CONTROL OF ADRENAL SECRETION OF ELECTROLYTE-ACTIVE STEROIDS*

BY

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PART I

Addison (1868) in his original report records the case of a tide waiter whose disease was exacerbated when living on salt provisions which would have been pickled with nitre—that is, potassium nitrate.

Loeb, Atchley, Benedict, and Leland (1933) showed that adrenalectomy caused urinary loss of Na^+ and a decrease of Na^+ and increase of K^+ in the plasma. The demonstration of Na^+ -retaining action by deoxycortone acetate (D.C.A.) led to a search for this or a similarly active substance in the adrenals. The discovery of aldosterone, with 25 to 120 times the activity of D.C.A., not only in extracts but in the adrenal venous blood, has led to the acceptance of it as by far the most electrolyte-active steroid (Simpson, Tait, Wettstein, Neher, von Euw, and Reichstein, 1953). It is 500 to 1,000 times more active than cortisol, and 4,000 times more active than corticosterone in causing renal Na^+ retention in rats (Swingle, Maxwell, Ben, Baker, LeBrie, and Eisler, 1954; Desaulles, 1958). It is also 100 times more active than these compounds in stimulating K^+ excretion.

The finding that there is increased urinary excretion of aldosterone in congestive cardiac failure (Deming and Luetscher, 1950; Singer and Wener, 1953) as in other oedematous states (Chart and Shipley, 1953) has raised the question whether increased adrenal cortical activity is a contributory cause of the development of oedema. Such an inference, however, like that proposing a causal relation between intravascular or extracellular volume and aldosterone secretion (Bartter, 1956; Bartter, Biglieri, Pronove, and Delea, 1958) depends on the assumption that the rate of urinary excretion of aldosterone is proportional to the rate of adrenal secretion *in all conditions*, and is not determined by other factors—for example, altered renal activity or liver oedematism (Wolff, Koczorek, and Buchborn, 1958). The amount of aldosterone excreted in the urine is about 5% of the amount secreted (Ayres, Garrod, Simpson, and Tait, 1957), but studies with labelled aldosterone by Ayres, Barlow, Garrod, Kellie, Tait, Tait, and Walker (1958) suggest that this small proportion may be a valid basis for inference in the instance of simple Na^+ deprivation.

The understanding of the role of the adrenal cortex in a complex situation like congestive heart failure would probably be simpler if the control of aldosterone

secretion in the ostensibly simple situation of Na^+ depletion were known. This involves three principal questions: (1) the site of the receptor (or receptors) sensitive to the state of Na^+ balance; (2) the exact nature of the stimulus to it; and (3) the site of origin and the mode of transmission of the stimulus to the adrenal.

Desiderata for Valid Experimental Investigation of the Causation and Manner of Secretion of a Hormone

In setting out to find what it is precisely which causes secretion of a hormonal material *under physiological conditions* there are the following seven desiderata:

1. All observations should be made on conscious undisturbed animals in normal correspondence with their environment. It was shown by Verney (1947) that the normal hormonal mechanism regulating osmotic pressure is interfered with by anaesthesia or fright, and, as we will recount, this is true also in the case of hormonal factors contributing to control of Na^+ balance. This places strictures on the methods of experimental approach, but solution of the problems by stage-by-stage surgery ultimately carries the advantage that the results derived are those holding for a normal animal.
2. The physiological stresses causing a response dependent upon the gland should be precisely defined and standardized, and be capable of graded variation.
3. The arterial blood supply of the endocrine gland under investigation should be exclusively accessible so that the effect of supposed active agents can be tested by direct local injection.
4. The nerve supply of the gland, when relevant, should be accessible for stimulation or blocking.
5. The venous effluent exclusively from the gland should be accessible.
6. There should be one or more biological systems for quantitative assay of the activity of the venous effluent.
7. The chemical assay of the venous effluent must be developed until it detects quantitatively each substance known to be active on the biological indicator, and the overall effect of these substances must correspond quantitatively to the action of the entire venous effluent.

In the case of the electrolyte-active secretion of the adrenal gland, inferences concerning regulation have been based on urinary excretion of aldosterone, or upon measurement of the adrenal secretion by cannulation of the adrenal vein in an anaesthetized animal or one which has been operated upon very recently. It remains to be shown in these latter instances whether changes of adrenal secretion evoked under these conditions reflect the physiological mechanism of fine

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adjustment of Na^+ balance in the normal animal or the effects of anaesthesia and trauma.

The desiderata are met in the case of electrolyte-active steroids by the stage-by-stage preparative surgery on sheep which has been developed in this laboratory.

