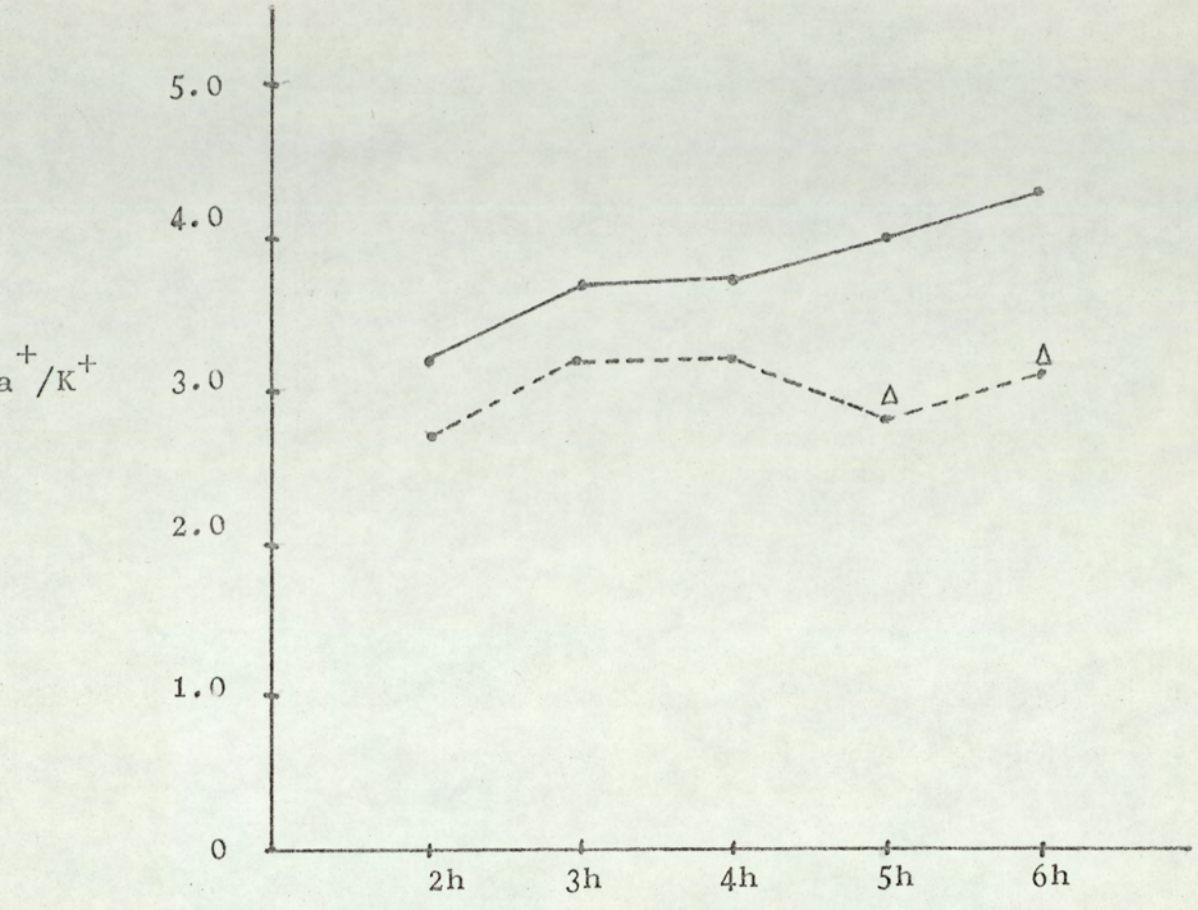
The Action of Spironolac tone on Urinary Sodium Concentration and Excretion Rates in Dioestrous Rats.

FIG 7c



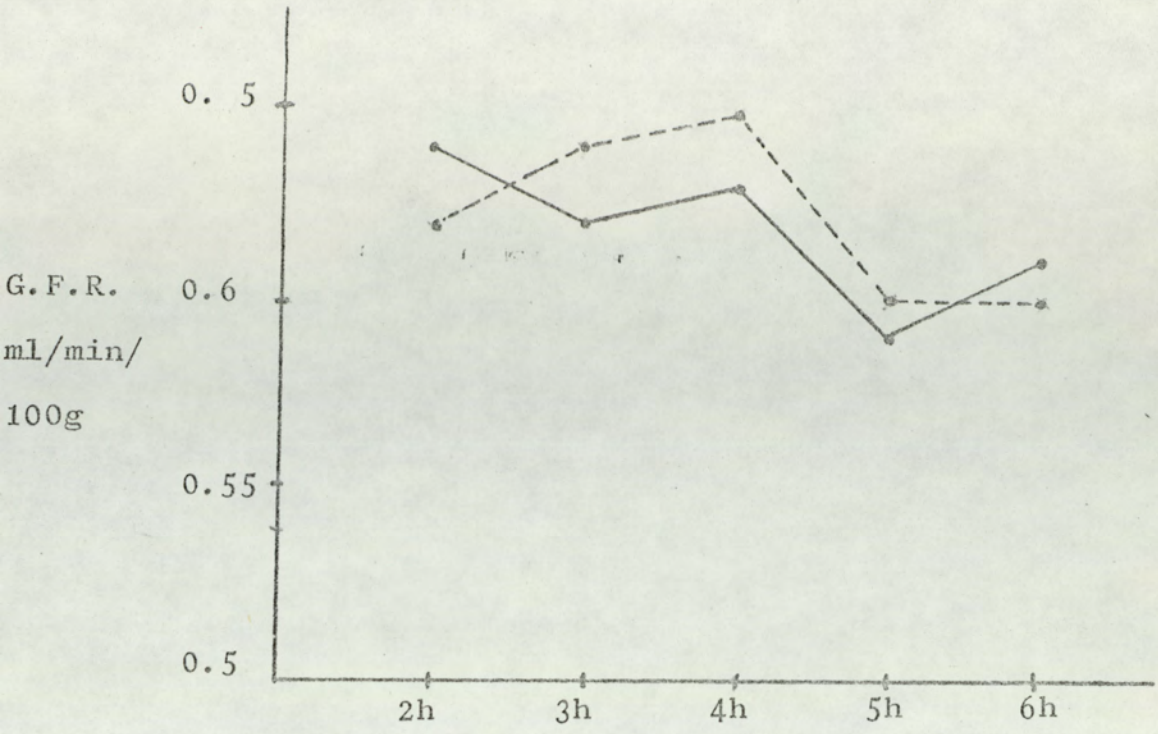
The Action of Spironolactone on Urinary Potassium Concentration and Excretion Rates in Dioestrous Rats

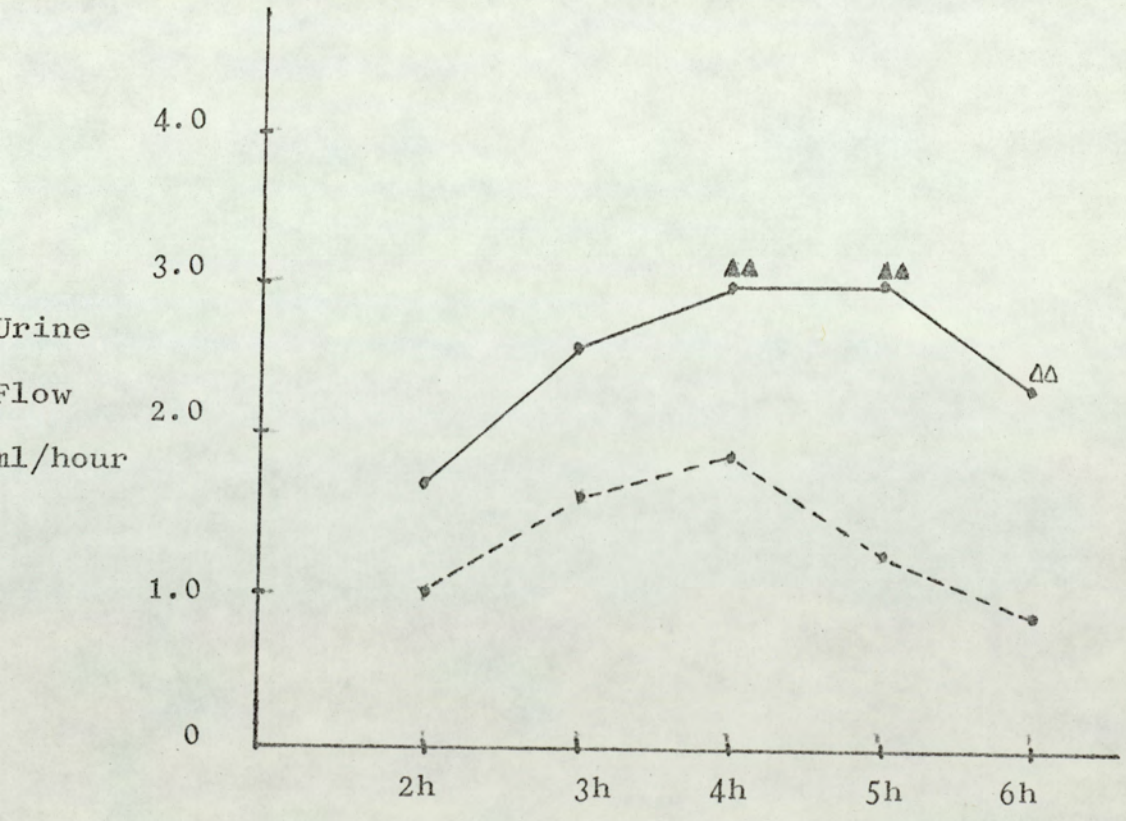
FIG. 7d



The Action of Spironolactone on the Sodium-Potassium Ratio in Dioestrous Rats

FIG. 8a

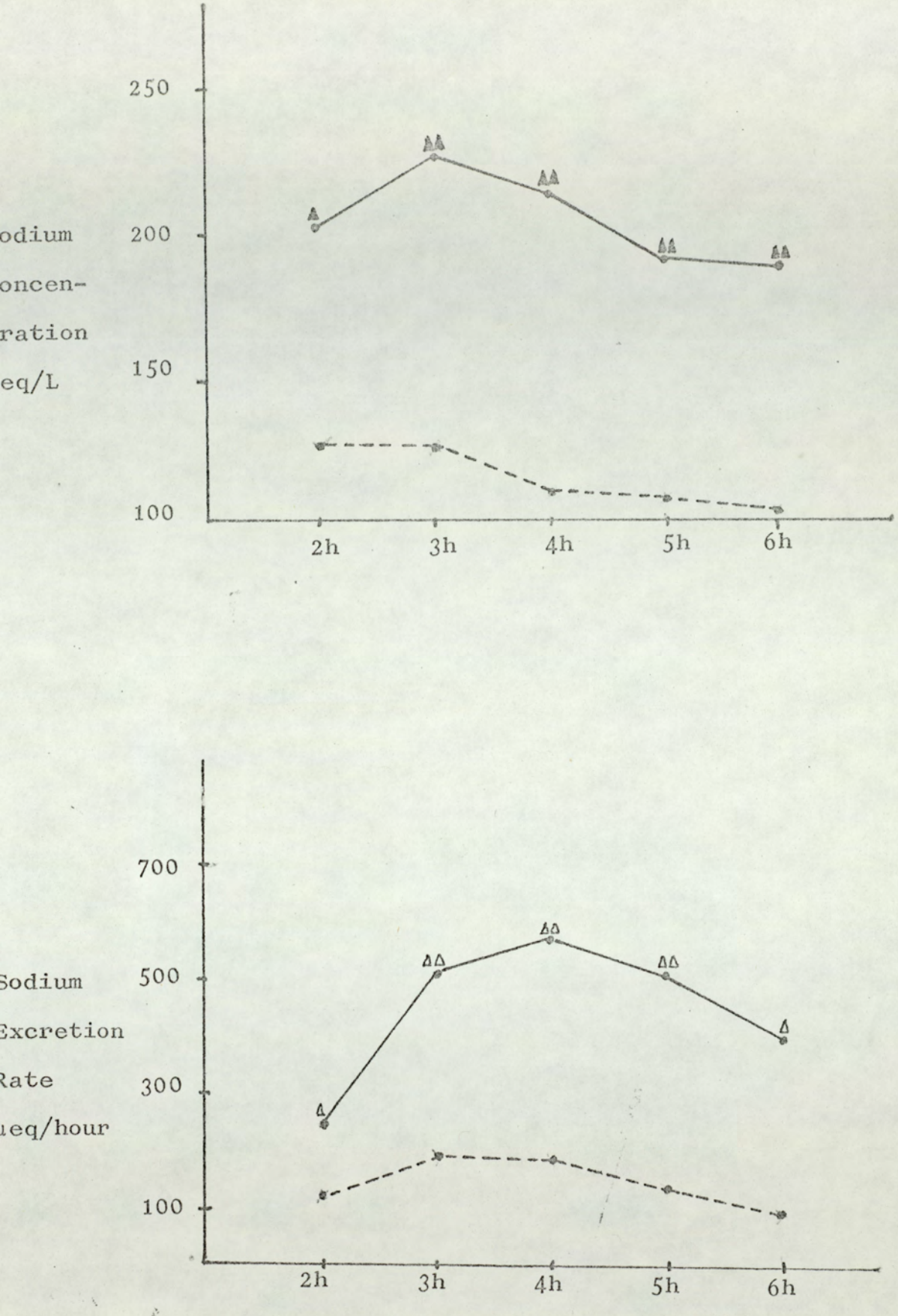




The Action of Spironolactone on G.F.R. and Urine Flow in Oestrous Rats.

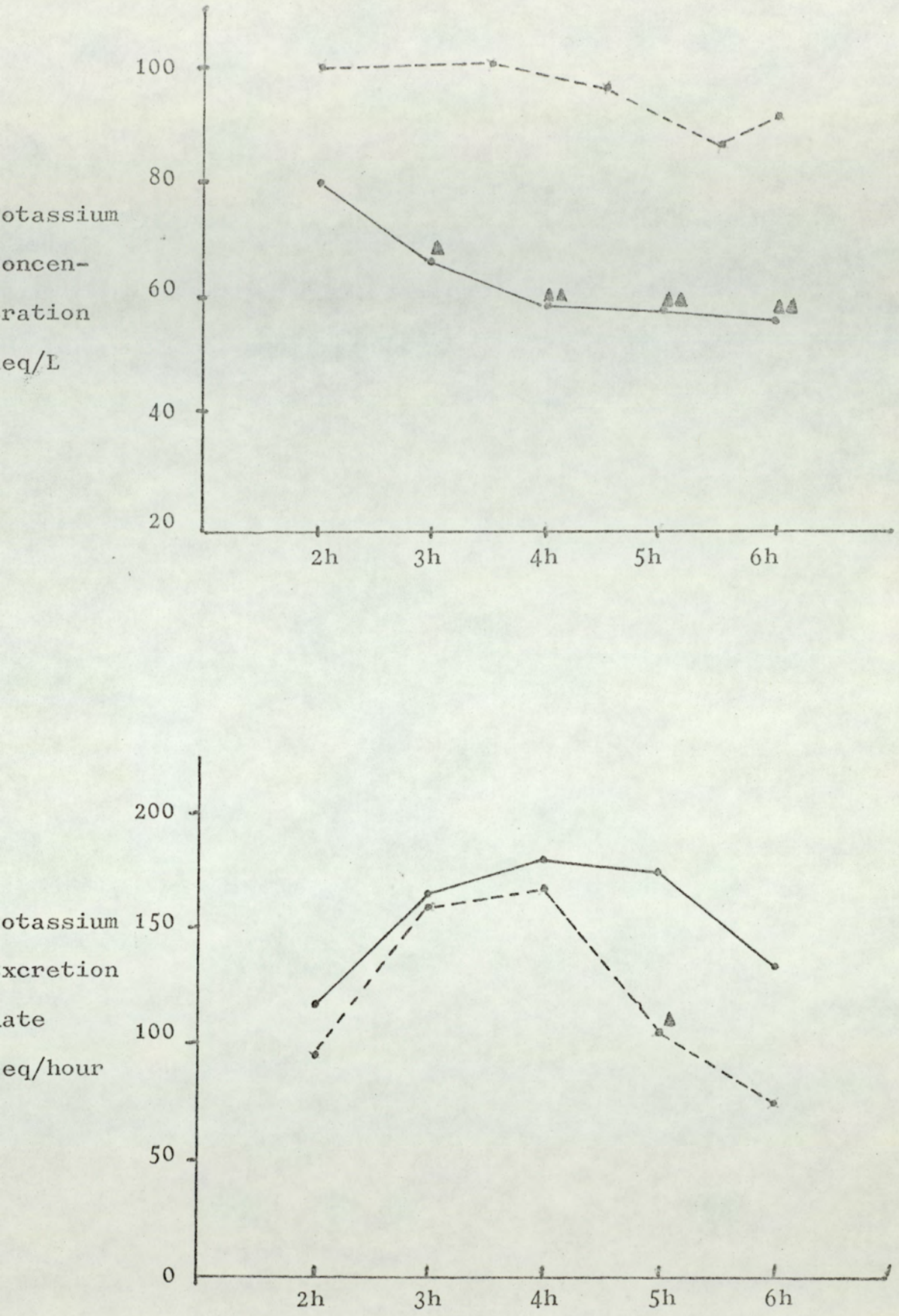
Spironolactone Treated ——— Oestrous Control - - - - - -

FIG. 8b



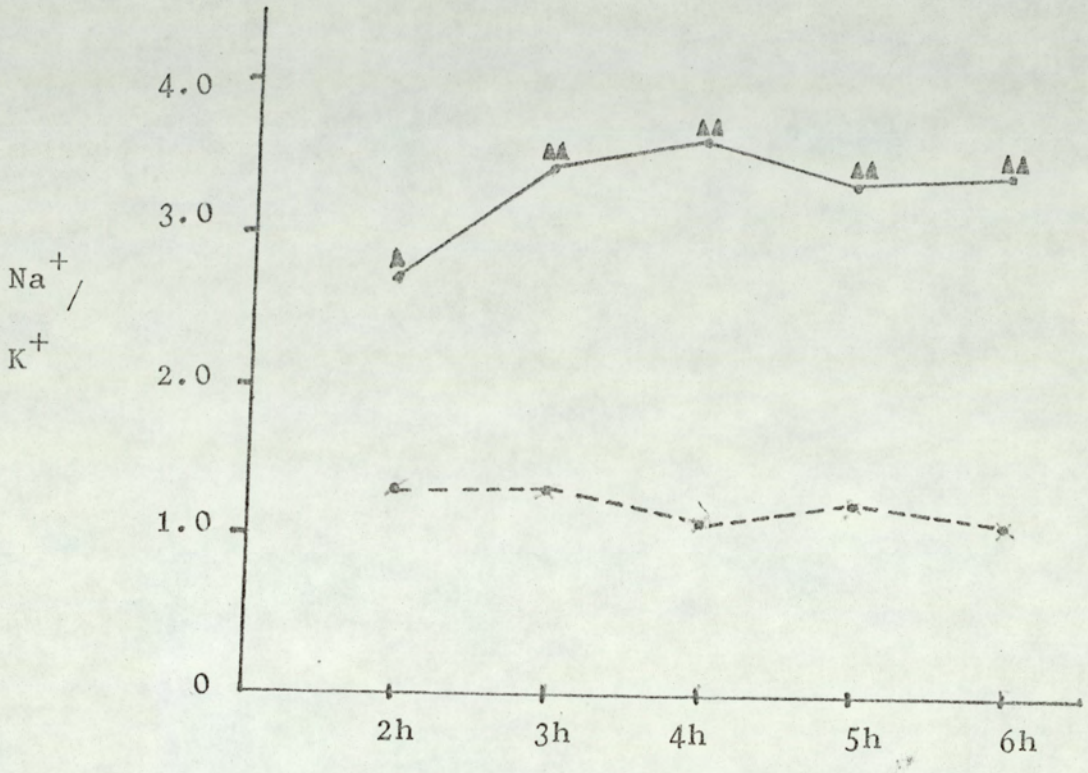
The Action of Spironolactone on Sodium Concentration and Excretion Rate in Oestrous Rats.

FIG 8c



The Ac tion of Spironolactone on Urinary Potassium Concentration and Excretion Rates in Oestrous Rats

FIG. 8d



The Action of Spironolactone on the Sodium: Potassium Ratio in Oestrous Rats

be seen that spironolactone does not significantly alter renal electrolyte function in ovariectomised rats compared with untreated ovariectomised controls. These results would indicate spironolactone does not affect normal kidney function and does not block the action of resting levels of mineralocorticoids.

Dioestrous Animals

Fig. 7 shows the effect of spironolactone on renal function in rats at dioestrus.

Urine Flow

It can be seen that there was no change in glomerular filtration rate with spironolactone treatment. Urine flow was found to be marginally higher five to six hours after starting saline infusion, with spironolactone treatment.

Sodium Excretion

No effect was observed on sodium concentration in the spironolactone treated group compared with the untreated control animals. However, sodium excretion rates were significantly increased 5 to 6 hours after starting saline infusion. This was probably due to an increase in urine flow at this time.

Sodium:Potassium Ratio

The sodium:potassium ratio was not influenced by spironolactone treatment. However, spironolactone did increase the sodium:potassium ratio 5 and 6 hours after starting saline infusion, indicating a decrease in mineralocorticoid action on the kidney at this time. This would suggest that there was an increase in mineralocorticoid

activity at this time. This may have been a result of the prolonged experimental procedure.

Potassium Excretion

The results presented in Fig. 7 show that spironolactone did not significantly alter potassium concentrations or excretion rates in dioestrous animals.

Oestrous Animals

The effect of spironolactone on renal electrolyte function at oestrus is shown in Fig. 8.

Urine Flow

A significant increase in urine flow was observed with spironolactone treatment in oestrous animals compared with vehicle blank injected, control oestrous animals. Urine flow in the spironolactone treated animals 4 hours after starting saline infusion was 3.0 ± 0.1 ml/hour compared with 1.7 ± 0.2 ml/hour in control oestrous animals ($p < 0.001$). Again no significant change in glomerular filtration rate was observed in the spironolactone treated group compared with the control animals.

Sodium Excretion

Sodium concentration and excretion rates were 213.3 ± 15.9 meq/L and 563.9 µeq/hour respectively, 4 hours after starting saline infusion in spironolactone treated oestrous animals. In the control oestrous animals, sodium concentration and excretion rates were 109.9 ± 5.8 meq/L and 188.9 ± 17.3 µeq/hour respectively at this time ($p < 0.001$ and $p < 0.02$). The results presented in Fig. 8 show that spironolactone increased sodium concentration and excretion

rates by 100% throughout the experimental period in oestrous animals, to levels which were comparable with dioestrous values.

Potassium Excretion

Spironolactone decreased the high levels of potassium concentration and excretion rates normally observed at oestrus. Potassium concentration and excretion rates were 59.6±4.8 meq/L and 179.3±14.2 µeq/hour in the spironolactone treated animals 4 hours after starting saline infusion, compared with potassium concentration and excretion rates of 97.4±3.8 meq/L and 169.5±21.7 µeq/hour in the untreated oestrous animals ($p<0.01$ and $p<0.70$ respectively).

These results indicate that the dose of spironolactone administered was sufficient to block the action of increased aldosterone. It can be seen therefore, that spironolactone treatment reverses the pattern in electrolyte and water excretion previously observed at oestrus, but has little effect on dioestrous animals. Since spironolactone has been shown to block the action of mineralocorticoids on the kidney, it is suggested that the retention of sodium and water at oestrus may reflect an increase in mineralocorticoid activity at oestrus compared with dioestrus.

Summary

1. Spironolactone, in the dose administered, had no significant effect on renal electrolyte function in ovariectomised or dioestrous animals.
2. Spironolactone blocked the sodium and water retention, and increased potassium excretion observed at oestrus.

3. Therefore, mineralocorticoids are implicated in the variation in renal electrolyte function observed during the oestrous cycle.

The Action of Oestrogen and Progesterone on Renal Electrolyte Function

Introduction

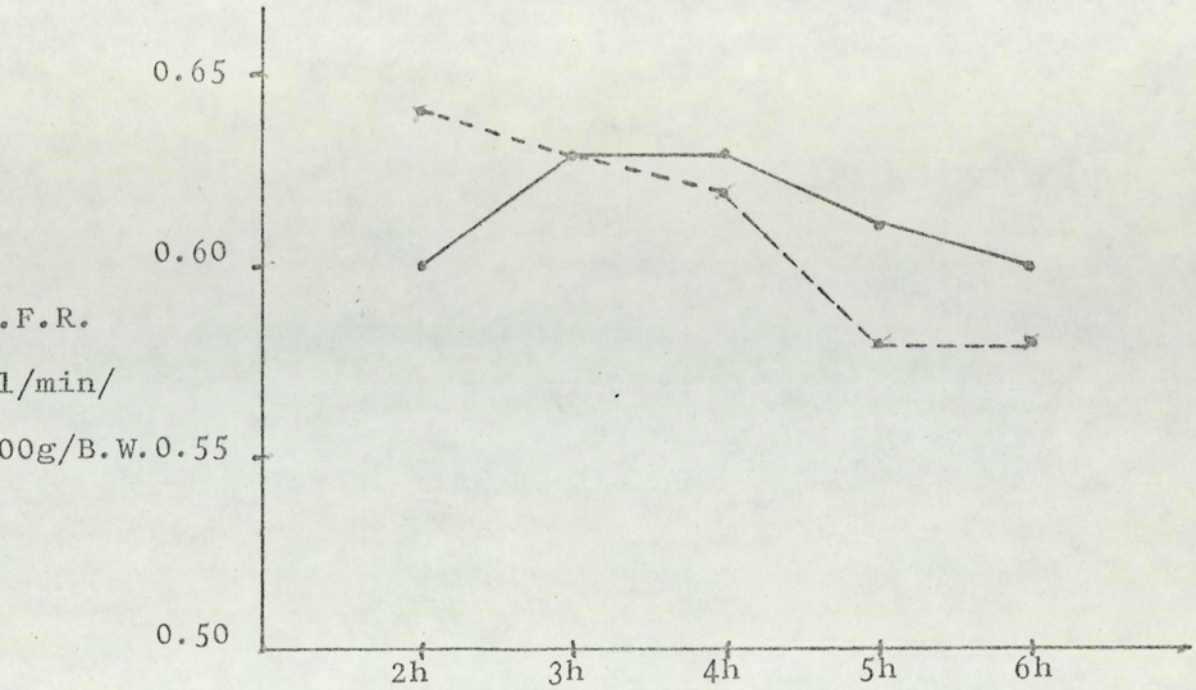
The experiments described in the preceding sections demonstrate a variation in renal electrolyte activity, possibly influenced by a change in mineralocorticoid activity associated with variations in the levels of oestrogen and progesterone during the oestrous cycle. However, previous work on the action of oestrogen and progesterone on renal function has been contradictory (see literature review). Therefore in this section the action of oestrogen, progesterone and oestrogen + progesterone on renal function in ovariectomised animals was investigated.

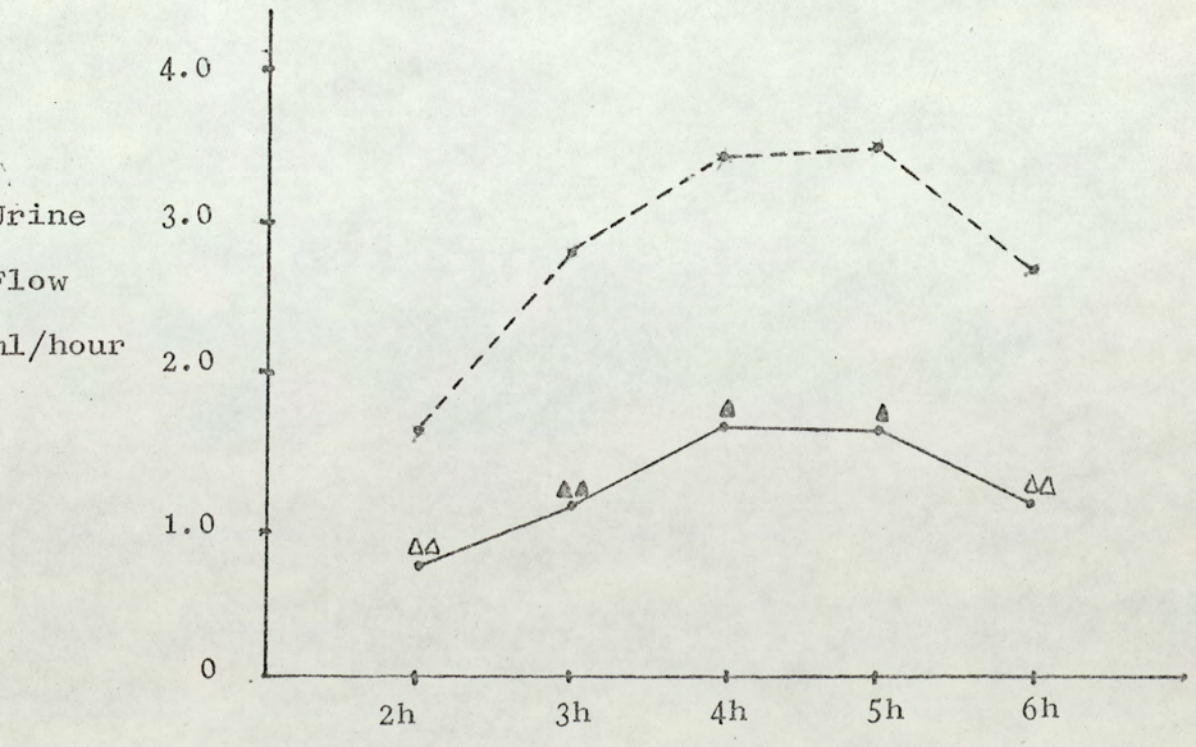
A daily intramuscular injection of 5µg of oestradiol, progesterone or a combination of the two was administered to ovariectomised animals for 14 days. Renal electrolyte function was determined on the 14th day of treatment and the results compared with vehicle blank injected, control animals.

Urine Flow

The effect of oestrogen therapy for 14 days is shown in Fig. 9B. It was found that urine flow was low with oestrogen treatment, 1.7 ± 0.3 ml/hour, 4 hours after starting saline infusion, compared with ovariectomised control levels, 3.45 ± 0.30 ml/hour 4 hours after starting saline infusion ($p < 0.01$). Glomerular filtration rate remained unchanged with oestrogen treatment compared with control animals. It was found that Urine flow in animals given oestrogen + progesterone and progesterone therapy was also significantly lower than that observed in control animals, but in the absence of any alteration in glomerular filtration rate,

FIG. 9a

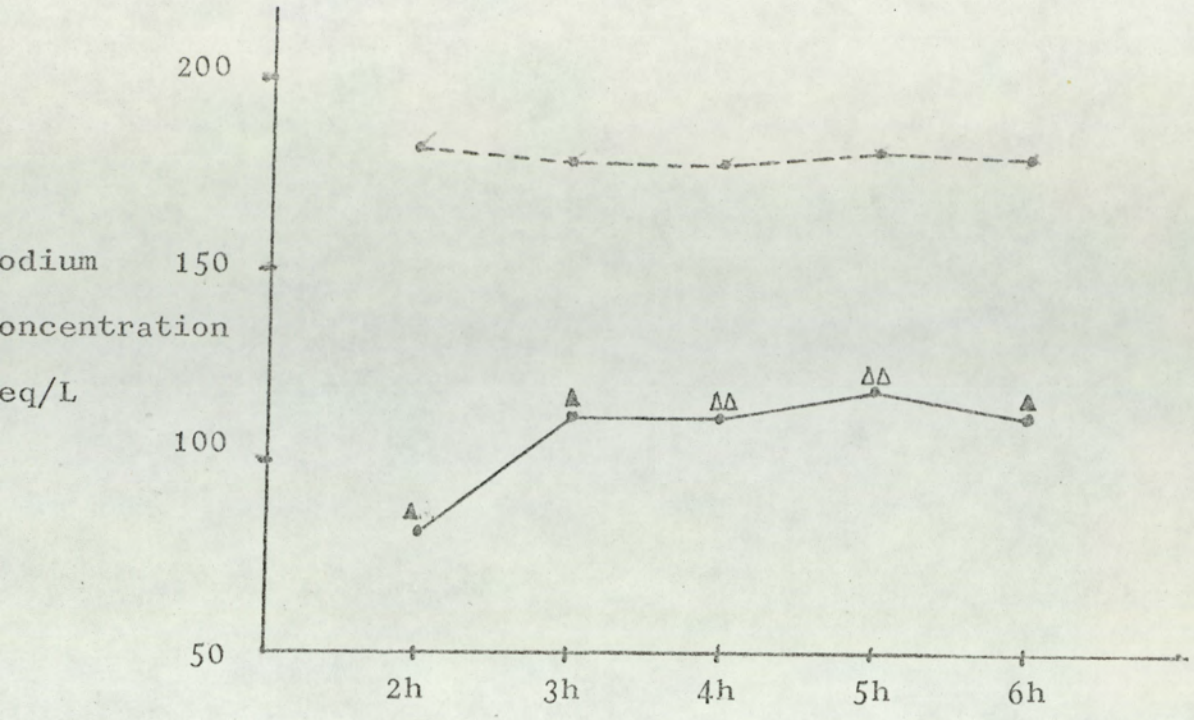


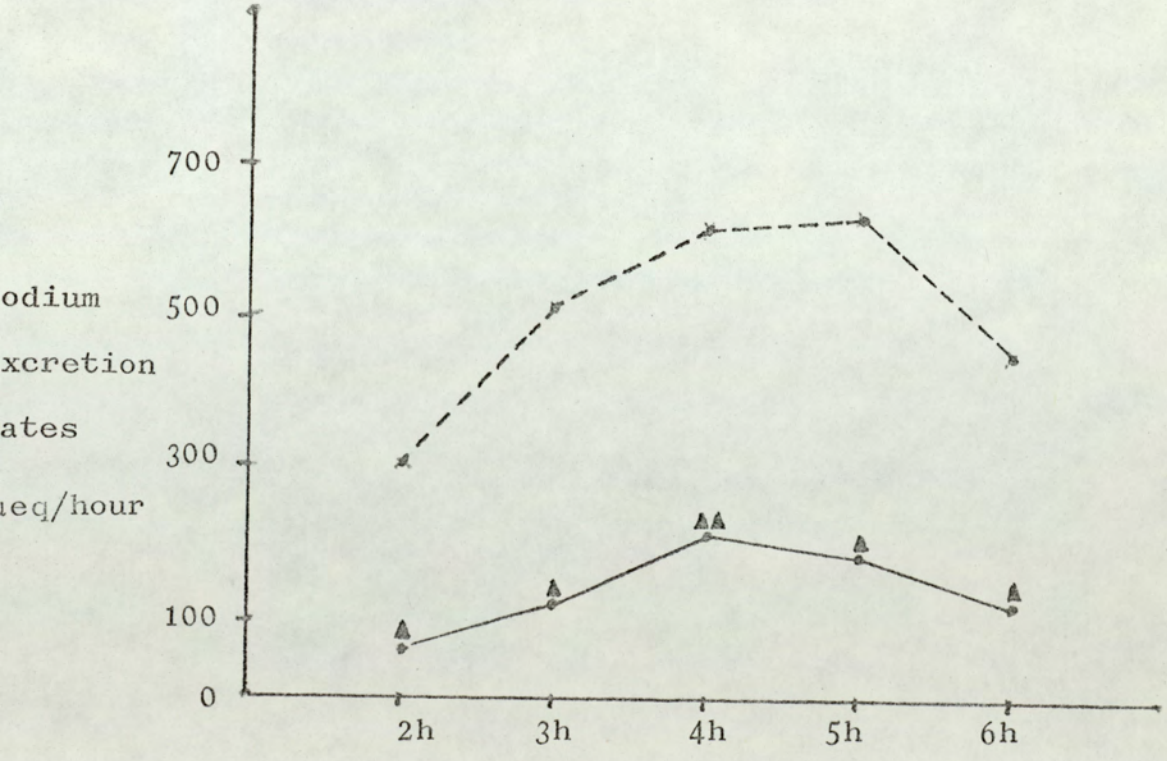


The Action of Oestradiol Administration for 14 days on G.F.R. and Urine Flow in Ovariectomised Rats.

Oestradiol Treated ——— Ovx. Control. – – – –

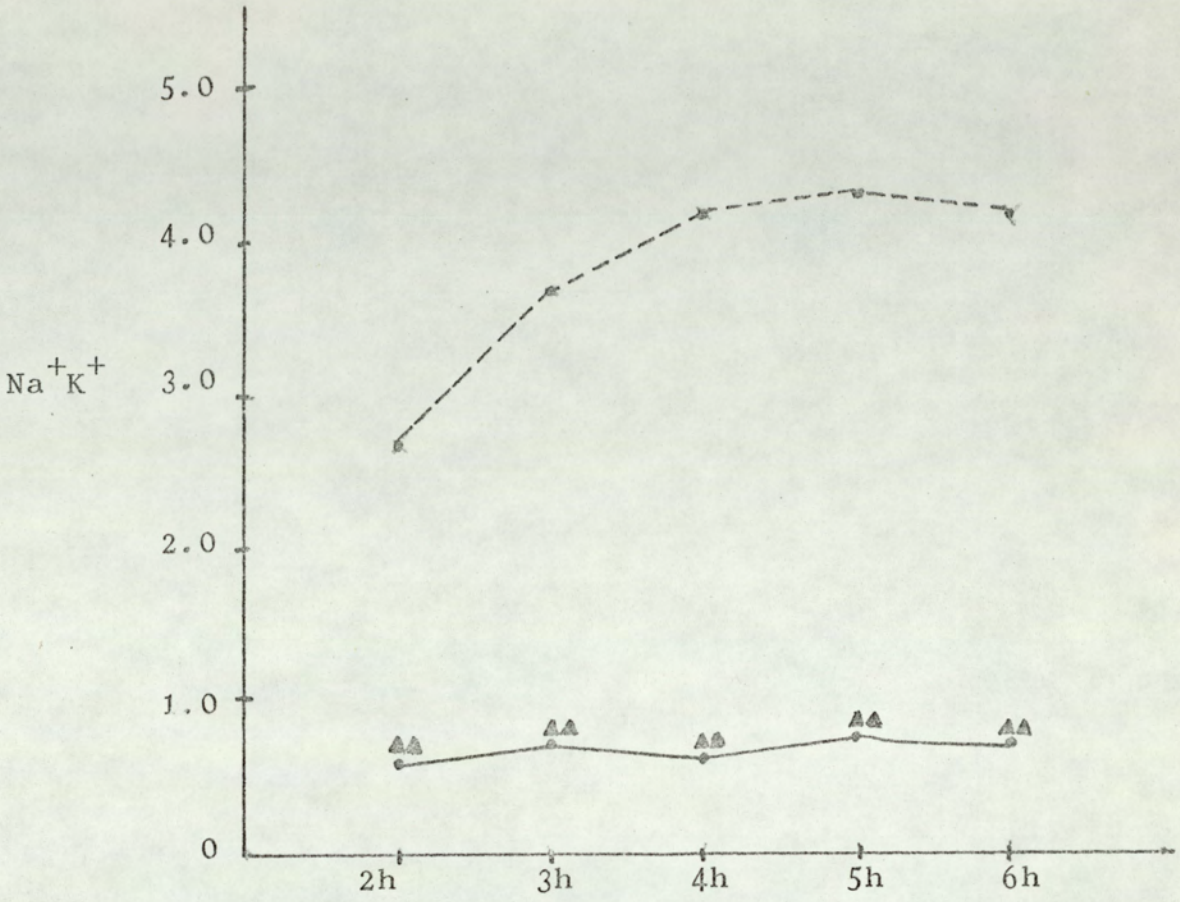
FIG. 9b





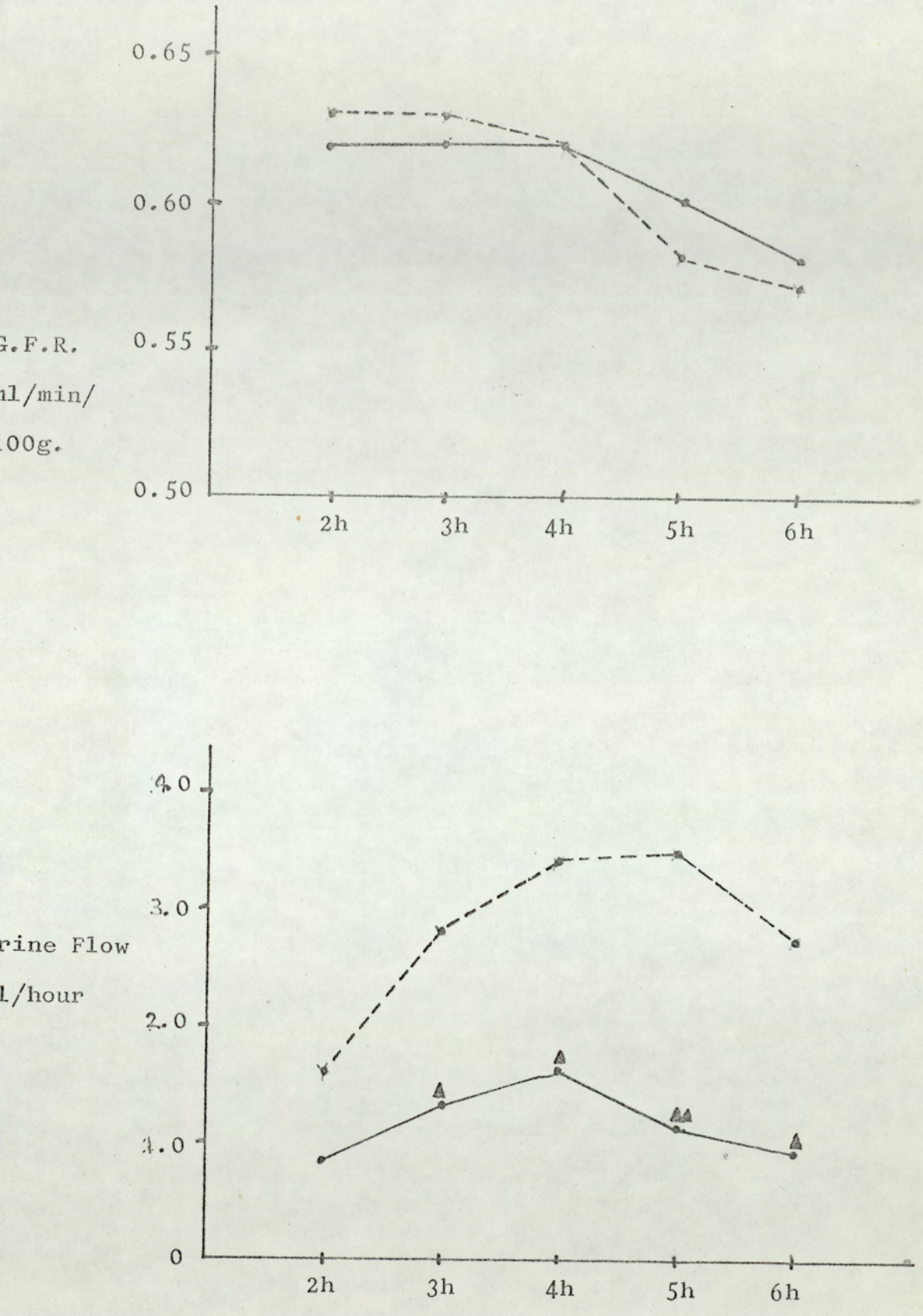
The Action of Oestradiol Administration for 14 days on Urinary Sodium Concentration and Excretion Rates In Ovariectomised Rats

FIG. 9d



The Action of Oestradiol Administration for 14 days on the Sodium:Potassium Ratio in Ovariectomised Rats

