# SOME ACTIONS OF GROWTH HORMONE ON THE PERFUSED CAT KIDNEY

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Two defects in the function of perfused cat kidneys result from the use of blood from headless or hypophysectomized animals. First, kidneys perfused with such blood excrete an increased proportion of the filtered load of sodium at raised rates of urine flow (de Lima & Lockett, 1961). The defect in the reabsorption of salt and water is in the proximal parts of the nephron (de Lima & Lockett, 1963) and cannot be corrected by the addition of neurohypophysial hormones alone. Secondly, these kidneys respond to aldosterone by diuresis when perfused with blood from hypophysectomized or headless animals (Davey & Lockett, 1960) as do hypophysectomized rats (Lockett & Roberts, 1963) and the diuresis is often sufficient to bring about increased excretion of sodium in spite of an accompanying reduction in the sodium concentration of the urine (Davey & Lockett, 1960).

Oxytocin, 4–10 m-u./150 ml. blood, can convert the diuretic natriurietic effect of aldosterone in blood from headless animals into an action of salt and water retention, such as is seen when these kidneys are perfused with blood from intact animals (Davey & Lockett, 1960). Oxytocin does not, however, reduce the abnormally high rate of sodium excretion shown by these kidneys when perfused with blood from headless donor animals. We have therefore examined the influence of three adenohypophysial hormones on sodium reabsorption and on the response to physiological concentrations of aldosterone in kidneys perfused with blood from headless animals.

## METHODS

Twenty-two heart-lung-kidney preparations were made as described by Davey & Lockett (1960) from kittens weighing 0·7–1·8 kg. Blood for perfusion was collected from male, female or neutered cats not less than 12 min after ligature of the brachiocephalic and left subclavian arteries for all but three experiments. For these three the intracranial circuit remained undisturbed. Each animal used for a single experiment was either made spinal or was given chloralose intravenously, 8 ml. 1·0 % (in NaCl solution 0·9 g/100 ml.)/kg. Heparin, 2000 u./100 ml. was used as anticoagulant.

*Chemical procedures.* Concentrations of creatinine, sodium and potassium in urine and plasma were determined as previously described (Davey & Lockett, 1960). A correction

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of 0·26 ml./10 g kidney was applied to serial urine samples to allow for the dead space of the renal pelvis and the ureteric catheter when urine flow exceeded 0·2 ml./min. This dead space was measured in eleven kidneys after 2·75 min arrest of urine flow during mannitol diureses having urine/plasma ($U/P$) creatinine values of 3·5–5·0 as the mean cumulative weight, $263 \pm 18\cdot2$ mg/10 g kidney, of those urine samples which emerged serially in advance of reduction in $U/P_{Na}/U/P_{creatinine}$. Dead space corrections were reduced proportionately at lower flows.

*Hormones.* The following were obtained commercially, already prepared for injection: dextro-aldosterone (Ciba Laboratories Ltd); thyrotropic hormone (Armour Products Ltd); adrenocorticotropic hormone (Organon Laboratories Ltd and Armour Products Ltd). A slightly impure sample of bovine growth hormone, kindly supplied by Parke Davis and Co. Ltd, was used for two experiments. Fully purified samples of bovine and ovine growth hormone, kindly sent to us by Professor Wilhelmi, were used on fourteen preparations. Weighed amounts of growth hormone were dissolved just before use in 0·9 % NaCl solution which had been adjusted to pH 11·5 with NaOH. All solutions of hormones were added dropwise to the venous blood as it returned to the reservoir.

## RESULTS

Whereas $1\cdot7 \pm 0\cdot5\%$ of the filtered sodium has been excreted by ten kidneys perfused with blood from intact donors, the corresponding value given by eighteen kidneys perfused with blood from headless animals has been $6\cdot8 \pm 2\cdot7\%$ in our experiments. These observations confirm the findings of de Lima & Lockett (1961).

In each of three preparations perfused with blood from *intact donor animals* physiological concentrations (20–160 $\mu$g/150 ml.) of pure bovine growth hormone reduced the rate of urine flow, the concentrations of sodium and potassium in the urine and the proportion of the filtered sodium which subsequently became excreted (Fig. 1). Slight increase in the renal blood flow (RBF) was unaccompanied by measurable change in the glomerular filtration rate (GFR). Maximum effects were reached in 25–35 min; there was no initial latency.