1 and 2. Defined Stress in a Confident Trained Animal

Sodium deficit causes increased secretion of electrolyte-active steroids. Na^+ deficiency can be readily produced in a sheep. The parotid glands of the sheep, a ruminant, secrete 4–8 litres of alkaline saliva each day. This fluid contains 180 mEq of Na^+ /l. and 5–10 mEq of K^+ /l. ($\text{Na}^+/\text{K}^+=36\text{--}18$), and HCO_3^- (100–140 mEq/l.) is the main anion (Fig. 1). The saliva enters the rumen, where it buffers the volatile fatty acids produced by the microbial fermentation of the grass, chaff, etc., which the animal eats. If a permanent

FIG. 2.—Adrenal transplant 2. Photograph shows a Merino sheep with its left adrenal gland transplanted into a combined loop of carotid artery and jugular vein on left side. Loop is occluded cranial to transplant by pneumatic cuff inflated to 300 mm. Hg. An infusion of 660 mEq of NaCl /l. is being made at 0.2 ml./minute through a needle inserted in carotid artery caudal to transplant. This caused a 27 mEq/l. rise in adrenal arterial Na^+ concentration. Carotid artery loop and Wright type parotid fistula are shown on right side.

FIG. 1.—Composition of parotid saliva of sheep compared with plasma and pancreatic juice of dog.

unilateral parotid fistula is made (Fig. 2) the sheep loses 2–4 l. of this fluid a day. Normally the animal is given 50 g. of NaHCO_3 (600 mEq of Na^+) each day to replace the Na^+ lost from the fistula, and it remains in good condition indefinitely. Digestive processes proceed normally, though the secretion of only one parotid enters the alimentary canal. If the daily Na^+ supplement is withdrawn the animal becomes depleted of 500–900 mEq of Na^+ over a period of 4 to 10 days (Denton, 1956, 1957a). Commensurate with this Na^+ deficit, the parotid salivary Na^+ concentration falls, and the K^+ rises reciprocally, so that by the time the Na^+ deficit is 900 mEq the ratio has decreased to 40/140 = 0.3 (Fig. 3). There is virtually complete conservation of Na^+ in the urine. This phenomenon has occurred without exception in all of 60 sheep with parotid fistulae. The extent of the Na^+ deficit can be controlled by the extent of replacement of NaHCO_3 by rumen tube or by giving the animal NaCl or NaHCO_3 to drink.

Effects of the Na^+ depletion are manifested in the salivary composition and the urinary composition. We have studied the effect on salivary composition most extensively because, with the Wright fistula, consecutive

specimens of clear saliva can be collected at frequent intervals over prolonged periods and are easy to assay for Na^+ and K^+ with the flame photometer. Secretion of saliva in the sheep is continuous, so that the parotid gland both depletes the animal of Na^+ and reflects the extent of Na^+ deficiency so produced. The change of concentration of both ions is at least 10 times greater than that occurring in human saliva in Na^+ deficiency (McCance, 1938; Thorn, Schwartz, and Thaysen, 1956). Moreover, in contrast to the human parotid (Thorn *et al.* 1956), the salivary composition varies little with secretion rate when the animal is in normal Na^+ balance (Denton, 1957b). Hence a normal salivary Na^+/K^+ ratio at any secretion rate may be taken confidently to indicate normal Na^+ balance.

The parotid response to Na^+ deficiency does not occur if the animal is adrenalectomized and is adrenally insufficient, or is given only sufficient D.C.A. to maintain it when in normal Na^+ balance. However, it does occur if there is a fourfold increase of D.C.A. dosage (Goding and Denton, 1956, 1957, 1959). Thus there is a defined general stress with a commensurate response dependent upon adrenal secretion of electrolyte-active hormone.

FIG. 3.—Effect of Na^+ deficiency on Na^+ and K^+ concentrations of parotid saliva. A negative balance of 970 mEq of Na^+ developed over a period of 14 days.

The question which naturally arises is whether the adrenal responds directly to the lowered blood Na^+ or K^+ or other local factors in these circumstances, or to a substance liberated at a distance, or to nervous action. In order to analyse these questions it was desirable to meet the desiderata 3, 4, and 5.

3, 4, and 5. Access to the Arterial, Venous, and Nervous Connexions of the Adrenal

All these can be met by transplanting the adrenal gland into a skin loop containing the carotid artery and jugular vein in the neck of a sheep (McDonald, Goding, and Wright, 1958). The left renal artery usually gives off an adrenal branch sufficient for viability of that organ, and the main adrenal vein drains into the left renal vein. These renal vessels are anastomosed end-to-side with the carotid artery and jugular vein (Figs. 2 and 4). The kidney is discarded. The gland is presumed to be denervated.

The right adrenal gland is removed later and the animal tested for normal parotid response to Na^+

FIG. 4.—Diagram of method of transplantation of left adrenal gland (McDonald *et al.*, 1958).

deficiency. A full response shows that the transplanted adrenal gland is functioning normally. When the animal is Na^+ -deficient, the total adrenal venous blood is drained for 3 to 10 hours, and if the salivary ratio rises to normal it indicates that the transplant is the only adrenal cortical tissue in the body.