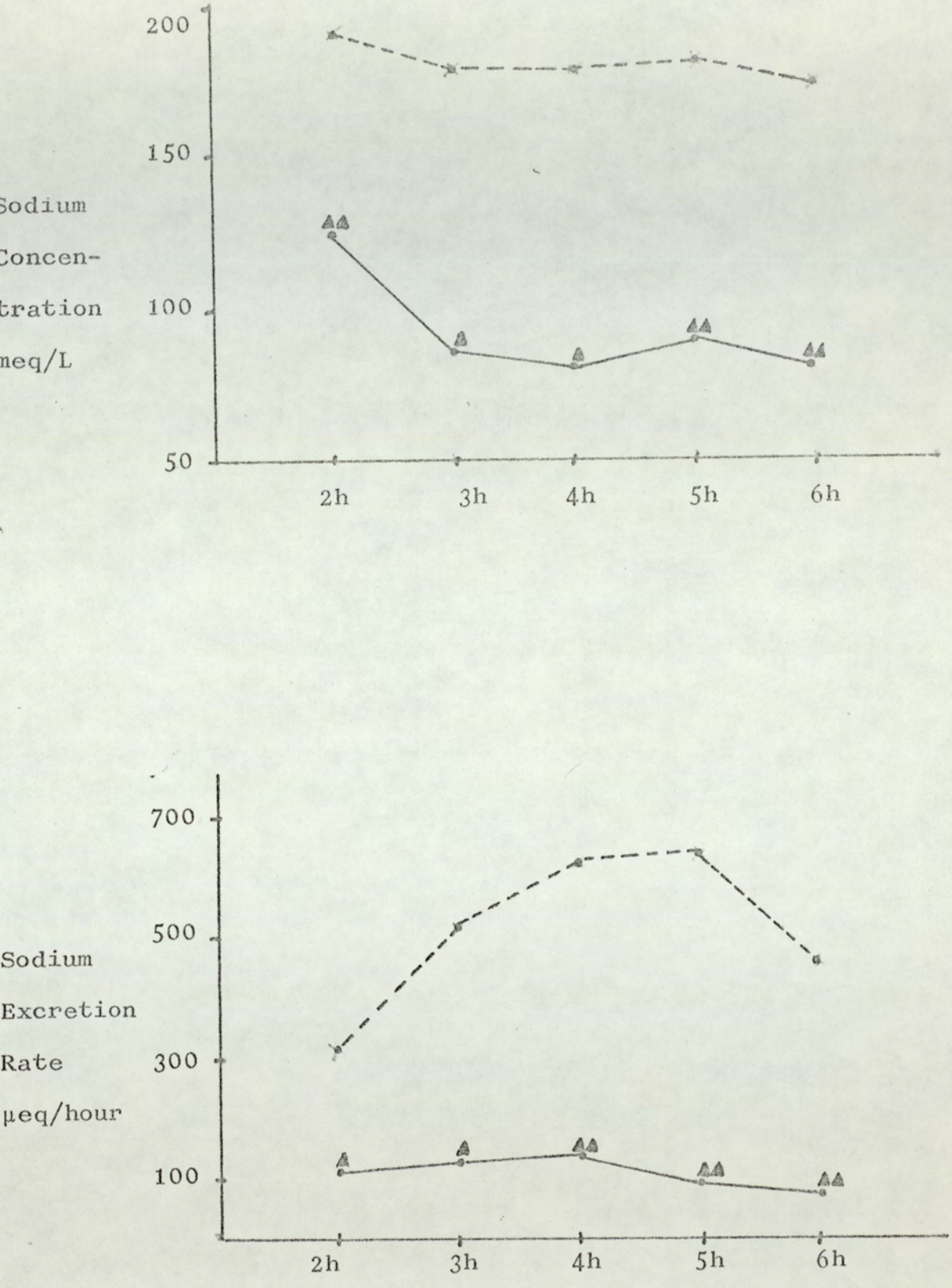
FIG 10a



The Action of Progesterone Administration for 14 days on G.F.R. and Urine Flow in Ovariectomised Rats

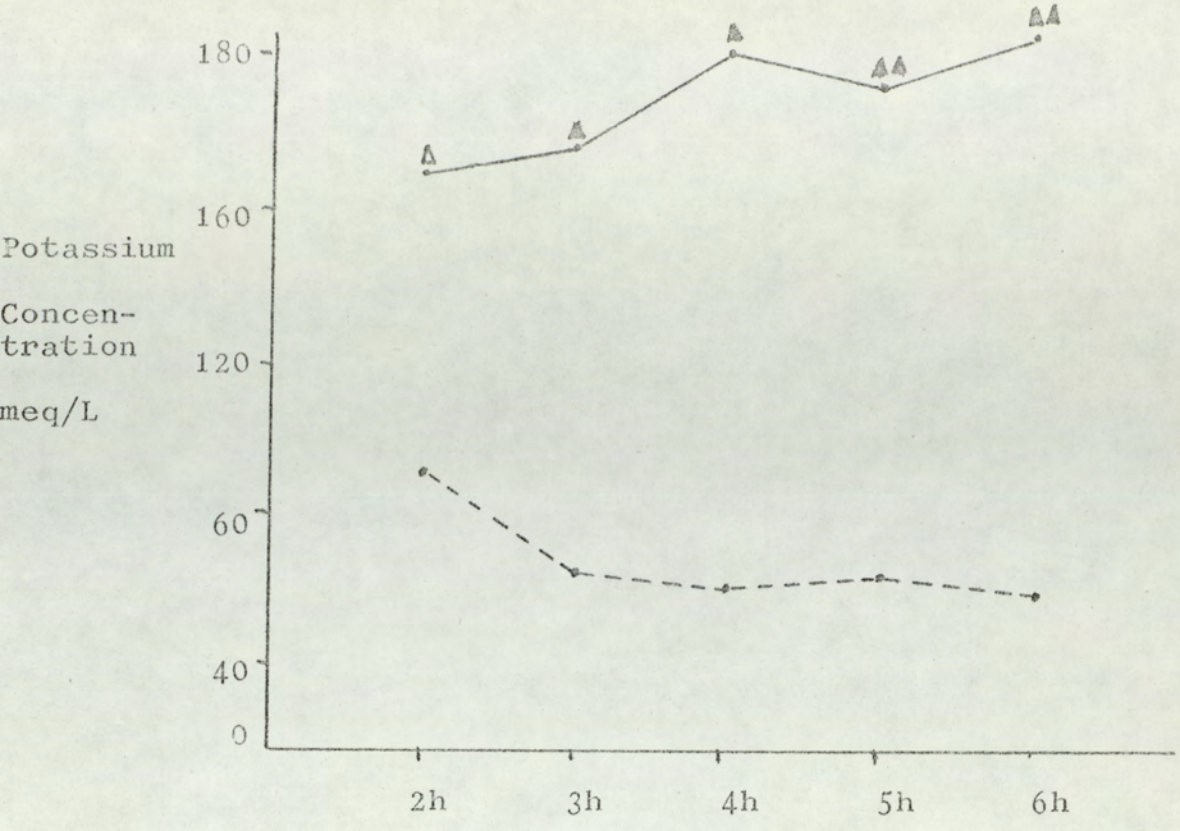
Progesterone Treated ——— Ovx. Control – – – –

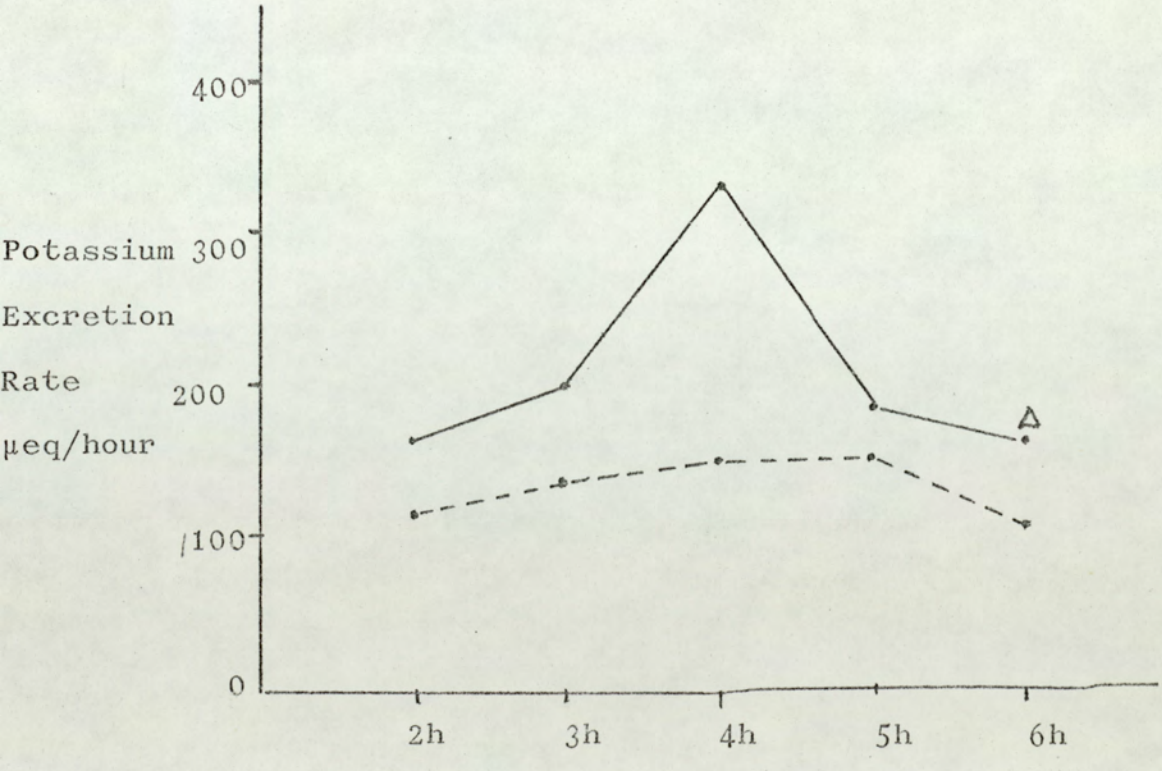
FIG. 10b



The Action of Progesterone Administration for 14 days on Urinary sodium Concentration and Excretion Rates in Ovariectomised Rats.

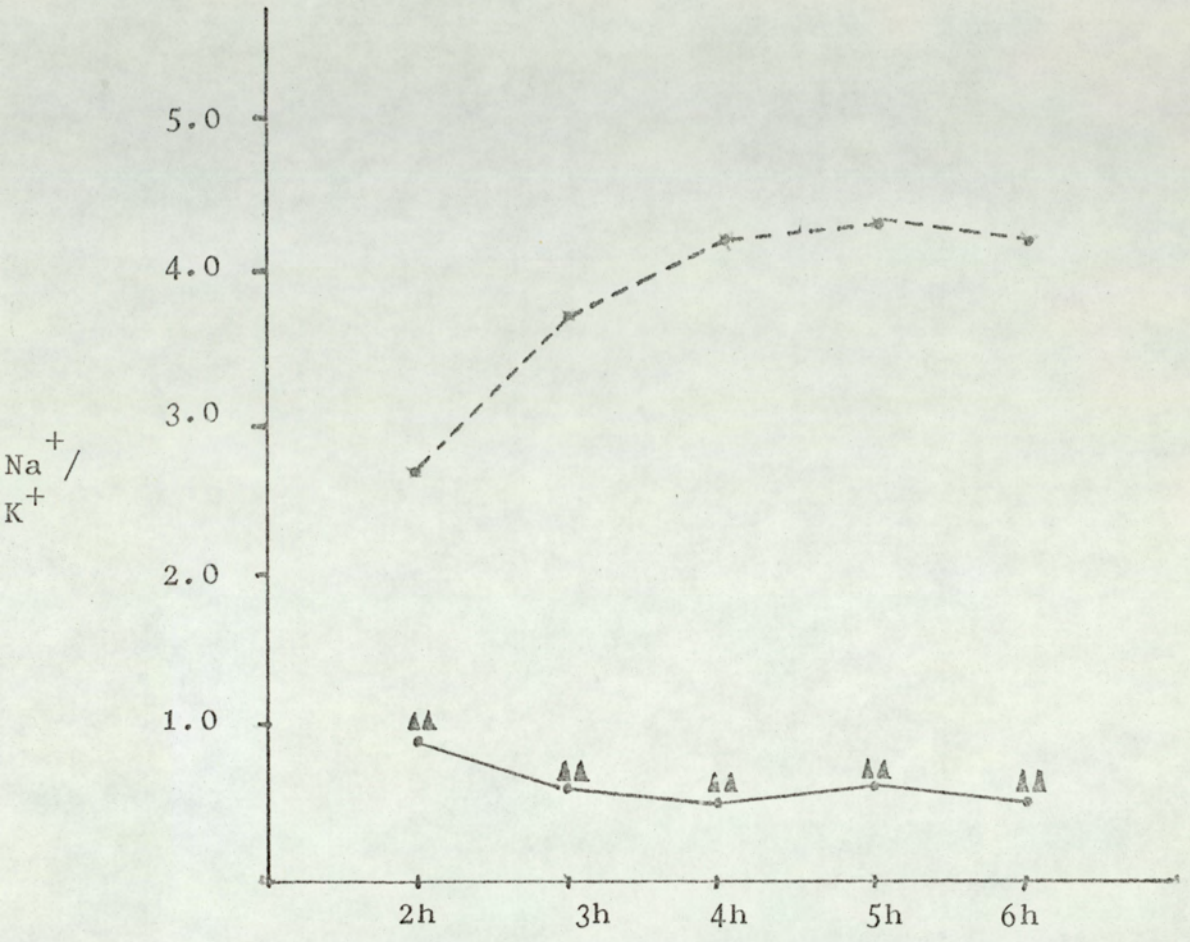
FIG. 10c





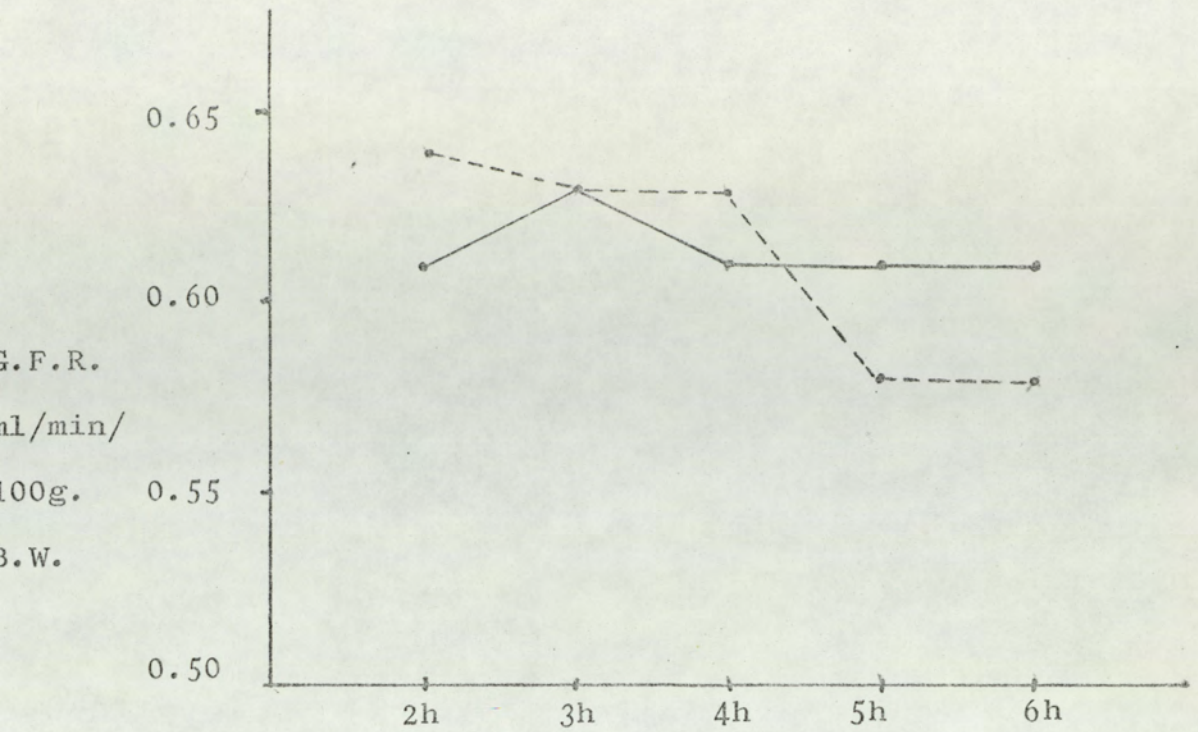
The Action of Progesterone Administration for 14 days on the Sodium:Potassium Ratio in Ovariectomised Rats i

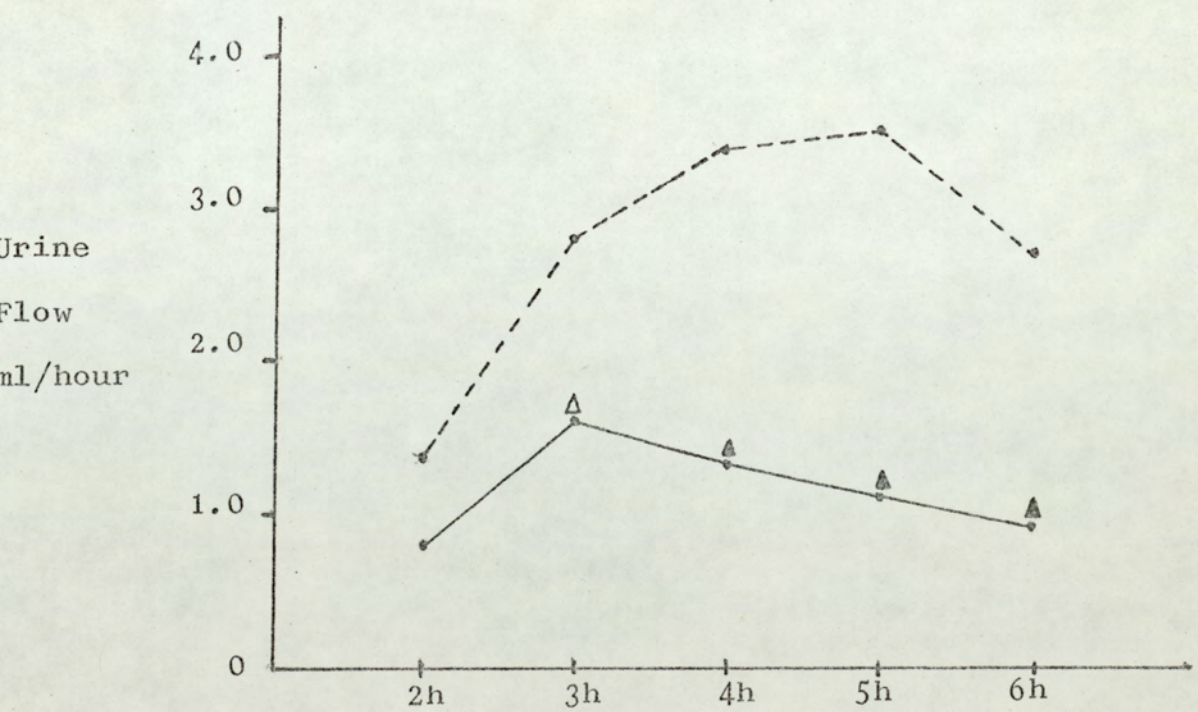
FIG. 10d



The Action of Progesterone Administration for 14 days on Sodium Potassium Ratio in Ovariectomised Rats.

FIG. 11a

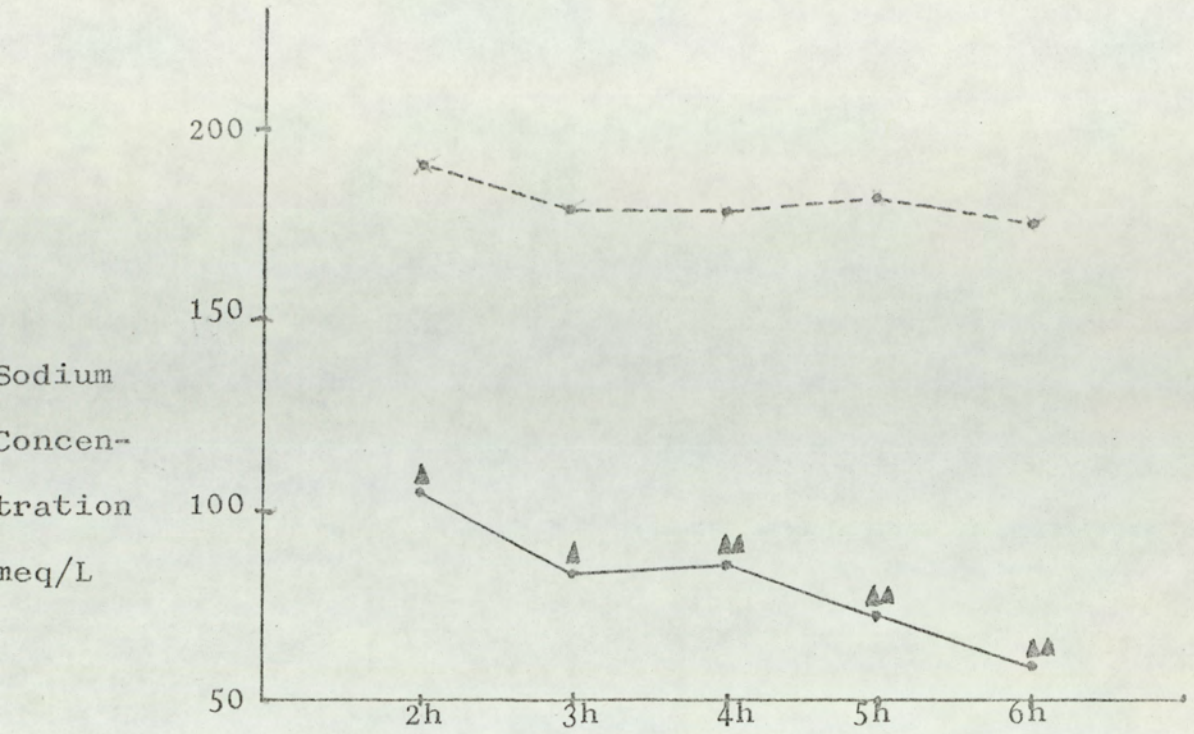


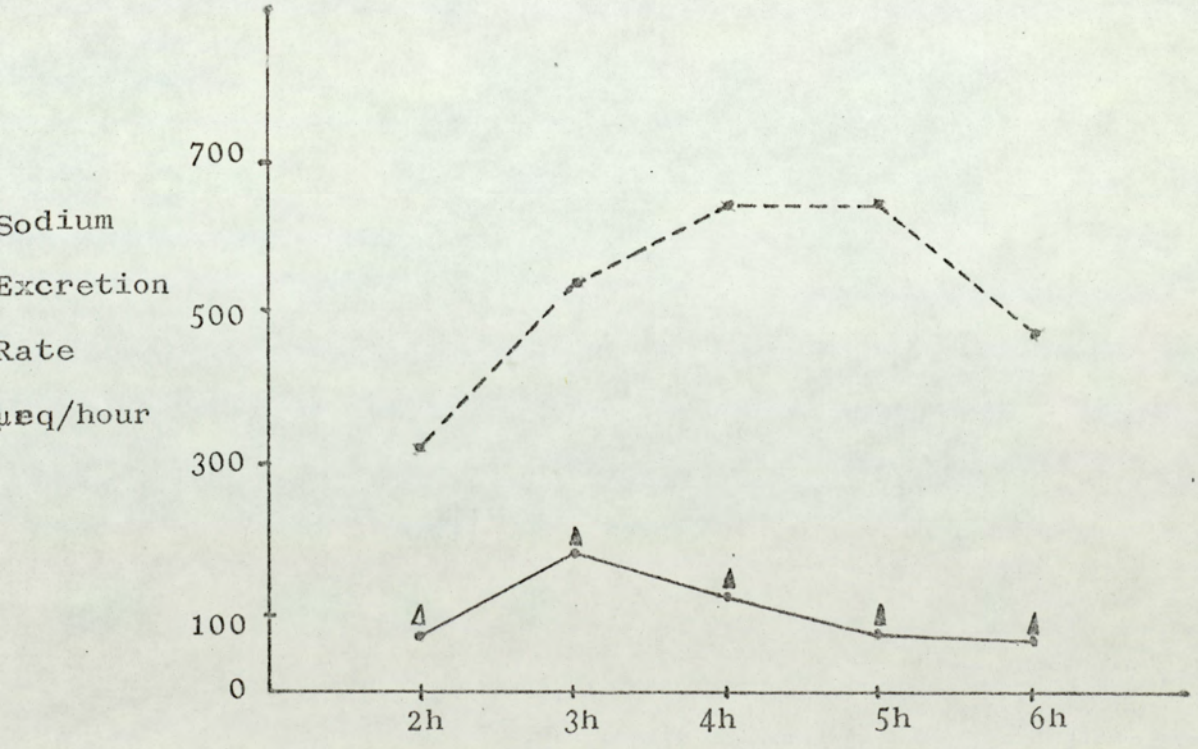


The Action of Progesterone and Oestradiol Administration for 14 days on G.F.R. and Urine Flow in Ovariectomised Rats.

Progesterone and Oestradiol Treated ———— Ovx. Control – – – – –

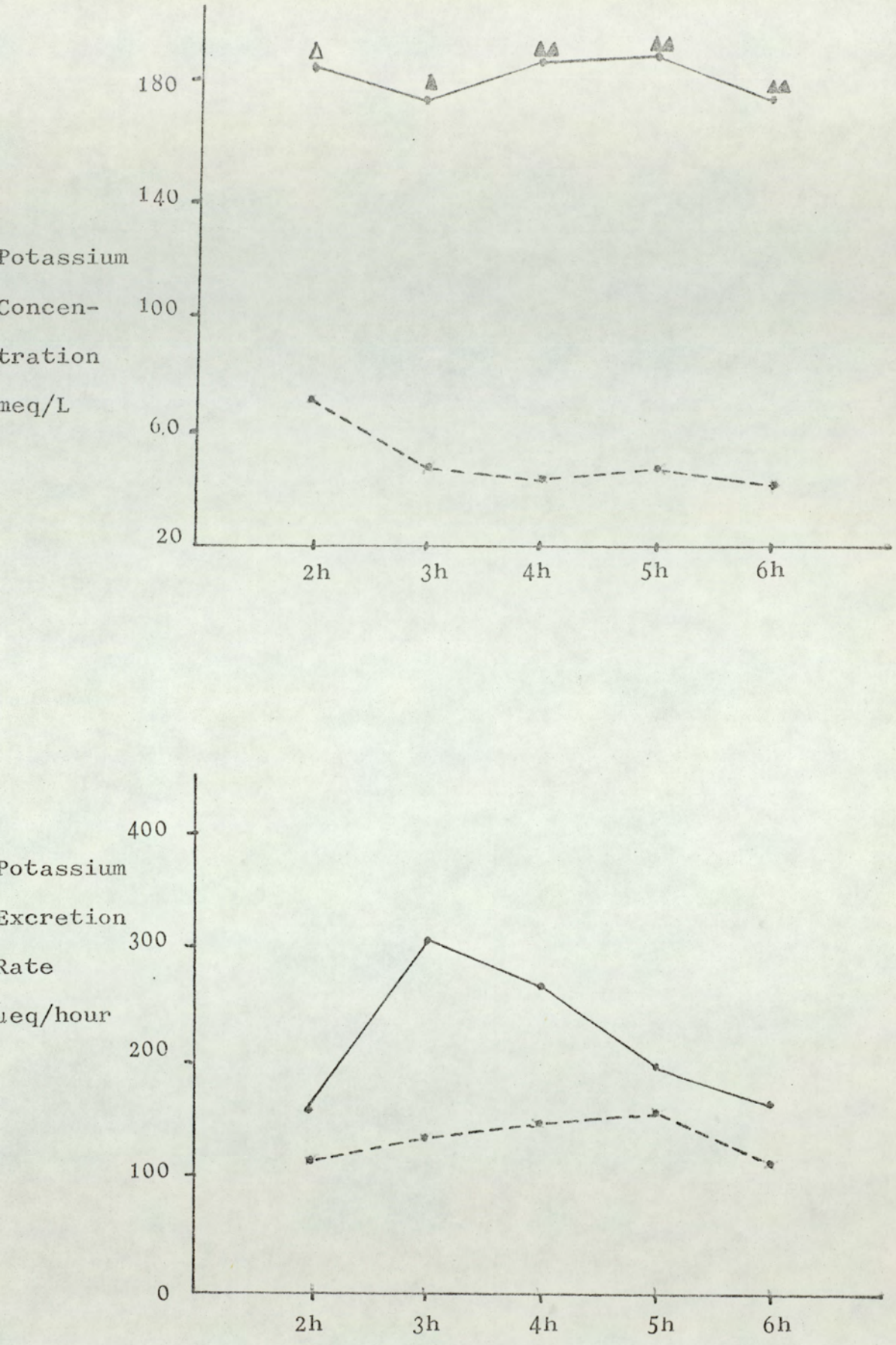
FIG. 11b





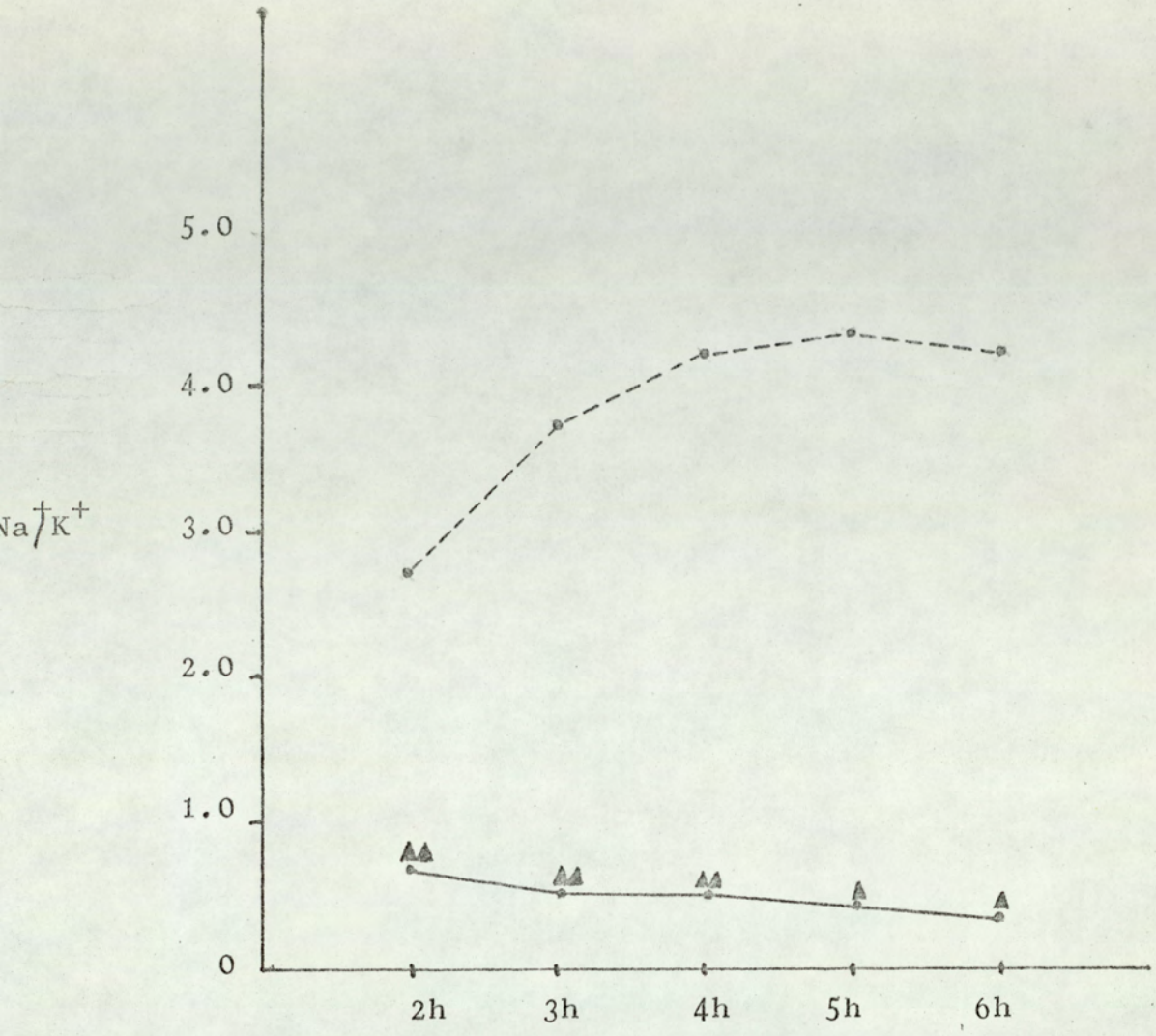
The Action of Progesterone and Oestradiol Administration for 14 days on Urinary Sodium Concentration and Excretion Rate in Ovariectomised Rats.

FIG. 11c



The Action of Progesterone and Oestradiol Administration for 14 days on Urinary Potassium Concentration and Excretion Rate in Ovariectomised Rats.

FIG. 11d



The Action of Progesterone and Oestradiol Administration for 14 days on the Sodium:Potassium Ratio in Ovariectomised Rats.

filtration rate (Figs. 10A and 11A).

Sodium Excretion

Oestrogen, progesterone and combined oestrogen + Progesterone treatment resulted in massive sodium retention. Four hours after starting saline infusion sodium concentrations were 113.7±15.6 meq/L, 78.9±15.3 meq/L and 86.9±6.8 meq/L with oestrogen, progesterone and combined oestrogen and progesterone treatment respectively, compared with sodium concentration of 179.3±11.6 meq/L in vehicle blank injected, ovariectomised animals ($p<0.02$, $p<0.01$, $p<0.001$ respectively). Oestrogen, progesterone and combined oestrogen + progesterone treatment resulted in sodium excretion rates of 214.4±56.4 µeq/hour, 133.3±33.7 µeq/hour and 111.9±17.2 µeq/hour respectively, 4 hours after starting saline infusion, compared with a sodium excretion rate of 627.5±91.6 µeq/hour in the control ovariectomised animals ($p<0.001$, $p<0.001$, $p<0.01$ respectively) (Figs. 9B, 10B and 11B).

Sodium:Potassium Ratio

The results presented in Figs.9D, 10D and 11D show that oestrogen, progesterone and combined oestrogen + progesterone treatment induced a significant reduction in the sodium: potassium ratio compared with ovariectomised control values. The sodium:potassium ratios 4 hours after starting saline infusion were 0.64±0.1, 0.52±0.1, and 0.48±0.07 respectively with oestrogen, progesterone and combined oestrogen + progesterone treatment compared with 4.2±0.4 in the control animals ($p<0.001$, $p<0.001$, $p<0.001$ respectively).

The sodium:potassium ratios observed after ovarian hormone treatment were lower than those observed in oestrous animals.

Potassium Excretion

The sodium retention observed with ovarian hormone treatment was accompanied by an increase in urinary potassium concentration. Oestrogen, progesterone and combined oestrogen + progesterone treatment resulted in potassium concentrations of 172.2±18.8 meq/L, 182.0±22.0 meq/L, and 191.0±23.3 meq/L respectively, 4 hours after starting saline infusion, compared with ovariectomised, control a level of 43.6±2.8 meq/L at this time ($p<0.001$, $p<0.01$, $p<0.001$ respectively).

Potassium excretion rates were not significantly different from control values (Figs. 9,10 & 11c).

These results show that oestrogen and progesterone cause a massive sodium and water retention in ovariectomised rats. The reduction in the sodium:potassium ratio with ovarian hormone treatment suggests that their action on kidney function may be via mineralocorticoids.

These findings are in accordance with the early work of Thorn & Harrap (1938) in dogs. Although they suggested that the similarity in structure of oestrogen, progesterone and mineralocorticoids might mean that they have certain physiological properties in common, Landau & Luigibihl (1961) have shown that progesterone antagonises the action of aldosterone on sodium retention in human beings. They suggested that this natriuretic action of progesterone could stimulate aldosterone secretion and hence induce sodium and water retention.

Summary

1. Oestrogen and progesterone treatment significantly reduced urine flow compared with vehicle blank injected, control animals.
2. Oestrogen and progesterone had no significant influence in glomerular filtration rate.
3. Oestrogen and progesterone significantly reduced sodium concentration and excretion rates compared with control animals.
4. Oestrogen and progesterone increased potassium excretion compared with control values.

The Action of Spironolactone on Sodium and Water Retention Induced by Oestrogen and Progesterone

Introduction

The previous findings showed that oestrogen and progesterone caused sodium and water retention, with an associated increase in urinary potassium concentration and again suggested that the action of these hormones might be mediated by mineralocorticoids. Therefore, experiments were carried out to discover what effect spironolactone had on the action of these sex hormones on the kidney.

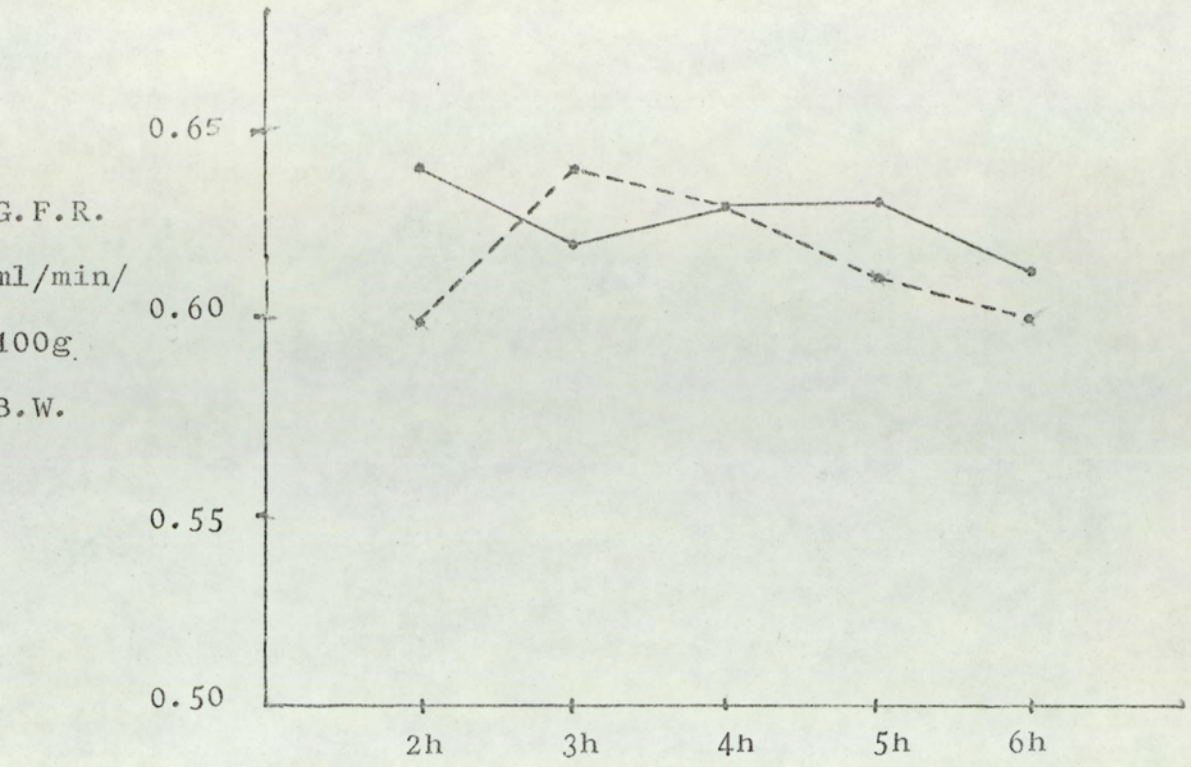
The same experimental procedures were used for the administration of ovarian hormones and spironolactone as described in the preceding sections.

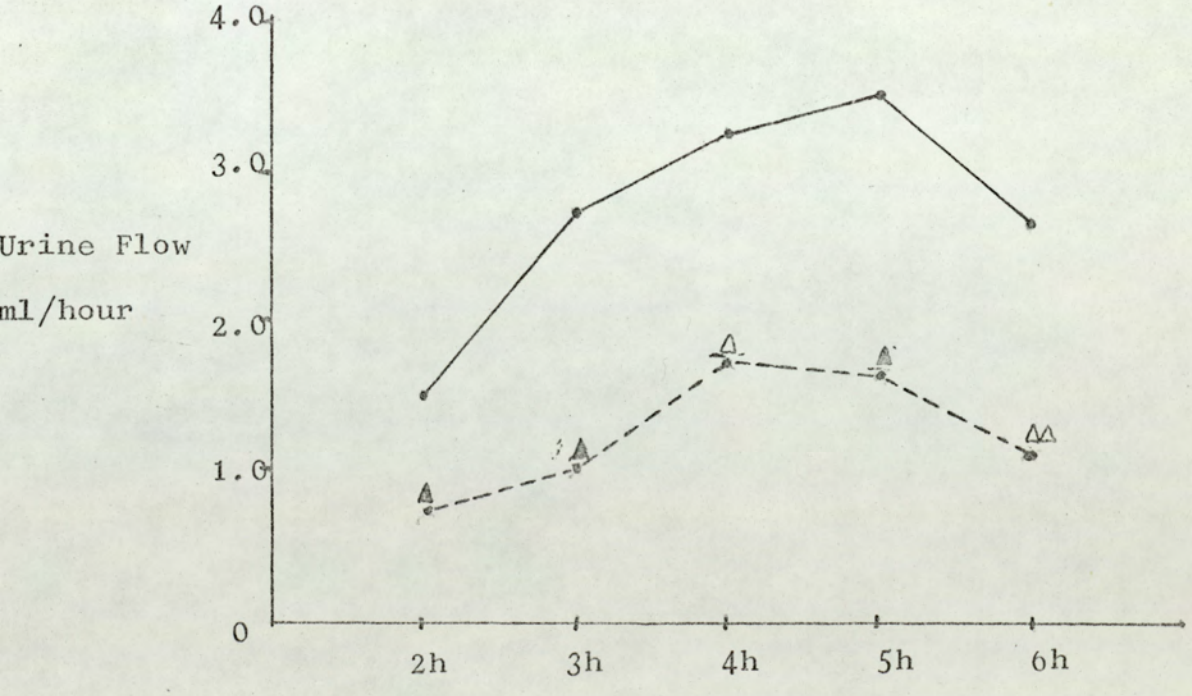
The results presented in Figs. 12, 13 and 14 A-D show that effect of spironolactone treatment on renal electrolyte function after oestrogen, progesterone and combined oestrogen + progesterone treatment.