The effects of physiological concentrations of bovine growth hormone in the absence of any other added hormone were tested on five preparations perfused with blood from *headless animals* (Figs. 2 and 3). Growth hormone again reduced the urine flow and caused a slight increase in RBF, unaccompanied by change in GFR. However, the concentrations of sodium and potassium in the urine then rose (compare Figs. 2 and 3 with Fig. 1), but the rate of excretion of sodium still fell because the percentage of the filtered load which was reabsorbed increased. The rate of excretion of potassium remained unchanged or showed a small decrease. Higher concentrations of growth hormone (Fig. 5) had a similar effect on blood and urine flow and on the excretion of potassium, but decreased the urinary concentration of potassium, thereby markedly reducing the rate of potassium excretion. The time course of these effects did not differ from those of growth hormone in blood from intact animals. The actions of ovine

growth hormone, examined in two further preparations perfused with blood from headless animals, appeared qualitatively and quantitatively similar to those of bovine growth hormone.
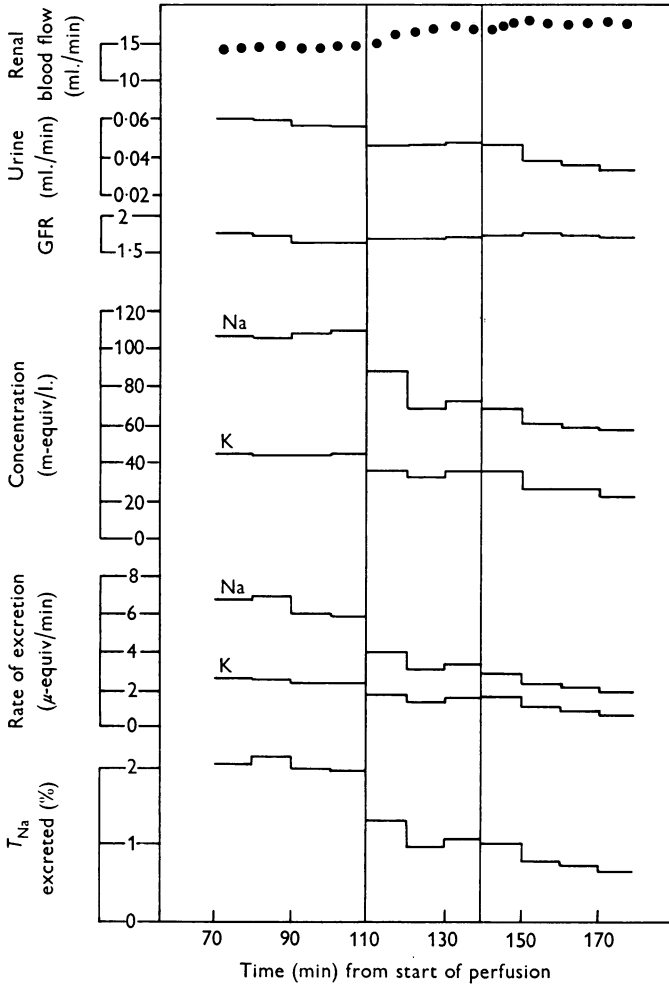


Fig. 1. Cat kidney, 8·5 g, perfused at 114 mm Hg and 36° C with 150 ml. blood from intact chloralosed animals by a heart-lung preparation. Plasma Na and K concentrations, 187 and 3·3 m-equiv/l. respectively. At the first vertical line 30 μg pure bovine growth hormone, and at the second 5 μg aldosterone, were added to the venous blood.