6 and 7. Biological and Chemical Assay

The salivary gland of the sheep can be used as the assay tissue for electrolyte-active steroid. 1 mg. of DL-aldosterone monoacetate intramuscularly in a Na^+ -depleted adrenalectomized sheep weighing 30 kg. reduces the Na^+/K^+ from 15 to 0.5 for a period of six hours. It is over 40 times more active than D.C.A. 200 mg. of hydrocortisone acetate intramuscularly or 130 mg. of hydrocortisone hemisuccinate intravenously during 450 minutes had little or no effect on the ratio (cf. Fig. 22, in Part II). Hence it is many hundred times more active than cortisol (cf. Swingle *et al.*, 1954; Desaulles, 1958).

Chromatography of peripheral arterial and adrenal venous blood has been done (Reich and McDonald, 1958) but is not yet at a stage where full arithmetical correspondence with the biological effect can be certified.

The results of experiments on the transplanted adrenal gland have given an indication that one factor acting on the adrenal in Na^+ deficiency may be a hitherto unrecognized humoral stimulus.

EXPERIMENTAL RESULTS

Analysis of the Mechanism of Parotid Response to Na^+ Depletion

Effect of Decreased Parotid Secretion Rate During Na^+ Depletion

The following investigations were made:

When this investigation began, the first step taken in seeking to define the stimulus which caused the parotid salivary Na^+/K^+ to change with Na^+ balance was to find out whether local parotid factors caused the response. Experiments showed that the decrease of secretion rate which occurred during Na^+ depletion was a factor, but not the major factor, in causing the fall of Na^+/K^+ ratio. During the course of a day the parotid secretion rate varied from 0.3 to 4 ml./minute (Denton, 1957c) as a result of psychic, ruminal, and buccal stimuli. When the Na^+ balance was normal, such rapid and large variation had little effect on the salivary Na^+/K^+ ratio. In the Na^+ -depleted animal, however, a highly significant linear relation between secretion rate and salivary Na^+/K^+ was observed (Fig. 5). With the object of finding whether the salivary Na^+/K^+ could be made to return to normal by maximal stimulation of secretion rate, acetylcholine infusions (2×10^{-5} in 5% glucose at 0.5–1 ml./minute) were made into a carotid loop on the same side as the fistula. As shown in Fig. 5, the line relating secretion rate and salivary Na^+/K^+ became asymptotic with the secretion rate axis. Even though in one experiment acetylcholine stimulated the secretion rate to 11 ml./minute, the Na^+/K^+ ratio did not rise above 1.5. The result of these experiments (Denton, 1957b) established that decline of secretion rate was one factor in the parotid response to Na^+ depletion, but it accounted for only 20–40% of the fall, and the main cause (X on Fig. 5) would have to be sought elsewhere.

Reduction of Na^+ Concentration and Na^+ Load to the Parotid as a Possible Cause of the Response

During Na^+ depletion the plasma Na^+ concentration falls by 5–20 mEq/l. (Denton, 1958). Also the plasma volume decreases by 20–40% (Denton, 1957a). One of the possibilities first considered in seeking the cause of this remarkable parotid response was that Na^+ deficiency resulted in a reduction in the supply of Na^+ presenting to the parotid cells. Therefore experiments were made in which 4 M NaCl was infused into a carotid artery loop on the same side as the fistula. An acetylcholine infusion was made also into this carotid artery loop. These procedures increased both Na^+ concentration (by 20 mEq/l.) and blood flow, and

FIG. 5.—Effect of secretion rate on parotid salivary Na^+/K^+ ratio during moderate Na^+ deficiency. ● = Ipsilateral intracarotid infusion of ACh. ▲ = Ipsilateral intracarotid injection of 1/50 gr. (1.3 mg.) of atropine. Y indicates range of ratio change attributable to secretion rate variation. X indicates effect attributable to adrenal cortical secretion.

contrived that the Na^+ supply to the parotid secretory cells was greater than under control conditions with normal Na^+ balance. The 4 M NaCl infusion had little immediate effect on the salivary Na^+/K^+ ratio (Fig. 6, ●—●), but if the amount of Na^+ infused was adequate to alter significantly the animal's Na^+ balance—for example, 60 ml. of a 4 M NaCl=240 mEq Na^+

FIG. 6.—Effect on salivary Na^+/K^+ ratio of ipsilateral intracarotid injection of various solutions during Na^+ deficiency. ●—● P.F.16. 60 ml. of 4 M NaCl in 40 minutes=240 mEq of Na^+ . ○—○ P.F.16. 69 ml. of 50% dextrose (2.7 M) in 46 minutes. ▲—▲ P.F.7. 15 ml. of 4 M NaCl in 10 minutes=60 mEq of Na^+ .