Urine Flow

Spironolactone treatment completely blocked the water retention observed with ovarian hormone treatment. Four hours after starting saline infusion the urine flow was 3.2 ± 0.5 ml/hour in animals treated with oestrogen + spironolactone, compared with 1.7 ± 0.3 ml/hour in animals treated with oestrogen alone ($p < 0.05$). In the progesterone + spironolactone treated group urineflow was 3.4 ± 0.2 ml/hour compared with 1.6 ± 0.3 ml/hour with progesterone treatment alone ($p < 0.001$). Similar values were observed with combined oestrogen and progesterone therapy. No significant

FIG. 12a

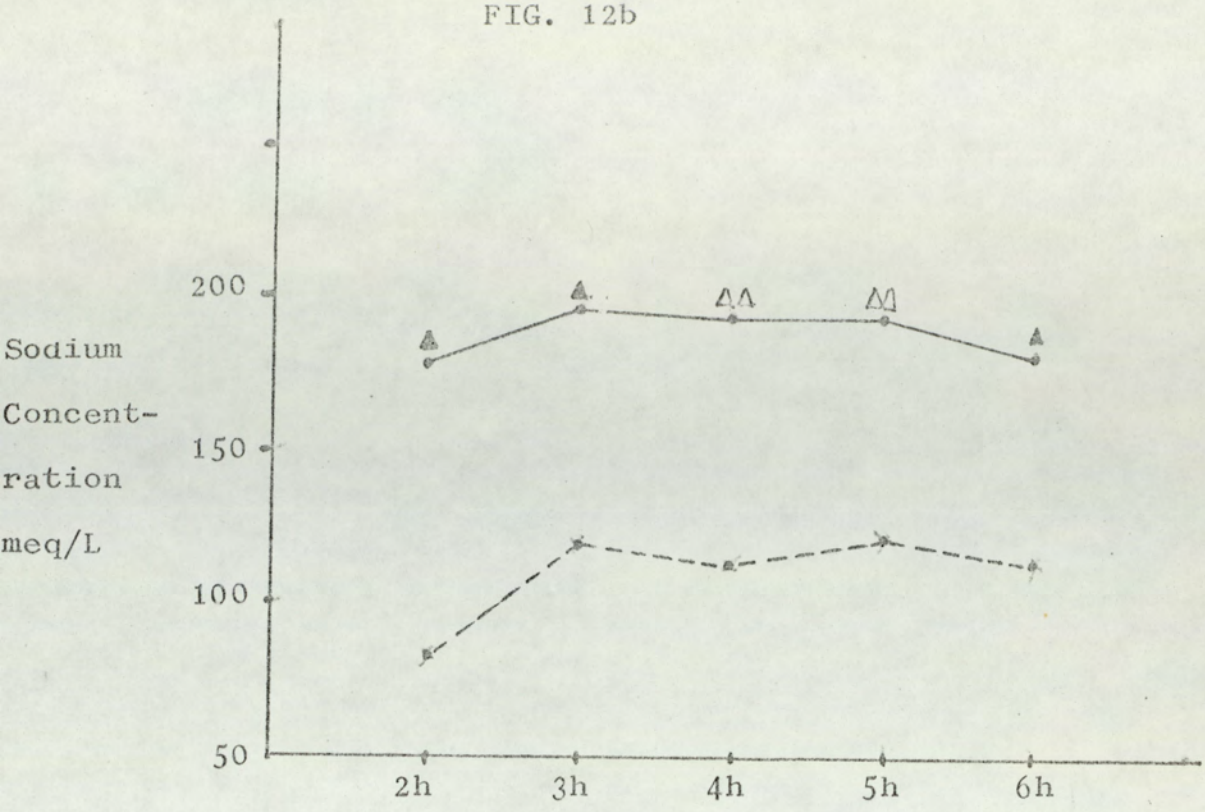


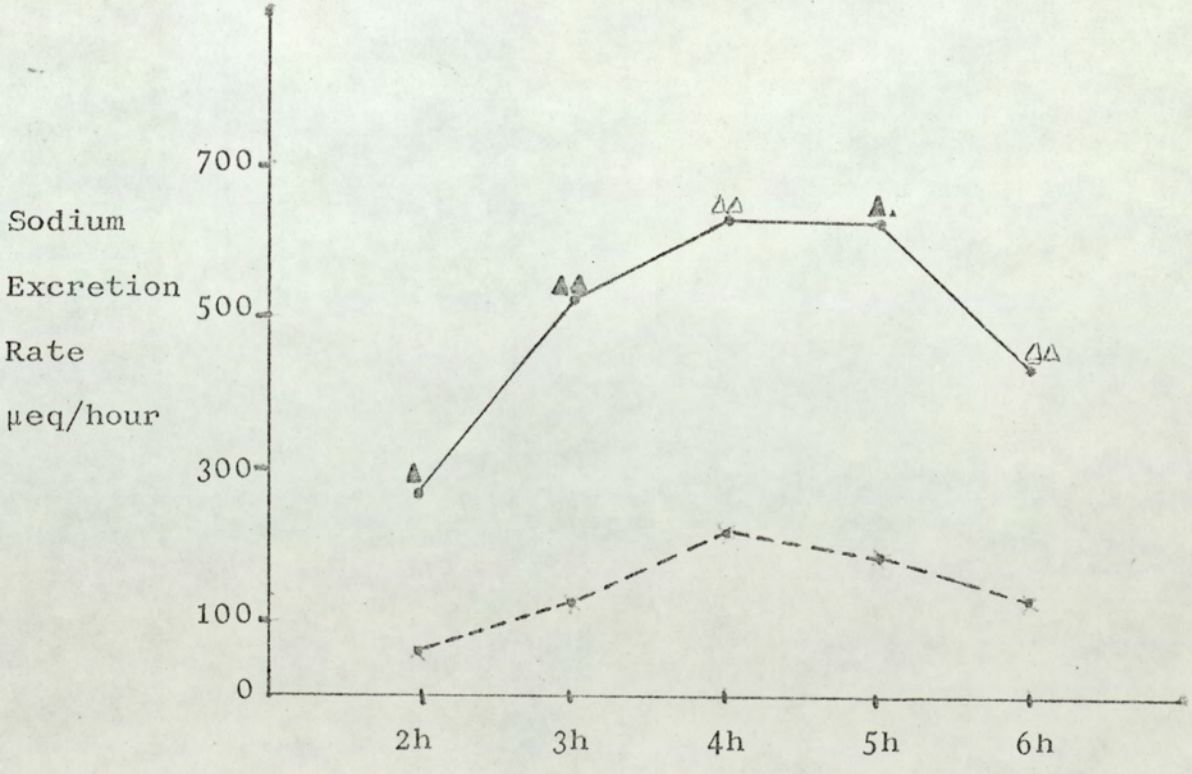


The Action of Spironolactone on G.F.R. and Urine Flow in Ovariectomised Rats following Prolonged Oestradiol Administration.

Spironolactone Treated Oestradiol Treated Control

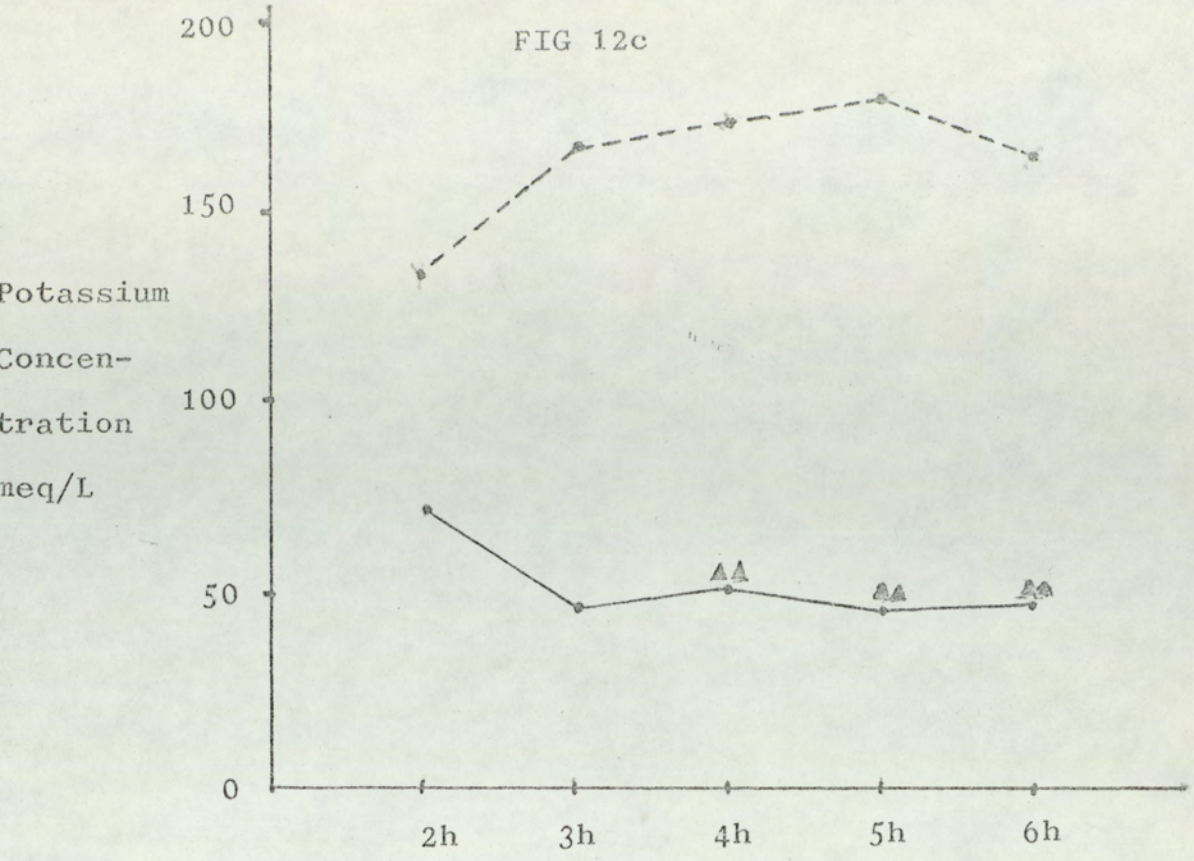
FIG. 12b

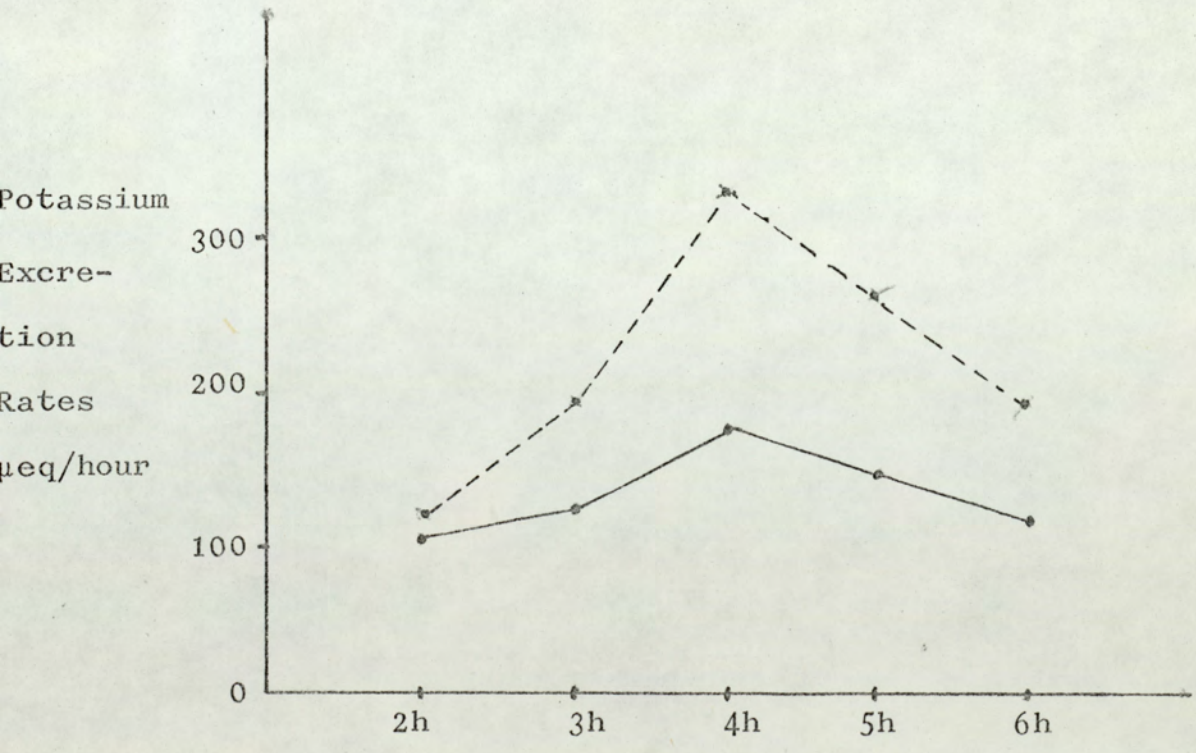




The Action of Spironolactone on Urine Sodium Concentration and Excretion Rate in Ovariectomised Rats following Prolonged Oestradiol Administration

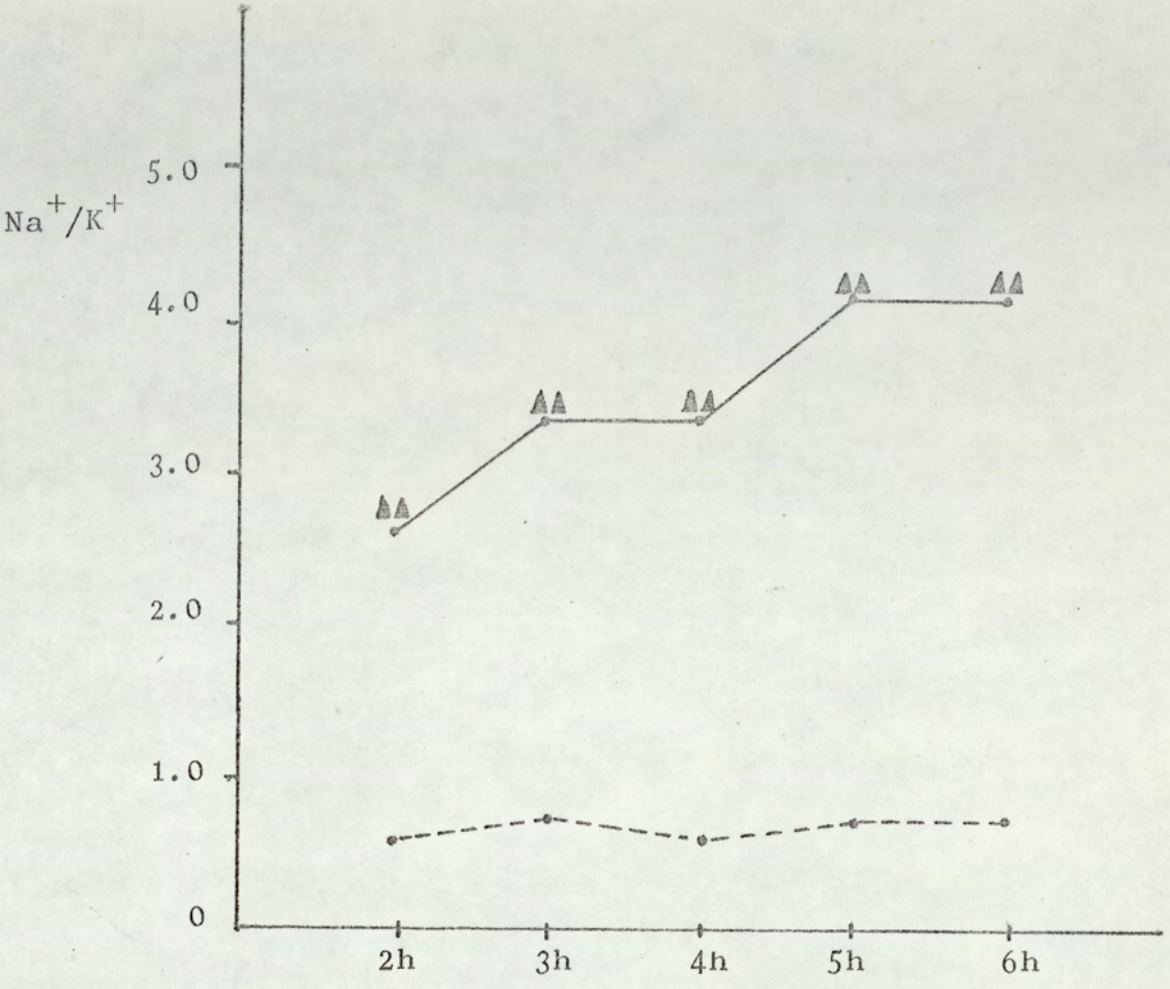
FIG 12c





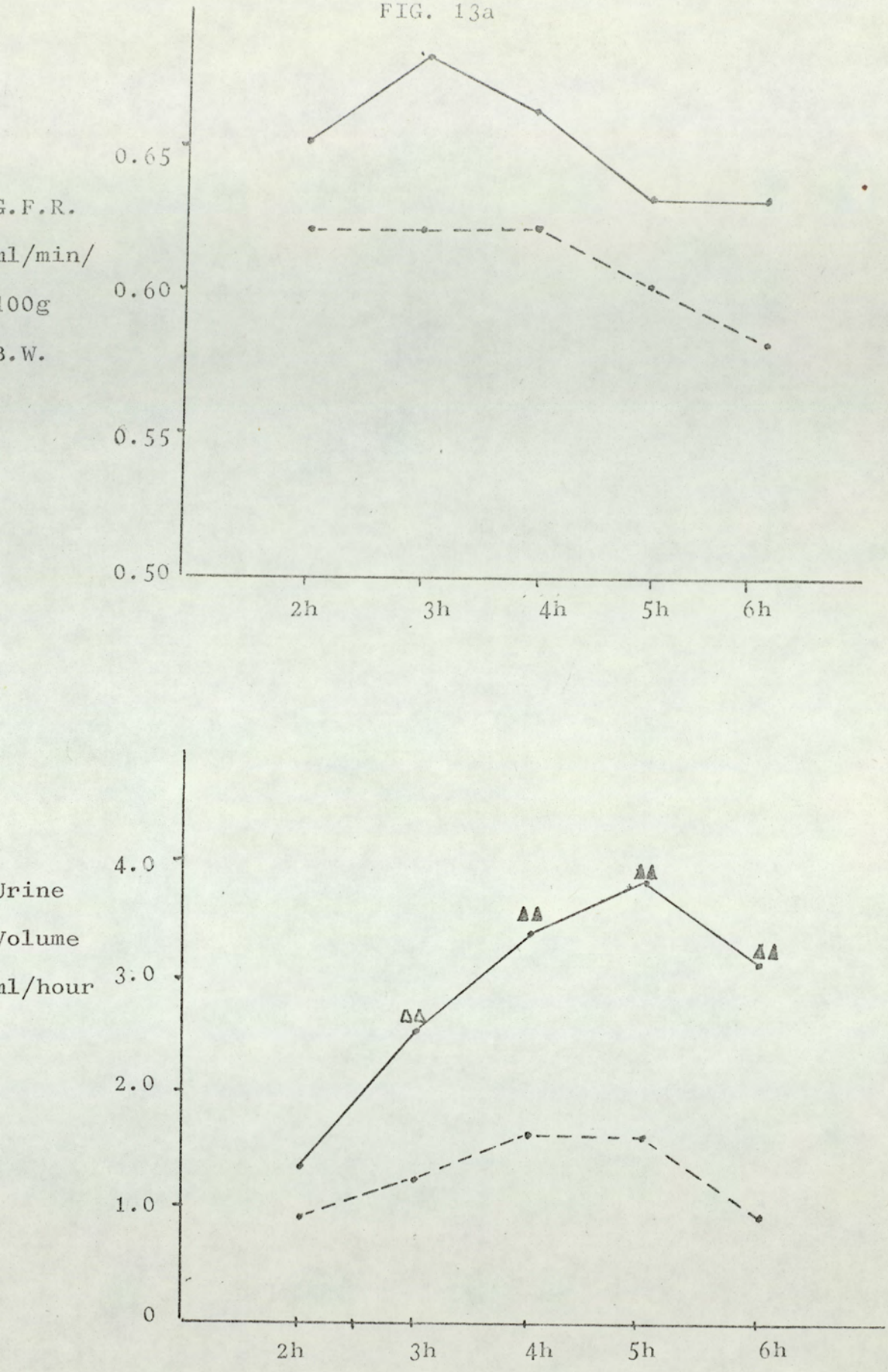
The Action of Spironolactone on Urinary Potassium Concentration and Excretion Rate in Ovariectomised Rats Following Prolonged Administration of Oestradiol.

FIG. 12d



The Action of Spironolactone on the Sodium: Potassium Ratio in Ovariectomised Rats Following Prolonged Oestradiol Administration.

FIG. 13a

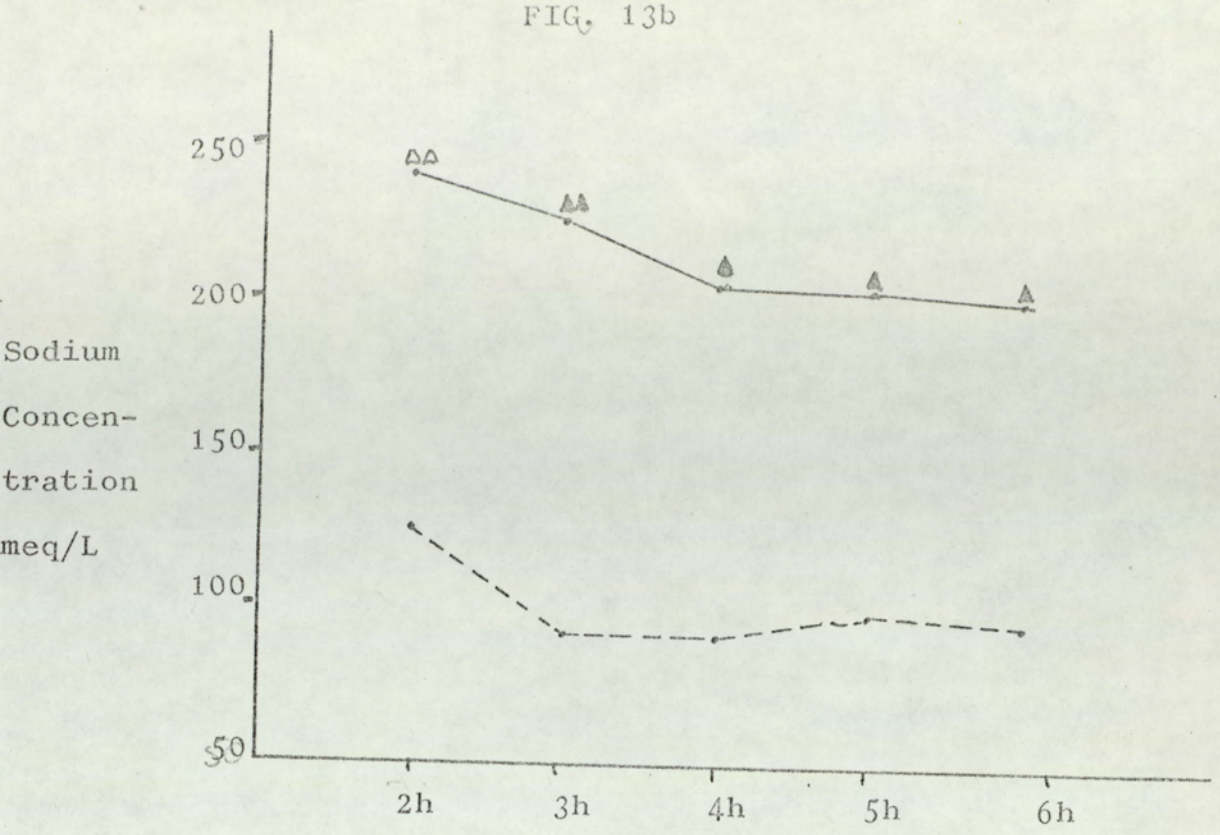


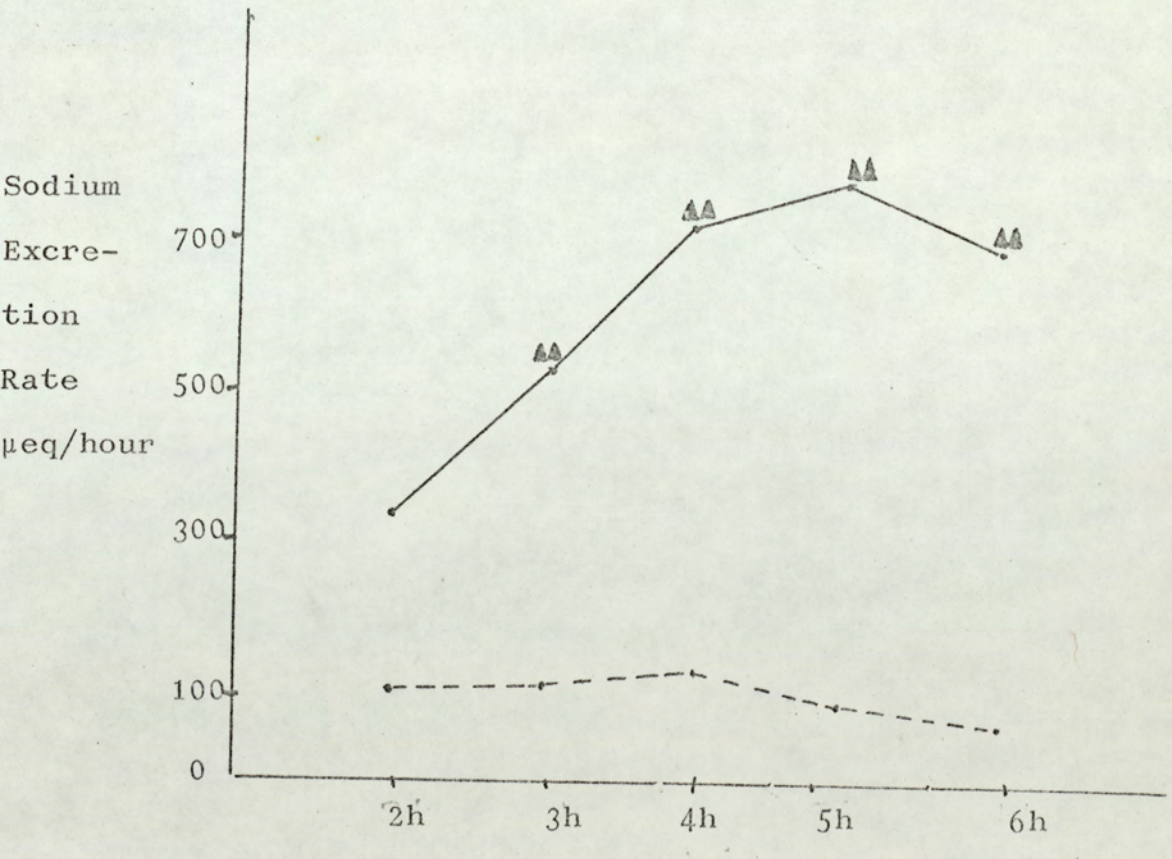
The Action of Spironolactone on G.F.R. and Urine Volume in Ovariectomised Rats Treated with Progesterone

Spironolactone Treated

Control

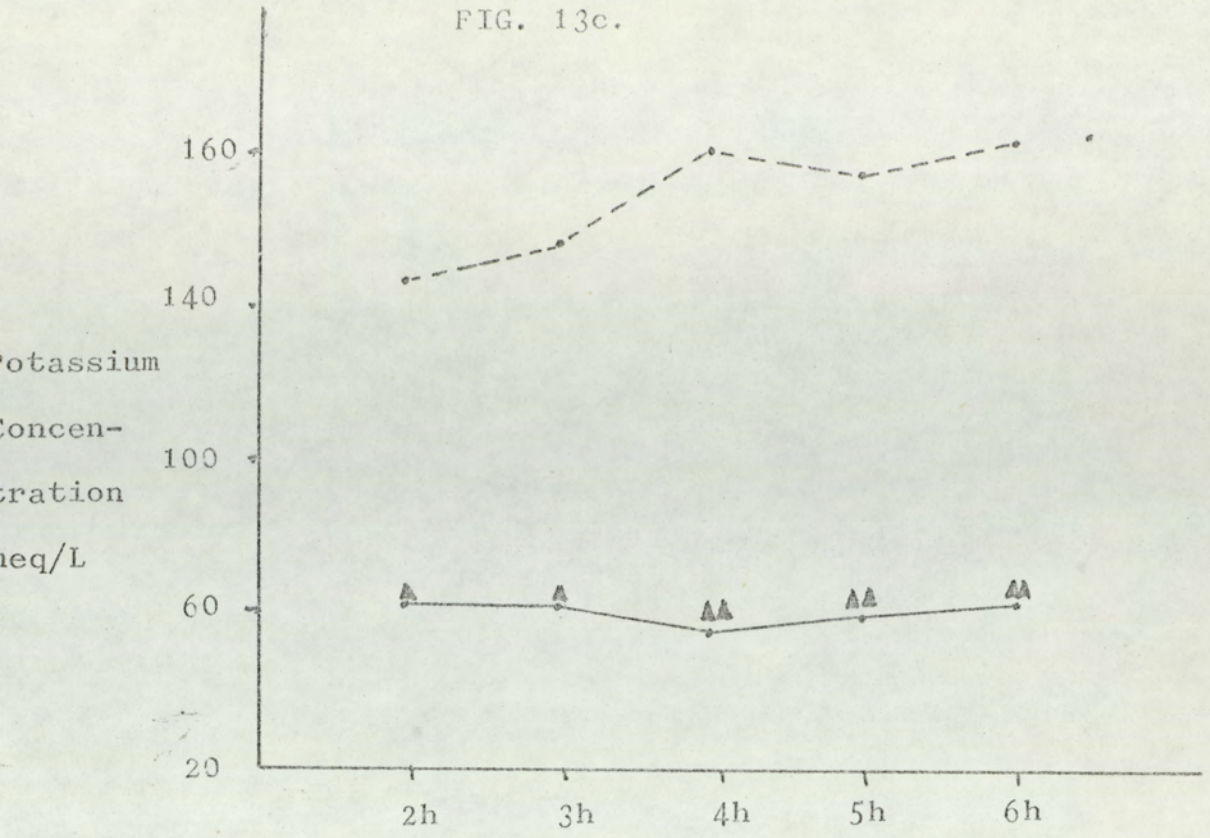
FIG. 13b

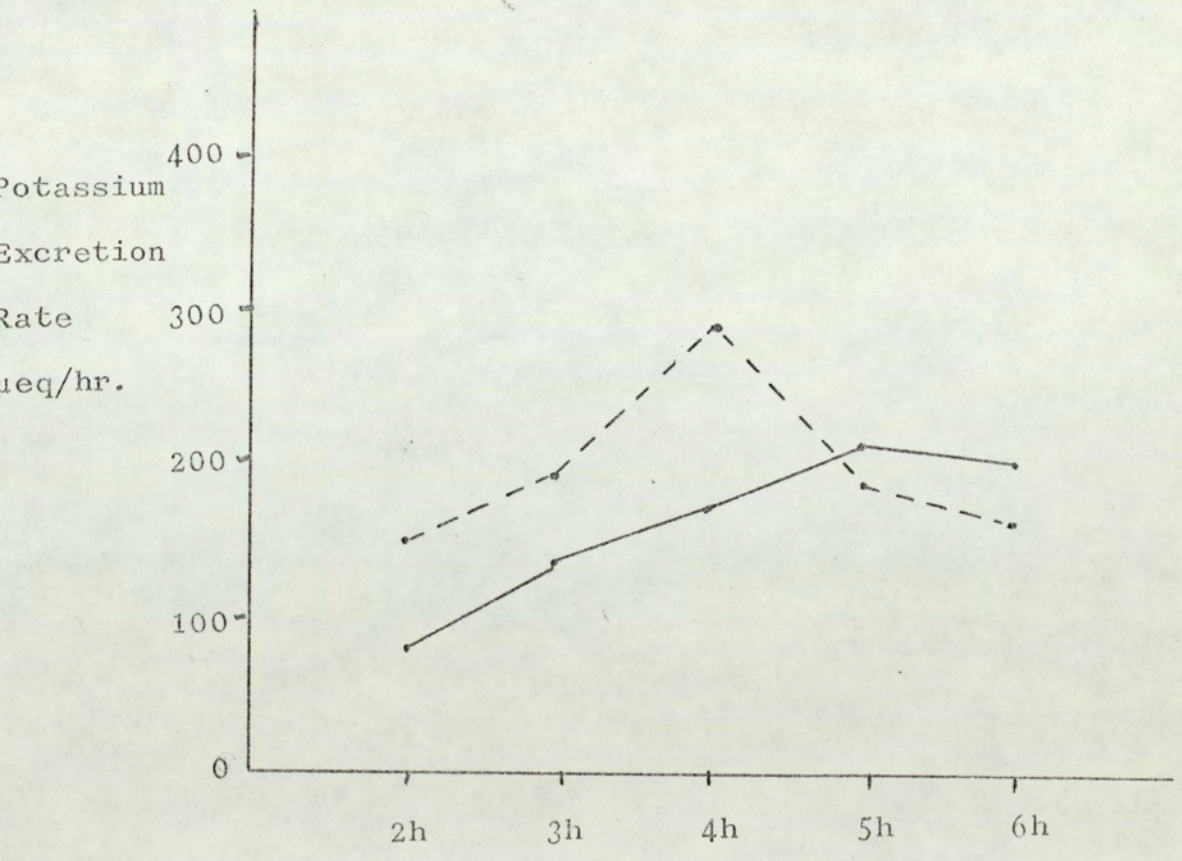




The Action of Spironolactone on Sodium Concentration and Excretion Rate in Ovariectomised Rats Treated with Progesterone.

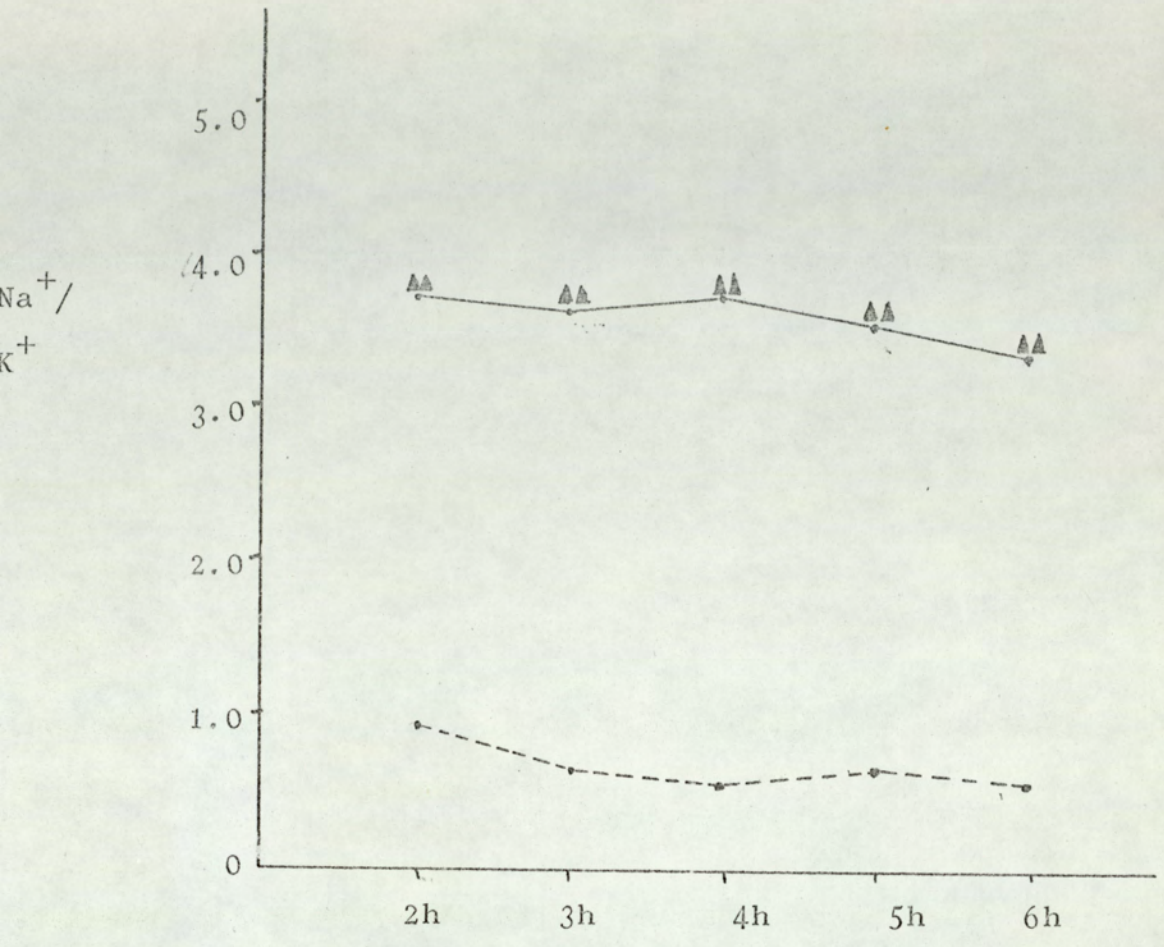
FIG. 13c.





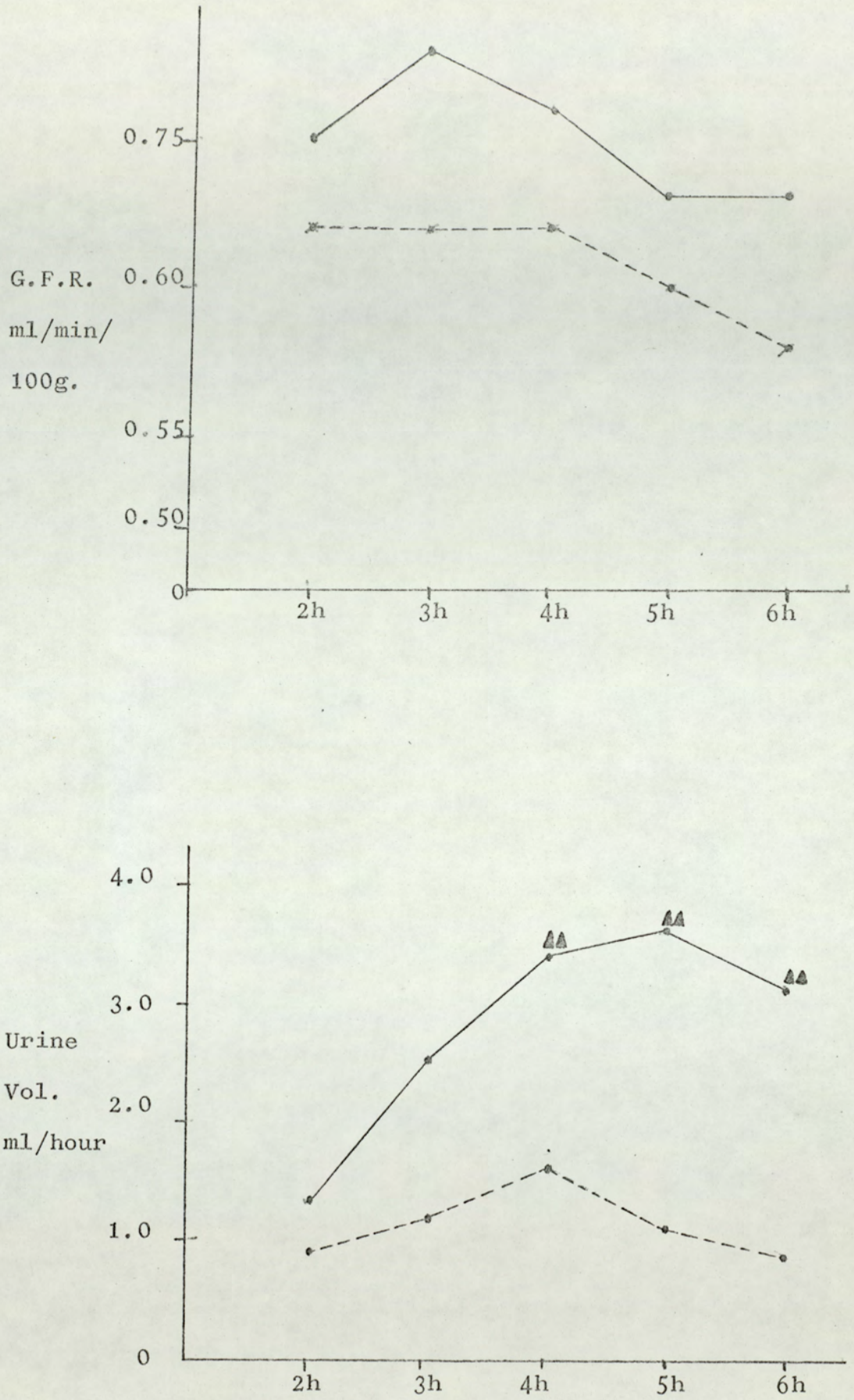
The Action of Spironolactone on Potassium Concentration and Excretion Rate in Ovariectomised Rats treated with Progesterone.

FIG. 13d



The Action of Spironolactone on the Sodium: Potassium Ratio on Ovariectomised Rats Treated with Progesterone.

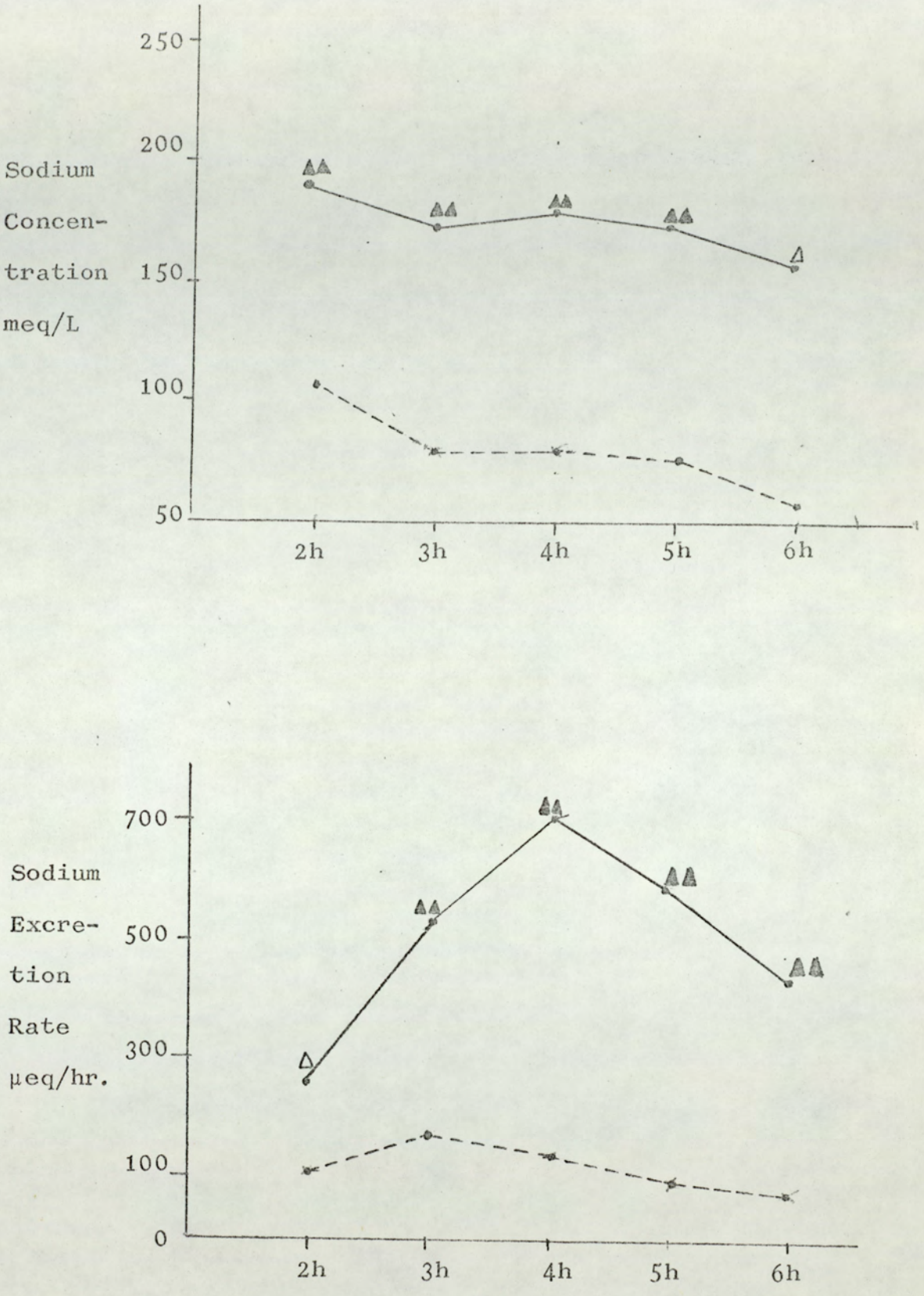
FIG. 14a.



The Effect of Spironolactone on GFR and Urine Volume in Ovariectomised Rats Given Prolonged Oestrogen and Progesterone.