*The influence of growth hormone on the direct renal actions of aldosterone* has been examined in ten preparations. Cat kidneys perfused with blood from *intact donors* respond to physiological concentrations of bovine growth

hormone (Fig. 1) or of aldosterone (Davey & Lockett, 1960) by retention of water, sodium and potassium. Figure 1 demonstrates the additive effects of physiological concentrations of these two hormones on such preparations. Each hormone shows its own characteristic latency of action.
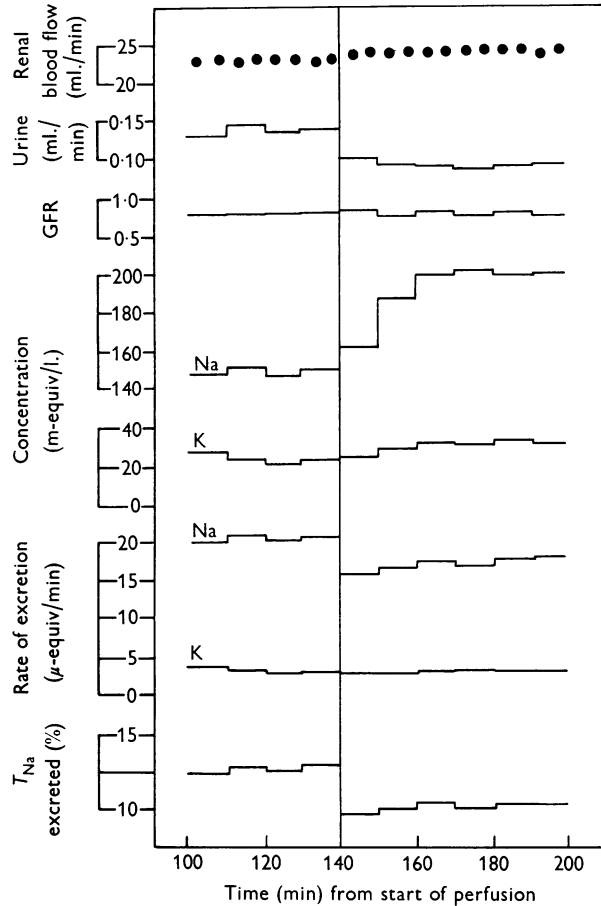


Fig. 2. Cat kidney, 8·4 g, perfused at 130 mm Hg and 36° C with 150 ml. blood from headless chloralosed animals. Plasma Na and K, 200 and 4·0 m-equiv/l. respectively. Abscissae as in Fig. 1. At the vertical line 30 µg pure bovine growth hormone was added to the venous blood.

Kidneys perfused with blood from *headless donors* respond to physiological concentrations of aldosterone (Davey & Lockett, 1960) by diuresis which, despite reduction in the sodium concentration in urine, is often (Fig. 4) but not always (Fig. 5) sufficient to cause net sodium loss. Physiological concentrations of growth hormone in blood from headless donors

(Fig. 2) reduce urine flow, the percentage of the filtered sodium excreted and net sodium loss, although the concentration of sodium in the urine rises. In each of four preparations, in which a physiological concentration of growth hormone had already been added to blood from headless donors,
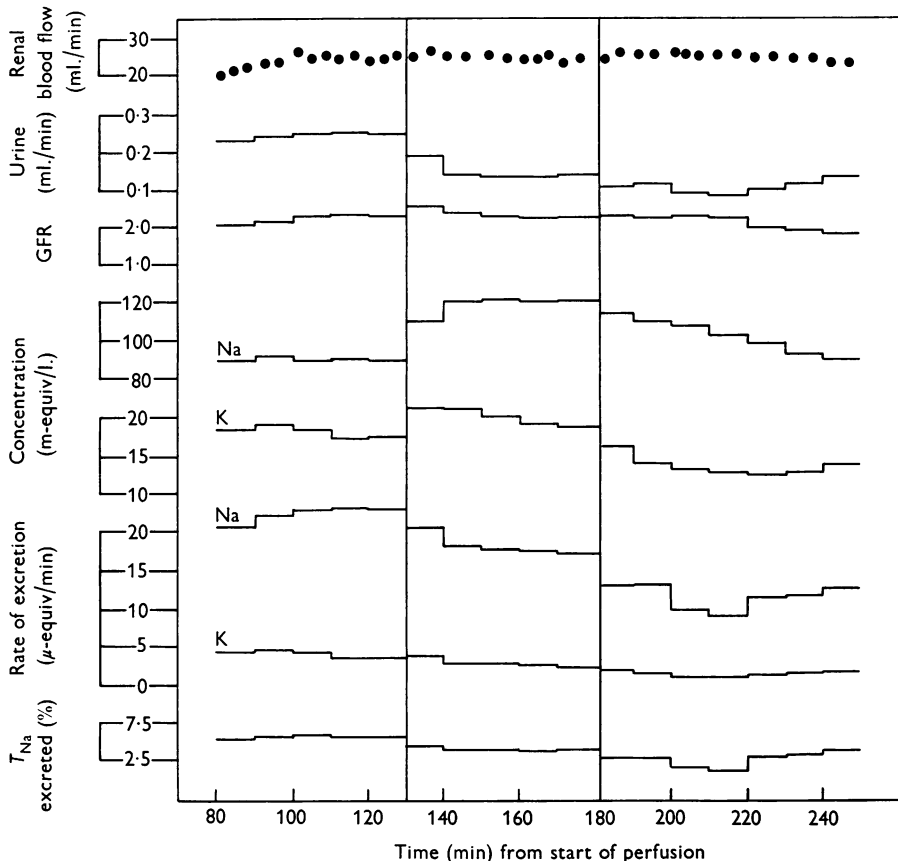