site of action of the injected Na^+ was elsewhere in the body than the parotid cells, and that it inhibited the production of a humoral agent evoked by Na^+ deficiency. This agent acted upon the parotid cells. The time delay included the time necessary for destruction of the amount of the agent already in the circulation. This parotid response to a rapid correction of Na^+ deficiency could not be elicited in an anaesthetized sheep or in a sheep which was excited or disturbed. The effect of a rapid correction of Na^+ deficiency could be reproduced by rapid bilateral adrenalectomy in an anaesthetized sheep, and a series of experiments on bilaterally adrenalectomized sheep with parotid fistulae showed that the humoral agent acting upon the parotid was the electrolyte-active secretion of the adrenal gland (Goding and Denton, 1956, 1957, 1959), and that the response in the normal animal depended upon an increased secretion by the adrenal of electrolyte-active steroid. In fact, there were only two major components in the mechanism of parotid response to Na^+ deficiency, and, if the secretion rate factor were accounted for, the salivary Na^+/K^+ ratio could be used as an index of adrenal secretion of electrolyte-active steroid.

In considering this inhibition of adrenal secretion which followed a rapid change of Na^+ balance, the fact that little or no response occurred if only 60 mEq of Na^+ was infused over 10 minutes (Fig. 6, ▲—▲) suggested that it was necessary to infuse an amount which altered significantly some property of the *milieu intérieur*. It did not appear that the response was osmotically determined (Verney, 1947), since no effect was produced if hypertonic glucose—50% (2.7M)—was infused into the ipsilateral carotid artery at the same rate for the same period (Fig. 6, ○—○). The great advantage emerging from finding this parotid response to adrenal inhibition following rapid correction of Na^+ deficiency was that a very large quantitative change occurred over a short time interval, and this provided an excellent test system for experimental analyses of the control of adrenal secretion. Moreover, the experimental animal

was, in effect, also the bioassay preparation, and the same trained animal could be used over a period of years, both for investigations and as its own experimental control.

In the light of the evidence that some tissue of the body other than the parotid was sensitive to change of Na^+ balance or some consequence of it, it seemed legitimate tentatively to use the term "receptor" on the basis that this sensitive function would be localized. An immediate question was whether the adrenal itself was the site of the receptor function.

Site and Nature of Stimulus to the Putative Receptor Controlling Secretion of Aldosterone

When electrolyte-active adrenal secretion is inhibited by rapid correction of Na^+ deficiency in a depleted sheep the following are some of the physiological factors which would change in the direction of correcting the deviation from normal caused by Na^+ deficiency. There would be an increase in plasma Na^+ concentration, plasma Na^+/K^+ ratio, osmotic pressure of extracellular and intracellular fluid, the volume of plasma and extracellular fluid, systemic blood pressure, atrial pressure, the blood flow through the organs including the adrenal, and hence the supply of solutes to them. Probably intracellular Na^+ concentration would increase. Plasma K^+ , protein osmotic pressure, haematocrit, and intracellular volume would decrease. The usual response to rapid correction of Na^+ deficiency may follow systemic 4M NaCl injection without any change of blood pH or plasma HCO_3^- -occurring.

The evidence for or against any one of these factors being the specific stimulus to the receptor is complicated (Bartter, 1956; Bartter *et al.* 1958; Laragh and Stoerk, 1957; Homer Smith, 1957; Denton, 1958). Consideration of this, and the evident difficulty of isolating one factor without affecting a number of others, led to the view that an essential and possibly decisive step to the object of answering both the question of the locus and the stimulus to the receptor-regulating aldosterone secretion might be the devising of a preparation in which there was local access to the arterial supply of the adrenal in a conscious undisturbed animal. Thus, allowing the relatively small blood flow through the adrenal gland, it would be possible to insert a needle into the artery and impose for some hours a significant local alteration in the concentration of Na^+ or any other electrolyte perfusing the gland, yet cause only negligible change in the Na^+ balance of the animal. This might decide (1) whether or not the adrenal gland itself responded directly to change of Na^+ or K^+ concentration, or any other condition induced locally by Na^+ deficiency, or (2) whether the gland responded to a stimulus from a distance.

The stipulation of a conscious undisturbed animal was crucial, since experiments had shown that the anaesthetized or frightened animal did not respond to rapid correction of Na^+ deficiency, and this ruled out conduct of these experiments by acute surgical approach under anaesthesia. Dissections showed that the renal artery usually supplies one branch to the adrenal gland on the left side, and that the left adrenal vein drains into the left renal artery, and not the inferior vena cava. In the first instance the construction of a left renal artery loop by the method of Lockett, O'Connor, and Verney (1942), with

subsequent removal of the left kidney, was considered. However, the ensuing great reduction in blood flow in the renal artery would have made the insertion of a needle for long periods difficult, and the desired access to the adrenal vein blood would have been extremely difficult. Therefore it was decided to attempt an adrenal transplant into a combined skin loop of carotid artery and jugular vein on the left side of the neck (McDonald *et al.*, 1958).