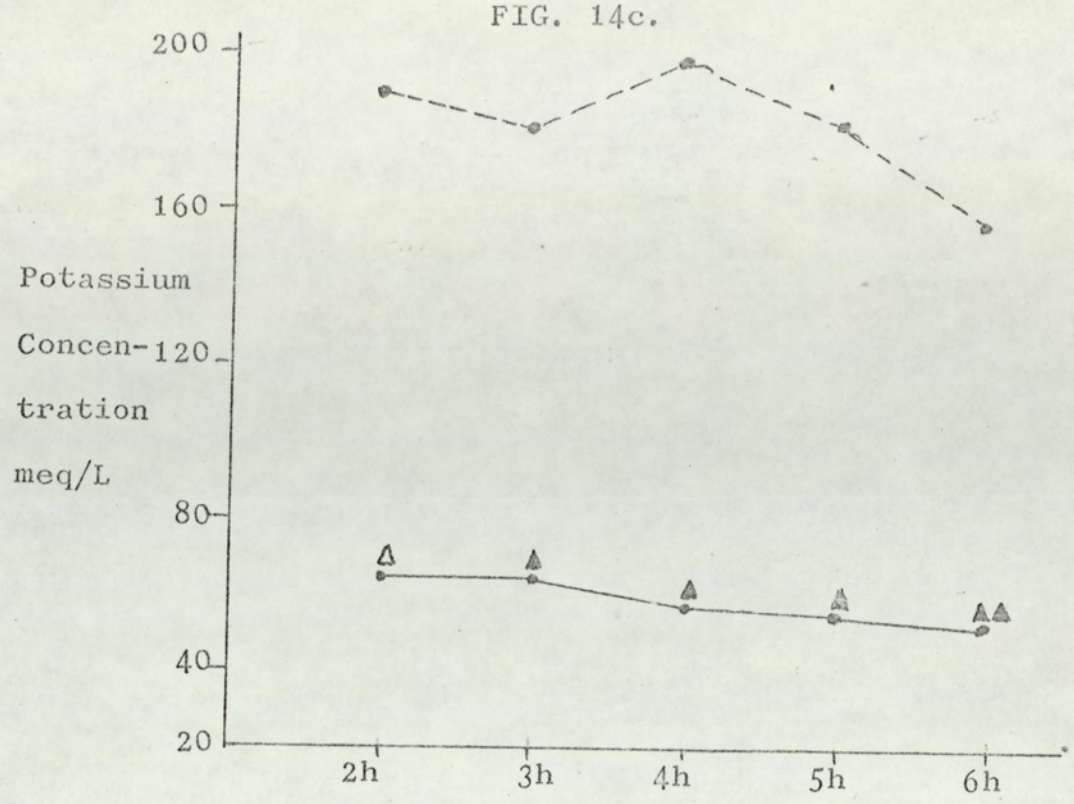
Oestrogen + Progesterone Treated ——— Control - - - -

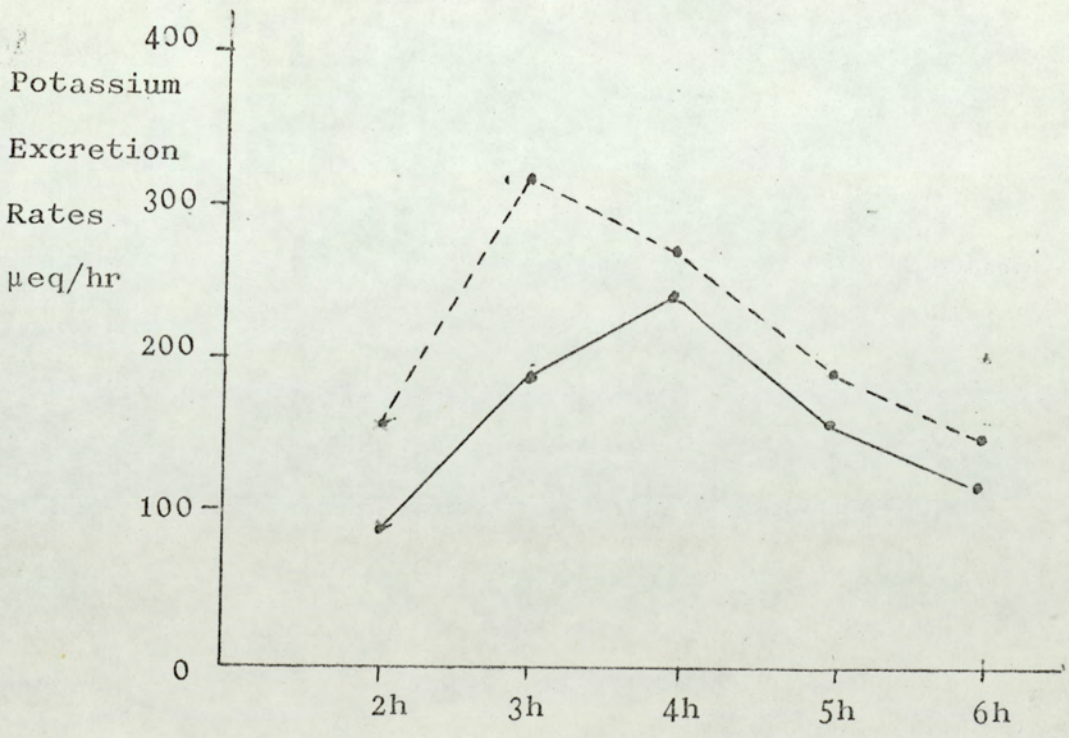
FIG. 14b



The Effect of Spironolactone on Sodium Concentration and Excretion Rate in Ovariectomised Rats Given Prolonged Oestrogen + Progesterone Therapy.

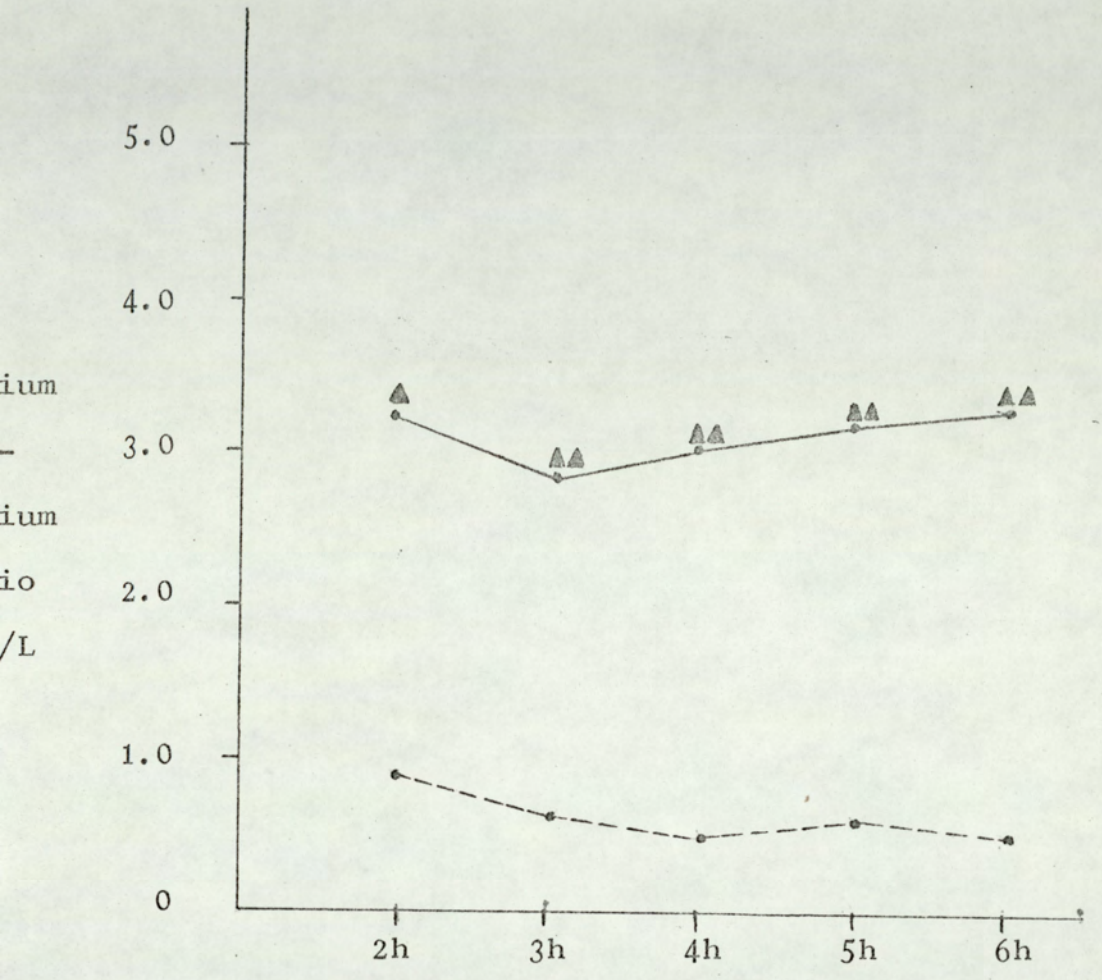
FIG. 14c.





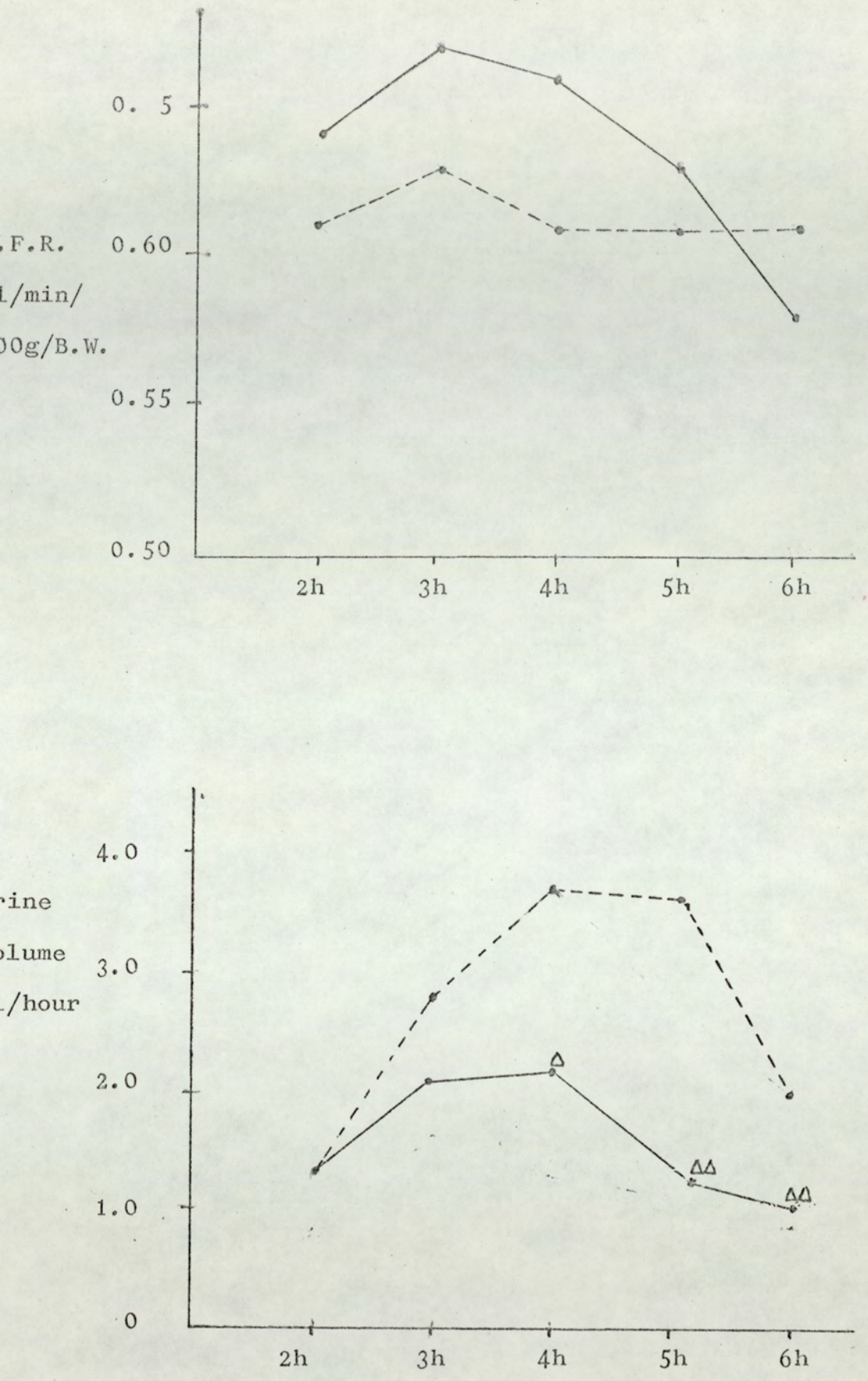
The Effect of Spironolactone on Potassium Concentratio and Excretion Rate in Ovariectomised Rats Given Oestrogen and Progesterone Therapy.

FIG. 14d



The Effect of Spironolactone on the Sodium Potassium Ratio in Ovariectomised Rats Given Prolonged Oestrogen and Progesterone Therapy.

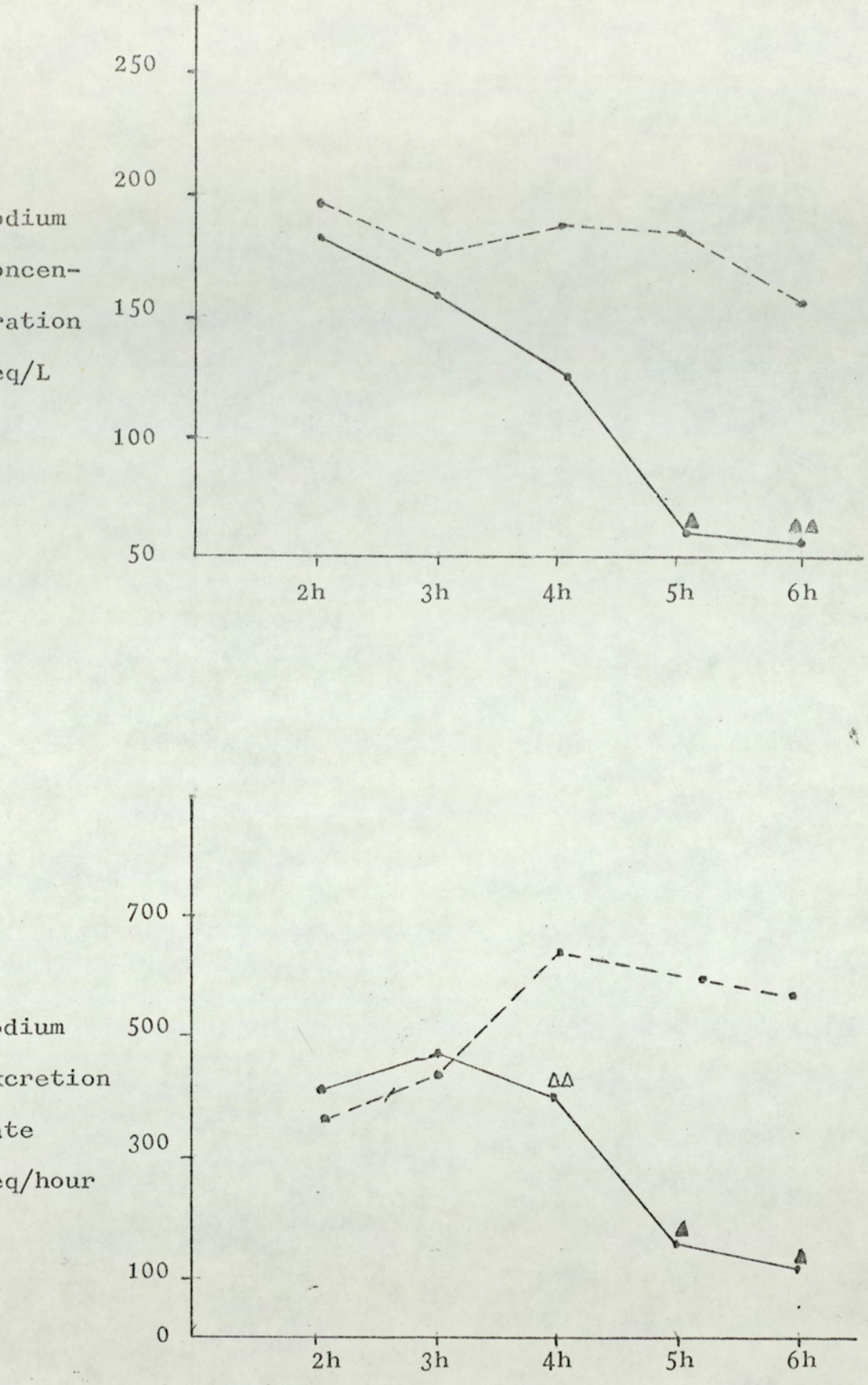
FIG. 15a



G.F.R. and Urine Volume in Ovariectomised Rats Following Oestrogen Administration 2 hours prior to Experimentation.

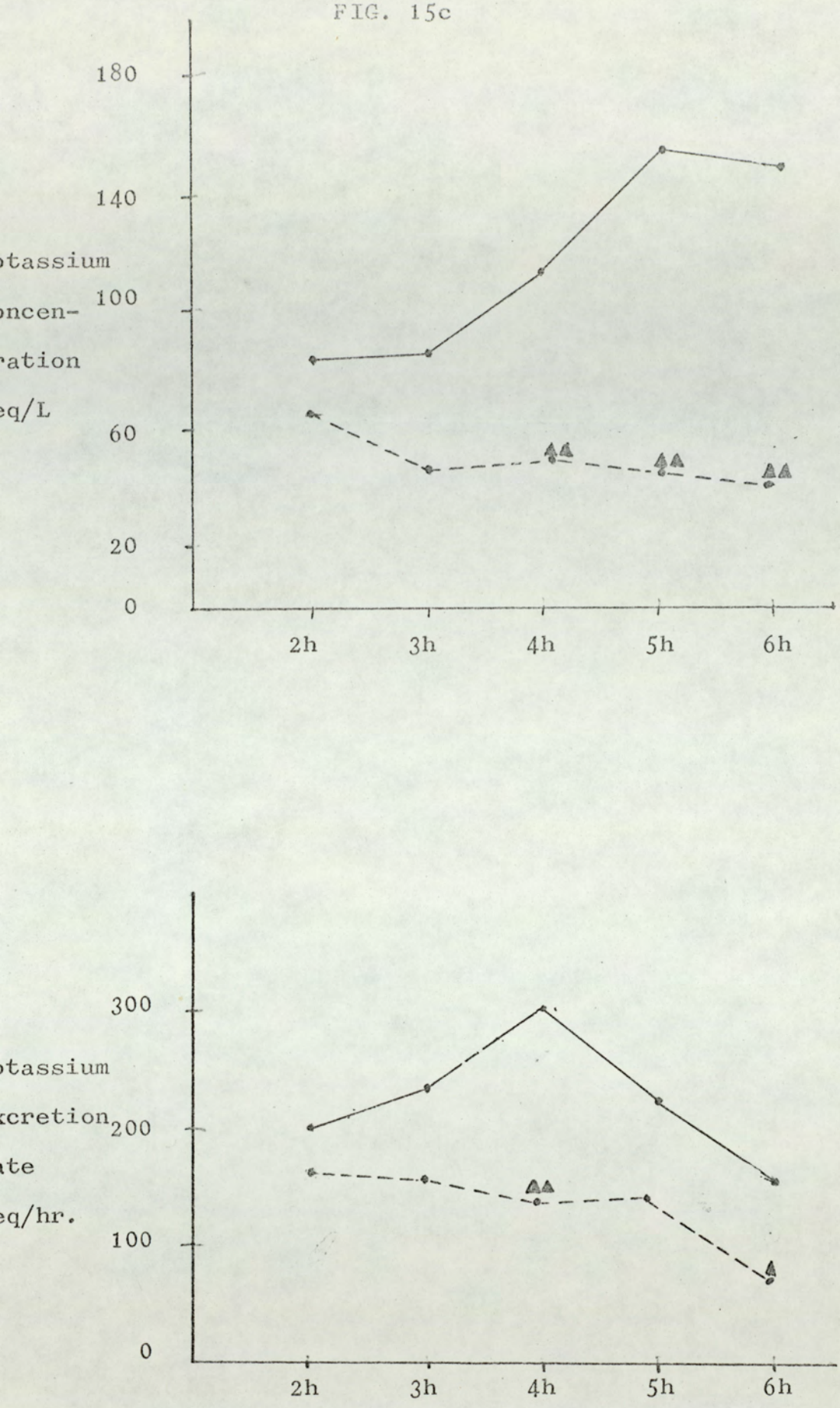
Oestrogen Treated ——— Control – – –

FIG. 15b



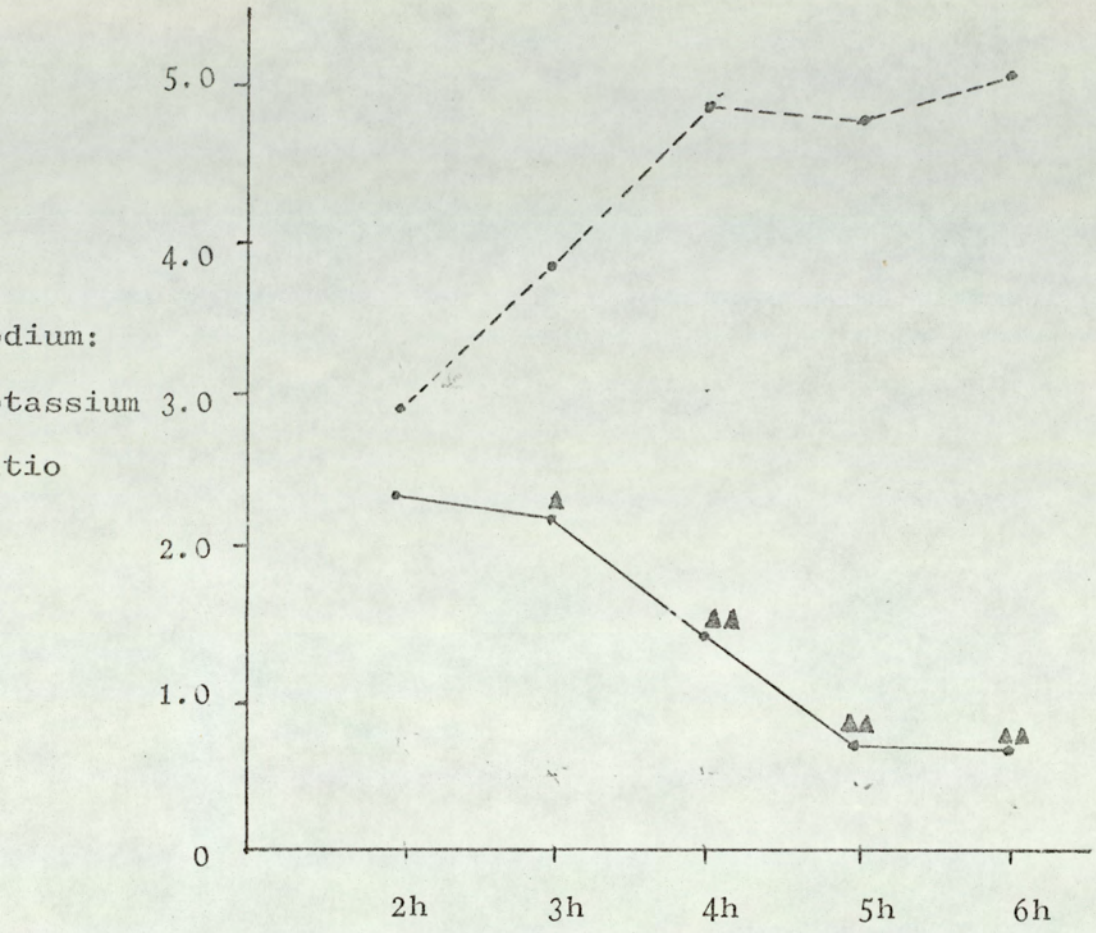
Sodium Concentration and Excretion Rates in Ovariectomised Rats Following Oestrogen Administration 2 hours prior to Experimentation.

FIG. 15c



Potassium Concentration and Excretion Rates in Ovariectomised Rats Following Oestrogen Administration prior to Experimentation.

FIG. 15d



The Sodium Potassium Ratio in Ovariectomised Rats Following Oestrogen Administration 2 hours Prior to Experimentation.

alterations in glomerular filtration rates were observed.

Sodium Excretion

The significant reduction in sodium concentration and excretion rates previously observed with oestrogen, progesterone and oestrogen + progesterone was not observed in animals treated with spironolactone. Four hours after starting saline infusion, sodium concentration and excretion rates in oestrogen + spironolactone treated animals were 186.9±13.4 meq/L and 701.2±82.3 µeq/hour respectively, compared with a sodium concentration of 86.9±6.9 meq/L and sodium excretion rate of 111.9±17.2 µeq/hour in animals treated with oestrogen alone ($p<0.001$) for both values). In animals treated with progesterone + spironolactone sodium concentration was 207.2±26.4 meq/L and sodium excretion rate 701.0±82.3 µeq/hour compared with sodium concentration and excretion rates of 79.0±15.3 meq/L and 133.3±33.7 µeq/hour respectively in animals treated with progesterone alone ($p<0.01$, $p<0.001$ respectively). In animals treated with both oestrogen and progesterone + spironolactone sodium concentration and excretion rates were 186.9 meq/L and 701.2±82.3 µeq/hour respectively compared with 86.9± 6.8 meq/L and 111.9 µeq/hour in animals treated with oestrogen + progesterone alone ($p<0.001$ for both values).

Sodium:Potassium Ratio

The sodium to potassium ratio was significantly increased with spironolactone in all three groups of animals, suggesting a decrease in mineralocorticoid activity at the kidney tubules. Four hours after starting saline infusion

the sodium:potassium ratio was 3.8±0.4 in animals treated with oestrogen and spironolactone compared with 0.60±0.1 in animals treated with oestrogen alone (p 0.001). In the group of animals treated with progesterone and spironolactone the sodium:potassium ratio was 3.7±0.3 compared with 0.5±0.2 in animals treated with spironolactone alone ($p<0.001$).

The sodium to potassium ratio in animals given combined oestrogen and progesterone treatment with spironolactone was 3.0±0.2 compared with 0.5±0.1 in animals treated with oestrogen + progesterone alone ($p<0.001$).

Potassium Excretion

Potassium concentrations and excretion rates were significantly reduced with spironolactone administration compared with ovarian hormone treatment alone. Four hours after starting saline infusion potassium concentration and excretion rates were 57.8±5.9 meq/L and 172.2±18.8 µeq/hour respectively, in animals treated with oestrogen and spironolactone, compared with 175.4 meq/L and 335.6±70.1 µeq/hour in animals treated with oestrogen alone ($p<0.001$ and $p<0.1$ respectively). In animals treated with progesterone with spironolactone potassium concentration and excretion rates were 182.0±22.0 meq/L and 331.4±82.5 µeq/hour respectively compared with 56.8 meq/L and 175.2±28.5 µeq/hour in animals treated with progesterone alone ($p<0.001$ and $p<0.1$ respectively). The potassium concentration and excretion rates in animals treated with oestrogen + progesterone with spironolactone treatment were 191.0±23.3 meq/ L and 263.0±70.7 µeq/hour respectively, compared with 61.5±3.8 meq/L and 230±24.4 µeq/hour ($p<0.01$ and $p<0.7$ respectively).

The sodium and water retention, low sodium:potassium ratio and increased potassium excretion observed with prolonged administration of oestrogen and progesterone is blocked by spironolactone administration. This evidence suggested that oestrogen and progesterone exerted their influece on renal electrolytes and water excretion via mineralocorticoids.

However, results obtained from prolonged administration of oestrogen and progesterone are difficult to relate to the events which occur during the oestrous cycle when oestrogen and progesterone levels are increased for relatively short periods of time. For this reason, and in order to investigate further their separate influence on the kidney, experiments were carried out to determine the effect of single separate injections of oestrogen and progesterone on renal electrolyte function.

Summary

Therefore the results of spironolactone treatment with prolonged oestrogen and progesterone administration show that:-

1. Glomerular filtration rates remained unchanged.
2. The significant decrease in urine flow observed with ovarian hormone treatment was restored to ovariectomised levels with spironolactone.
3. The decrease in sodium excretion rates and concentration observed after ovarian hormone treatment was absent when spironolactone was also administered.
4. The increase in potassium concentration and excretion rates observed with ovarian hormone treatment was not observed when spironolactone was also administered.

The Action of a Single Injection of Oestrogen on Renal Electrolyte Function

Introduction

Allen et al. (1944) demonstrated a delay of 4 to 6 hours in the action of oestradiol on uterine water uptake. Therefore, ovariectomised animals were given a priming injection of 2 µg oestradiol, 48 hours prior to experimentation, followed by a further injection of 5 µg of oestradiol 2 hours prior to commencing saline infusion. The results obtained were compared with vehicle blank injected control animals.

The effects of a single injection of oestradiol on renal electrolyte function in ovariectomised rats are presented in Fig.15.

Urine Flow

Urine flow was not significantly different from that observed in vehicle blank injected control animals 2 hours after starting saline infusion. However, 3 hours after starting saline infusion urine flow was 2.5±0.3 ml/hour in the oestradiol treated group compared with 2.9±0.3 ml/hour in control group ($p<0.5$). Four hours after starting saline infusion urine flow was 2.5±0.2 ml/hour in the oestradiol treated group compared with 3.3±0.3 ml/hour in the control animals ($p<0.05$). From 4 to 6 hours after starting saline infusion urine flow decreased from 2.5±0.2 ml/hour to 1.0±0.1 ml/hour in the oestradiol treated group compared with from 3.3±0.3 ml/hour to 2.1±0.4 ml/hour in the control group ($p<0.02$ at 6 hours).

No significant difference was found in glomerular filtration rates between the oestradiol treated and control

injected animals.

Sodium Excretion

Sodium excretion followed a similar pattern to that of urine flow. Sodium concentration and excretion rates in the oestradiol treated animals were not significantly different from those of the control animals 3 hours after starting saline infusion. From 4 to 6 hours after starting saline infusion, sodium concentration decreased from 158.1±14.5 meq/L to 102.9±5.9 meq/L in the oestradiol treated animals, compared with a decrease from 195.6±81 meq/L to 186.9±7.7 meq/L in the control animals ($p<0.1$ and $p<0.001$ respectively 4 and 6 hours after starting saline infusion).

Sodium excretion rates decreased from 403.4±49.8 µeq/hour four hours after starting saline infusion to 105.1±10.1 µeq/hour, 6 hours after starting saline infusion, in the oestradiol treated group, compared with a decrease from 640.3±56.4 µeq/hour to 390.3±76.1 µeq/hour, over the same time in control animals ($p<0.02$ and $p<0.01$ respectively).

Sodium:Potassium Ratio

A significant decrease in the sodium:potassium ratio was observed 3 hours after starting saline infusion. The sodium to potassium ratio decreased from 2.2±0.2 3 hours after starting saline infusion, to 0.7±0.03 6 hours after starting saline infusion, compared with 3.8±0.5 to 5.1±0.7 over the same time in control animals ($p<0.01$ and $p<0.001$ respectively).

Potassium Excretion

Potassium concentration was significantly increased with oestradiol treatment, compared with control animals.

Potassium concentration increased from 118.4±9.7 meq/L to 156.4±4.5 meq/L, from 4 to 6 hours after starting saline infusion in oestradiol treated animals compared with a slight increase from 40.8±4.5 meq/L to 41.9±11.3 meq/L in the control group over the same period ($p<0.001$ and $p<0.001$ respectively) (Fig. 15C).

Potassium excretion rate was 293.6±16.6 µeq/hour in the oestradiol treated group, compared with 136.1±18.6 µeq/hour in the control group 4 hours after starting saline infusion ($p<0.001$). Six hours after starting saline infusion the potassium excretion rate in the oestradiol treated animals was 160.9±17.2 µeq/hour compared with 76.2±10.9 in the control animals ($p<0.01$).

These results indicate that there is a delay of 6 to 7 hours in the action of oestradiol on the kidney. The changes in electrolyte excretion and urine flow observed with the injection of oestradiol were similar to those observed at oestrus. The significant reduction in sodium to potassium ratio suggests that mineralocorticoids may be involved in bringing about these changes.

Summary

1. There is a delay of 6 to 7 hours in the action of oestradiol on the kidney.
2. A single injection of oestradiol reduces urine volume in the absence of any change in glomerular filtration rate compared with vehicle blank injected control animals.
3. Oestradiol significantly reduced sodium concentration

and excretion rate compared with control animals.

4. Oestradiol significantly reduced the sodium:potassium ratio compared with control animals.

5. Oestradiol significantly increased potassium concentration and excretion rates compared with control animals.

The Action of Spironolactone on Renal Electrolyte Function Following a Single Injection of Oestradiol

Introduction

Spironolactone was administered in an attempt to block the retention of sodium and water observed after oestradiol treatment.

The procedures for spironolactone and single injection of oestradiol were identical to those previously described.

The results presented in Fig. 16 A-D show the effect of spironolactone treatment on renal electrolyte function in ovariectomised rats injected with 5μg of oestradiol 2 hours prior to experimentation.

Urine Flow

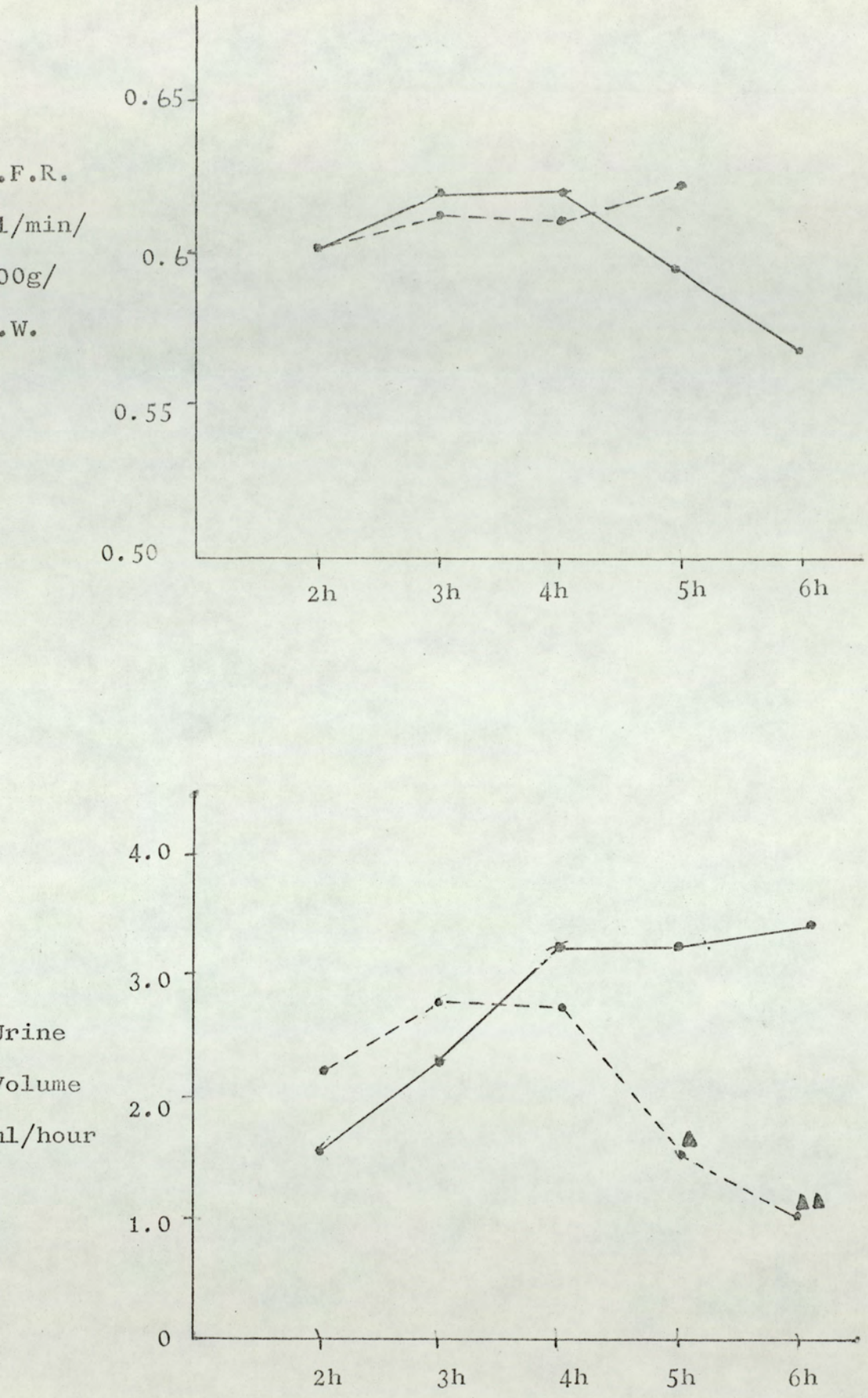
Spironolactone treatment blocked the reduction in urine flow observed after oestradiol injection. Four hours after starting saline infusion urine flow was 3.1±0.4 ml/hour in the animals treated with spironolactone and oestrogen, compared with 2.5±0.2 ml/hour in animals treated with oestrogen alone ($p<0.3$). Six hours after starting saline infusion urine flow was 3.2±0.6 ml/hour in animals treated with spironolactone and oestradiol, compared with 1.0±0.1 ml/hour in animals treated with oestradiol alone ($p<0.001$).

There was no significant difference in glomerular filtration rates between the two groups of animals (Fig.16A).

Sodium Excretion

Sodium concentration was not decreased in the animals treated with spironolactone and oestradiol, as it was in animals treated with oestradiol alone. Sodium concentration

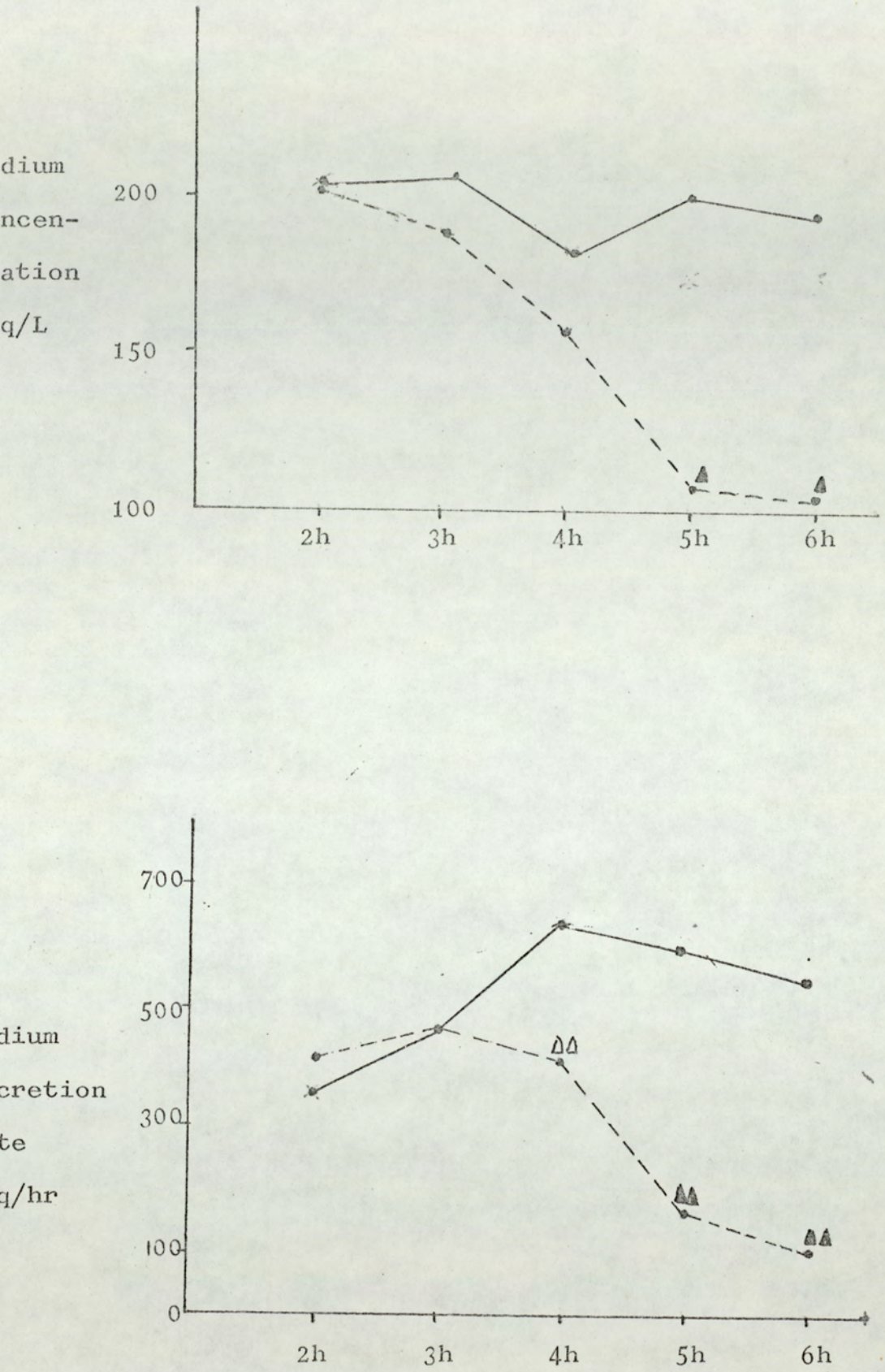
FIG. 16a



The Effect of Spironolactone on G.F.R. and Urine Volume in Ovariectomised Rats Following Oestrogen Administration 2 hours prior to Experimentation

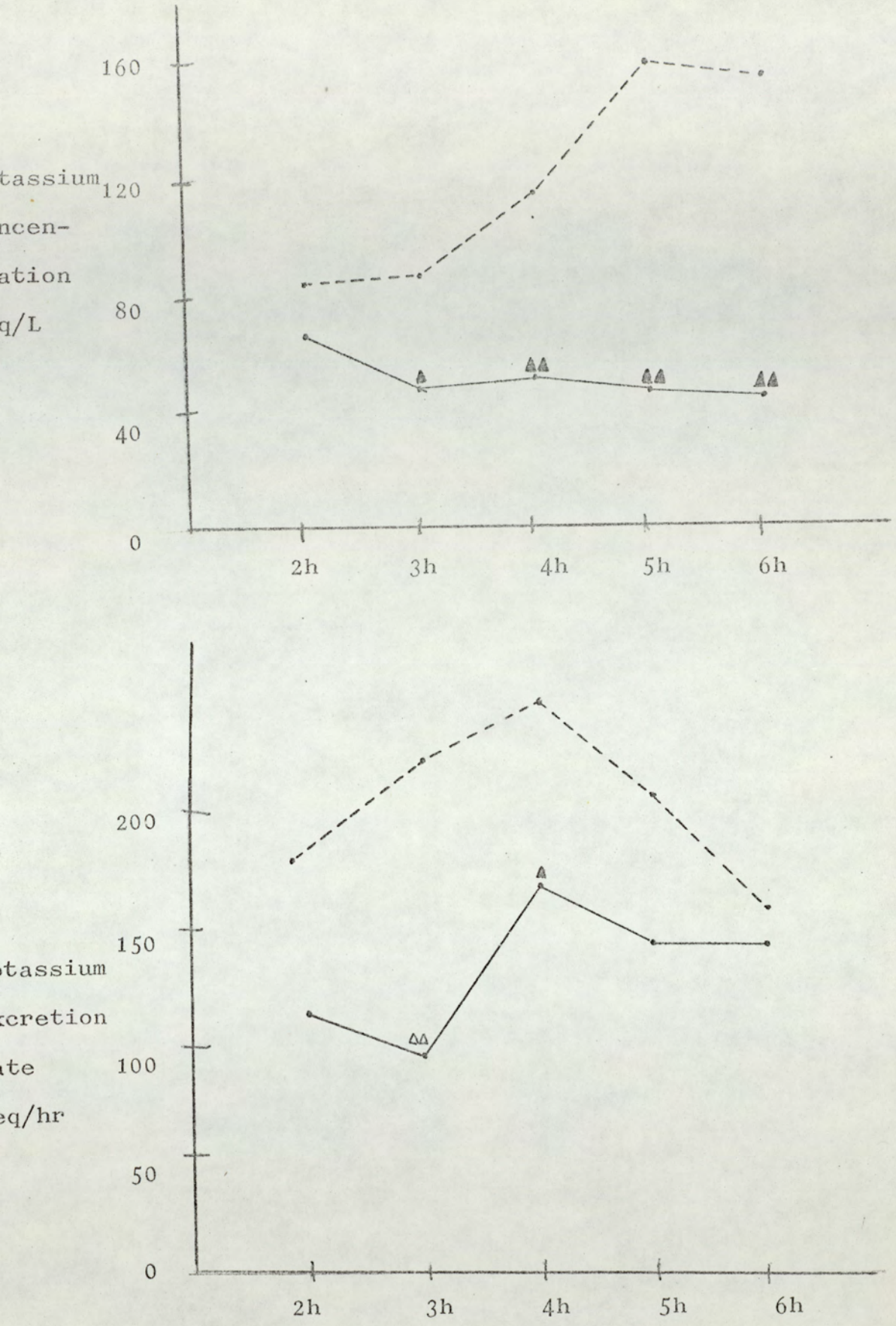
Spironolactone Treated ——— Control - - - - -