Fig. 3. Cat kidney, 7·9 g, perfused at 118 mm Hg and 36° C with 150 ml. blood from headless chloralosed animals. Plasma Na and K, 180 and 2·9 m-equiv/l. respectively. At the first vertical line 100 µg pure bovine growth hormone, and at the second 5 µg aldosterone, were added to the venous blood.

aldosterone caused antidiuresis and reduced the concentrations of sodium and potassium in the urine (Fig. 3). This effect of aldosterone resembled, qualitatively and in time course, the action of aldosterone on cat kidneys perfused with blood from intact (Fig. 1) and not from headless (Fig. 4) animals. Thus the diuretic natriuretic effect typical of aldosterone in blood from headless animals (Fig. 4) had been converted to the action of aldosterone

in blood from intact animals (Fig. 1) by prior addition of growth hormone to blood from headless donors (Fig. 3). By contrast, no evidence was obtained of the interaction of aldosterone and growth hormone in the kidney when aldosterone was given before growth hormone. Once the natriuretic diuretic effect of aldosterone in blood from headless animals
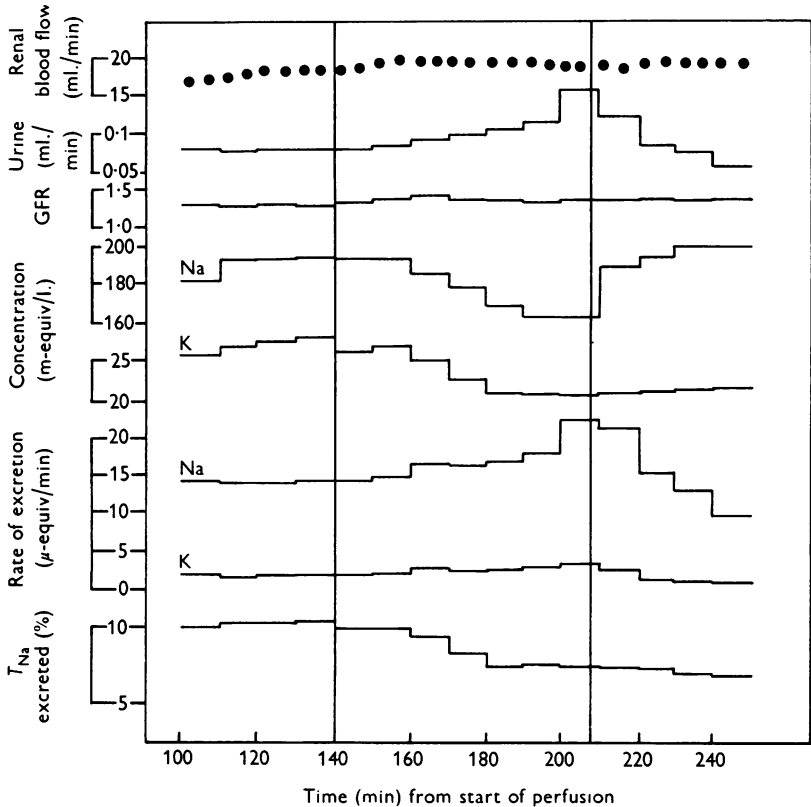