An end-to-side anastomosis was made between the left renal artery and the carotid artery, and the left renal vein and the jugular vein, respectively. The renal artery was ligated distal to the origin of the adrenal artery and the kidney was discarded. This transplant procedure succeeded 7 out of the 13 times it was carried out. At a later operation the right adrenal gland was removed. A parotid fistula and carotid loop were made on the right side. Inflation to 300 mm. Hg. of a pneumatic cuff applied to the loop cranial to the transplant makes the carotid artery supply only the adrenal gland, and simultaneous application of a cuff at 20 mm. Hg. caudal to the transplant contrives that a jugular vein cannula drains only adrenal vein blood. A needle can be inserted into the carotid artery caudal to the transplant and the composition of the adrenal arterial blood altered as desired for 10 to 12 hours (Fig. 2).

The great practical advantage of this preparation with end-to-side anastomoses and the inflation of the cuff cranial to the transplant lies in the fact that infusions are inserted into and blood is withdrawn from vessels of large calibre which usually carry 100–200 ml. of blood each minute. By cannulation of the jugular vein and application of cuffs cranial and caudal to the transplant the total adrenal venous effluent can be collected in sufficient amounts for chromatographic analysis and the pattern of steroids can be compared during Na^+ depletion and normal Na^+ balance. Once again the stipulated advantage of use of a conscious undisturbed animal is of cardinal importance, since anaesthesia may provoke corticotrophin release (Sandberg, Eik-Ness, Samuels, and Tyler, 1954), and hence non-specific effects on the adrenal effluent pattern would occur.

Evidence bearing upon the animal's survival, response to Na^+ depletion, histological study, and chromatographic demonstration of the presence of aldosterone, cortisol, and corticosterone in adrenal venous blood was cited to show that the transplanted gland was normal (McDonald *et al.*, 1958). Subsequently, Reich and McDonald (1958) reported that in normal Na^+ balance the gland secretes mainly cortisol, and small amounts only of corticosterone and cortisone. Aldosterone was not detected in 40–70 ml. of adrenal venous plasma. In moderate Na^+ depletion aldosterone was easily detected at 0.006–0.025 mg./hour. There was little or no change of cortisol or corticosterone in moderate Na^+ depletion. Corticotrophin caused the cortisol secretion rate to rise to 3–4 mg./hour.

Method of Graphing Results of Experiments on Transplants

The experiments on sheep with transplants were conducted in the following manner.

Prior to the adrenal infusion there was a control period of 150–250 minutes, during which, in the Na^+ -depleted

animal, the relation between parotid secretion rate and both the salivary Na^+/K^+ ratio and the salivary K^+ concentration was determined. When salivary Na^+/K^+ ratio or K^+ concentration is graphed against an arithmetical ordinate scale, there is a highly significant linear relation with secretion rate (Denton, 1957b; Goding and Denton, 1959). During the experiment the position of each point relative to this control period line (as statistically determined) was measured, and the results were plotted as salivary Na^+/K^+ or K^+ concentration "corrected for secretion rate."

A sustained deviation from the control period line during the period of experimental alteration of the adrenal ionic environment was interpreted as reflecting a change of electrolyte-active adrenal secretion. A rise above the control period line indicated an inhibition, and a fall below the line indicated stimulation of adrenal cortical secretion. The reason for graphing corrected K^+ concentration as well as the Na^+/K^+ ratio is that the ratio may be misleading in quantitative comparison of effects, since it tends to exaggerate reciprocal small changes of Na^+ and K^+ concentration when the ratio is high—for example, $170/17=10$, to $177/10=17$ —and minimize large changes of concentration when it is low—for example, $60/120=0.5$, to, $90/90=1$. In the experiments where the sheep were in normal Na^+ balance, variation of secretion rate had no significant effect on salivary Na^+/K^+ (Denton, 1957b) relative to the magnitude of changes under consideration in this paper. Thus in experiments which began with the sheep in normal Na^+ balance the salivary Na^+/K^+ was graphed against a logarithmic ordinate scale.

Adrenal Blood Flow

A necessary condition for the imposition of a precise change in the composition of the adrenal arterial blood was knowledge of the rate of adrenal blood flow. The flow was measured either by cannulation of the jugular vein with "polythene" tubing or by timed withdrawal of blood with a wide-bore needle and syringe subsequent to occlusion of the jugular vein at the caudal end of the carotid–jugular loop. The adrenal blood flow of the sheep with transplants varied from 4 to 12 ml./minute (T.P.1=8 ml./minute; T.P.2=about 4–6 ml./minute; T.P.9=10 ml./minute; T.P.12=12 ml./minute), and severe Na^+ depletion caused a 25–40% reduction of it.