FIG 16b



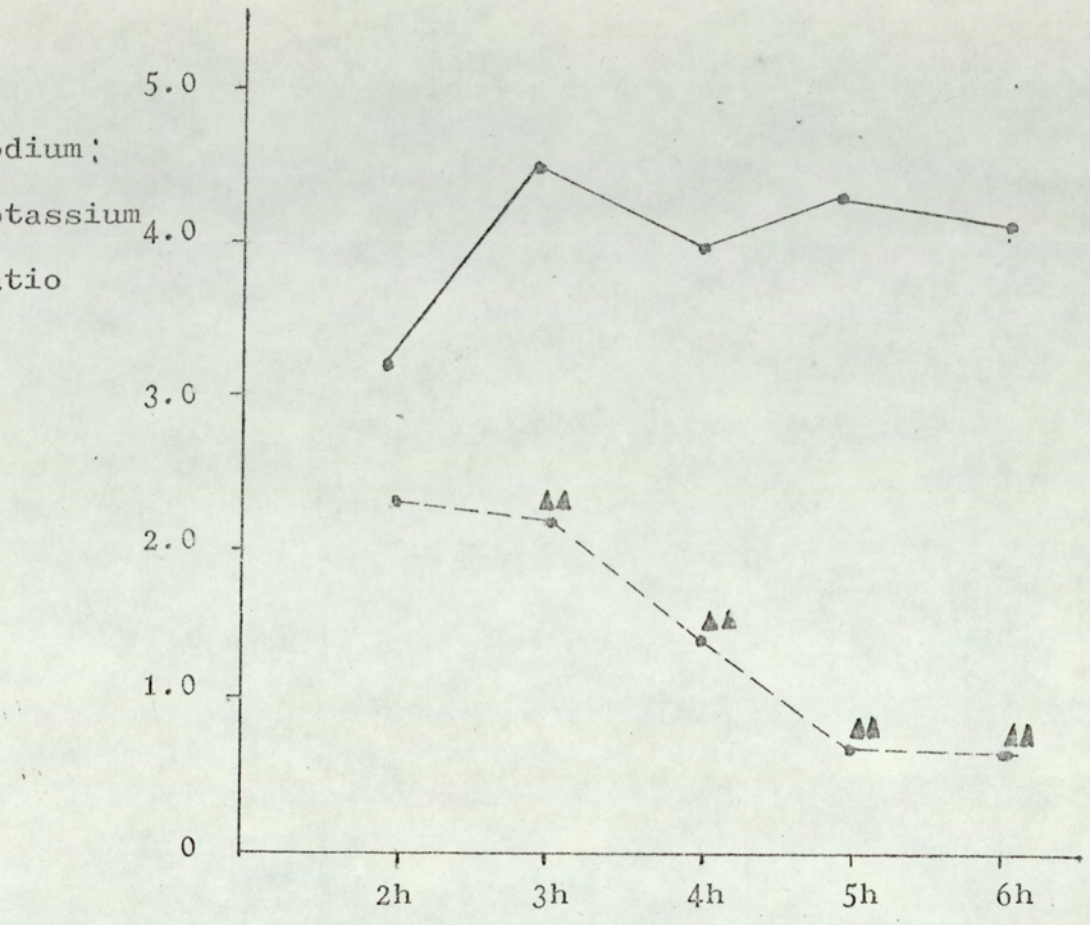
The Effect of Spironolactone on G.F.R. and Urine Volume in Ovariectomised Rats Following Oestrogen Administration 2 hours prior to Experimentation

FIG. 16c



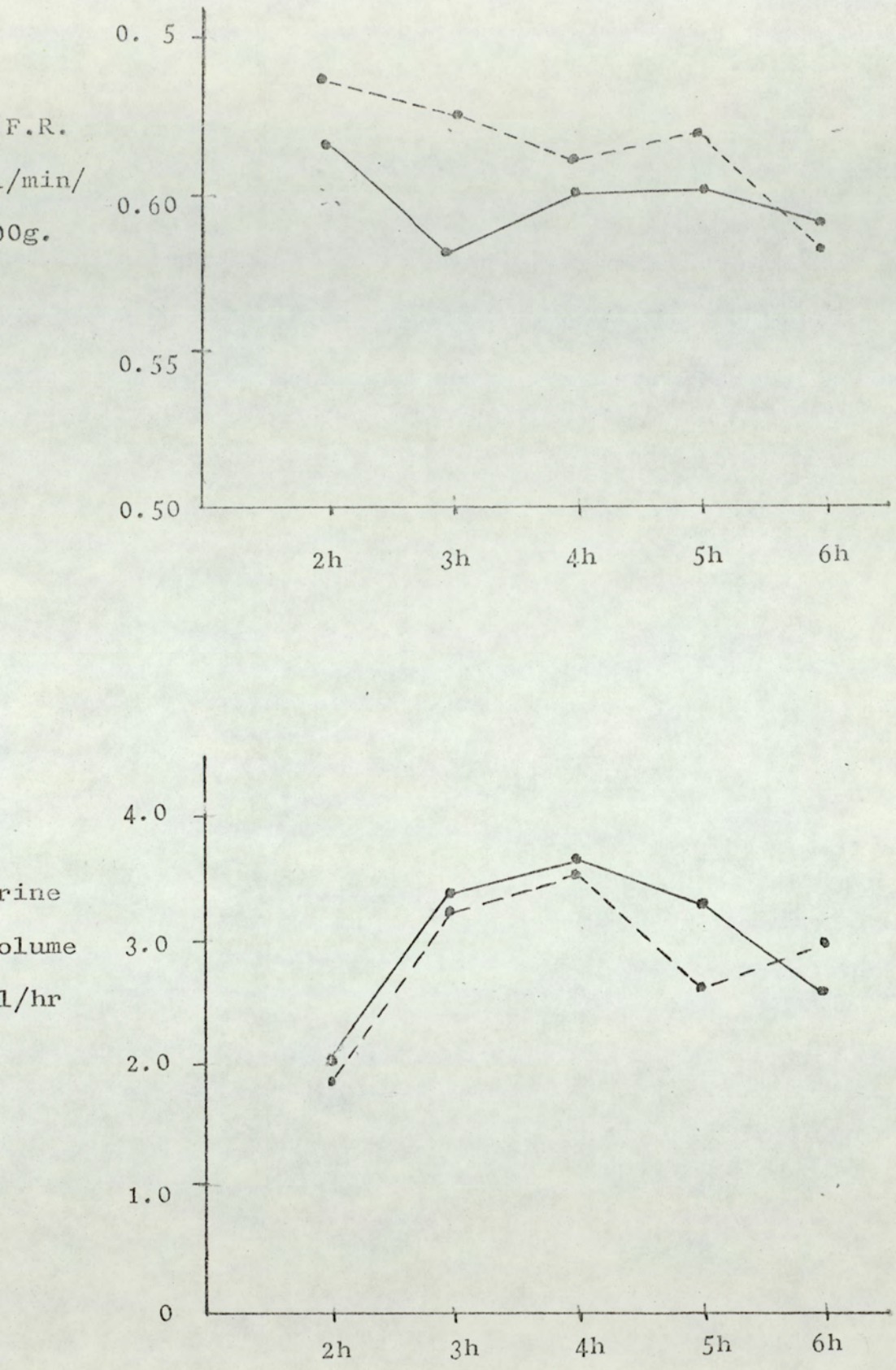
The Effect of Spironolactone on Potassium Concentratio and Excretion Rates in Ovariectomised Rats with Oestrogen 2 hours prior to Experimentation

FIG. 16d



The Effect of Spironolactone of the Sodium Potassium Ratio in Ovariectomised Rats Following Oestrogen Administration 2 hours prior to Experimentation.

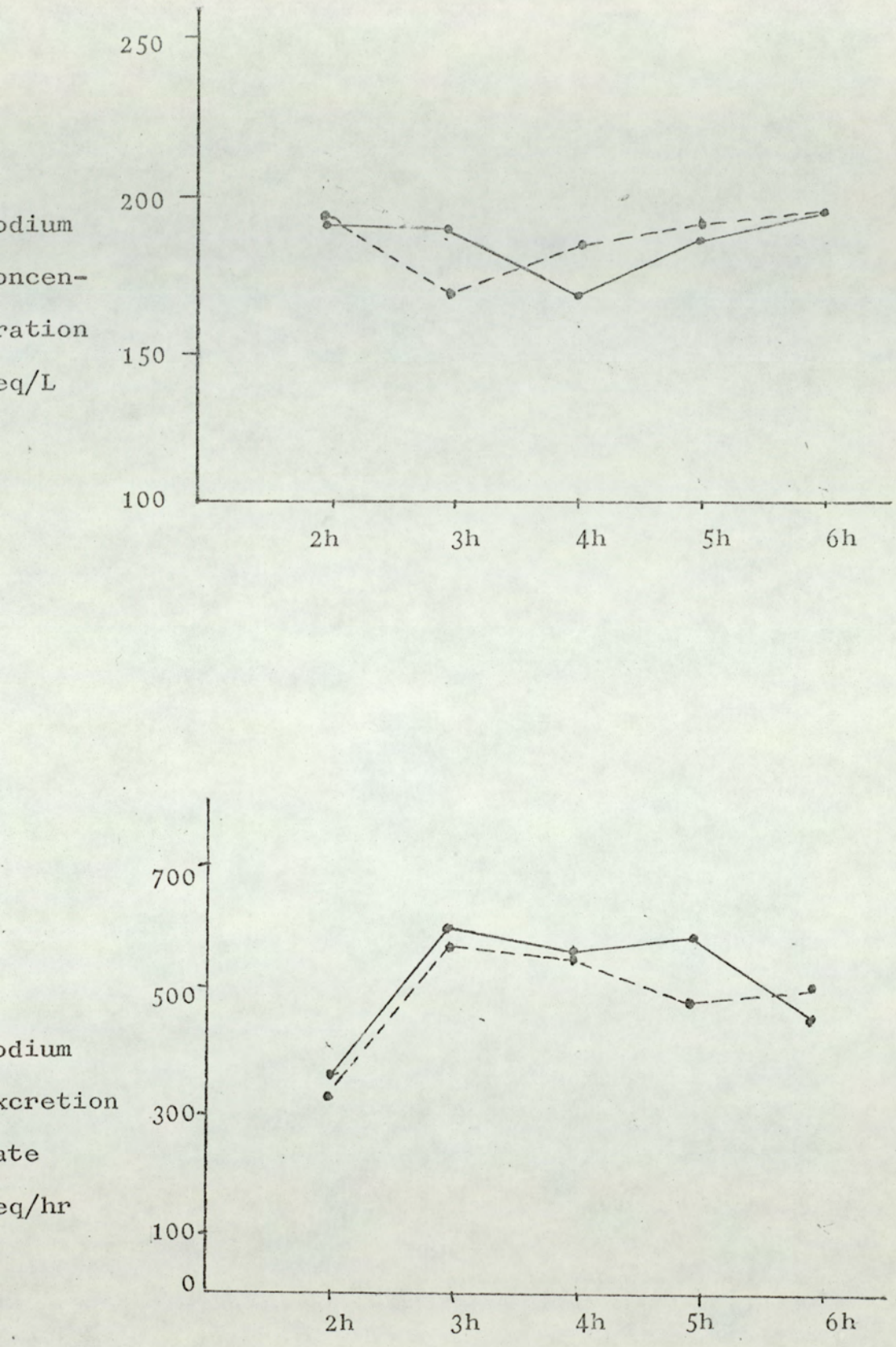
FIG. 17a



G.F.R. and Urine Volume in Ovariectomised Rats Treated with Oestrogen 12 hours prior to Experimentation.

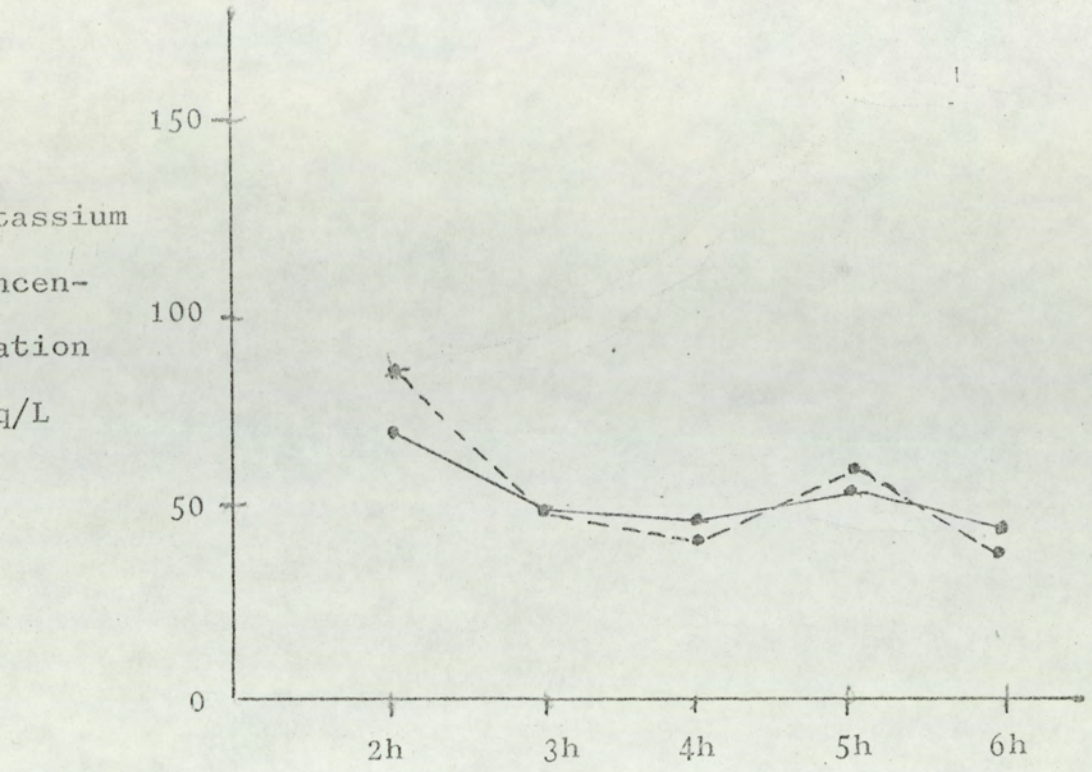
Oestrogen treated ——— Control - - - -

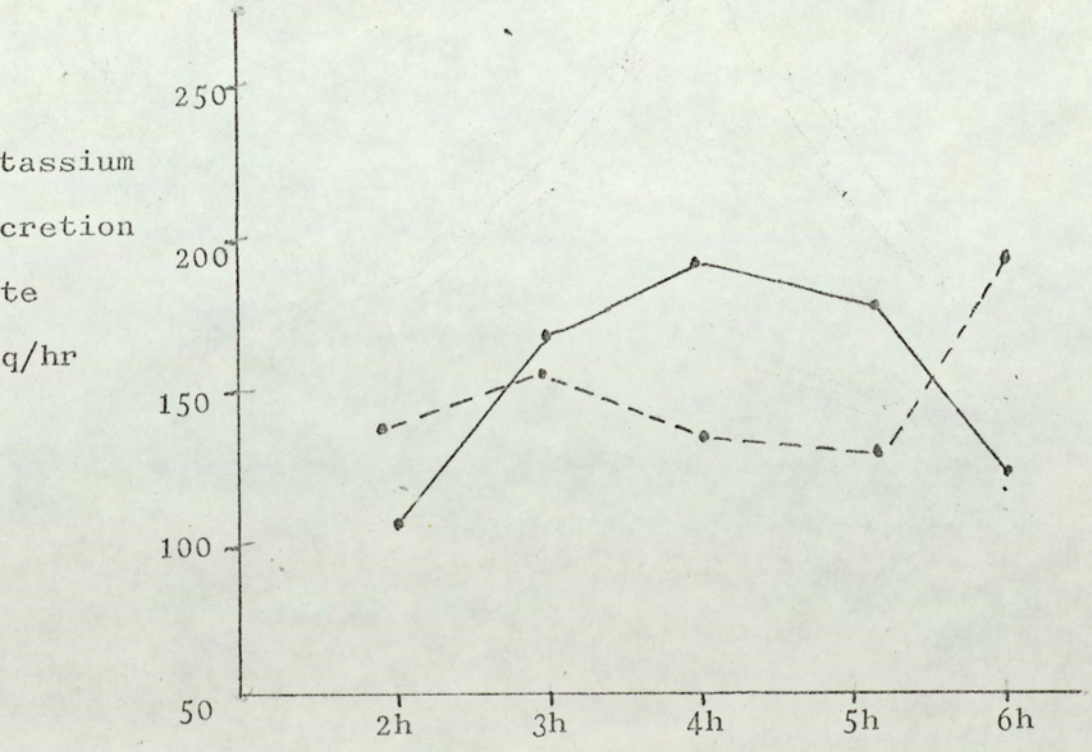
FIG. 17b



Sodium Concentration and Excretion Rate in Ovariectomised Rats Treated with Oestrogen 12 hours prior to Experimentation.

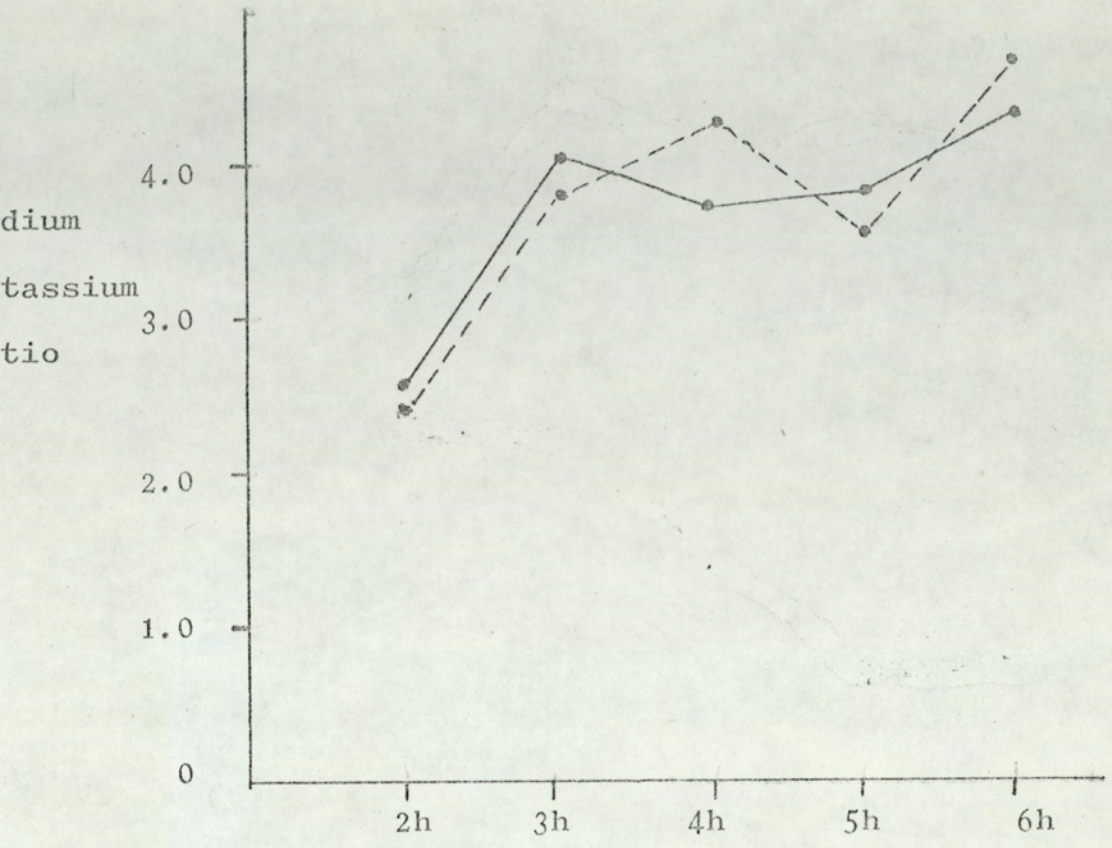
FIG. 17c





Potassium Concentration and Excretion Rate in Ovariectomised Rats with Oestrogen Treatment 12 hours prior to Experimentation.

FIG. 17d



The Sodium Potassium Ratio in Ovariectomised Rats Treated with Oestradiol 12 hours prior to Experimentation.

varied between 177.2±13.3 meq/L, 4 hours after starting saline infusion, and 180.8 meq/hour, 6 hours after starting saline infusion in animals treated with spironolactone and oestradiol. In animals treated with oestradiol alone sodium concentration was 158.1±14.1 meq/L and 102.9±5.9 meq/L respectively at these times ($p<0.4$ and $p<0.01$ respectively).

Sodium excretion rates followed a similar pattern. Sodium excretion rates, 4 hours after starting saline infusion, were 643.4±49.2 µeq/hour in animals treated with spironolactone and oestradiol compared with 403.4±49.8 µeq/hour in animals treated with oestradiol alone ($p<0.02$) Six hours after starting saline infusion sodium excretion rates were 562.2±50.1 µeq/hour in the animals treated with spironolactone and oestrogen, compared with 105.1±10.1 µeq/hour in animals treated with oestradiol alone ($p<0.001$).

Sodium:Potassium Ratio

The sodium:potassium ratio was found to be significantly higher in the spironolactone treated group, 3 hours after starting saline infusion, compared with the oestradiol injected control animals. Three hours after starting saline infusion the sodium:potassium ratio was 4.5±0.4 in the spironolactone treated group, compared with 2.2±0.2 in animals treated with oestradiol alone ($p<0.001$). Six hours after starting saline infusion the sodium:potassium ratio was 4.0±0.5 in the animals treated with oestrogen and spironolactone compared with 0.7±0.03 in animals treated with oestradiol alone ($p<0.001$).

Potassium Excretion

Fig. 16 also shows the action of spironolactone treatment on the urine potassium concentrations and excretion rates in oestradiol injected animals. It was found that spironolactone blocked the significant increase in potassium concentration and excretion rates previously observed with oestradiol treatment. Four hours after starting saline infusion potassium concentration was 54.1 meq/L in the animals treated with spironolactone and oestradiol compared with 118.3±9.7 meq/L in animals treated with oestradiol alone ($p<0.001$). Six hours after starting saline infusion potassium concentration was 45.5±3.0 meq/L in animals treated with spironolactone and oestradiol compared with 156.4±4.6 meq/L in animals treated with oestradiol alone (p 0.001).

Potassium excretion rates were 170.4±30.5 µeq/hour in animals treated with spironolactone and oestradiol, compared with 293.6±16.6 µeq/hour in animals treated with oestradiol alone ($p<0.01$).

Spironolactone therefore blocked the significant changes in urine flow and electrolyte excretion observed 5 to 8 hours after a single injection of oestradiol. These findings again suggest that the action of oestrogens on the kidney might be mediated via mineralocorticoids.

Summary

The progressive increase in sodium and water retention, and potassium excretion, observed 5 to 8 hours after oestradiol injection was blocked by administration of spironolactone.

The "Long-Term" Action of Oestradiol on Renal Electrolyte Function

It has been demonstrated that oestrogen therapy induces prolonged retention of sodium in other species (Thorn, Nelson & Thorn, 1939). The results presented in the previous section show that sodium and water excretion is significantly reduced 5 to 8 hours after oestradiol injection. The effect of a single injection of oestradiol 12 hours prior to experimentation was therefore investigated.

5 µg of oestradiol in arachisol was administered intramuscularly 12 hours prior to the start of saline infusion into oestrogen primed ovariectomised rats. The results obtained from the oestradiol injected rats were compared with those from blank injected control animals.

In Fig. 17 the results are given showing the effects of 12 hour oestradiol injection on urine flow and G.F.R. in ovariectomised rats. Urine flow and G.F.R. were not significantly different from the control levels.

The results presented in Fig. 17 also demonstrate that oestradiol did not affect the sodium to potassium ratio, urine sodium concentration or excretion rate. It may be concluded, therefore, that no significant alteration in electrolyte or water excretion can be detected 12 to 18 hours after oestradiol administration.

The Action of Progesterone on Renal Electrolyte Function

A single injection of 5 µg of progesterone was administered to ovariectomised animals 2 hours prior to experimentation.

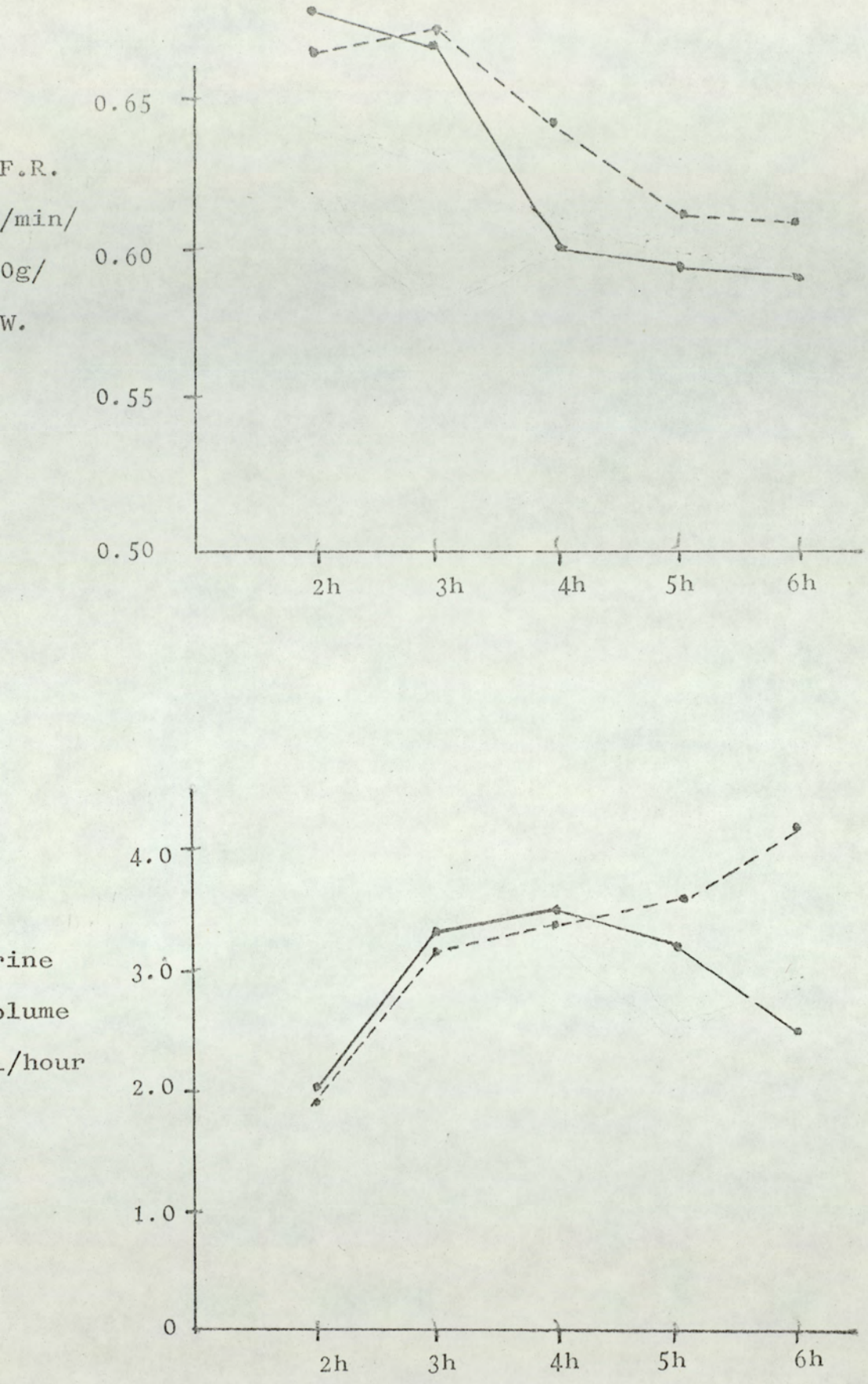
Fig.18A shows the mean values of urine volume and glomerular filtration rate in progesterone treated and control injected animals. No significant difference was found in either urine flow or glomerular filtration rate between the two groups of animals.

Progesterone treatment had no significant effect on sodium or potassium concentration and excretion rates.(Figs.18A-D

Therefore, the effect of a single injection of 5 µg of progesterone, administered at + 2 hours, was investigated. Figs. 19 A-D shows that progesterone administration 2 hours after commencing saline infusion had no significant effect on renal electrolyte function. Possible reasons for the lack of effect of progesterone are, firstly, rapid excretion of the hormone from the body, and, secondly, rapid conversion of the hormone to inactive metabolites.

Therefore, the effect of constant infusion of progesterone, throughout the six hour experimental period was investigated. Progesterone was infused in the saline solution at the rate of 1 µg/hour. Fig 20 shows that progesterone infusion induced a marked reduction in the sodium to potassium ratio and potassium excretion. However, there was little alteration in the other parameters measured (Figs.20A-C).These results would indicate that progesterone induces an increase in mineralocorticoid activity, but since sodium diuresis was not observed, it would appear unlikely that progesterone is acting via this mechanism.

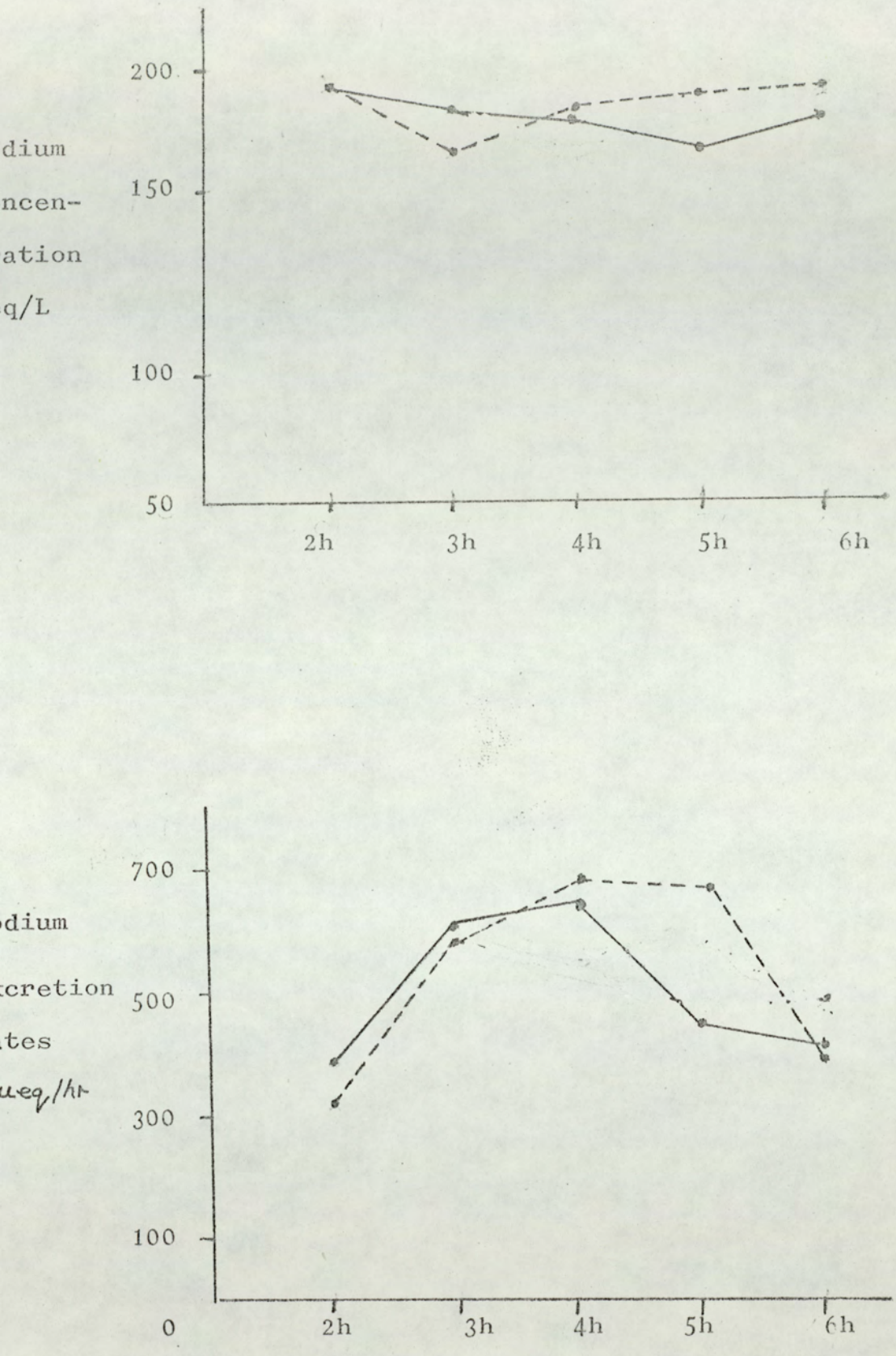
FIG. 18a



G.F.R. and Urine Volume in Ovariectomised Rats Treated with Progesterone 2 hours prior to Experimentation.

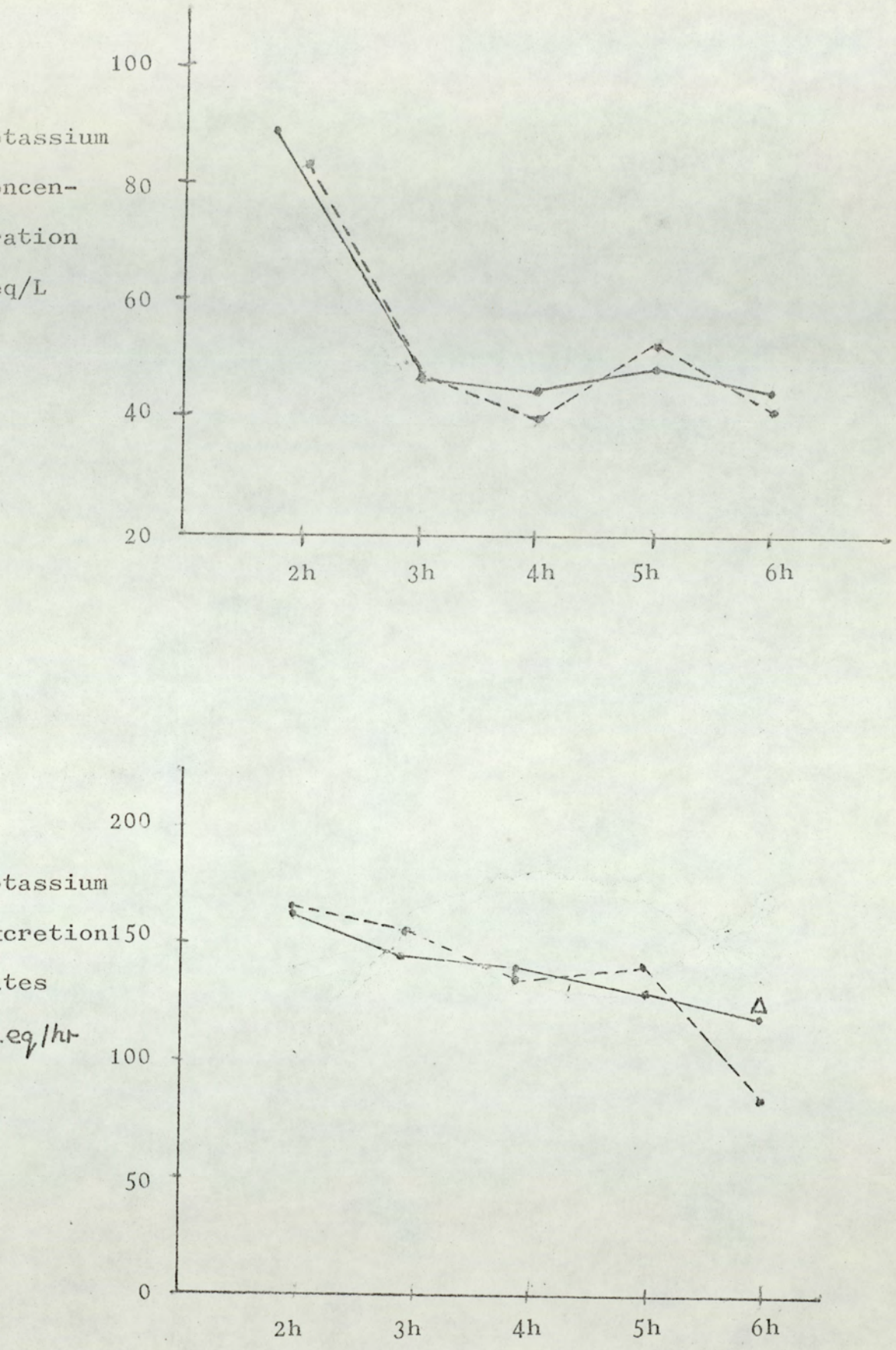
Progesterone treated ——— Control- - - -

FIG. 18b



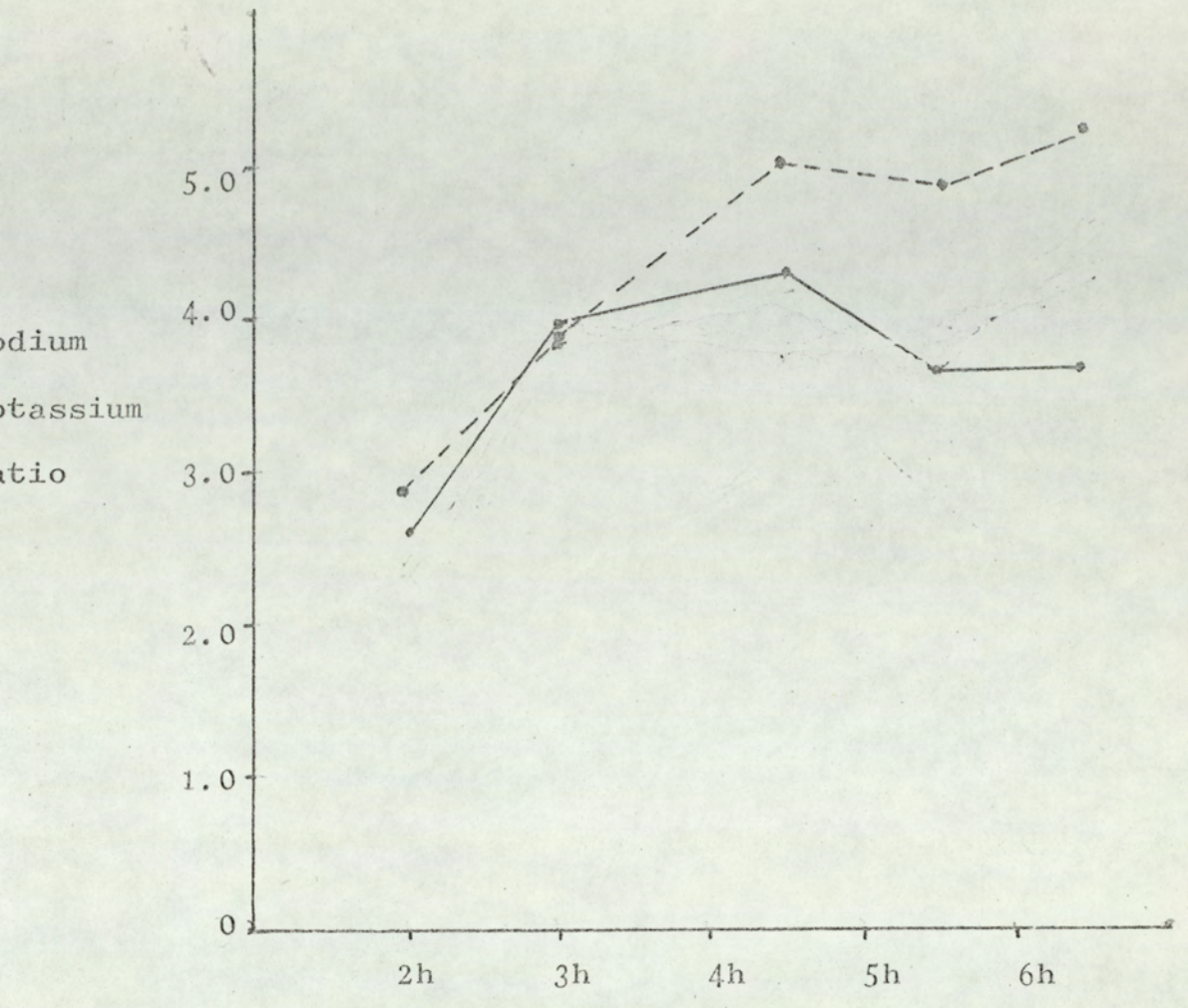
Sodium Concentration and Excretion Rate in Ovariectomised Rats Treated with Progesterone 2 hours prior to Experimentation.

FIG. 18c



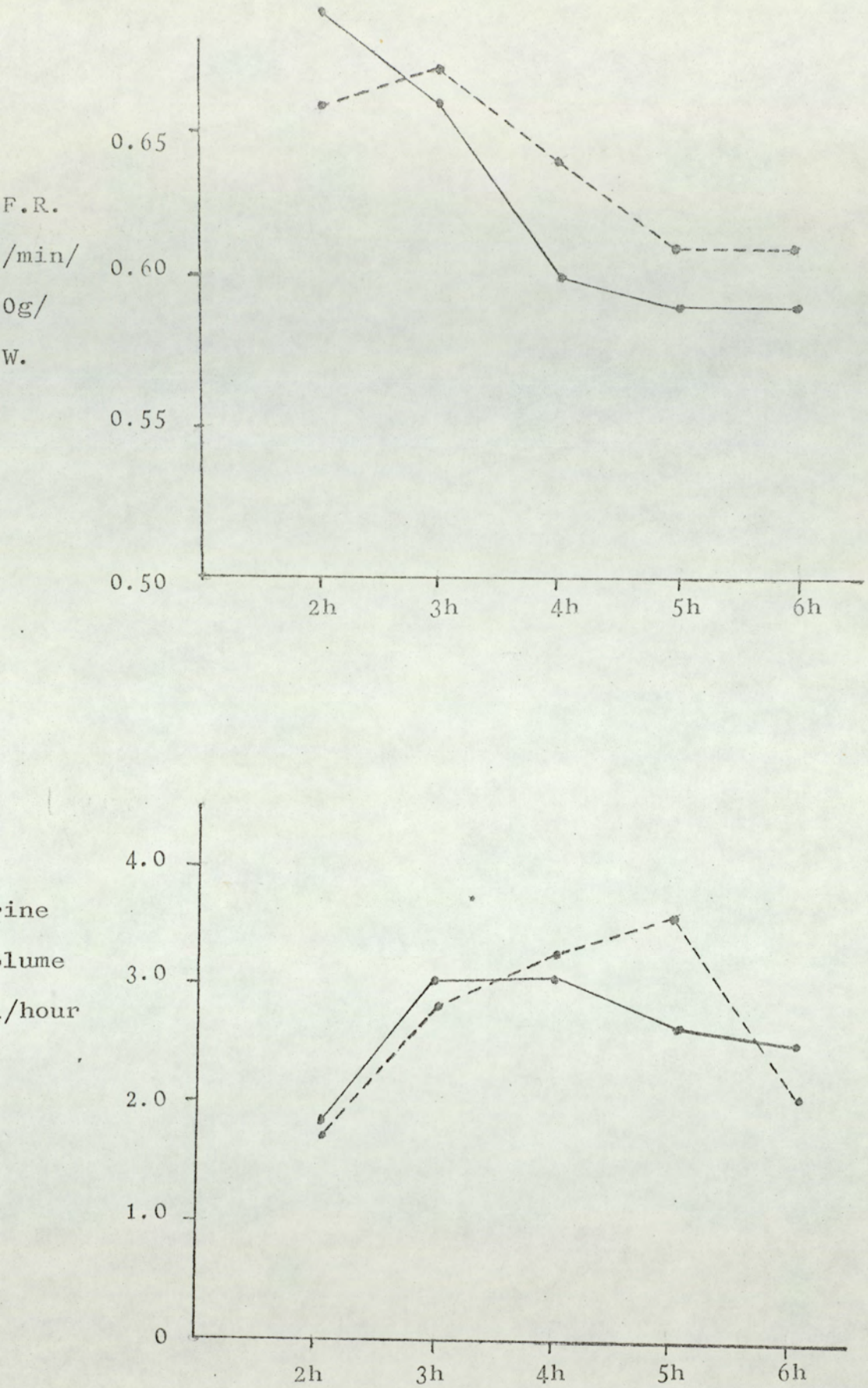
Potassium Concentration and Excretion Rates in Ovariectomised Rats Treated with Progesterone 2 hours prior to Experimentation

FIG. 18d



The Sodium Potassium Ratio in Ovariectomised Rats Treated with Progesterone 2 hours prior to Experimentation.