Fig. 4. Cat kidney, 7·4 g, perfused at 119 mm Hg and 36·5° C with blood from headless spinal animals. Plasma Na and K, 200 and 3·6 m-equiv/l. respectively. At the first vertical line 0·25 μg aldosterone, and at the second 20 μg pure bovine growth hormone, were added to the venous blood.

had developed (Fig. 4) physiological (six preparations, Fig. 4) or excessive (two preparations, Fig. 5) concentrations of growth hormone caused an almost immediate increase in the sodium concentration of the urine which was accompanied by antidiuresis. The extent to which the percentage of the filtered sodium excreted fell varied. The concentration of potassium in the urine remained unchanged (Fig. 4), except when excessive amounts of growth hormone were added (Fig. 5); it then fell. Hence the renal

actions of growth hormone in blood from headless animals were not significantly modified by the previous addition of physiological concentrations of exogenous aldosterone (compare Figs. 2 and 4).

Whereas chloralose anaesthesia reduces the sensitivity of perfused
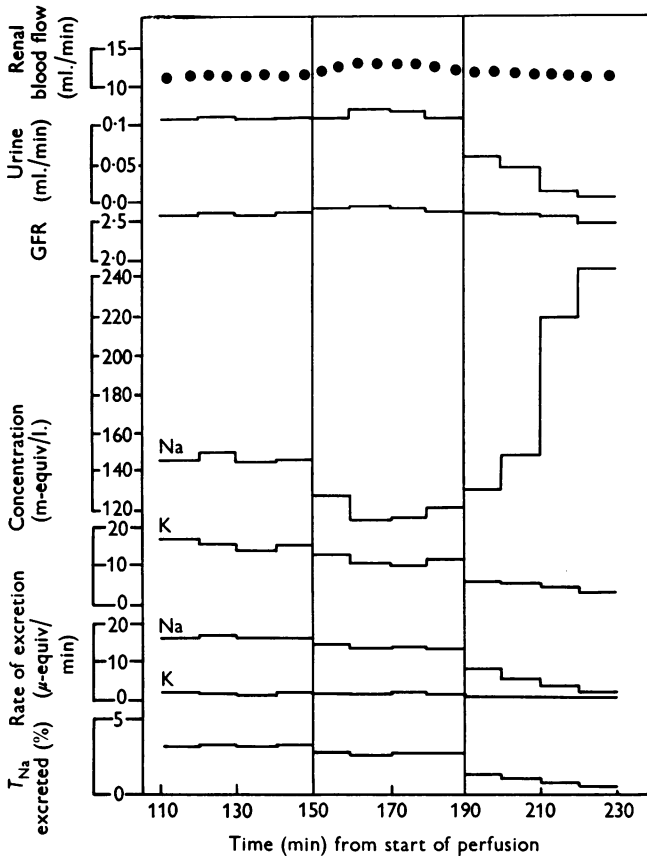


Fig. 5. Cat kidney, 5·1 g, perfused at 118 mm Hg and 36° C with 150 ml. blood from chloralosed headless animals. Plasma Na and K, 190 and 3·5 m-equiv/l. respectively. At the first vertical line 5 μg aldosterone, and at the second 10 mg impure bovine growth hormone, were added to the venous blood.

kidneys to aldosterone (Davey & Lockett, 1960; compare Figs. 3 and 4 above), chloralose does not modify sensitivity to growth hormone (Figs. 2 and 4) to any significant extent.

*Thyrotropic and adrenocorticotropic hormones.* Neither thyrotropic nor adrenocorticotropic hormone, 5 u./150 ml., decreased the high proportion of the filtered sodium which is excreted by kidneys perfused with

blood from headless donors. Nor did the presence of either hormone modify the diuretic natriuretic action of aldosterone in blood from headless animals.

<div align="center">DISCUSSION</div>

The direct renal actions of growth hormone were predicted by Whitney, Bennett & Li (1952) and have been demonstrated on the perfused cat kidney (Figs. 1 and 2). These preparations respond to physiological concentrations of growth hormone (Fig. 1) in blood from intact animals by antidiuresis accompanied by reduction in the concentration of sodium (Na) and potassium (K) in the urine without change in the rate of glomerular filtration (GFR). This response resembles that produced in man by a single injection of human growth hormone (Biglieri, Watlington & Forsham, 1961).