Physiological Adrenalectomy

Experiments involving insertion of polythene tubing into the jugular vein, and drainage of the total adrenal venous effluent during Na^+ deficiency, showed that the salivary Na^+/K^+ ratio rose rapidly approximately 90 minutes after drainage began (McDonald *et al.*, 1958). The results of such an experiment on T.P.2 when Na^+ -deficient are shown in Fig. 7, and may be compared with results reported below, in which local adrenal changes inhibited secretion of the electrolyte-active steroids.

The sheep was Na^+ -depleted, and the adrenal venous blood was drained for 581 minutes. The salivary Na^+/K^+ ratio rose rapidly and reached normal after 220 minutes. This involved approximately 70 mEq/l. rise in K^+ concentration. During the first 325 minutes the animal was given two infusions of blood to cover the loss by adrenal drainage. This blood was taken from another sheep in normal Na^+ balance, on the assumption that this blood would contain negligible amounts of electrolyte-active steroid. After 375 minutes we began to return to the depleted sheep its adrenal venous effluent by infusion into the right carotid artery loop, which supplied the parotid fistula (Fig. 3). After 40 minutes the salivary Na^+/K^+ ratio began to fall and by 500 minutes, when 200 ml. of

adrenal venous blood had been infused, the ratio had returned to the level obtaining before drainage started. Chromatographic analysis of this blood by Miss Reich and Dr. McDonald showed that the rate of intracarotid infusion was: aldosterone, 24 $\mu\text{g./hour}$; corticosterone,

Fig. 7.—T.P.2 (Na⁺-depleted), 27/9/57. "Physiological adrenal-ectomy." Effect, corrected for variation of secretion rate, on parotid salivary Na⁺/K⁺ ratio (O—O—O) and salivary K⁺ concentration (X—X—X) of drainage of total adrenal venous effluent for 581 minutes. Drainage began at time 0. At +100 and +300 minutes, an infusion of compatible peripheral blood from a sheep in normal Na⁺ balance was given into right jugular vein to repair blood loss of T.P.2. At +375 minutes, adrenal venous blood was infused into right carotid artery loop, which was ipsilateral to parotid fistula (see Fig. 2). At +500 minutes, after 206 ml. had been infused, the remaining adrenal venous blood was infused into right jugular vein. At conclusion of adrenal venous drainage, the sheep was given 595 mEq of NaHCO₃ in 1 litre of H₂O into rumen.

84 $\mu\text{g./hour}$; and cortisol, 660 $\mu\text{g./hour}$. Subsequent to return of the remainder of the adrenal venous blood by intravenous infusion, the drainage of adrenal venous blood was stopped and the sheep was given 595 mEq of NaHCO₃ in 1 litre of water into the rumen.

This experiment demonstrated that the transplanted left adrenal was the source of the material which caused the salivary Na⁺/K⁺ ratio to fall during Na⁺ deficiency, and that it was the only adrenal cortical tissue in the body. To some extent this experiment tended to differentiate the three main factors likely to be involved in the 120-minute time delay in parotid response to rapid correction of Na⁺ deficiency—namely, time for the receptor response resulting in adrenal inhibition; time for destruction of circulating steroid in peripheral blood (20-minute half-life for aldosterone (Ayes *et al.*, 1958)); and time for the biological indicator, the parotid, to register this peripheral blood change. The ratio had risen significantly by 90 minutes, an effect consistent with bypassing the receptor of the system. The time delay of about 30–40 minutes in parotid response when adrenal venous blood was infused into the carotid artery indicated that a latent period in the biological indicator also contributed to the 120-minute delay period following rapid correction of Na⁺ deficiency.

Effect of Sustained Local Change of Composition of Adrenal Arterial Blood on Electrolyte-active Steroid Secretion

Altogether some 60 experiments have been made so far in this series.

Section I. Local Reduction of Na⁺ Concentration and Increase of K⁺ Concentration of the Adrenal Arterial Blood

These experiments, which aimed to reproduce the effect of Na⁺ depletion, have been made when the sheep were either in normal Na⁺ balance or when they were mildly Na⁺-depleted. The reason for the latter modification lay in the possibility that a "threshold" concentration of electrolyte-active steroid was required in the peripheral blood before there was an effect on the parotid gland. Thus the parotid of a mildly Na⁺-depleted sheep with salivary Na⁺/K⁺ of, for example, 140/30=4.7 would be responding already to corticosteroid and presumably would register more readily an increased stimulus if a decrease of Na⁺ concentration in adrenal arterial blood provoked an increased production. The Na⁺ concentration of adrenal arterial blood was lowered by 8–20 mEq/l. for 120–405 minutes, and the K⁺ concentration was raised by 0.2–5.5 mEq/l. for 120–571 minutes. This result, which also lowered the plasma

Fig. 8.—T.P.2 (mild Na⁺ deficiency), 9/4/57. Effect on corrected salivary Na⁺/K⁺ ratio (O—O—O) and corrected salivary K⁺ concentration (X—X—X) of infusion into adrenal arterial blood of 4% glucose with 20 mEq of KCl/l. added at 0.65 ml./minute for 281 minutes. Infusion began at time 0. Effect of such an infusion on composition of adrenal venous blood is shown also.