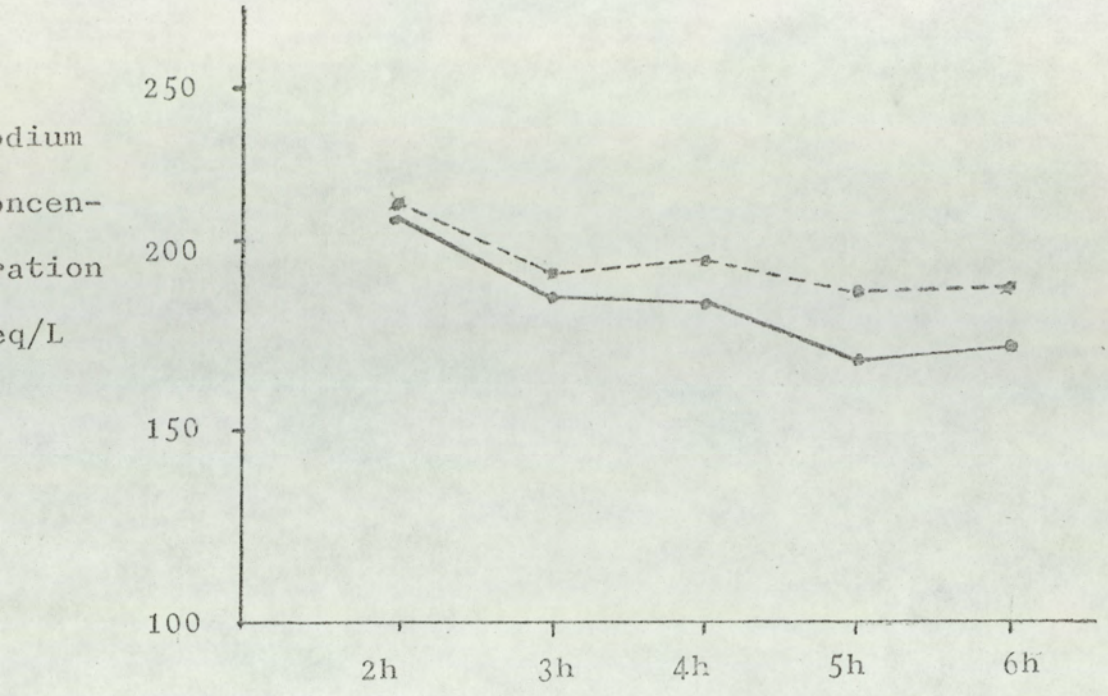
FIG. 19a

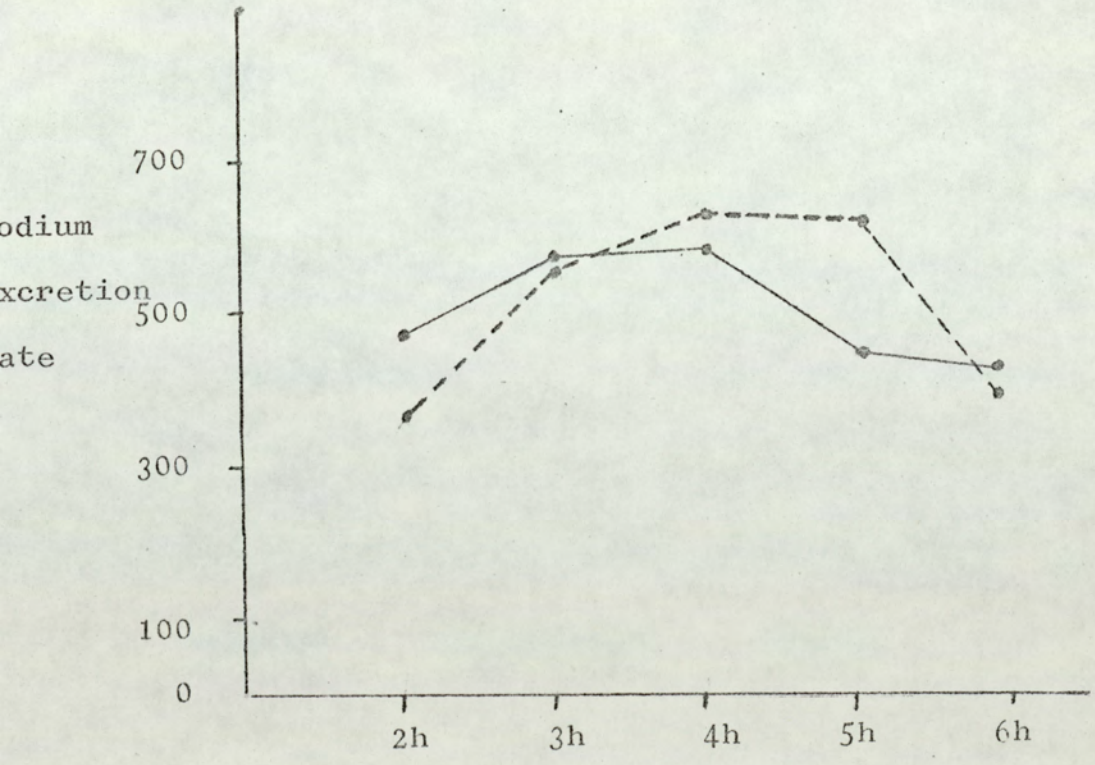


G.F.R. and Urine Volume in Ovariectomised Rats Treated with Progesterone 2 hours after Beginning Saline Infusion.

Progesterone Treated ——— Control - - - - -

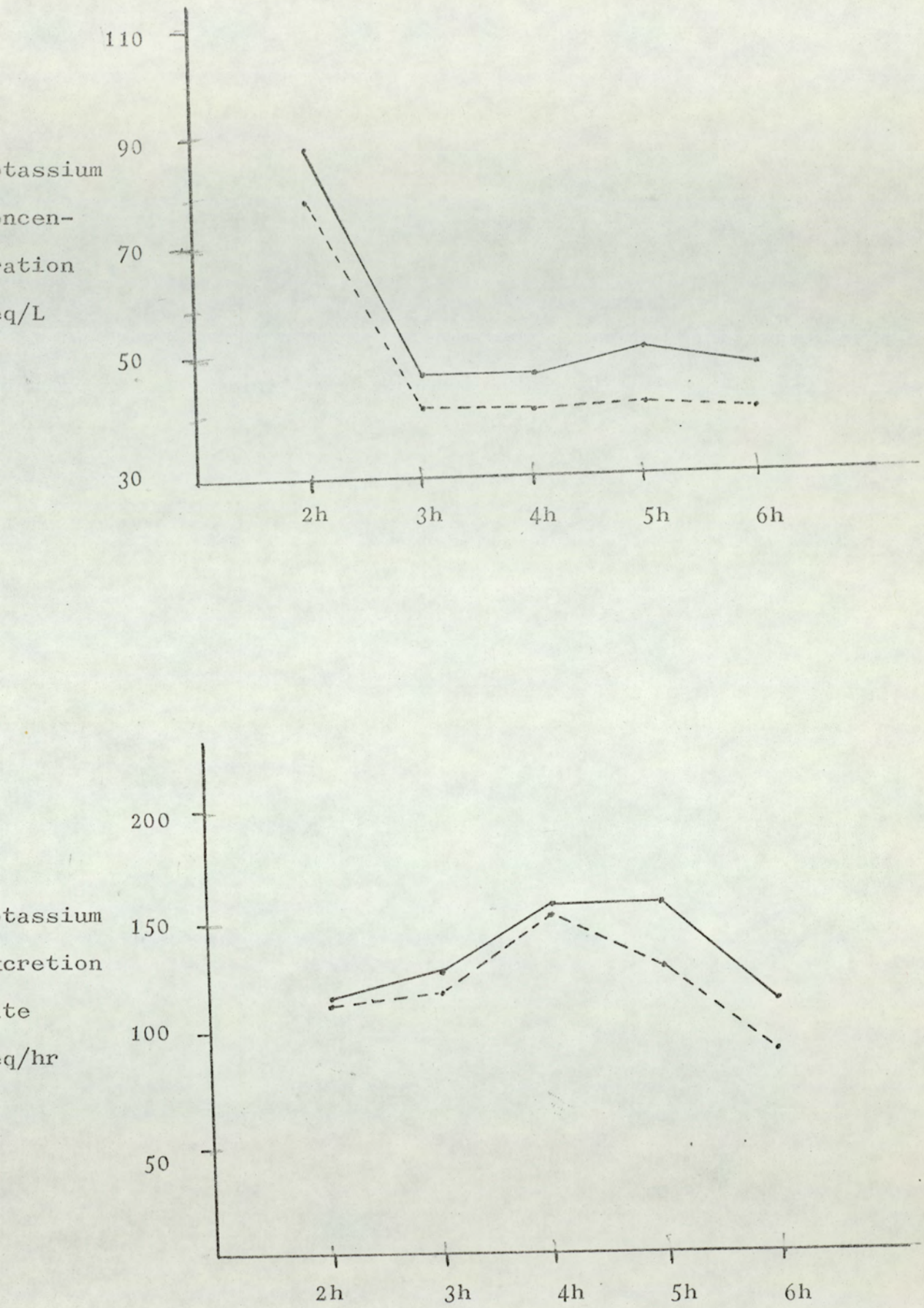
FIG. 19b





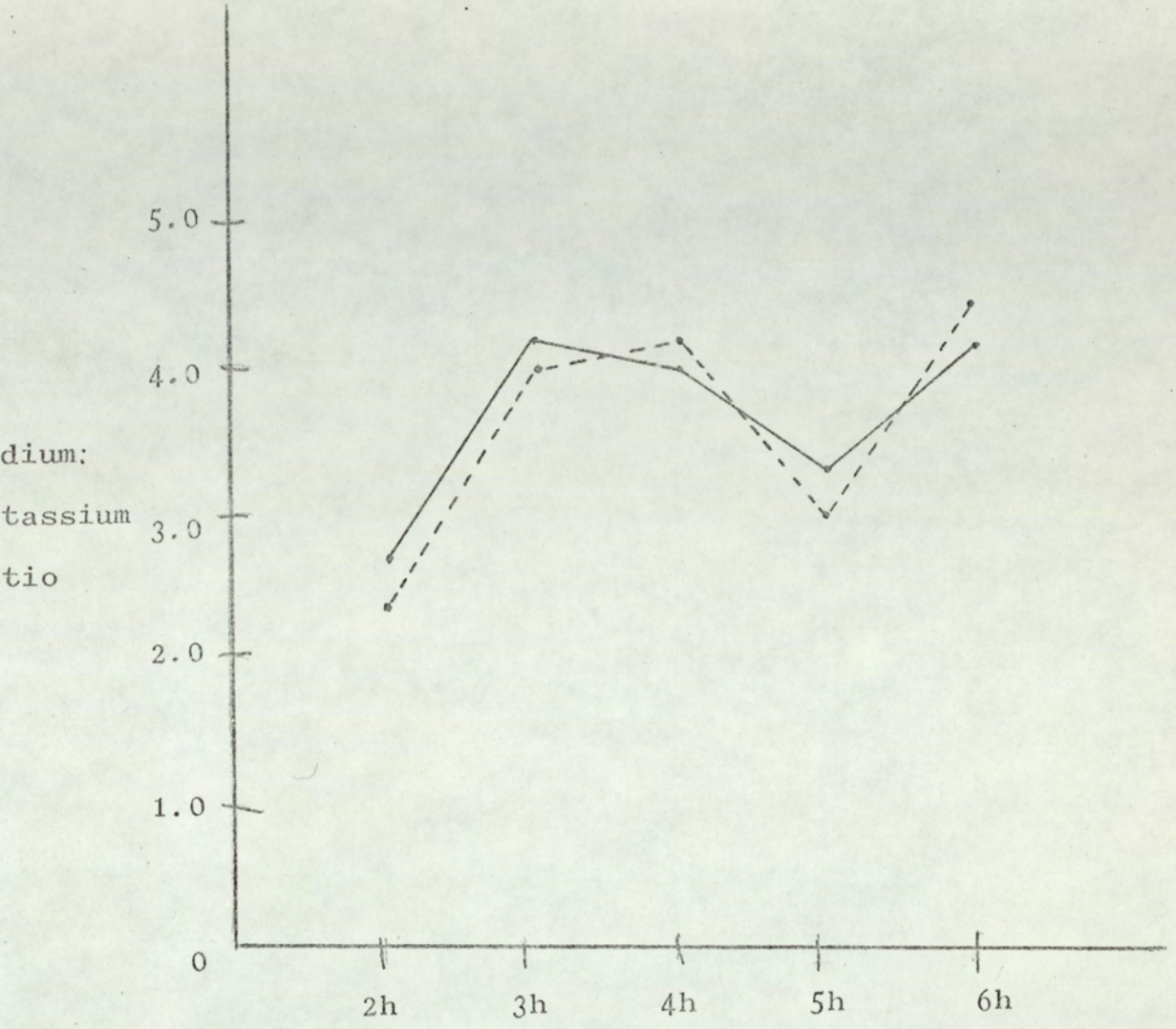
Sodium Concentration and Excretion Rate in Ovariectomised Rats Treated with Progesterone 2 hours after beginning Saline Infusion

FIG. 19c



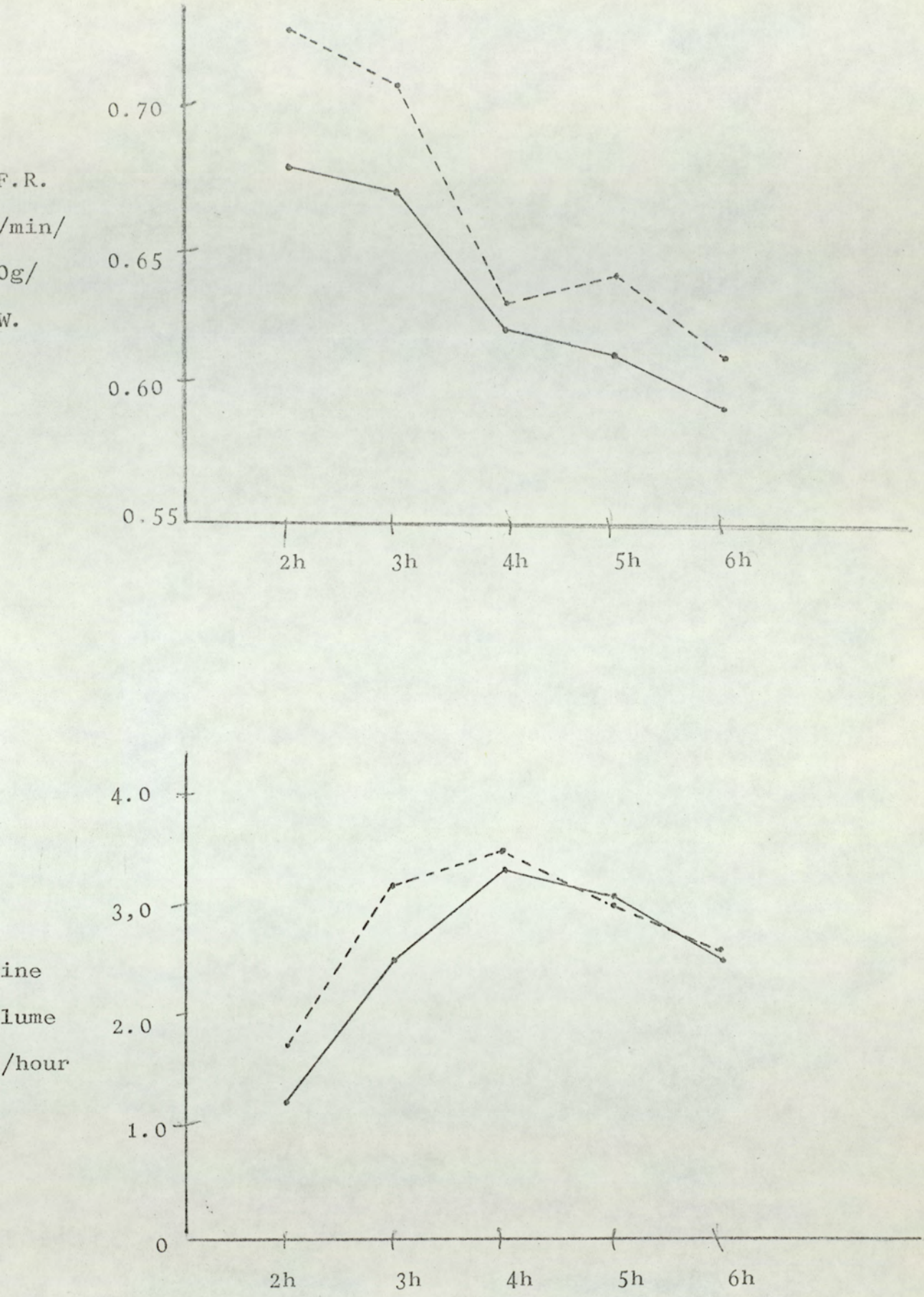
Potassium Concentration and Excretion Rates in Ovariectomised Rats Treated with Progesterone 2 hours after starting Saline Infusion

FIG. 19d



The Sodium Potassium Ratio in Ovariectomised Rats Treated with Progesterone 2 hours after starting Saline Infusion

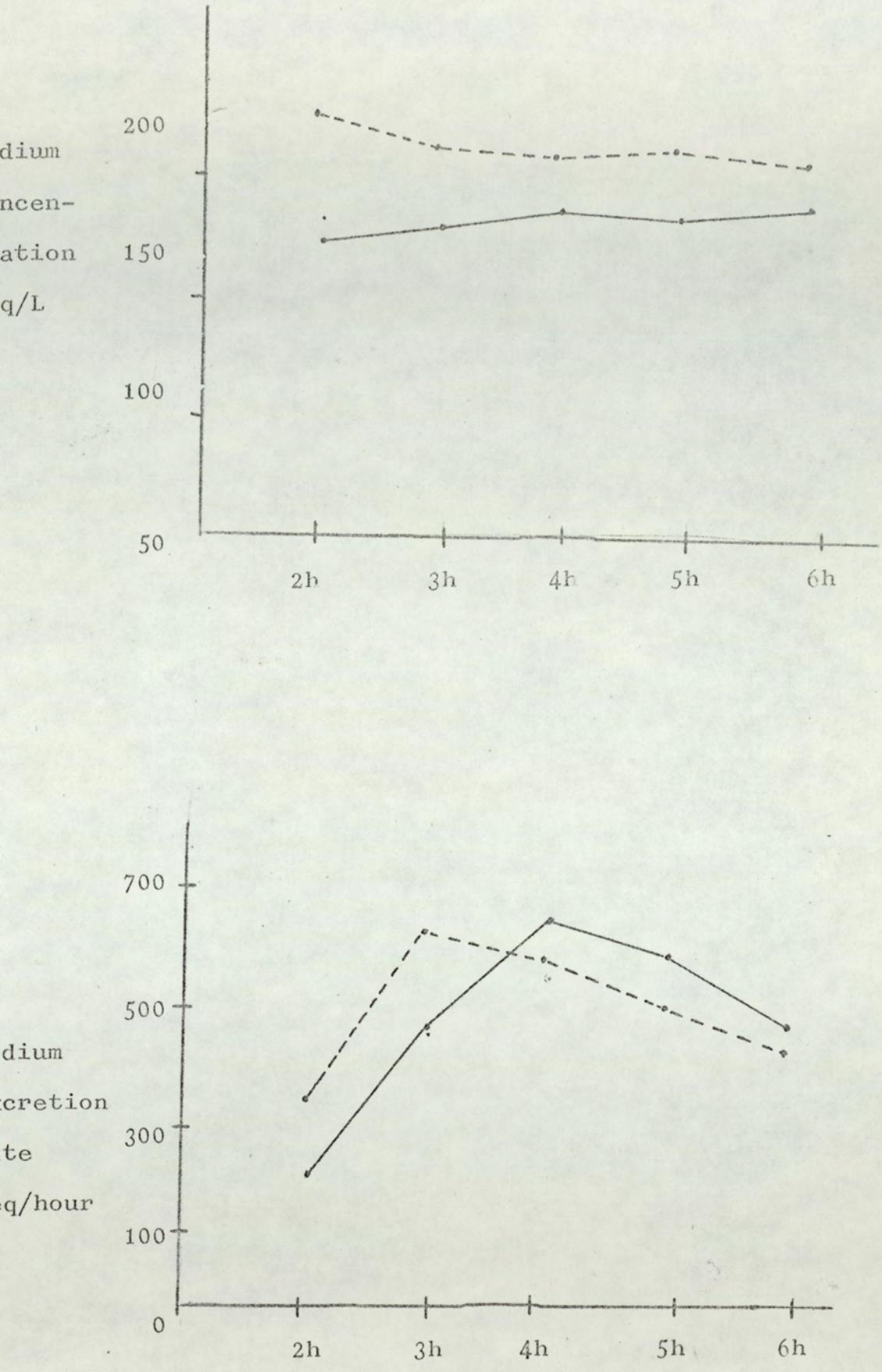
FIG. 20a



G.F.R. and Urine Flow in Ovariectomised Rats Given a Progesterone Infusion

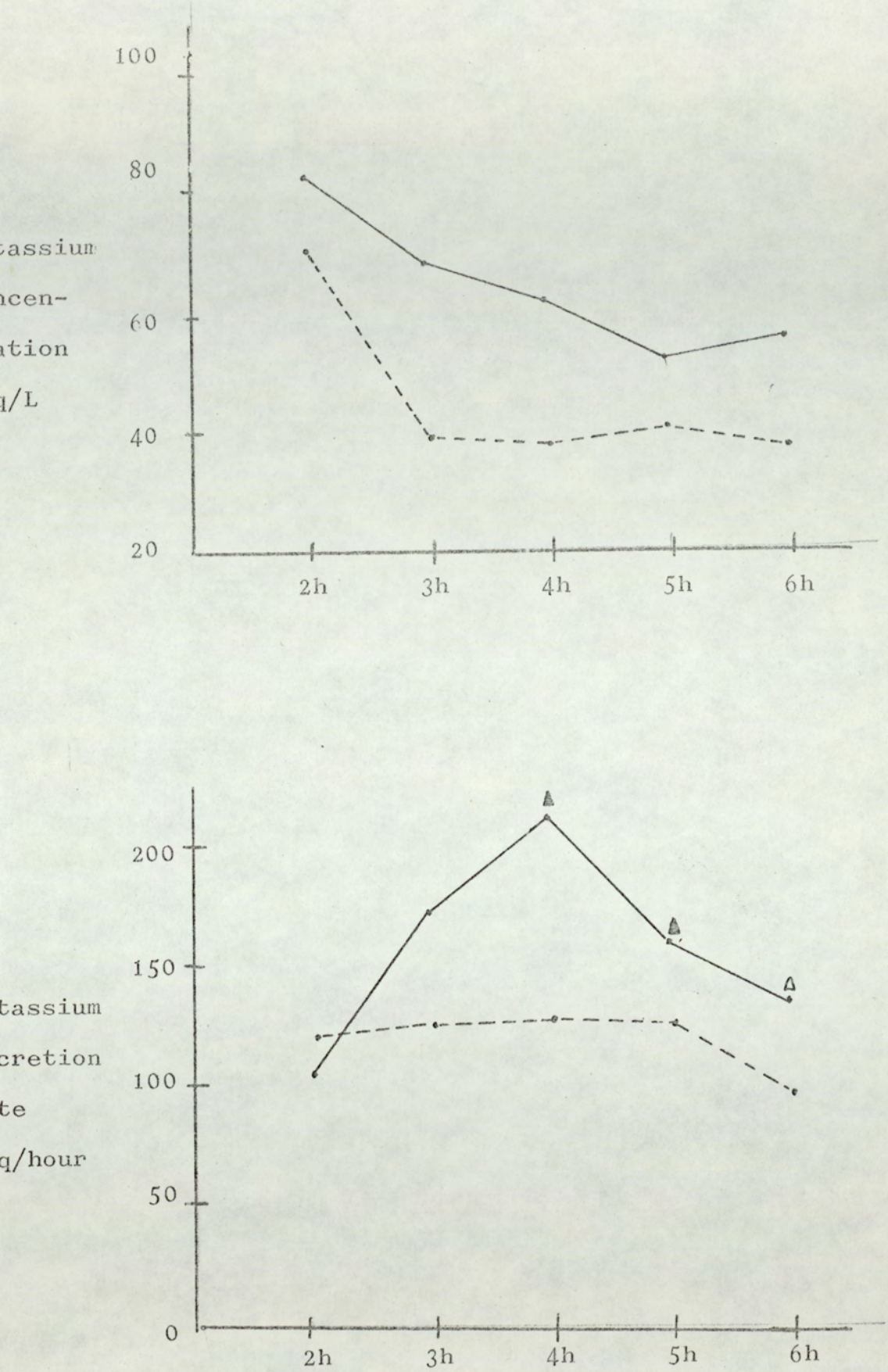
Progesterone Treated ——— Control - - - - -

FIG. 20b



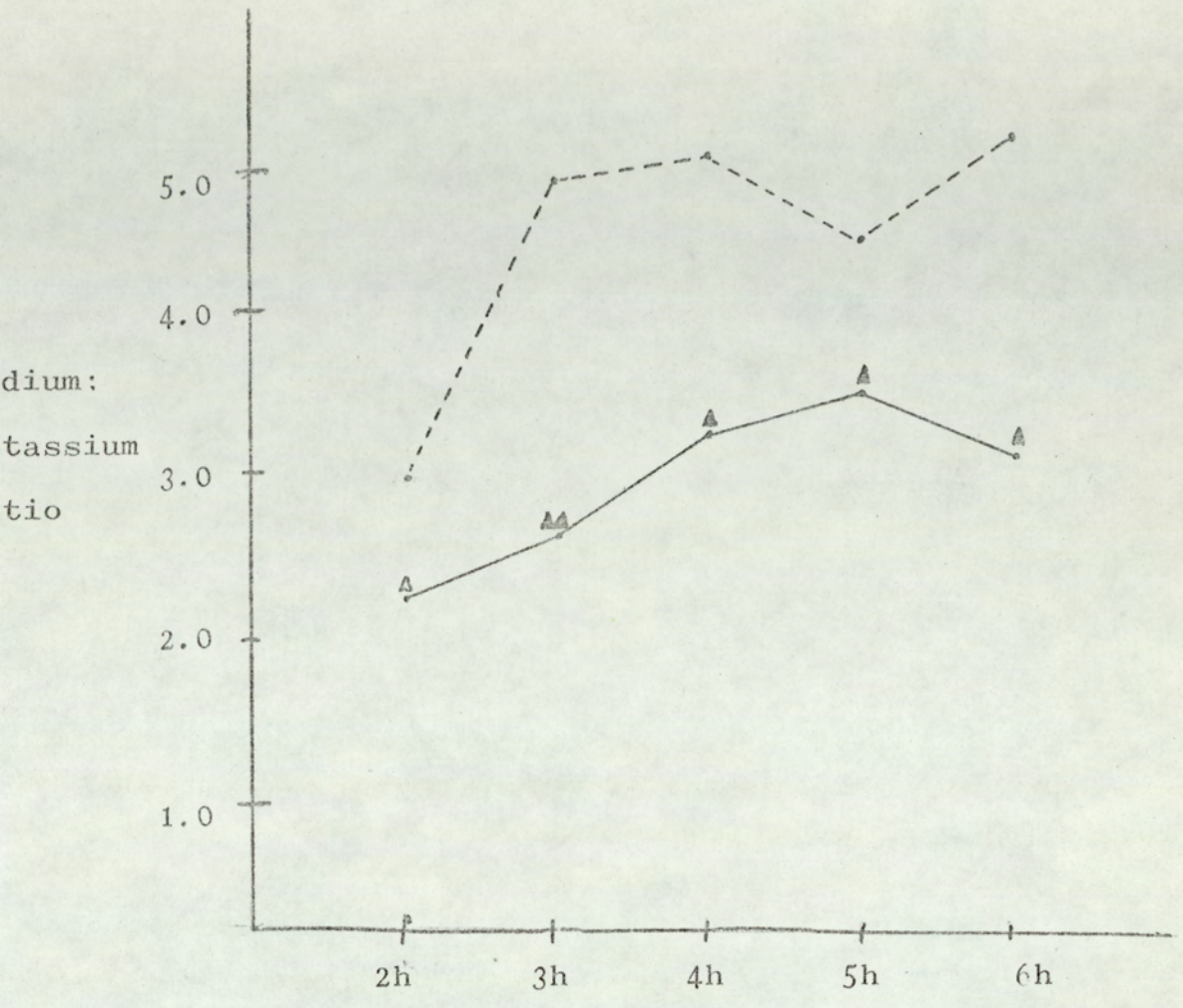
Sodium Concentration and Excretion Rate in Ovariectomised Rats Given a Progesterone Infusion

FIG. 20c



Potassium Concentration and Excretion Rate in Ovariectomised Rats Given Progesterone Infusion.

FIG. 20d



Sodium:Potassium Ratio in Ovariectomised Rats Given . Progesterone Infusion

SECTION III

Aldosterone and Corticosterone Secretion Rates During the Rat Oestrous Cycle

Introduction

The results presented in Section I demonstrated a variation in tissue water and sodium content during the oestrous cycle. This suggested a variation in sodium metabolism and, therefore, necessarily implicated the kidney in this variation. Investigations on kidney electrolyte function during the oestrous cycle showed a significant retention of sodium and water, with a parallel increase in potassium excretion at oestrus compared with dioestrus.

When the mineralocorticoid blocking agent spironolactone was administered, it was found that sodium, potassium and water excretion values were comparable to those observed in dioestrus animals.

Since the variation in renal electrolyte function was absent in ovariectomised animals, this evidence suggested that the ovarian hormones influence renal electrolyte function, probably via an influence on adrenal cortical activity. Conclusive evidence of this could be obtained only by measuring adrenal steroid secretion rates during the oestrous cycle. Since the evidence for the separate roles of oestrogen and progesterone in the observed variation is conflicting, it was important to determine aldosterone and corticosterone secretion rates at each of the four stages of the oestrous cycle, in order to relate them to the oestrogen and progesterone secretion rates at these times.

Therefore, samples of adrenal venous blood were collected for exactly 15 min. between 9.30 a.m. and 11.00 a.m. at the various stages of the oestrous cycle in the rat and from normal male animals.

Aldosterone and corticosterone levels were determined by a modification of the double-isotope derivative technique of Kliman & Peterson (1960).

Aldosterone Secretion Rates

The results presented in Table 1 show that aldosterone secretion rates were 1.93±0.02 µg/Kg/hr/adr. and 1.32±0.04µg/Kg/hr adr. at pro-oestrus and oestrus respectively compared with 0.89±0.06 µg/Kg/hour at dioestrus ($p<0.001$ and $p<0.001$ respectively). This was an increase of 104% in aldosterone secretion rate at pro-oestrus compared with dioestrus and secretion rates were raised by 47% at oestrus compared with dioestrus.

No significant difference was found between aldosterone secretion rates in dioestrous female and normal male rats.

Corticosterone Secretion Rates

Table 2 shows the secretion rates of corticosterone during the oestrous cycle. Corticosterone levels were found to follow a similar pattern to the aldosterone levels, with the highest values at pro-oestrus. The corticosterone secretion rate was 464.7±23.4 µg/Kg/hr/adr. at pro-oestrus compared with 212.2±15.92 µg/Kg/hr/adr. at dioestrus ($p<0.001$). At oestrus the corticosterone secretion rate was 422.1±20.5 µg/Kg/hr/adr. compared with 212.2±15.9 µg/Kg/hr/adr. at dioestrus ($p<0.001$).

These results show, therefore, that there is a variation in adrenal cortical secretion during the oestrous cycle in the rat. The parallel increases in corticosterone and aldosterone secretion rates would suggest an overall stimulation of the adrenal gland at oestrus and pro-oestrus compared with di-oestrus.

Summary

1. Aldosterone and corticosterone secretion rates were determined at the various stages of the oestrous cycle.
2. Aldosterone secretion rates were highest at pro-oestrus and significantly increased at oestrus compared with dioestrus.
3. Corticosterone secretion rates followed a similar pattern to those of aldosterone during the oestrous cycle.

TABLE 1

Variations in Aldosterone Secretion Rates During the Oestrous Cycle

Group	Aldosterone Secretion Rate (µg/Kg/hr/adr.)		p.
Male	0.98 ± 0.04	(6)	
			0.20
Dioestrus	0.899 ± 0.06	(6)	
			0.001
Pro-oestrus	1.93 ± 0.02	(8)	
			0.001
Oestrus	1.32 ± 0.04	(8)	
			0.01
Metoestrus	1.10 ± 0.04	(7)	
			0.001
Dioestrus	0.899 ± 0.06	(6)	
			0.001
Oestrus	1.32 ± 0.04	(8)	

Results expressed as means ± S.E.

Number of animals in parentheses.

TABLE 2

Variations in Corticosterone Secretion Rates During the Oestrous Cycle

Group	Corticosterone Secretion Rate (µg/Kg/hr/adr.)		p.
Dioestrus	212.2 ± 15.92	(4)	
			0.001
Pro-oestrus	464.7 ± 23.4	(4)	
			0.20
Oestrus	422.1 ± 20.5	(3)	
			0.01
Metoestrus	300.7 ± 7.3	(4)	
			0.01
Dioestrus	212.2 ± 15.9	(4)	
			0.001
Oestrus	422.1 ± 20.5	(3)	

Results expressed as means ± S.E.

Number of animals in parenthesis.

GENERAL SUMMARY

1. A variation was observed in the sodium content of uterus, kidney, liver, plasma, heart and skeletal muscle during the oestrous cycle, the sodium content of these tissues being higher at oestrus than at dioestrus.
2. The water and potassium content of tissues showed a less marked variation during the oestrous cycle.
3. A variation was found in renal electrolyte function during the oestrous cycle in the rat.
4. Urine flow was significantly lower at oestrus than at dioestrus but no significant variation in glomerular filtration rate was observed during the oestrous cycle.
5. Sodium concentration and excretion rates were significantly lower at oestrus than at dioestrus.
6. Potassium concentration and excretion rates were higher at oestrus than at dioestrus.
7. Spironolactone blocked the sodium and water retention and increased potassium excretion at oestrus but had little effect on renal electrolyte function in dioestrus or ovariectomised rats.
8. Prolonged injection of oestrogen, progesterone and combined oestrogen and progesterone significantly reduced urine flow, urinary sodium concentration, rate of sodium excretion and increased potassium excretion but had little effect on glomerular filtration rate in ovariectomised rats.
9. Spironolactone treatment blocked the effects of prolonged ovarian hormone treatment on renal electrolyte function.

10. It was found that a single injection of 5 µg of oestradiol reduced urine flow, urinary sodium concentration, rate of sodium excretion, sodium:potassium ratio and increased urinary potassium concentration and excretion rate after a delay of 4-5 hours.
11. The progressive increase in sodium and water retention and increase in potassium excretion observed 5-8 hours after oestradiol injection was blocked by administration of spironolactone.
12. No significant alteration in renal electrolyte function was observed 12 to 18 hours after oestradiol administration.
13. Single injections of progesterone, 2 hours prior to saline infusion and 2 hours after starting saline infusion, had no effect on renal electrolyte function.
14. Infusion of 1 µg/hour of progesterone over a 6 hour period significantly reduced the sodium:potassium ratio compared with control ovariectomised animals in the absence of sodium diuresis.
15. A significant variation was found in aldosterone and corticosterone secretion rates during the rat oestrous cycle. The highest rates of secretion were observed at pro-oestrus.
16. It is suggested therefore, that the action of ovarian hormones on renal electrolyte function is a result of an initial stimulation of adrenal cortical hormone secretion by the ovarian hormones

DISCUSSION

Introduction

The early work of Thorn et al. (1938) and Thorn & Emerson (1940) demonstrated a variation in electrolyte excretion during the human menstrual cycle, with a pronounced decrease in electrolyte excretion and urine volume during the luteal phase of the menstrual cycle. The more recent evidence presented by Reich (1962) and Gray et al. (1968) suggested that the variation in electrolyte excretion during the menstrual cycle is associated with a variation in aldosterone excretion and secretion rates. The findings reported by these workers therefore suggest an association between ovarian hormone levels and adrenal corticol activity. However, the separate actions of oestrogens and progesterone on renal electrolyte function and adrenal gland activity have not been clearly defined.

Several workers have demonstrated a decrease in sodium and water excretion following oestrogen administration to a number of species (Thorn & Engel, 1938, Dance et al., 1959, Zelewski, 1965, Nocenti & Cizek, 1964, Preedy & Aitkin, 1956, Dignam et al., 1963 and Landau et al., 1967). However, the decrease in sodium and water excretion was observed after prolonged administration of pharmacological doses of oestrogens. The recent work of Johnson et al. (1970) has shown that administration of a physiological dose of oestradiol (100 µg/day) to dogs resulted in only slight sodium retention.

Reports on the action of progesterone on renal electrolyte function have suggested a species difference in renal

response to progesterone administration. The evidence presented by Landau et al. 1957, Landau & Lugibihl, 1958 and Jacobs, 1969, has consistantly shown that progesterone has a natriuretic action in man. In contrast, O'Connell et al., (1969) and Johnson et al. (1970) reported that progesterone had no effect on sodium excretion in dogs.

The evidence presented for the action of progesterone on renal function in rats has been contradictory. Gaunt et al.(1940) and Emery & Greco (1940) have demonstrated that progesterone has a mineralocorticoid action on renal function in rats. Rosemberg & Engel (1961) and Kagawa (1958) have contradicted this work by demonstrating that progesterone administration blocked the sodium retaining action of D.C.A. on the rat kidney tubules.

In the present study renal electrolyte function and adrenal cortical activity were determined during the rat oestrous cycle and the separate actions of oestrogen and progesterone on renal function were investigated.

The Action of Ovarian Hormones on Tissue Electrolytes

There are many reports suggesting that ovarian hormones affect sodium homeostasis but few of these have considered the effects of ovarian hormones on plasma and tissue electrolyte levels.

The results presented in Section 1 show that the levels of sodium, potassium and water in the uterus were significantly higher at pro-oestrus and oestrus than at dioestrus. It was also found that there is a significant increase in the sodium content of heart and skeletal muscle at oestrus compared with dioestrus and that plasma sodium levels were higher at oestrus than at dioestrus.

There was a similar variation in the sodium content of liver and kidney during the oestrous cycle, but these results were not significantly different from each other. The variations in potassium and water content during the oestrous cycle, in tissues other than the uterus, were not significantly different from each other.

The levels of sodium and potassium found in muscle and plasma agreed well with the findings of MacIntyre &

Davidsson (1958), demonstrating sodium concentrations of 128.6±4.6 meq/Kg in muscle and 140.4±5.5 meq/L in plasma, and potassium concentrations of 500.5±6.0 meq/Kg and 5.5±1.0 meq/L respectively.

No evidence has been presented by previous workers to show that there is a variation in plasma or tissue electrolytes during the rat oestrous cycle. However, Michelakis (1971) has demonstrated an increase in serum sodium levels during the luteal phase of the menstrual cycle when ovarian hormone levels are increased. In the present study plasma sodium levels were increased at oestrus when plasma ovarian hormone levels are also raised.

The variation in sodium content of tissues observed during the oestrous cycle may be a reflection of increased sodium content of extracellular or intercellular fluid or may reflect an increase in extracellular fluid content.

Richards et al. (1966) have shown that incubation of laryngeal cells *in vitro* with aldosterone significantly reduces intracellular sodium concentration, in the absence of any alteration in intracellular potassium concentration. These observations would suggest, therefore, that high plasma levels of mineralocorticoids *in vivo* may induce salt and water transfer from the intracellular to the extracellular fluid. Richards et al. have also suggested that high levels of aldosterone *in vivo* may result in a "swamping" of the action of aldosterone on intracellular sodium, because the action of aldosterone on the kidney results in an increased extracellular sodium.

Gray et al. (1968) have demonstrated an increase in aldosterone secretion rates during the luteal phase of the menstrual cycle, when plasma progesterone levels are raised (Woolever, 1963). The work presented by Pye & Matty (1969) demonstrated a decrease in urinary sodium:potassium ratio at oestrus, compared with dioestrus, again when plasma ovarian hormone levels are raised, thus indicating an increase in adrenal corticol activity at this time in the rat. Therefore the increased sodium content of tissues and plasma at oestrus, compared with dioestrus, may be a reflection of increased aldosterone levels at this time, inducing renal sodium retention and hence increased sodium content in the extracellular fluid.

The Action of Ovarian Hormones on Renal Electrolyte Function

Introduction

The findings that tissue and plasma sodium levels varied during the oestrous cycle led to an investigation into renal electrolyte function during the oestrous cycle.

In studying kidney function it is essential to determine glomerular filtration rate (G.F.R.) in order to establish any alteration in the rate of plasma filtration by the kidney. Previous evidence for the action of ovarian hormones on G.F.R. has been contradictory. A number of workers failed to detect any alteration in G.F.R. after administration of oestrogens in man (Dean et al., 1945), in dogs (Richardson & Houck, 1951) or in rats (Nocenti & Cizek, 1964). However, Dance et al. (1959) reported increased G.F.R. in dogs after oestrogen administration. Progesterone has been found to have no effect on G.F.R. except when administered in large doses (Chesley & Tepper, 1967).

In the present study inulin clearance was determined as a measure of glomerular filtration rate since it is freely filtered by the kidney; it is non-toxic and biologically inert and it is not reabsorbed or secreted by the kidney tubules and is easily and accurately determined in plasma and urine.

Inulin is hydrolysed to fructose in the gastrointestinal tract and is poorly absorbed from muscle or subcutaneous tissue, therefore, it was administered to the experimental animal intravenously. A primer dose of inulin was given

at the beginning of the experimental period and sustained by administration of 1% inulin in saline which was infused into the external jugular vein at the rate of 3 mls/hour in order to maintain a high rate of urine flow for G.F.R. determination. The saline solution contained the components necessary to keep the animal in salt and water balance throughout the experimental period. Urine and blood samples were taken at 60 minute intervals following a 2 hour equilibration period. Urine flow, glomerular filtration rate, urinary sodium and potassium concentrations and excretion rates were determined.

Keeler & Schreiden (1955) have shown that intravenous infusions of isotonic saline induced natriuresis and diuresis in rats. It was, therefore, necessary to establish the pattern of renal electrolyte function during saline infusion, in ovariectomised rats prior to investigating the association between renal electrolyte function and the levels of ovarian hormones.