The renal responses to the same concentration of growth hormone in blood from headless donors differ (compare Figs. 2 and 1). Antidiuresis without change in GFR is then accompanied by increase in the urinary concentrations of Na and K (Fig. 2). The rates of excretion of sodium and potassium fall slightly (Figs. 2 and 3), as does the proportion of the filtered sodium ($T_{Na}$) excreted, the latter to a varying extent. Over all, the raised values for the percentage of $T_{Na}$ excreted which result from the use of blood from headless animals (de Lima & Lockett, 1961, Fig. 1; and above) are reduced by physiological concentrations of growth hormone (Fig. 2 above) to levels characteristic for these preparations when perfused with blood from intact animals. Moreover, the presence of physiological concentrations of growth hormone in blood from headless animals (Fig. 3) converts the diuretic natriuretic effect of aldosterone in such blood into the antidiuretic sodium-retaining action of aldosterone in blood from intact animals (Davey & Lockett, 1960). Since the raised rates of Na loss from kidneys perfused with blood from headless donors and the inability of such preparations to respond to aldosterone by retention of water and salt result from functional changes in the proximal parts of the nephrons (de Lima & Lockett, 1963), it may be inferred that growth hormone has its effect at this site. The stimulus given by growth hormone in blood from headless donors to the reabsorption of water exceeds that to the reabsorption of Na, for the urinary concentration of Na rises (Fig. 2). Dissociation in the movements of salt and water in the proximal segments of the nephrons could lend support to Morel & Guinnebault's concept (1960) of the proximal tubular secretion of sodium. Alternatively, growth hormone may additionally stimulate distal water reabsorption more effectively in blood from headless than from intact animals. Antagonism between the actions of the antidiuretic hormone and growth hormone could not explain the greater antidiuretic effect of growth hormone in blood from headless

animals, since the antidiuretic actions of growth hormone are not affected (compare Figs. 2 and 3), but that of vasopressin is greatly reduced by chloralose anaesthesia (Davey & Lockett, 1960).

Physiological concentrations of either oxytocin (Davey & Lockett, 1961) or of growth hormone (Fig. 3) can replace the diuretic natriurietic effect of aldosterone in blood from headless animals by an antidiuretic sodium-retaining action like that of aldosterone in blood from intact animals. Oxytocin is effective whether added in the 12 min preceding, with, or after the addition of aldosterone (Davey & Lockett, 1960, Figs. 6 and 7). Two additional observations suggest that a 'bound form' of oxytocin is not the factor missing from the blood of headless animals which enables the kidney to respond to aldosterone by retention of salt and water. First, infusion of 400 m-u. of oxytocin into the blood stream of each headless donor during bleeding does not cause the appearance of this factor in the blood. Secondly, prior evisceration of donor animals with intact intracranial circulations does not cause the disappearance of this factor from the blood (Lockett and Roberts, unpublished observations). Growth hormone bears the greater resemblance to the missing factor because of its long-lasting effect in a perfusion circuit and in stored blood, and because it also reduces the excessive loss of Na by kidneys perfused with blood from headless animals (Fig. 2). Moreover, the ability of oxytocin to enhance the Na-retaining action of aldosterone in blood from intact animals may be of physiological importance (de Lima & Lockett, 1963). The direct renal effect of the lactogenic hormone, immunologically identical with (Irie & Barrett, 1962) and electrophoretically similar to (Ferguson & Wallace, 1961) growth hormone awaits our investigation.

### SUMMARY

1. Cat kidneys, perfused at constant temperature and pressure, respond to physiological concentrations (20–100 $\mu$g/150 ml.) pure bovine (or ovine) growth hormone by reduction in the rates of excretion of water, Na and K without change in glomerular filtration rate. These changes are accompanied by reduction in the urinary concentrations of Na and K when blood from intact donors is in use but by rise in the concentrations of these ions when blood from headless donors is circulating.

2. Physiological concentrations of growth hormone convert the diuretic natriuretic effect typical of aldosterone in blood from intact headless donors into the antidiuretic Na-retaining action of aldosterone in blood from intact animals.

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