Na⁺/K⁺ ratio, was produced by infusion of 4% glucose, with 10–40 mEq of KCl/l. added, at 0.4–1.5 ml./minute.

In none of nine experiments did this procedure of abruptly lowering the Na⁺ and raising the K⁺ of adrenal blood cause the parotid salivary Na⁺/K⁺ to decrease. Fig. 8 is an example. However, when the adrenal infusions were continued for 580–820 minutes, and the rate of infusion was increased in stepwise manner, so to an extent reproducing over this time the gradual change occurring over several days during progressive Na⁺ deficiency, a stimulation of adrenal steroid output occurred. Fig. 9 is an example. Control experiments with comparable Na⁺ intake were evidence against the effect produced being attributable to Na⁺ deficiency developing during the course of an experiment as long as 800–1,000 minutes.

In the course of this series some experiments done during mild Na⁺ depletion had an effect opposite to that which might have been expected. In one a small

FIG. 9.—T.P.9 (normal Na^+ balance), 2-3/7/58. Effect on salivary Na^+/K^+ ratio (O—O) of infusion into adrenal arterial blood of 4% glucose with 10 mEq of KCl/l added at 0.2–1.5 ml./minute for 444 minutes. Two control experiments made under same conditions of Na^+ balance are shown also (x—x, ▲—▲). The sheep was given NaHCO_3 during course of experiments to exclude possibility of Na^+ deficiency developing because of fistula loss. It received slightly more (400 mEq) at beginning of experiment involving infusion than it did in controls (300 mEq).

amount of haemolysis of adrenal arterial blood was produced during an attempt to lower Na^+ concentration without the use of glucose, and in another the K^+ concentration was raised above physiological levels—that is, to 10.4 mEq/l. In both examples the adrenal secretion was inhibited. These findings suggested that the adrenal cortex is a sensitive structure and the subtleties of its regulation are unlikely to be revealed by crude procedures.

Section II. Local Increase of Adrenal Arterial Na^+ Concentration in Na^+ -depleted Sheep

This experiment has been made 14 times during mild to gross Na^+ deficiency. The systemic arterial Na^+ concentration was reduced by Na^+ depletion from the normal value of 148 to 130–139 mEq/l. Solutions of NaCl (340–800 mEq/l.) were infused at 0.1–0.38 ml./minute into the adrenal arterial supply. Measurement of the Na^+ concentration in the adrenal venous effluent showed that it was raised by 7–36 mEq/l. according to rate of Na^+ infusion. Thus the change produced was of the order caused by a rapid infusion of 240 mEq of

FIG. 10.—T.P.9 (Na^+ -depleted), 7/2/58 and 31/1/58. Effect on corrected parotid salivary Na^+/K^+ ratio (▲—▲) of infusion into adrenal arterial blood of solutions of 400 and 800 mEq of NaCl/l . at 0.2 ml./minute for 290 minutes. Effect of this infusion on composition of adrenal venous blood is recorded also. A control experiment (T.P.9) shows effect on corrected salivary Na^+/K^+ ratio (O—O) of systemic infusion of 150 mEq of 4M NaCl under some conditions of Na^+ deficiency.

hypertonic NaCl systemically (Denton and McDonald, 1957), or a dose of 600 mEq of NaHCO_3 into the rumen. The infusions were made for 137 to 462 minutes (nine instances for more than 250 minutes), which was adequate time for complete development of the parotid response when systemic Na^+ concentration was raised in the course of correction of Na^+ deficiency by the standard systemic dose of Na^+ cited above. The total amount of NaCl infused to the adrenal varied from 13 to 62 mEq in the longest experiment, an amount which would have little or no systemic effect in view of the period over which it was given.

Local sustained rise of Na^+ concentration of the adrenal arterial blood in Na^+ -depleted sheep did not reproduce the effect of rapid correction of Na^+ deficit made by systemic arterial, intravenous, or intraruminal administration

of Na^+ . Fig. 10 shows a typical experiment on T.P.9. A control experiment (O—O) with a normal parotid response to the injection of 156 mEq of NaCl into the opposite carotid artery is also shown. The adrenal infusion raised the adrenal arterial Na^+ by 17 mEq/l., yet there was no response (▲—▲). An experiment on T.P.2 (Fig. 11), where the adrenal arterial Na^+ was raised gradually

FIG. 11.—T.P.2 (Na^+ -depleted), 4/9/57. Effect on corrected salivary Na^+/K^+ ratio (O—O) and corrected salivary K^+ concentration (x—x) of infusion into adrenal arterial blood of 400, 610, 700, 810, and 138 mEq of NaCl/l . at 0.2 ml./minute for 415 minutes. Effect on composition of adrenal venous blood is shown also.