The Action of Saline Infusion on Renal Electrolyte Function

Introduction

Inulin, in isotonic saline, was infused into the external jugular vein of ovariectomised rats at the rate of 3 mls/hour as previously described. The bladder and right carotid artery were cannulated and urine and blood samples collected at 60 min. intervals for 8 hours. The results presented in Section II demonstrate that there is a progressive rise in urine flow and sodium and potassium excretion during the initial 2 hour period of experimentation thus confirming the work of Keller & Schreiden. Sodium concentration decreased to a constant level 3 hours after starting saline infusion, while a parallel decrease in potassium concentration was also observed.

Since no change in glomerular filtration rate was observed during saline infusion the observed changes in sodium and water excretion must reflect changes in renal tubular function. It is possible that the increased extracellular fluid volume, induced by saline infusion, may play a part in the mechanism of saline diuresis. However, Keller & Schreiden have shown that infusion of 6% albumin, in saline, did not induce the same type of diuresis, although the expansion of plasma volume was the same.

Severance of the vagi does not impair the renal response to saline infusion. This would suggest that volume receptors are not involved in the response. Bartter et al. (1956) have shown that an increase in extracellular volume depresses mineralocorticoid secretion and the progressive increase in

sodium:potassium ratio observed in the present experiments supports this. Therefore decreased mineralocorticoid activity may be important in the response of the kidney to saline infusion. However, Leeber et al. (1958) have refuted this theory and postulate that saline infusion results in inhibition of sodium reabsorption from the loop of Henlé. Unfortunately, this suggestion does not account for the progressive decrease in potassium excretion with saline infusion. Neither can it account for later results presented in Section II demonstrating an inhibition of sodium and water diuresis, induced by saline infusion at oestrus, when later work showed (Section III) that aldosterone levels are raised.

Therefore the renal response to saline infusion is confirmed but the mechanism remains unclear.

It is possible that the renal response to saline infusion may affect the observed action of ovarian hormones on renal electrolyte function, especially the initial 3 hours of equilibration when a progressive increase in sodium and water excretion occurs. Nevertheless a number of workers (Dance et al., 1959, Dignam et al., 1956 and Lichton, 1963) have attempted to determine the action of female sex hormones on renal electrolyte function during this period. The observation on the affect of saline infusion on kidney function suggests that it is essential, when investigating renal function, to continue infusion beyond the three hour equilibration period.

In the present study renal function was determined over a six hour period since the results presented in section II show that 6 to 8 hours after starting saline infusion G.F.R. and urine flow began to decline, indicating renal failure.

The Role of Diurnal Variation in Renal Function over an 8 Hour Experimental Period

Bellamy, Goulding & Griffiths (1969) have demonstrated a diurnal rhythm in salt retention by the kidney in normal male rat Urinary sodium and potassium concentrations were found to be highest between 08.00-12.00 hours. These results suggested that diurnal variation in kidney function could play an important role in renal response to saline infusion over an 8 hour period. This was further investigated by determining renal electrolyte function in ovariectomised rats, beginning saline infusion at 09.00 hours (expt. 1) and 15.00 hours (expt. 2).

The results presented in section II show that there was no significant difference in renal electrolyte function between the two groups of animals. Urine flow and sodium and potassium excretion rates progressively increased during the initial 3 hours of saline infusion in both experimental groups. Therefore this response must be a result of saline infusion rather than an inherent diurnal variation.

It may be concluded, therefore, that saline infusion, under anaesthesia, masks normal diurnal variation in kidney function.

Variation in Renal Electrolyte Function During the Oestrous Cycle

In order to extend the evidence of a variation in electrolyte metabolism during the oestrous cycle, indicated in section I, renal electrolyte function was determined during the rat oestrous cycle.

The various stages of the oestrous cycle were determined by the vaginal smear technique. The reliability of this technique for assessing the stage of the oestrous cycle, and therefore indicating the levels of ovarian hormones, has been established by Yoshinaga et al. (1969) and Feder et al. (1971).

Renal electrolyte function was determined on the days of dioestrus and oestrus as previously described.

The results presented in section II show that there is a significant decrease in urine volume, sodium concentration and excretion rate and sodium:potassium ratio at oestrus compared with dioestrus. Potassium concentration and excretion rates were higher at oestrus than at dioestrus. No significant difference was found in G.F.R. at oestrus compared with dioestrus.

The observed decrease in sodium:potassium ratio at oestrus compared with dioestrus confirms the earlier findings of Pye & Matty (1967) demonstrating a decreased sodium:potassium ratio at pro-oestrus and oestrus compared with dioestrus in conscious rats not undergoing saline infusion. This would suggest that anaesthesia and saline infusion do not mask any variation in renal function during the oestrous cycle.

Thorn et al. (1939), Thorn & Emerson (1940) and Michelakis et al. (1971) have demonstrated a reduction in sodium and water excretion during the luteal phase of the cycle.

Therefore these findings demonstrate a similar variation in renal electrolyte function during the rat oestrous cycle to that which has been observed during the human menstrual cycle.

Increased sodium and water retention, and increased potassium excretion were observed at oestrus when progesterone levels are highest (Feder et al., 1971) and when oestrogen levels are also raised (Yoshinaga et al., 1969). In the present study a variation in renal electrolyte function was not observed in ovariectomised animals, therefore the variations which are present during the oestrous cycle must be related to the variation in plasma ovarian hormone levels.

The variation in the sodium:potassium ratio suggests that the adrenal glands are involved in the variation in renal function observed during the oestrous cycle. Therefore, in order to investigate the mechanism of this variation further it was necessary to remove the influence of the adrenals in this system.

The Action of Spironolactone on Renal Electrolyte Function During the Oestrous Cycle

The results presented in the previous section suggested an association between the variation in renal electrolyte function, the ovarian hormones and adrenal function during the rat oestrous cycle. In order to further investigate the part played by the adrenal glands in the sodium and water retention observed at oestrus compared with dioestrus, it was necessary to remove endogenous adrenal cortical activity.

Two ways in which the endogenol adrenal cortical hormone action on the kidney may be blocked are: either by adrenalectomy or by administration of spironolactone. Kagawa et al. (1957) have shown that the synthetic steroid spironolactone specifically blocks the sodium retaining action of the mineralocorticoids, particularly aldosterone, on the distal tubules of the kidney. In contrast adrenalectomy results in decreased adrenal steroid levels, decreased plasma corticosteroidss resulting in increased adrenocorticotrophin, and therefore, a general deterioration in the physiological condition of the animal. Feder et al. (1971) have shown that progesterone, originating from the adrenal gland, facilitates luteinizing hormone release from the pituitary at pro-oestrus. This would suggest that removal of the adrenal glands might lead to disruption of the oestrous cycle. Therefore administration of spironolactone was chosen as a tool for investigating the role played by the adrenal glands in the observed variation in renal electrolyte function.

The Effect of Spironolactone on Renal Electrolyte Function During the Oestrous Cycle

Edmonds & Marriott (1968) demonstrated that 5 mg/day of spironolactone had no effect on normal sodium handling by the intestinal tract but blocked the effects of increased aldosterone levels, on sodium handling by the intestine, after sodium depletion. Therefore, in the present study, 2.5 mg of spironolactone was administered at 24, 12 and 2 hours prior to experimentation to ovariectomised animals. The renal electrolyte function was determined over a 6 hour period, as previously described and the results compared with those from control ovariectomised animals.

The results presented in Section II show that, although the dose of spironolactone administered had little effect on renal electrolyte function in dioestrous rats, it was effective in abolishing the sodium and water retention observed at oestrus. Since Kagawa (1967) has shown that spironolactone blocks the action of mineralocorticoids on the distal tubules of the kidney, these results suggest that the sodium and water retention observed at oestrus may be due to increased levels of mineralocorticoids at this time.

It is well established that plasma oestrogen and progesterone levels are raised at oestrus (Yoshinaga et al., 1969 and Feder et al., 1971), when sodium and water retention were observed. Therefore, since a variation in renal electrolyte function was not observed in ovariectomised animals, it may be postulated that the ovarian hormones induce sodium and water retention at oestrus via the stimulation of the adrenal

cortex. However, the evidence for the separate actions of oestrogens and progesterone on renal electrolyte function remains unclear.

A number of workers have reported a decrease in renal excretion of sodium and water after oestrogen administration in dogs (Dance et al., 1959; Richardson & Houck, 1951; Thorn & Harrap, 1937 and Johnson et al., 1970), in rats (Zelewski, 1961; Nocenti & Cizek, 1964) and in man (Preedy & Aitkin, 1956 and Landau et al., 1957). However, with the exception of Johnson et al. (1970), the previous reports have involved the use of high doses of oestrogen for prolonged periods of time. These experiments do not reproduce the physiological changes in oestrogen levels entirely and the findings cannot be directly related to the variations in renal electrolyte function.

Landau et al. (1957), Landau & Luigibihl, 1958 and Jacobs, (1969) have presented evidence to suggest that, in human subjects, progesterone antagonises the action of aldosterone on the kidney tubules thus inducing natriuresis. However, the evidence concerning the action of progesterone on sodium excretion in other species is contradictory. O'Connell et al. (1969) and Johnson et al. (1970) were unable to detect any effect of progesterone on sodium excretion in dogs, but Thorn & Engel (1938) and Thorn & Harrap (1937) found that the administration of large doses of progesterone to dogs induced sodium retention.

Workers investigating the action of progesterone on renal function in rats have administered extremely high doses of the hormone, thus making physiological interpretation of their

results difficult. Emery & Greco (1940) and Gaunt et al. (1940) found that administration of 1-2 mg/day of progesterone prolonged the life-span of adrenalectomised rats. However, Rosemberg & Engel (1961) found that administration of similar doses of progesterone antagonised the action of D.C.A. on the kidney.

The evidence concerning the action of oestrogens or progesterone on renal electrolyte function is contradictory and therefore experiments were carried out in this study to investigate the action of these steroids on rat renal electrolyte function.

The Action of Ovarian Hormones on Renal Electrolyte Function

The results presented in section II suggest that the variation in plasma ovarian hormone levels during the oestrous cycle may influence renal electrolyte function. In order to investigate further the separate actions of ovarian hormones on kidney function, 5μg/day of oestradiol - 17β and 5 μg/day of progesterone were administered separately and in combination to ovariectomised rats for 14 days. Renal electrolyte function was determined, as previously described, over a 6 hour experimental period on the 14th day.

The results presented in section II show that prolonged administration of oestradiol, progesterone and oestradiol + progesterone resulted in very similar alterations in renal function. A significant reduction in urinary sodium concentration, sodium and water excretion and sodium: potassium ratio and increased potassium excretion rates and urinary potassium concentrationwere observed. Glomerular filtration rate was not significantly different from that observed in ovariectomised control animals.

These results confirm the earlier findings of Thorn & Engel (1938) and Thorn & Harrap (1937) demonstrating sodium and water retention in dogs following progesterone administration. They also support the findings of Emery & Greco (1940) and Gaunt et al. (1940) demonstrating a mineralocorticoid-action with prolonged progesterone administration in rats.

The sodium and water retention observed after oestrogen administration are in agreement with the work of Zelewski

(1961) and Nocenti & Cizek(1964) demonstrating a reduction in sodium and water excretion in rats following administration of oestrogens.

These findings suggest that both oestradiol and progesterone therapy results in a similar change in renal electrolyte function.

Since no significant change in glomerular filtration rate was observed with oestradiol or progesterone administration, this suggested that the decrease in sodium and water excretion, with parallel increase in potassium excretion, must take place via a change in renal tubular function.

The significant reduction in sodium:potassium ratio observed after ovarian hormone administration suggested that mineralocorticoids were involved in the observed response. Thorn & Harrap (1937) have suggested that the close structural relationship between the ovarian hormones and the mineralocorticoids indicate that the ovarian hormones may have similar properties to mineralocorticoids, especially when administered for prolonged periods . Therefore, it is possible that the ovarian hormones may mimick the action of mineralocorticoids and thus have a direct action on the distal tubules of the kidney. Thus two of the possible mechanisms of action of the ovarian hormones on the kidney are - 1) by a direct effect on the kidney tubules, similar to that observed with mineralocorticoid administration, or, 2) by an indirect action in stimulating mineralocorticoid secretion by the adrenal cortex.

The Action of Spironolactone on Renal Electrolyte Function in Ovariectomised Rats Treated with Oestradiol and Progesterone

The results presented in section II suggested that the adrenal cortex may be involved in the renal response to oestradiol and progesterone therapy. Therefore spironolactone was administered to block mineralocorticoid activity. 2.5 mg of spironolactone was administered 24 hr, 12 hr and 2 hr, prior to experimentation, to ovariectomised animals injected with 5 µg/day for 14 days with oestradiol, progesterone or oestradiol + progesterone. Renal electrolyte function was determined over a 6 hour period as previously described.

It was found that spironolactone administration blocked the sodium and water retention, and increased potassium excretion observed after oestradiol, progesterone and oestradiol + progesterone therapy. These results strongly suggested that the ovarian hormones influence renal electrolyte function via stimulation of the adrenal cortex. However, since the ovarian hormones were administered for 14 days it is possible that, because of their close structural relationship with mineralocorticoids, they show a similar salt-retaining action. If this is so, it is possible that spironolactone would also inhibit the action of the ovarian hormones on the distal tubules of the kidney. It was therefore necessary to investigate the effect of a single injection of a physiological dose of oestradiol and progesterone on renal electrolyte function.

The Action of a Single Injection of Oestradiol on Renal Electrolyte Function

The results presented in the preceding sections suggest that oestradiol may induce renal sodium and water retention with increased potassium excretion. In order to investigate this possibility further, and to determine the role of oestrogens in the variations in renal electrolyte function observed during the rat oestrous cycle, renal electrolyte function was determined after administration of a single, low dose of oestradiol.

Renal electrolyte function was determined 2 hours and 12 hours after administration of 5 µg of oestradiol to ovariectomised rats. The results presented in section II show that there was a delay in the action of oestradiol on the kidney of 6-7 hours. This is in accordance with the work of Beato (1960) demonstrating a delay of 4-6 hours in the action of oestrogen on the uterus. Six hours after oestradiol administration it was found that urinary sodium concentration and rate of excretion and the sodium:potassium ratio were significantly lower, and urinary potassium concentration significantly higher than observed in blank injected control animals.

No observable effect on renal electrolyte function was obtained with oestradiol administration 12 hours prior to experimentation. This is in contrast with the work of Dignam et al. (1956) showing a decrease in sodium and water excretion for 72 hours after cessation of oestradiol therapy. However, Dignam et al. (1956) administered

8 mg/day and, therefore, the prolonged action which they observed may be due to this high dosage.

These findings show that a single injection of oestradiol induced sodium and water retention, and increased potassium excretion, comparable with that observed at oestrus. No significant difference was found between glomerular filtration rate in the oestradiol treated and ovariectomised animals. This suggests that oestradiol exerts the observed effect on renal electrolyte function via an action on the kidney tubules. The reduction in sodium:potassium ratio following oestradiol administration implicated the adrenal cortex in the action of oestradiol on renal electrolyte function.

The Action of Spironolactone on Renal Electrolyte Function in Ovariectomised Rats Treated with Oestradiol

The conclusions arrived at in the preceding section suggest that oestradiol administration influenced renal electrolyte. Therefore spironolactone was administered to ovariectomised animals treated with oestradiol, in an attempt to block the action of mineralocorticoids on the distal tubules of the kidney, and thus determine the role played by mineralocorticoids in the observed action of oestradiol on renal function.

2.5 mg of spironolactone was administered 24 hours, 12 hours and 2 hours prior to experimentation; in addition, 5µg of oestradiol was given to ovariectomised rats 2 hours prior to experimentation and renal electrolyte function determined

Spironolactone administration was found to inhibit the action of oestradiol on renal electrolyte function. It is postulated, therefore, that oestradiol therapy results in increased plasma levels of mineralocorticoids which act on the distal tubules of the kidney, causing sodium and water retention, with increased potassium excretion. These results would also suggest that the increased plasma levels of oestrogens observed at oestrus (Yoshinaga et al., 1969) play an important role in the sodium and water retention observed at oestrus in the rat.

The Action of Progesterone on Renal Electrolyte Function in Ovariectomised Rats

Feder et al. (1971) have shown that progesterone levels are raised at oestrus when sodium and water retention was observed. It is possible, therefore, that progesterone is involved in the variation in renal electrolyte function observed during the oestrous cycle. However, work published by other workers (see literature review, page 3) on the action of progesterone on renal function has been contradictory. Therefore further evidence for the action of progesterone on renal function is required.

In order to investigate the action of progesterone on renal electrolyte function, 5 µg of progesterone was administered to ovariectomised animals 2 hours prior to experimentation and renal function determined over a 6 hour period.

The results presented in section II show that progesterone had no significant effect on renal electrolyte function when administered 2 hours prior to experimentation.

Progesterone, administered 2 hours after starting saline infusion, had no significant action on renal electrolyte function in ovariectomised rats compared with blank-injected control, ovariectomised animals. It is possible, however, that rapid excretion of the hormone from the body or rapid conversion of the hormone to inactive metabolites could account for the lack of effect of progesterone on renal electrolyte function. Therefore, the effect of constant infusion of 1 µg/hr of progesterone, for 6 hours, on renal

electrolyte function was investigated.

The results presented in section II show that, although progesterone infusion resulted in a significant reduction in the sodium:potassium ratio compared with control animals, little effect was observed on the other parameters measured. The reduction in sodium:potassium ratio indicates an increase in mineralocorticoid activity. However, since progesterone infusion had no significant effect on sodium excretion, it is improbable that progesterone stimulated the adrenal cortex via the natriuretic effect postulated by Landau & Lugibihl (1961).

It is possible that progesterone acts as a precursor in the biosynthesis of mineralocorticoids at the adrenal. Alternatively, progesterone may decrease the metabolic breakdown of the mineralocorticoids; (Layne et al., 1962).

In the preceding section it was found that when oestradiol and progesterone were administered for long periods to ovariectomised rats, both hormones had a similar, profound effect on renal electrolyte function. However, when oestradiol or progesterone were administered as a single injection, oestradiol administration was found to result in a decrease in sodium concentration, excretion rate and sodium:potassium ratio and an increase in potassium concentration, whereas progesterone administration resulted in a decrease in the sodium:potassium ratio. Therefore oestradiol appears to have a more profound effect on renal electrolyte function than progesterone does.

Aldosterone and Corticosterone Secretion Rates During the Oestrous Cycle in the Rat

The results presented in the previous sections have suggested that there is a variation in electrolyte metabolism during the rat oestrous cycle. Later experiments have implicated oestrogens, particularly in this variation, but have suggested that progesterone plays only a minor role in this variation. Therefore it was essential to determine aldosterone and corticosterone secretion rates during the oestrous cycle, particularly in relation to the separate peaks in plasma oestrogen and progesterone levels.

Blood samples were collected from the adrenal vein over a 15 minute period at various stages of the oestrous cycle. The blood samples were assayed for aldosterone and corticosterone levels by a modification of the double-isotope derivative assay of Kliman & Peterson (1960).

The results presented by Kinson (1968) showed an insignificant difference between blanks estimated for water and blood from adrenalectomised animals, thus demonstrating a lack of interference from extra-adrenal substances and other steroids. It was found that recovery of known amounts of steroids was 104.9% $\pm$ 3.9% and 100.3% $\pm$ 0.8%, indicating accurate determination of aldosterone and corticosterone. The standard errors for replicate samples were 3.4% for aldosterone and 1.3% for corticosterone, indicating the reproducibility associated with the procedure.

The results presented in section III show that there is a variation in aldosterone and corticosterone secretion

rates during the rat oestrous cycle. It was found that aldosterone secretion rates were increased by 104% at pro-oestrus compared with dioestrus and by 47% at oestrus compared with dioestrus. No significant difference was found between aldosterone secretion rates in dioestrus female and normal male animals. Corticosterone secretion rates followed a similar pattern with an increase of 119% at pro-oestrus compared with dioestrus and an increase of 52% at oestrus compared with dioestrus.

The aldosterone and corticosterone secretion rates in dioestrus female and normal male rats agree with the aldosterone secretion rates of 0.72 ± 0.17 µg/Kg/adr/hr and corticosterone secretion rates of 172 ± 11.5 µg/Kg/adr/hr reported by Singer et al. (1963).

The observed variation in corticosterone secretion rates during the oestrous cycle are in accordance with those reported by Dean et al. (1956) and Raps et al. (1971) in rats and Zondek & Burnstein (1952) in guinea-pigs.

The results presented in section III show that aldosterone and corticosterone secretion rates are increased to a similar extent at pro-oestrus and oestrus, indicating that there is an overall stimulation of the adrenal cortex. The increased steroid secretion rates were observed at the time of maximum oestrogen secretion (Yoshinaga et al., 1969) and when progesterone levels were also raised (Feder et al., 1971) indicating that the increase in aldosterone and corticosterone levels may be a result of the increased levels of oestrogen and progesterone.

It has been suggested that increased secretion and excretion rates of aldosterone observed during the luteal phase of the menstrual cycle and during pregnancy (see literature review, page 3), are induced by increased levels of progesterone which occur at this time. This theory is based on evidence presented by several workers (Landau et al., 1957; Landau & Lugibihl, 1958 and Jacobs, 1969) that, in human subjects, progesterone antagonises the action of aldosterone on the kidney tubules, thus indicating natriuresis. It has been postulated that the rise in renin activity observed during the luteal phase of the cycle (Brown, 1963) is induced by the natriuretic effect of progesterone and results in the stimulation of aldosterone secretion.

The results presented in section II show that a single injection of progesterone, at the dose administered in the present study, did not result in natriuresis in ovariectomised rats and did not mimick the sodium and water retention o observed at oestrus, as a single injection of oestradiol did.

The mechanism of increased adrenal function during the oestrous cycle may be further elaborated by relating the levels of aldosterone and corticosterone during the oestrous cycle to the levels of oestrogen and progesterone. Yoshinaga et al. (1969) showed that plasma oestrogen levels

progressively increase during the night of dioestrus/pro-oestrus to reach a peak between 0.5.00 hr to 10.00 hr on the morning of pro-oestrus, when aldosterone and corticosterone levels are highest. Feder et al. (1971) reported that progesterone levels reach a peak at 21.00 hr on the day of pro-oestrus, that is 11 hours after the highest levels of aldosterone and corticosterone were detected.

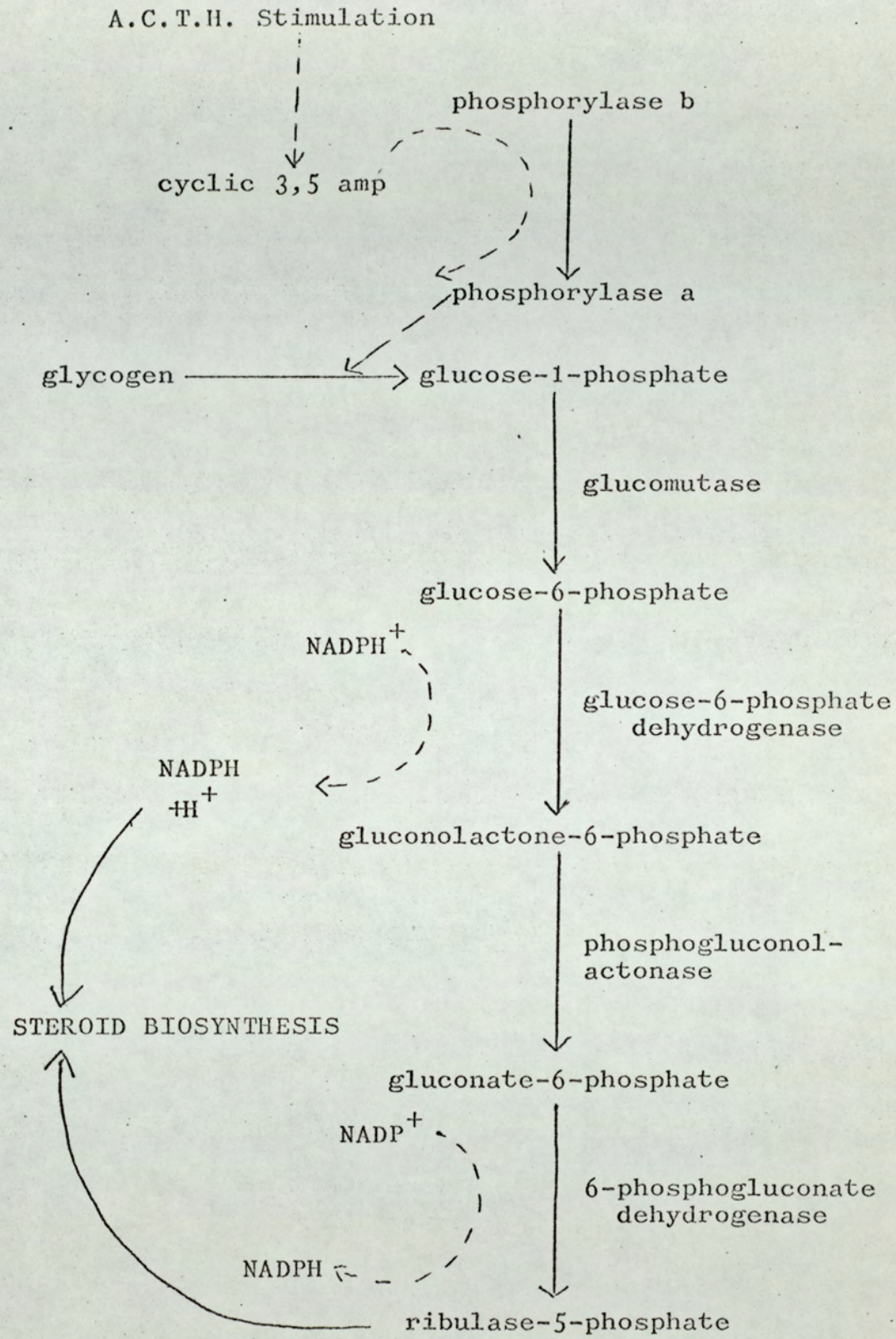
In contrast the results presented in section II show that oestradiol administration results in sodium and water retention similar to that observed at oestrus. The observed reduction in sodium:potassium ratio and the ability of spironolactone to block the sodium and water retention observed after oestradiol administration suggest that oestrogens induce the observed variation in sodium and water retention during the oestrous cycle. The observation that aldosterone and corticosterone secretion rates are highest at the time of the peak in plasma oestrogen levels during the oestrous cycle support the theory that oestrogens are primarily involved in the variation in renal and adrenal function.

Earlier workers have presented evidence to suggest that oestrogens may stimulate the secretion of adrenal corticol hormones. Zondek & Burnstein (1952) have demonstrated a variation in corticosteroids during the guinea-pig oestrous cycle. This cyclic variation was absent in male and ovariectomised female animals but corticosterone levels were increased in ovariectomised animals after oestrogen therapy. The effect of oestrogen therapy in human sugjects has been

investigated by Layne et al. (1962) and Bradley & Waterhouse (1966) who found that oestrogen therapy resulted in increased plasma cortisol levels and increased plasma binding of cortisol. It is possible that the increased protein binding may protect the adrenal steroids from metabolic break-down by the liver, thus further increasing the plasma concentration of steroids (Layne et al., 1962 and Peterson et al., 1960).

The ability of oestrogens to increase plasma protein binding of renin may result in the increased renin substrate activity observed by Crane & Harris (1969) after ethynyl oestradiol administration to women. However, the size of the renin molecule would appear to preclude this hypothesis. There is further well documented evidence, however, to suggest that oestrogens may influence adrenal activity via the renin-angiotensin system in rats. Helmer & Griffiths (1952) and Nasjletti et al. (1969) have proposed that oestrogens inhibit the breakdown of renin resulting in increased plasma angiotensinogen concentration.

A.C.T.H. is vital in adrenal cortical function. Haynes & Barthet (1957) postulated that A.C.T.H. stimulates steroidogenesis:-


A.C.T.H. Stimulation
phosphorylase b
cyclic 3,5 amp
phosphorylase a
glycogen
glucose-1-phosphate
glucomutase
glucose-6-phosphate
NADPH+
glucose-6-phosphate dehydrogenase
NADPH +H+
gluconolactone-6-phosphate
phosphogluconol-actonase
STEROID BIOSYNTHESIS
gluconate-6-phosphate
NADP+
6-phosphogluconate dehydrogenase
NADPH
ribulase-5-phosphate

Vogt (1955) and Holzbauer (1957) have reported that hexoestrol inhibits corticosterone secretion by the rat adrenal, possibly by inhibiting NADP specific dehydrogenase enzyme activity in vivo (McKerns, 1957). However, the more recent work of Marks & Banks (1960) has shown that stilboestrol had no inhibitory effect on rat adrenal glucose-6-phosphate activity.

It has been shown that oestrogens may have a direct effect on A.C.T.H. production. Ovariectomy has been shown to result in a 25% decrease in A.C.T.H. secretion rates but the secretion rates were restored after administration of oestrogens (Coyne & Kitay, 1970). In male rats, oestrogen administration resulted in increased A.C.T.H. content of the pituitaries. These effects were observed in the absence of the adrenal glands, therefore the inhibition of adrenal corticosteroids by oestrogens, resulting in A.C.T.H. stimulation, suggested by Vogt (1955) and the stimulation of A.C.T.H. via increased hepatic metabolism of steroids, induced by oestrogen administration, postulated by Urquart (1959) cannot be acting.

The evidence presented by Holub (1962) showed that A.C.T.H. synthesis was decreased after ovariectomy, suggesting a decrease in response in the hypothalamus to stimulation. It has been shown, however, that A.C.T.H. production may be restored by implanting oestradiol into the hypothalamus or the anterior pituitary (Chowers & McCann, 1964). Therefore the site of oestrogen stimulation in the brain remains undefined.

There is also evidence to suggest that, as well as a direct action on A.C.T.H. production, oestrogens augment the action of A.C.T.H. on the adrenal cortex (Carter, 1955). Peterson et al. (1960) have shown that subjects given both A.C.T.H. and oestrogens have plasma corticoid levels which are two to three times higher than those observed with A.C.T.H. therapy alone.

The anterior pituitary hormones, growth hormone and prolactin may also be involved in the observed action of ovarian hormones on adrenal and renal function. Lockett & Nail (1965) have demonstrated that both bovine growth hormone and prolactin have similar actions in reducing sodium excretion in rats, in the absence of any alteration in glomerular filtration rate. However, Enthoven, Swanson and van der Werfften Bosch (1966) have demonstrated that growth hormone is enhanced by ovariectomy in rats. Therefore, since ovarian hormones were raised at the time of sodium retention during the oestrous cycle and sodium retention was observed af ter ovarian hormone therapy, in the present study, it is improbable that growth hormone is involved in the observed response of the kidneys and adrenal glands to variations in plasma ovarian hormone levels.

It has been demonstrated that oestrogens increase the manufacture of prolactin, either by a direct action on the pars distalis (Nicholls & Meites, 1962) or via the hypothalamus (Kanematsu & Sawyer, 1963). It is possible, therefore that the increased levels of oestrogens at pro-oestrus, or oestrogen therapy, may result in increased prolactin levels which may contribute to the decrease in sodium excretion

observed at oestrus and after oestrogen therapy.

However, Lockett & Roberts (1963) has shown that growth hormone and prolactin have a direct action on the perfused cat kidney to induce sodium retention. Therefore it would be unlikely that prolactin is involved in the increase in aldosterone and corticosterone secretion rates observed at pro-oestrus and oestrus in rats.

The evidence presented by Ruhmann-Wennhold et al. (1970) suggests that oestradiol administration results in increased 11β and 18-hydroxylation by the adrenal cortex. Therefore oestrogens may have an additional direct action on the adrenal cortex. This action of oestrogens is not elucidated without A.C.T.H. but cannot be acting via A.C.T.H. since A.C.T.H. stimulation is already maximal due to stress in the system used by these workers. This supports the earlier findings of Kitay et al. (1965) demonstrating a decrease in adrenal activity after ovariectomy in hypophysectomised animals.

It may be concluded from this evidence that oestrogens may act on the adrenal cortex through a wide range of mechanisms. However, since aldosterone and corticosterone secretion rates are increased by a similar extent at pro-oestrus, this would implicate a mechanism which would induce a rise in both aldosterone and corticosterone secretion rates. Kinson & Singer (1969) have presented evidence to suggest that hypophysectomy results in decreased corticosterone secretion rates, but has little effect on aldosterone secretion rates. The same workers have also shown that

increased plasma angiotensin II levels stimulate aldosterone secretion rates but not corticosterone secretion rates. This evidence precludes stimulation of the adrenal cortex by oestrogens via A.C.T.H. or the renin-angiotensin system alone. However, oestrogens may act on both A.C.T.H. and the renin-angiotensin system, therefore stimulating aldosterone and corticosterone secretion rates by separate mechanisms. Since aldosterone and corticosterone secretion rates are increased by a similar degree, however, the possibility of oestrogenic stimulation via two separate mechanisms would appear improbable.

Therefore oestrogens may act on the adrenal cortex via increased protein binding or via a direct stimulation of steroid biosynthesis. Steroid secretion rates were determined during the oestrous cycle, and not plasma steroid concentrations. Therefore, it is unlikely that oestrogens increase aldosterone and corticosterone secretion rates via increased protein binding or decreased metabolic breakdown of the steroids.

Since the peaks in aldosterone and corticosterone secretion rates were observed 11 hours before the peak in plasma progesterone levels, this would suggest that progesterone does not play a major role in the variation in aldosterone and corticosterone secretion rates observed during the rat oestrous cycle. It is possible, however, that the high levels of plasma progesterone of oestrus may serve as a precursor of aldosterone and corticosterone and thus contribute to the 50% increase in aldosterone and

corticosterone secretion rates observed at oestrus compared with dioestrus.

In order to investigate further the action of ovarian hormones on the adrenal cortex it will be necessary to determine adrenal corticol secretion rates after oestrogen and progesterone therapy in ovariectomised rats. The rate of A.C.T.H. and the renin-angiotensin in this system would be examined by determining adrenal corticol secretion rates in hypophysectomised and nephrectomised animals after oestrogen and progesterone administration. However, in order to establish the action of oestrogen on aldosterone and corticosterone biosynthesis and the suggestion that progesterone may act as a precursor for these hormones, it will be necessary to incubate adrenal tissue in vitro with oestrogens and progesterone and determine the rate of aldosterone and corticosterone biosynthesis from labelled precursors.

The results presented in the present study suggest that the adrenal and renal response to increased plasma levels of ovarian hormones may differ greatly in different species. It is possible, however, that the role oestrogen plays in the increased adrenal activity observed during the luteal phase of the human menstrual cycle and pregnancy is of greater significance than was initially indicated by earlier workers. Since ovarian hormones are so widely used clinically, especially in their capacity as oral contraceptives, it is of considerable importance that their relation to other endocrine glands is fully investigated.

Conclusions

The evidence presented in the present study suggests that there is a variation in electrolyte metabolism during the rat oestrous cycle, with a significant sodium and water retent at oestrous compared with dioestrus. Since sodium and water retention were observed at the time of increased plasma ovarian hormone levels, this would suggest that the ovarian hormones are involved in the variation in renal electrolyte function observed during the oestrous cycle.

It was found that oestradiol had a more profound effect on renal electrolyte function than progesterone. This suggested that oestrogens are important in the variation in renal electrolyte function observed during the oestrous cycle. This theory was confirmed by the observation that aldosterone and corticosterone secretion rates were significantly increased at the time of maximum plasma oestrogen levels but eleven hours before the observed peak in plasma progesterone levels.

APPENDIX I

The Action of Ovarian Hormones on Renal Electrolyte Function

TABLE I

The Action of Saline Infusion on Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats

I. Glomerular Filtration Rate ml/min/100g

Time after starting Saline Infusion	Expt.1	Expt.2	p.
2h	(9) 0.73±0.02	(8) 0.68±0.05	<0.40
3h	(9) 0.71±0.03	(8) 0.69±0.05	<0.80
4h	(9) 0.60±0.04	(8) 0.69±0.05	<0.20
5h	(9) 0.64±0.03	(8) 0.65±0.03	<0.70
6h	(9) 0.61±0.03	(8) 0.58±0.04	<0.50
7h	(8) 0.59±0.03	(8) 0.54±0.02	<0.70
8h	(6) 0.47±0.05	(8) 0.52±0.04	<0.50

II. Urine Flow ml/h

Time after starting Saline Infusion	Expt.1	Expt.2	p.
2h	1.7±0.3	1.0±0.2	<0.05
3h	3.2±0.5	3.4±0.3	<0.80
4h	3.5±0.4	3.9±0.3	<0.90
5h	3.0±0.2	3.2±0.5	<0.70
6h	2.6±0.2	2.8±0.4	<0.70
7h	2.1±0.2	2.4±0.2	<0.20
8h	1.4±0.3	1.7±0.2	<0.20

Results expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE II

The Action of Saline Infusion on Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats

I. Sodium Concentration meq/L

Time after starting	Expt.1	Expt.2	p.
Saline Infusion			
0h	287.9±23.4	195.1±20.8	<0.02
2h	202.8±12.2	175.4±13.4	<0.20
3h	188.4±11.4	183.0± 8.7	<0.60
4h	183.7±10.3	182.6± 5.4	<0.90
5h	186.6±11.4	204.0± 8.3	<0.20
6h	184.7± 6.4	210.8±14.5	<0.20
7h	194.4±10.1	202.7±12.5	<0.70
8h	199.9± 7.9	196.7± 9.5	<0.90

II. Sodium Excretion Rate µeq/h

Time after starting	Expt.1	Expt.2	p.
Saline Infusion			
2h	354.6±66.8	185.5±34.5	<0.05
3h	604.4±89.2	603.3±57.9	<0.90
4h	623.1±56.7	721.3±58.2	<0.30
5h	559.2±41.1	635.4±85.5	<0.50
6h	477.2±29.9	566.5±45.8	<0.20
7h	392.9±31.5	481.4±26.1	<0.05
8h	283.2±47.6	360.6±12.1	<0.10

TABLE II (Cont.)

III. Sodium:Potassium Ratio

Time after starting	Expt. 1.	Expt. 2.	p.
Saline Infusion			
0h	3.3±0.3	1.9±0.2	<0.01
2h	3.0±0.4	1.8±0.3	<0.05
3h	4.9±0.4	3.6±0.2	<0.05
4h	5.1±0.4	3.8±0.1	<0.02
5h	4.5±0.3	4.2±0.3	<0.50
6h	5.2±0.4	4.9±0.5	<0.70
7h	5.6±0.7	4.3±0.2	<0.10
8h	5.2±0.7	4.7±0.3	<0.50

TABLE III

The Action of Saline Infusion on Urinary Potassium Concentration and Potassium Excretion Rates in Ovariectomised Rats

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Expt. 1.	Expt. 2.	p.
0h	95.5±11.2	106.1± 6.2	<0.50
2h	74.3± 8.2	105.4±11.2	<0.05
3h	40.3± 3.8	51.9± 6.2	<0.20
4h	38.2± 3.7	48.4± 2.0	<0.05
5h	42.2± 2.5	50.0± 4.3	<0.20
6h	37.2± 2.7	46.5± 6.0	<0.20
7h	37.6± 5.4	47.7± 4.7	<0.30
8h	40.4± 6.1	43.2± 4.0	<0.30

II. Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Expt. 1.	Expt. 2.	p.
2h	120.2±20.7	100.5±13.1	<0.50
3h	122.7±16.8	168.5±24.4	<0.20
4h	125.4±11.6	190.9±16.5	<0.01
5h	124.0± 7.8	152.8±19.4	<0.01
6h	93.9± 5.3	119.2±12.8	<0.10
7h	72.3± 6.3	111.9± 6.6	<0.01
8h	53.3± 4.1	79.4± 4.7	<0.01

TABLE IV

Glomerular Filtration Rate and Urine Flow at Different Stages of the Oestrous Cycle

I. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Dioestrus	Oestrus	p.
2h	(8) 0.57±0.06	(8) 0.68±0.09	< 0.30
3h	(8) 0.63±0.04	(8) 0.65±0.04	< 0.70
4h	(8) 0.63±0.08	(8) 0.60±0.04	< 0.70
5h	(8) 0.58±0.06	(8) 0.50±0.04	< 0.30
6h	(8) 0.52±0.04	(8) 0.53±0.04	< 0.80

II. Urine Flow (ml/hr)

Time after starting Saline Infusion	Dioestrus	Oestrus	p.
2h	2.0±0.3	0.8±0.06	< 0.01
3h	2.7±0.7	1.4±0.3	< 0.20
4h	2.8±0.3	1.3±0.2	< 0.001
5h	1.9±0.3	0.7±0.1	< 0.01
6h	1.4±0.3	0.7±0.3	< 0.30

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE V

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio at Different Stages of the Oestrous Cycle

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Dioestrus	Oestrus	p.
2h	171.7±70.8	130.5±16.6	< 0.30
3h	190.7±18.8	122.4±10.3	< 0.01
4h	175.1±19.1	117.7± 5.4	< 0.02
5h	172.0±16.0	97.8±12.7	< 0.01
6h	178.2±19.2	110.1±10.4	< 0.01

II. Rate of Sodium Excretion (μeq/hr)

Time after starting Saline Infusion	Dioestrus	Oestrus	p.
2h	331.0± 56.4	100.3±10.8	<0.01
3h	551.2±125.2	175.3±38.6	<0.02
4h	520.3± 69.7	156.9±28.4	<0.01
5h	360.0± 70.6	78.2±16.0	<0.01
6h	229.3± 42.8	83.7± 4.2	<0.10

TABLE V (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Dioestrus	Oestrus	p.
2h	2.3±0.4	1.8±0.5	< 0.10
3h	4.3±0.8	1.2±0.3	< 0.001
4h	4.2±0.8	1.3±0.3	< 0.001
5h	3.9±0.8	1.1±0.4	< 0.02
6h	3.1±0.6	0.9±0.1	< 0.02

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE VI

Urinary Potassium Concentration and Potassium Excretion Rate at Different Stages of the Oestrous Cycle

I. Potassium Concentration (meq/L)

Time after starting	Dioestrus	Oestrus	p.
Saline Infusion			
2h	86.5±16.1	100.3±18.7	<0.60
3h	52.1± 7.6	117.2±22.6	<0.01
4h	54.8±14.2	111.1±18.9	<0.05
5h	54.2± 9.8	104.5±17.8	<0.10
6h	78.4±18.2	117.9±20.7	<0.30

II. Potassium Excretion Rate (μeq/hr)

Time after starting	Dioestrus	Oestrus	p.
Saline Infusion			
2h	146.3±21.8	80.5±17.1	<0.60
3h	121.2±17.8	136.5±17.5	<0.60
4h	147.7±39.4	134.7±24.0	<0.80
5h	122.4±34.0	74.3±15.1	<0.20
6h	75.6±23.7	89.4±14.1	<0.60

TABLE VII

Glomerular Filtration Rate and Urine flow in Ovariectomised Rats Treated with Spironolactone Compared with Ovariectomised Control Animals

I. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Spironolactone Treated Ovx Rats	Control Ovx Rats	p.
2h	(6) 0.62±0.05	(9) 0.73±0.02	<0.20
3h	(6) 0.64±0.02	(9) 0.70±0.03	<0 10
4h	(6) 0.62±0.01	(9) 0.60±0.04	<0.70
5h	(6) 0.64±0.01	(9) 0.64±0.03	<0.60
6h	(6) 0.59±0.01	(9) 0.61±0.03	<0.90

II. Urine Flow (ml/hr)

Time after starting Saline Infusion	Spironolactone Treated Ovx Rats	Control Ovx Rats	p.
2h	1.6±0.2	1.7±0.3	<0.90
3h	2.4±0.2	3.2±0.5	<0.20
4h	3.2±0.2	3.5±0.4	<0.70
5h	3.1±0.4	3.0±0.2	<0.90
6h	2.9±0.3	2.6±0.2	<0.40

Results are expressed as mean ± standard error.

(Numbers of animals in parenthesis).

TABLE VIII

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Spironolactone Compared with Ovariectomised Control Animals

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Ovx Rats	Control Ovx Rats	p.
2h	(6) 209.9± 2.2	(9) 202.8±12.2	<0.80
3h	(6) 204.7±11.8	(9) 188.4±11.4	<0.40
4h	(6) 188.6±12.8	(9) 183.8±10.3	<0.90
5h	(6) 187.2±12.3	(9) 186.6±11.4	<0.90
6h	(6) 184.8± 7.2	(9) 184.8± 6.4	<0.30

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Ovx Rats	Control Ovx Rats	p.
2h	369.7±86.2	354.7±66.8	<0.90
3h	490.1±55.3	604.4±89.2	<0.40
4h	614.6±73.8	623.1±56.7	<0.90
5h	582.1±85.2	559.2±41.1	<0.70
6h	541.9±62.6	477.2±29.9	<0.40

TABLE VIII (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Spironolactone Treated Ovx Rats	Control Ovx Rats	p.
2h	2.4±0.5	3.0±0.1	<0.40
3h	4.7±0.7	4.9±0.4	<0.90
4h	4.4±0.4	5.1±0.4	<0.30
5h	4.2±0.5	4.5±0.3	<0.60
6h	4.2±0.5	5.2±0.5	<0.20

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE IX

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Animals Treated with Spironolactone Compared with Ovariectomised Control Animals.

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion		Spironolactone Treated Ovx Rats		Control Ovx Rats	p.
2h	(6)	104.3±20.5	(9)	74.3±8.2	<0.20
3h	(6)	46.6± 6.4	(9)	40.3±3.8	<0.40
4h	(6)	46.1± 6.9	(9)	38.2±3.7	<0.30
5h	(6)	47.8± 6.3	(9)	42.2±2.5	<0.40
6h	(6)	46.6± 4.9	(9)	37.2±2.7	<0.20

II. Potassium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Ovx Rats	Control Ovx Rats	p.
2h	152.3±14.5	120.2±20.7	<0.30
3h	106.6±11.1	122.7±16.8	<0.50
4h	149.9±25.1	125.4±11.6	<0.40
5h	140.2±14.6	124.0± 7.8	<0.40
6h	130.6± 9.1	93.9± 5.3	<0.01

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE X

Glomerular Filtration Rate and Urine Flow in Rats at Dioestrus Treated with Spironolactone Compared with Dioestrous Control Animals

I. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion		Spironolactone Treated Dioestrous Rats		Control Dioestrous Rats	p.
2h	(6)	0.62+0.06	(6)	0.60+0.05	<0.80
3h	(6)	0.64+0.06	(6)	0.62+0.04	<0.90
4h	(6)	0.58+0.04	(6)	0.61+0.05	<0.80
5h	(6)	0.59+0.05	(6)	0.61+0.03	<0.90
6h	(6)	0.58+0.04	(6)	0.59+0.06	<0.90

II. Urine Flow (ml/hr)

Time after starting Saline Infusion	Spironolactone Treated Dioestrous Rats	Control Dioestrous Rats	p.
2h	1.8+0.3	2.0+0.2	<0.70
3h	2.7+0.2	2.4+0.2	<0.40
4h	3.2+0.5	3.3+0.2	<0.90
5h	2.9+0.3	2.2+0.2	<0.10
6h	2.6+0.2	1.7+0.2	<0.01

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XI

Urinary Sodium Concentration, Rate of Sodium Excretion and Sodium:Potassium Ratio in Rats at Dioestrus, Treated with Spironolactone, Compared with Dioestrous Control Animals

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion		Spironolactone Treated Dioestrous Rats		Dioestrous Control Rats	p.
2h	(6)	208.7±18.4	(6)	167.4±11.8	<0.10
3h	(6)	184.8± 9.6	(6)	189.5±16.7	<0.90
4h	(6)	182.6±10.2	(6)	182.6± 9.2	<0.90
5h	(6)	183.6±12.6	(6)	171.7±17.6	<0.60
6h	(6)	173.2± 7.5	(6)	172.8±16.7	<0.90

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Dioestrous Rats	Control Dioestrous Rats	p.
2h	355.5± 35.7	356.7±76.4	<0.90
3h	490.8± 41.8	465.1±49.6	<0.80
4h	602.1±105.1	600.7±70.4	<0.90
5h	536.7± 77.5	357.8±37.5	<0.20
6h	454.9± 50.0	285.0±37.4	<0.05

TABLE XI (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Spironolactone Treated Dioestrous Rats	Control Dioestrous Rats	p.
2h	3.2±0.2	2.7±0.3	<0.20
3h	3.7±0.4	3.2±0.4	<0.40
4h	3.7±0.3	3.2±0.3	<0.20
5h	4.0±0.3	2.8±0.2	<0.05
6h	4.3±0.3	3.1±0.2	<0.05

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XII

Urinary Potassium Concentration and Potassium Excretion Rate in Rats at Dioestrus, Treated with Spironolactone, Compared with Dioestrous Control Animals

I. Urinary Potassium Concentration (meq/L)

Time after starting Saline Infusion		Spironolactone Treated Dioestrous Rats		Dioestrous Control Rats	p.
2h	(6)	64.7±6.4	(6)	64.2±4.6	<0.9
3h	(6)	52.3±5.9	(6)	61.2±6.0	<0.1
4h	(6)	50.5±5.7	(6)	58.6±7.9	<0.9
5h	(6)	46.9±5.4	(6)	60.9±4.1	<0.10
6h	(6)	40.9±4.0	(6)	58.0±7.0	<0.10

II. Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Dioestrous Rats	Control Dioestrous Rats	p.
2h	108.9± 8.7	126.1±12.6	<0.30
3h	129.5±15.9	150.4±22.0	<0.50
4h	171.2±38.3	195.9±33.5	<0.70
5h	137.0±24.3	128.3±15.5	<0.80
6h	108.2±16.7	95.1±12.5	<0.60

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XIII

Glomerular Filtration Rate and Urine Flow in Rats at Oestrus, Treated with Spironolactone, Compared with Control Oestrous Animals

I. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Spironolactone Treated Oestrous Rats	Control Oestrous Rats	p.
2h	(6) 0.64±0.04	(6) 0.62±0.06	<0.80
3h	(6) 0.62±0.04	(6) 0.64±0.05	<0.80
4h	(6) 0.63±0.05	(6) 0.65±0.04	<0.90
5h	(6) 0.59±0.03	(6) 0.60±0.04	<0.90
6h	(6) 0.61±0.04	(6) 0.60±0.03	<0.90

II. Urine Flow (ml/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestrous Rats	Control Oestrous Rats	p.
2h	1.7±0.4	1.0±0.2	<0.30
3h	2.6±0.4	1.6±0.3	<0.10
4h	3.0±0.1	1.7±0.2	<0.001
5h	3.0±0.2	1.2±0.2	<0.001
6h	2.3±0.4	0.8±0.1	<0.02

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XIV

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Rats at Oestrus, Treated with Spironolactone, Compared with Control Oestrous Animals

I. Urinary Sodium Concentration (meq/L)

Time after starting Saline Infusion		Spironolactone Treated Oestrous Rats	Control Oestrous Rats	p.
2h	(6)	203.6±16.9	(6) 128.2±3.8	<0.01
3h	(6)	228.5±22.7	(6) 126.3±9.1	<0.001
4h	(6)	217.7±15.9	(6) 109.9±5.8	<0.001
5h	(6)	191.3±13.8	(6) 108.0±4.3	<0.001
6h	(6)	189.8± 8.5	(6) 101.1±2.1	<0.001

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestrous Rats	Control Oestrous Rats	p.
2h	253.9±50.9	125.6±23.4	<0.05
3h	505.8±80.8	192.0±27.6	<0.02
4h	563.9±96.0	188.9±17.3	<0.02
5h	501.0±88.6	130.0±17.7	<0.02
6h	389.0±101.1	83.1± 6.7	<0.05

TABLE XIV (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Spironolactone Treated Oestrous Rats	Control Oestrous Rats	p.
2h	2.7±0.3	1.3±0.1	<0.01
3h	3.4±0.2	1.3±0.1	<0.001
4h	3.6±0.3	1.1±0.1	<0.001
5h	3.3±0.2	1.2±0.1	<0.001
6h	3.4±0.2	1.1±0.1	<0.001

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XV

The Action of Spironolactone on Urinary Potassium Concentration and Rate of Potassium Excretion in Rats at Oestrus

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Oestrous Rats	Control Oestrous Rats	p.
2h	80.7±12.7	100.0±9.5	<0.3
3h	67.7± 6.8	101.9±5.4	<0.01
4h	59.6± 4.8	97.4±3.8	<0.001
5h	58.1± 2.5	87.3±5.5	<0.001
6h	55.6± 4.3	92.9±5.6	<0.001

II. Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestrous Rats	Control Oestrous Rats	p.
2h	114.0±14.4	96.4±18.9	<0.40
3h	165.2±16.6	161.2±36.4	<0.90
4h	179.3±14.2	169.5±21.7	<0.70
5h	174.3±11.6	105.6±18.3	<0.01
6h	136.2±30.3	75.9± 5.9	<0.20

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XVI

Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Oestradiol for 14 days, Compared with Ovariectomised Control Rats

I. Urine Flow (ml/hr)

Time after starting Saline Infusion		Oestradiol Treated		Ovariectomised Controls	p.
2h	(6)	0.7±0.1	(6)	1.6±0.4	<0.02
3h	(6)	1.1±0.1	(6)	2.8±0.3	<0.001
4h	(6)	1.7±0.3	(6)	3.4±0.3	<0.01
5h	(6)	1.6±0.3	(6)	3.5±0.5	<0.01
6h	(6)	1.1±0.3	(6)	2.7±0.4	<0.02

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	0.60±0.05	0.64±0.03	<0.60
3h	0.63±0.04	0.63±0.03	<0.90
4h	0.63±0.04	0.62±0.03	<0.90
5h	0.61±0.03	0.58±0.02	<0.50
6h	0.60±0.03	0.58±0.04	<0.60

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XVII

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Oestradiol for 14 days Compared with Ovariectomised Rats

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	83.4±22.7	185.8± 7.8	<0.01
3h	112.3±12.8	179.7±18.2	<0.01
4h	113.8±15.6	179.3±11.6	<0.02
5h	121.0±16.5	183.7± 9.8	<0.02
6h	112.3±12.3	180.4± 5.7	<0.01

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	67.7±25.2	305.4± 72.3	<0.01
3h	122.8±25.8	521.4± 99.5	<0.01
4h	214.4±56.4	627.5± 91.6	<0.001
5h	194.2±41.5	640.5±103.8	<0.01
6h	129.7±33.6	466.7± 78.6	<0.01

TABLE XVII (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	0.6±0.2	2.7±0.1	<0.001
3h	0.7±0.2	3.7±0.3	<0.001
4h	0.6±0.1	4.2±0.4	<0.001
5h	0.7±0.1	4.3±0.9	<0.001
6h	0.7±0.1	4.2±0.2	<0.001

TABLE XVIII

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated with Oestradiol for 14 Days Compared with Ovariectomised Control Rats

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	166.6±16.2	71.4±4.0	<0.001
3h	180.3±25.0	48.1±1.9	<0.001
4h	179.1±14.6	43.6±2.8	<0.001
5h	163.2±11.4	46.2±6.8	<0.001
6h	158.1± 8.3	41.7±1.2	<0.001

II. Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	121.3±20.6	114.3±26.9	<0.90
3h	192.6±32.4	128.2±19.2	<0.30
4h	335.6±70.1	149.5±13.8	<0.10
5h	256.3±52.5	153.6±23.5	<0.20
6h	189.4±34.7	110.9±16.7	<0.20

TABLE XIX

Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Progesterone for 14 Days Compared with Ovariectomised Control Rats

I. Urine Flow (ml/hr)

Time after starting Saline Infusion		Progesterone Treated		Ovariectomised Controls	p.
2h	(6)	0.8±0.1	(6)	1.6±0.4	<0.10
3h	(6)	1.3±0.3	(6)	2.8±0.3	<0.01
4h	(6)	1.6±0.3	(6)	3.4±0.3	<0.01
5h	(6)	1.1±0.1	(6)	3.5±0.5	<0.001
6h	(6)	0.9±0.1	(6)	2.7±0.4	<0.01

II. Glomerular Filtration Rate (ml/min/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	0.62±0.05	0.63±0.03	<0.90
3h	0.62±0.05	0.63±0.03	<0.90
4h	0.62±0.03	0.62±0.03	<0.90
5h	0.60±0.03	0.58±0.02	<0.70
6h	0.58±0.02	0.57±0.04	<0.90

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XX

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated for 14 Days with Progesterone Compared with Ovariectomised Control Animals.

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	126.3± 5.2	191.2± 7.5	<0.001
3h	86.2±15.2	179.7±18.2	<0.01
4h	79.0±15.3	179.3±11.7	<0.01
5h	89.8±10.7	183.7± 9.8	<0.001
6h	81.9± 9.4	176.1± 9.5	<0.001

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	102.7±14.9	305.4± 72.3	<0.01
3h	108.2±28.1	521.4± 99.5	<0.01
4h	133.3±33.7	627.5± 91.6	<0.001
5h	93.9±10.7	640.5±103.8	<0.001
6h	71.3± 8.6	466.7± 78.6	<0.001

TABLE XX (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	0.9±0.2	2.7±0.1	<0.001
3h	0.6±0.2	3.7±0.2	<0.001
4h	0.5±0.2	4.2±0.4	<0.001
5h	0.6±0.1	4.3±0.9	<0.001
6h	0.5±0.1	4.2±0.3	<0.001

TABLE XXI

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated for 14 Days with Progesterone Compared with Ovariectomised Control Animals.

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	149.1±24.9	71.4±4.0	<0.05
3h	158.2±24.4	48.1±1.9	<0.01
4h	182.0±22.0	43.6±2.8	<0.01
5h	173.5±15.5	46.2±6.8	<0.001
6h	186.3±12.6	41.7±1.2	<0.001

II. Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	154.2±45.5	114.3±26.9	<0.5
3h	199.6±61.3	138.2±19.2	<0.5
4h	331.4±82.5	149.5±13.8	<0.1
5h	184.1±29.2	153.6±23.5	<0.5
6h	164.8±15.5	110.4±16.7	<0.05

TABLE XXII

Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Oestradiol and Progesterone for 14 Days Compared with Ovariectomised Control Rats.

I. Urine Flow (ml/hr)

Time after starting Saline Infusion	Oestradiol + Progesterone Treated	Ovariectomised Controls	p.
2h	(4) 0.8±0.2	(4) 1.6±0.4	<0.1
3h	(4) 1.6±0.3	(4) 2.8±0.3	<0.05
4h	(4) 1.3±0.3	(4) 3.4±0.3	<0.01
5h	(4) 1.1±0.2	(4) 3.5±0.5	<0.01
6h	(4) 0.9±0.1	(4) 2.7±0.4	<0.01

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Oestradiol + Progesterone Treated	Ovariectomised Controls	p.
2h	0.61±0.06	0.64±0.03	<0.80
3h	0.63±0.06	0.63±0.03	<0.90
4h	0.61±0.07	0.63±0.03	<0.90
5h	0.61±0.06	0.58±0.02	<0.80
6h	0.61±0.07	0.58±0.05	<0.80

Results are expressed as mean ± standard error

(Number of animals in parenthesis).

TABLE XXIII

Urinary Sodium Concentration, Rate of Sodium Excretion and Sodium:Potassium Ratio in Ovariectomised Rats Treated for 14 Days with Oestradiol + Progesterone compared with Ovariectomised Control Animals.

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Oestradiol + Progesterone Treated	Ovariectomised Controls	p.
2h	105.6±14.4	191.2 ± 7.8	<0.01
3h	83.9±10.4	179.7±18.2	<0.01
4h	86.9± 6.8	179.3±11.7	<0.001
5h	73.8±11.6	183.7± 9.8	<0.001
6h	59.8± 8.4	176.5± 5.7	<0.001

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Oestradiol + Progesterone Treated	Ovariectomised Controls	p.
2h	82.8± 22.8	305.4± 72.3	<0.05
3h	144. 4± 20.0	521.4± 99.5	<0.01
4h	111.9± 17.2	627.5± 91.6	<0.01
5h	80.8± 19.77	640.5±103.8	<0.01
6h	57.4± 14.0	466.7± 78.6	<0.01

TABLE XXIII (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Oestrogen + Progesterone Treated	Ovariectomised Controls	p.
2h	0.7±0.2	2.7±0.1	<0.001
3h	0.5±0.1	3.7±0.3	<0.001
4h	0.5±0.1	4.2±0.4	<0.001
5h	0.4±0.1	4.3±0.9	<0.01
6h	0.3±0.1	4.2±0.3	<0.01

TABLE XXIV

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated for 14 Days with Oestradiol and Progesterone Compared with Ovariectomised Control Rats

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Oestrogen + Progesterone Treated	Ovariectomised Controls	p.
2h	187.5±39.0	71.4±4.0	<0.05
3h	177.5±25.2	48.0±1.9	<0.01
4h	189.6±23.0	43.6±2.8	<0.001
5h	191.0±23.3	46.2±6.8	<0.001
6h	174.9±20.3	41.7±1.2	<0.001

Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Oestrogen + Progesterone	Ovariectomised Controls	p.
2h	157.4±55.2	114.3±26.9	<0.60
3h	307.1±88.1	138.2±19.2	<0.20
4h	263.0±70.7	149.5±13.8	<0.20
5h	198.2±15.4	153.6±23.5	<0.20
6h	159.3±21.7	110.4±16.7	<0.20

TABLE XXV

The Action of Spironolactone on Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Oestradiol for 14 Days

I. Urine Flow (ml/hr)

Time after starting Saline Infusion		Spironolactone Treated Oestradiol Injected		Oestradiol Injected Controls	p.
2h	(6)	1.5±0.3	(6)	0.7±0.1	<0.01
3h	(6)	2.7±0.5	(6)	1.0±0.1	<0.01
4h	(6)	3.2±0.4	(6)	1.7±0.3	<0.05
5h	(6)	3.5±0.4	(6)	1.6±0.3	<0.01
6h	(6)	2.6±0.4	(6)	1.1±0.3	<0.02

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	0.64±0.04	0.60±0.05	<0.70
3h	0.62±0.04	0.64±0.04	<0.90
4h	0.63±0.04	0.63±0.04	<0.90
5h	0.63±0.03	0.61±0.03	<0.70
6h	0.61±0.03	0.60±0.03	<0.80

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XXVI

The Action of Spironolactone on Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated for 14 Days with Oestradiol

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	177.4± 9.8	83.6±22.7	<0.01
3h	197.9±17.2	119.6±12.8	<0.01
4h	191.3±23.3	113.7±15.6	<0.02
5h	193.9±17.7	121.0±16.5	<0.02
6h	181.9±14.4	112.3±12.3	<0.01

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	263.3± 45.6	67.7±25.2	<0.01
3h	524.6± 84.6	122.8±25.8	<0.001
4h	633.4±136.2	214.4±56.4	<0.02
5h	631.0±134.5	194.2±41.5	<0.01
6h	442.5±102.3	129.7±33.6	<0.02

TABLE XXVI (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	2.6±0.1	0.6±0.1	<0.001
3h	3.8±0.5	0.7±0.2	<0.001
4h	3.8±0.4	0.6±0.1	<0.001
5h	4.1±0.3	0.7±0.1	<0.001
6h	4.1±0.6	0.7±0.1	<0.001

TABLE XXVII

The Action of Spironolactone on Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated for 14 Days with Oestradiol

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	71.8±1.6	132.5±42.2	<0.20
3h	46.1±1.4	166.7±16.2	<0.001
4h	51.8±5.9	172.2±18.8	<0.001
5h	45.6±3.3	179.1±14.1	<0.001
6h	46.7±4.0	163.2±11.4	<0.001

II. Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	105.1±15.0	121.3±20.6	<0.60
3h	123.7±21.1	192.5±32.4	<0.20
4h	175.4±43.2	335.6±70.1	<0.10
5h	148.8±27.9	256.3±52.5	<0.20
6h	114.7±24.1	189.4±34.7	<0.20

TABLE XXVIII

The Action of Spironolactone on Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated for 14 Days with Progesterone

I. Urine Flow (ml/hr)

Time after starting Saline Infusion		Spironolactone Treated Progesterone Injected		Progesterone Injected Controls	p.
2h	(6)	1.3±0.3	(6)	0.9±0.1	<0.20
3h	(6)	2.2±0.2	(6)	1.2±0.2	<0.02
4h	(6)	3.4±0.1	(6)	1.6±0.3	<0.001
5h	(6)	3.6±0.3	(6)	1.1±0.1	<0.001
6h	(6)	3.1±0.3	(6)	0.9±0.10	<0.001

II. Glomerular Filtration Rate (ml/min/hr)

Time after starting Saline Infusion	Spironolactone Treated Progesterone Injected	Progesterone Injected Controls	p.
2h	0.65±0.05	0.62±0.01	<0.70
3h	0.68±0.04	0.62±0.05	<0.40
4h	0.66±0.04	0.62±0.03	<0.50
5h	0.63±0.03	0.60±0.03	<0.60
6h	0.63±0.03	0.58±0.02	<0.40

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XXIX

The Action of Spironolactone on Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated for 14 Days with Progesterone

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Progesterone Injected	Progesterone Injected Controls	p.
2h	241.3±37.9	126.3± 5.2	<0.02
3h	225.1±20.2	81.4±13.8	<0.001
4h	207.2±26.4	79.0±15.3	<0.01
5h	204.6±26.9	89.8±10.7	<0.01
6h	199.5±26.0	81.9± 9.4	<0.01

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Progesterone Injected	Progesterone Injected Controls	p.
2h	377.0±134.8	102.7±14.9	<0.10
3h	523.0± 87.0	108.2±28.1	<0.001
4h	701.2± 82.3	133.3±33.7	<0.001
5h	770.7±166.0	93.9±10.7	<0.001
6h	674.2±142.8	71.3±20.9	<0.001

TABLE XXIX (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Spironolactone Treated Progesterone Injected	Progesterone Injected Controls	p.
2h	3.7±0.6	0.9±0.2	<0.001
3h	3.6±0.2	0.6±0.2	<0.001
4h	3.7±0.3	0.5±0.2	<0.001
5h	3.5±0.2	0.6±0.1	<0.001
6h	3.3±0.2	0.5±0.1	<0.001

TABLE XXX

The Action of Spironolactone on Urinary Potassium Concentration and Rate of Excretion in Ovariectomised Rats Treated with Progesterone for 14 days

I. Potassium Concentration (meq/L)

Time after Starting Saline Infusion	Spironolactone Treated + Pro-	Control Progesterone	p.
2h	64.4±3.7	149.1±24.9	<0.01
3h	63.7±7.5	158.2±24.4	<0.01
4h	56.8±7.3	182.0±22.0	<0.001
5h	58.3±6.8	173.5±15.5	<0.001
6h	61.1±6.7	186.3±12.6	<0.001

II. Potassium Excretion Rate (μeq/hr)

Time after Starting Saline Infusion	Spironolactone Treated + Pro-	Control Progesterone	p.
2h	87.5±21.7	154.2±45.5	<0.20
3h	138.6±21.7	199.6±61.3	<0.40
4h	175.2±28.5	298.9±82.5	<0.10
5h	216.9±40.9	184.1±29.2	<0.60
6h	202.4±35.9	164.8±15.5	<0.40

TABLE XXXI

The Action of Spironolactone on Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Oestradiol and Progesterone

I. Urine Flow (ml/hr)

Time after starting Saline Infusion		Spironolactone Treated Oestradiol + Progesterone		Control Oestradiol + Progesterone	p.
2h	(4)	1.3±0.3	(4)	0.8±0.2	<0.30
3h	(4)	2.8±0.4	(4)	1.6±0.3	<0.10
4h	(4)	3.7±0.3	(4)	1.3±0.3	<0.001
5h	(4)	2.8±0.1	(4)	1.1±0.2	<0.001
6h	(4)	2.0±0.2	(4)	0.9±0.1	<0.001

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol + Progesterone	Control Oestradiol + Progesterone	p.
2h	0.64±0.05	0.61±0.06	<0.80
3h	0.67±0.08	0.63±0.06	<0.70
4h	0.66±0.07	0.61±0.07	<0.60
5h	0.63±0.03	0.61±0.06	<0.80
6h	0.58±0.04	0.61±0.07	<0.80

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XXXII

The Action of Spironolactone on Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Oestradiol and Progesterone

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol + Progesterone	Control Oestradiol + Progesterone	p.
2h	194.9± 4.7	105.6±14.4	<0.001
3h	175.4±19.2	83.9±10.4	<0.001
4h	186.9±13.4	86.9± 6.8	<0.001
5h	183.7± 9.4	73.8±11.6	<0.001
6h	152.2±33.8	59.7± 8.4	<0.05

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol + Progesterone	Control Oestradiol + Progesterone	p.
2h	255.0±66.5	82.8± 22.8	<0.05
3h	509.3±94.8	144.4± 20.0	<0.001
4h	701.2±82.3	111.9± 17.2	<0.001
5h	516.4±41.2	80.8± 19.8	<0.001
6h	355.2±51.7	57.4± 14.0	<0.001

TABLE XXXII (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Spironolactone Treated Oestradiol + Progesterone	Control Oestradiol + Progesterone	p.
2h	3.2±0.4	0.7±0.1	<0.01
3h	2.8±0.2	0.5±0.1	<0.001
4h	3.0±0.2	0.5±0.1	<0.001
5h	3.2±0.3	0.4±0.1	<0.001
6h	3.3±0.1	0.3±0.1	<0.001

TABLE XXXIII

The Action of Spironolactone on Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated with Oestradiol and Progesterone

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol + Progesterone	Control Oestradiol + Progesterone	p.
2h	64.2±6.3	187.5±39.0	<0.05
3h	63.1±2.9	183.3±27.9	<0.01
4h	61.5±3.8	191.0±23.3	<0.01
5h	59.3±5.2	174.9±20.3	<0.01
6h	52.8±4.1	148.1±12.5	<0.001

II. Potassium Excretion Rate (μeq/hr)

Time After Starting Saline Infusion	Spironolactone Treated Oestradiol + Progesterone	Control Oestradiol + Progesterone	p.
2h	83.2±25.2	157.4±55.2	<0.30
3h	182.2±29.2	307.1±88.1	<0.30
4h	230.0±24.4	263.0±70.7	<0.70
5h	166.5±17.9	198.2±15.4	<0.30
6h	106.3±17.5	159.3±21.7	<0.20

TABLE XXXIV

Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Oestradiol 2 Hours Prior to Experimentation Compared with Ovariectomised Control Animals

I. Urine Flow (ml/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	(6) 1.8±0.4	(6) 1.8±0.2	<0.90
3h	(6) 2.3±0.4	(6) 2.9±0.3	<0.50
4h	(6) 2.6±0.2	(6) 3.3±0.3	<0.05
5h	(6) 1.5±0.2	(6) 3.6±0.8	<0.02
6h	(6) 1.0±0.1	(6) 2.1±0.4	<0.02

II. Glomerular Filtration Rate (ml/min/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	0.59±0.05	0.66±0.07	<0.40
3h	0.62±0.06	0.67±0.03	<0.50
4h	0.62±0.06	0.64±0.03	<0.90
5h	0.63±0.05	0.61±0.03	<0.90
6h	0.62±0.04	0.61±0.03	<0.70

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XXXV

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Oestradiol 2 Hours Prior to Experimentation, Compared with Ovariectomised Control Animals.

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Control	p.
2h	202.9±18.6	203.6± 9.6	<0.90
3h	186.5±16.1	189.1± 3.7	<0.60
4h	158.1±14.5	195.6± 8.1	<0.10
5h	104.3±10.6	185.8±18.8	<0.01
6h	102.9± 5.9	186.9± 7.7	<0.001

II. Sodium Excretion Rate (meq/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Control	p.
2h	402.6±47.5	366.5± 51.2	<0.50
3h	460.5±58.6	562.6± 62.6	<0.30
4h	403.4±49.8	640.3± 56.4	<0.02
5h	158.5±28.3	635.3±129.1	<0.01
6h	105.1±10.1	390.4± 76.1	<0.01

TABLE XXXV (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Control	p.
2h	2.3±0.1	2.9±0.3	<0.90
3h	2.2±0.2	3.8±0.5	<0.01
4h	1.4±0.2	4.9±0.8	<0.001
5h	0.7±0.1	4.8±0.7	<0.001
6h	0.7±0.1	5.1±0.7	<0.001

TABLE XXXVI

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated with Oestradiol 2 Hours Prior to Experimentation, Compared with Ovariectomised Control Animals

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Control	p.
2h	86,8± 5.5	83.4± 8.4	<0.80
3h	88.5± 6.1	46.8± 2.6	<0.30
4h	118.4± 9.7	40.8± 4.5	<0.001
5h	160.2±14.4	53.9± 7.6	<0.001
6h	156.4± 4.5	41.9±11.3	<0.001

II. Potassium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Control	p.
2h	180.0±29.5	158.1±16.7	<0.60
3h	220.9±28.9	152.1±27.7	<0.20
4h	293.6±16.6	136.1±18.6	<0.001
5h	213.3±25.7	140.9±33.2	<0.20
6h	160.9±17.2	76.2±10.9	<0.01

TABLE XXXVII

The Action of Spironolactone on Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Oestradiol 2 Hours Prior to Experimentation.

I. Urine Flow (ml/hr)

Time after starting Saline Infusion		Spironolactone Treated Oestradiol Injected		Oestradiol Injected Controls	p.
2h	(6)	1.6±0.3	(6)	2.1±0.3	<0.40
3h	(6)	2.1±0.6	(6)	2.6±0.4	<0.50
4h	(6)	3.1±0.4	(6)	2.5±0.2	<0.30
5h	(6)	3.1±0.5	(6)	1.5±0.2	<0.01
6h	(6)	3.2±0.6	(6)	1.0±0.1	<0.001

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	0.60±0.06	0.60±0.05	<0.90
3h	0.63±0.04	0.62±0.06	<0.90
4h	0.63±0.04	0.62±0.06	<0.90
5h	0.59±0.03	0.63±0.05	<0.70
6h	0.56±0.06	0.62±0.04	<0.50

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XXXVIII

The Action of Spironolactone on Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Oestradiol 2 Hours Prior to Experimentation

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	213.0±15.4	202.9±18.5	<0.80
3h	218.5± 9.6	186.5±16.0	<0.60
4h	177.2±13.3	158.1±14.1	<0.40
5h	192.4±18.1	104.3±10.6	<0.01
6h	180.8±17.1	102.9± 5.9	<0.01

II. Sodium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	352.0±53.7	402.6±47.5	<0.40
3h	430.4±92.3	460.5±58.6	<0.80
4h	643.4±49.2	403.4±49.8	<0.02
5h	600.3±81.5	158.4±28.2	<0.001
6h	562.2±50.1	105.1±10.1	<0.001

TABLE XXXVIII (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	3.2±0.4	2.3±0.1	<0.90
3h	4.5±0.4	2.2±0.2	<0.001
4h	4.0±0.5	1.4±0.2	<0.001
5h	4.3±0.5	0.7±0.1	<0.001
6h	4.0±0.5	0.7±0.1	<0.001

TABLE XXXIX

The Action of Spironolactone on Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated with Oestradiol 2 Hours Prior to Experimentation

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	69.9±4.6	86.8± 5.5	<0.10
3h	49.3±5.3	88.5± 6.1	<0.01
4h	54.1±4.2	118.3± 9.7	<0.001
5h	48.2±3.7	160.2±14.3	<0.001
6h	45.5±3.0	156.4± 4.6	<0.001

II. Potassium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Spironolactone Treated Oestradiol Injected	Oestradiol Injected Controls	p.
2h	116.7±23.4	180.0±29.5	<0.20
3h	97.6±24.2	220.9±28.9	<0.02
4h	170.4±30.5	293.6±16.6	<0.01
5h	144.7±23.4	213.3±25.7	<0.20
6h	144.0± 9.7	160.9±17.2	<0.50

TABLE XL

Glomerular Filtration Rate and Urine Flow in Ovariectomised Animals Treated with Oestradiol 12 Hours Prior to Experimentation, Compared with Ovariectomised Control Animals

I. Urine Flow (ml/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	(5) 2.0±0.4	(5) 1.9±0.5	< 0.70
3h	(5) 3.3±0.4	(5) 3.2±0.4	< 0.90
4h	(5) 3.5±0.6	(5) 3.4±0.5	< 0.90
5h	(5) 3.2±0.6	(5) 2.5±0.3	< 0.40
6h	(5) 2.5±0.4	(5) 2.9±0.3	< 0.50

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	0.62±0.05	0.64±0.03	< 0.90
3h	0.58±0.05	0.63±0.04	< 0.50
4h	0.60±0.03	0.61±0.04	< 0.90
5h	0.60±0.05	0.62±0.03	< 0.70
6h	0.59±0.05	0.58±0.04	< 0.90

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XLI

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Oestradiol 12 Hours Prior to Experimentation, Compared with Ovariectomised Control Animals

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	189.8±16.2	190.6±18.2	<0.90
3h	182.6± 7.3	166.3±12.3	<0.80
4h	166.9±15.0	183.7±15.6	<0.80
5h	185.2± 6.3	188.0± 5.7	<0.90
6h	189.6±13.1	189.1± 8.0	<0.90

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	374.4±79.5	323.5±105.8	<0.80
3h	605.9±87.6	584.3± 92.5	<0.90
4h	553.9±49.8	581.7±114.1	<0.90
5h	598.9±91.8	478.0± 83.9	<0.40
6h	460.1±65.3	496.8± 90.3	<0.80

TABLE XLI(Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Control	p.
2h	2.6±0.3	2.4±0.4	<0.80
3h	4.0±0.3	3.9±0.3	<0.80
4h	3.8±0.3	4.2±0.5	<0.60
5h	3.9±0.2	3.7±0.7	<0.80
6h	4.3±0.3	4.6±0.9	<0.80

TABLE XLII

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated with Oestradiol 12 Hours Prior to Experimentation, Compared with Ovariectomised Control Animals

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	74.9±3.4	83.4± 8.4	<0.40
3h	46.6±2.9	46.6± 2.6	<0.90
4h	44.3±4.7	40.8± 4.5	<0.70
5h	47.7±1.9	53.9± 7.6	<0.50
6h	44.6±1.0	41.9±11.3	<0.80

II. Potassium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Oestradiol Treated	Ovariectomised Controls	p.
2h	102.1±18.9	139.1± 41.8	<0.40
3h	164.9±16.7	152.2± 26.2	<0.70
4h	179.3±14.2	136.2± 18.8	<0.20
5h	174.4±11.6	132.9± 12.0	<0.05
6h	136.7±30.7	186.0± 20.0	<0.30

TABLE XLIII

Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Progesterone 2 Hours Prior to Experimentation Compared with Ovariectomised Control Animals

I. Urine Flow (ml/hr)

Time after starting Saline Infusion		Progesterone Treated		Ovariectomised Controls	p.
2h	(6)	1.9±0.4	(6)	1.8±0.2	<0.90
3h	(6)	3.1±0.5	(6)	2.9±0.3	<0.80
4h	(6)	3.7±0.2	(6)	3.3±0.3	<0.90
5h	(6)	2.7±0.2	(6)	3.6±0.8	<0.90
6h	(6)	2.5±0.2	(6)	2.1±0.4	<0.50

II. Glomerular Filtration Rate (ml/min/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomisedd Controls	p.
2h	0.69±0.04	0.66±0.07	<0.70
3h	0.66±0.02	0.67±0.03	<0.90
4h	0.60±0.02	0.64±0.04	<0.50
5h	0.59±0.01	0.61±0.03	<0.70
6h	0.59±0.01	0.61±0.03	<0.70

Results are expressed as mean ± standard error.

(Number of animals in parenthesis).

TABLE XLIV

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Progesterone 2 Hours Prior to Experimentation Compared with Ovariectomised Control Animals

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	206.9±11.6	203.6± 9.6	<0.90
3h	186.9± 7.7	189.1± 3.7	<0.90
4h	182.6±11.1	195.6± 8.1	<0.60
5h	168.5± 7.4	185.8±18.8	<0.60
6h	172.8± 6.7	186.9± 7.7	<0.50

II. Sodium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	474.7±92.9	366.5± 51.2	<0.50
3h	576.2±84.0	562.6± 62.6	<0.90
4h	582.7±58.9	640.3± 56.4	<0.40
5h	449.5±30.4	635.3±129.5	<0.20
6h	427.1±32.8	390.4± 76.1	<0.90

TABLE XLIV (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	2.6±0.4	2.9±0.3	<0.60
3h	3.8±0.1	3.8±0.5	<0.90
4h	4.1±0.3	4.9±0.8	<0.40
5h	3.6±0.4	4.8±0.7	<0.20
6h	3.6±0.3	5.1±0.7	<0.20

TABLE XLV

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated with Progesterone 2 Hours Prior to Experimentation Compared with Ovariectomised Control Animals

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	89.4±19.0	83.4± 8.4	<0.9
3h	48.7± 2.3	46.8± 2.6	<0.9
4h	45.5± 4.8	40.8± 4.5	<0.9
5h	47.4± 3.7	53.9± 7.6	<0.9
6h	50.6± 2.6	41.9±11.3	<0.8

II. Potassium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	157.0±13.8	158.1±16.7	<0.90
3h	148.6±18.2	152.1±27.7	<0.90
4h	144.3±16.3	136.1±18.6	<0.90
5h	125.7± 7.9	140.9±33.2	<0.70
6h	117.7±12.2	76.2±10.9	<0.05

TABLE XLVI

Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Treated with Progesterone 2 Hours After Starting Saline Infusion Compared with Ovariectomised Control Animals

I. Urine Flow (ml/min)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	(5) 1.3±0.3	(5) 1.4±0.3	<0.80
3h	(5) 2.8±0.3	(5) 2.4±0.6	<0.50
4h	(5) 3.5±0.3	(5) 3.7±0.6	<0.80
5h	(5) 3.0±0.1	(5) 3.1±0.7	<0.90
6h	(5) 2.3±0.2	(5) 2.0±0.3	<0.50

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	0.60±0.07	0.60±0.05	<0.90
3h	0.60±0.06	0.63±0.05	<0.90
4h	0.60±0.05	0.62±0.03	<0.70
5h	0.59±0.06	0.60±0.03	<0.90
6h	0.59±0.06	0.61±0.03	<0.90

Results are expressed as mean ± standard error

(Number of animals in parenthesis).

TABLE XLVII

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Treated with Progesterone 2 Hours after Starting Saline Infusion Compared with Ovariectomised Control Animals

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	206.8±11.6	201.0±11.1	<0.90
3h	186.9± 7.7	184.0± 9.0	<0.90
4h	182.6±11.1	185.0±10.0	<0.90
5h	168.5± 7.4	178.2±11.2	<0.50
6h	172.8± 6.7	181.3±18.1	<0.60

II. Sodium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	318.6±95.5	281.5±60.8	<0.80
3h	519.9±81.4	456.6±28.7	<0.60
4h	621.9±57.9	672.5±78.0	<0.50
5h	505.2±34.2	528.4±38.7	<0.80
6h	409.2±38.1	372.3±56.6	<0.80

TABLE XLVII (Cont.)

III. Sodium:Potassium Ratio

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	2.6±0.3	2.4±0.3	<0.50
3h	4.1±0.4	3.9±0.3	<0.80
4h	3.9±0.2	4.2±0.3	<0.40
5h	3.2±0.2	2.9±0.3	<0.50
6h	4.1±0.7	4.4±0.3	<0.90

TABLE XLVIII

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Treated with Progesterone 2 Hours After Starting Saline Infusion compared with Ovariectomised Control Animals

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	80.7±19.0	78.0±9.0	<0.70
3h	47.7± 4.2	42.1±4.0	<0.80
4h	46.0± 4.8	41.0±3.8	<0.80
5h	52.2± 3.7	42.2±2.8	<0.80
6h	48.1± 2.2	40.3±3.2	<0.20

II. Potassium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Progesterone Treated	Ovariectomised Controls	p.
2h	116.5±28.5	111.9±20.0	<0.9
3h	126.3±18.2	115.6±15.5	<0.8
4h	158.4±13.8	152.7±16.8	<0.9
5h	158.9±10.0	126.6±17.2	<0.2
6h	111.3±27.0	84.6±12.5	<0.5

TABLE XLIX

Glomerular Filtration Rate and Urine Flow in Ovariectomised Rats Infused with Progesterone for 6 Hours Compared with Ovariectomised Control Animals

I. Urine Flow (ml/min)

Time after starting Saline Infusion		Progesterone Infusion		Ovariectomised Controls	p.
2h	(6)	1.2±0.2	(9)	1.7±0.3	<0.20
3h	(6)	2.5±0.1	(9)	3.2±0.5	<0.20
4h	(6)	3.4±0.2	(9)	3.5±0.4	<0.90
5h	(6)	3.2±0.3	(9)	3.0±0.2	<0.90
6h	(6)	2.5±0.3	(8)	2.6±0.2	<0.90

II. Glomerular Filtration Rate (ml/min/100g)

Time after starting Saline Infusion	Progesterone Infusion	Ovariectomised Controls	p.
2h	0.68±0.03	0.73±0.02	<0.20
3h	0.67±0.03	0.71±0.03	<0.40
4h	0.62±0.03	0.63±0.04	<0.80
5h	0.61±0.03	0.64±0.03	<0.40
6h	0.59±0.03	0.61±0.03	<0.80

Results are expressed as mean ± standard error (Number of animals in parenthesis).

TABLE L

Urinary Sodium Concentration, Sodium Excretion Rate and Sodium:Potassium Ratio in Ovariectomised Rats Infused with Progesterone for 6 Hours Compared with Ovariectomised Control Animals

I. Sodium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Infusion	Ovariectomised Controls	p.
2h	172.5±18.3	202.8±12.2	<0.10
3h	180.8±15.3	188.4±11.4	<0.90
4h	186.3±12.1	183.8±10.3	<0.90
5h	179.1±11.9	186.6±11.4	<0.90
6h	184.2± 9.8	184.8± 6.4	<0.90

II. Sodium Excretion Rate (μeq/hr)

Time after starting Saline Infusion	Progesterone Infusion	Ovariectomised Controls	p.
2h	206.7± 24.6	334.4±69.2	<0.10
3h	459.8±109.8	623.1±56.7	<0.10
4h	642.4± 70.7	559.2±41.1	<0.20
5h	570.7± 71.3	477.2±29.9	<0.20
6h	462.1± 39.9	392.9±31.5	<0.20

TABLE LI

Urinary Potassium Concentration and Potassium Excretion Rate in Ovariectomised Rats Infused with Progesterone for 6 Hours Compared with Ovariectomised Control Animals

I. Potassium Concentration (meq/L)

Time after starting Saline Infusion	Progesterone Infusion	Ovariectomised Controls	p.
2h	83.6± 7.4	74.3±8.2	<0.80
3h	68.2± 4.2	40.3±3.8	<0.01
4h	62.6±10.6	38.2±3.7	<0.20
5h	52.3± 5.3	42.2±2.5	<0.20
6h	55.9± 5.2	37.2±2.7	<0.05

II. Potassium Excretion Rate (µeq/hr)

Time after starting Saline Infusion	Progesterone Infusion	Ovariectomised Controls	p.
2h	107.6±23.4	120.2±20.7	<0.60
3h	172.5±13.8	122.7±16.8	<0.10
4h	213.0±37.3	125.4±11.5	<0.01
5h	160.6± 6.2	124.0± 7.8	<0.01
6h	136.8± 5.3	93.9± 5.3	<0.05

III Sodium:Potassium Ratio

Time after starting Saline Infusion	Progesterone Infusion	Ovariectomised Controls	p.
2h	2.2±0.3	3.0±0.1	0.05
3h	2.6±0.2	4.9±0.4	0.001
4h	3.3±0.5	5.1±0.4	0.01
5h	3.5±0.4	4.5±0.3	0.01
6h	3.3±0.3	5.2±0.5	0.01

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