from 19 to 35 mEq/l. above the systemic Na^+ concentration, had a small effect only, and may be compared with a control experiment on the same sheep made two days later, when the Na^+ deficit was the same. In the control the carotid loop was occluded cranial to the transplant, but this time the NaCl infusion was given into the opposite carotid artery. The systemic infusion of slightly more Na^+ , 56 mEq in 9 minutes (Fig. 12) compared with 38 mEq in 350 minutes (Fig. 11), caused a 6 mEq/l. rise of systemic Na^+ concentration. There was a clear-cut response 100–120 minutes after this injection, but it was short-lived. When the systemic Na^+ concentration was raised 20 mEq/l. by infusion of 281 mEq/ NaCl into this opposite carotid, there was a complete inhibition of electrolyte-active secretion (Fig. 12) whereas the local adrenal rise of 35 mEq/l. (Fig. 11) had little effect.

Section III. Local Reduction of Na^+ Concentration and Increase of K^+ Concentration of Adrenal Arterial Blood with the Aim of Preventing the Normal Adrenal Inhibition Following a Rapid Correction of Na^+ Deficiency

The object of these experiments was the prevention in the local adrenal circulation of the rise of Na^+ concentration and the plasma Na^+/K^+ ratio which occurs systemically when Na^+ deficiency is corrected rapidly. Thus if the adrenal gland responded directly

FIG. 12.—T.P.2 (Na^+ -depleted), 6/9/57. Effect on corrected salivary Na^+/K^+ ratio ($\circ$ — $\circ$) and corrected salivary K^+ concentration ($\times$ — $\times$) of systemic infusion (right carotid artery) of (a) 56 of 4M NaCl in 9 minutes at time 0, (b) 281 of 4M NaCl in 50 minutes at +180 minutes. Effect of infusion on Na^+ concentration in systemic arterial blood is shown also. Left carotid artery was occluded cranial to the adrenal transplant throughout experiment.

to lowered Na^+ concentration or plasma Na^+/K^+ ratio in Na^+ deficiency, the increased output of electrolyte-active steroid should persist while the local infusion continued. Further, an immediate control would be provided if the normal response to the rapid correction of Na^+ deficiency did occur an appropriate time interval after the local adrenal infusion stopped.

An additional advantage was that these experiments circumvented the problem that it might be necessary to lower the adrenal arterial Na^+ concentration and raise the K^+ concentration for a very long time before the biological indicator (the parotid gland) unequivocally registered any increased steroid output if such change occurred. Here, continuation of output was involved rather than the initiation of the adrenal secretory process. Exclusive of control experiments, this experiment has been made fourteen times during moderate to gross Na^+ deficiency. An infusion of 3–4% glucose with 10–40 mEq of KCl /l. added was made into the adrenal circulation. This was done in two different ways and the results of the two methods differed.

An infusion was begun into the adrenal circulation before or immediately after a large dose of Na^+ . In the first method the infusion was made at a constant rate which was sufficient in the light of known adrenal blood flow to

reduce Na^+ concentration approximately 20 mEq/l. and increase K^+ concentration 1–4 mEq/l. Under these conditions the reduction of Na^+ concentration would be immediate, but as the systemic Na^+ concentration rose progressively as the result of correction of Na^+ deficiency, so would that of the adrenal blood. However, the difference between its Na^+ concentration and that of systemic blood would be maintained apart from a small decrease attributable to increased adrenal blood flow occurring with correction of the animal's Na^+ deficiency—for example, T.P.9 (March 4, 1958), Na^+ deficient, adrenal venous blood flow 7 ml./minute, 220 minutes after 50 g. of NaHCO_3 in 1 litre of H_2O given into rumen = 9.0–9.8 ml./minute, which was in the range of normal. In the second method the rate of adrenal infusion was increased in a stepwise fashion following the initiation of the change of Na^+ balance. Under these conditions the adrenal Na^+ concentration would decrease in a stepwise manner, and the steady rise of it caused by the systemic infusion (as occurs for the first method) would be largely avoided.

The effect of the two procedures, constant and stepwise infusion, on T.P.12 when Na^+ deficient are compared in Figs. 13 and 14. In both instances the systemic infusion caused a significant rise of systemic Na^+ concentration, and in both instances the final rate of adrenal infusion ensured that the adrenal arterial Na^+ concentration was much lower than the initial systemic concentration and the K^+ concentration was slightly higher. With the stepwise infusion offsetting the progressive rise of adrenal Na^+ concentration, the normal parotid response to a rapid change of Na^+ balance was prevented ($\circ$ — $\circ$) (Fig. 13). The response occurred 100 minutes after the adrenal infusion was stopped. With the constant rate infusion, however, the response occurred normally 120 minutes after

FIG. 13.—T.P.12 (Na^+ -depleted), 1/5/58. Effect on corrected salivary Na^+/K^+ ratio ($\circ$ — $\circ$) of stepwise infusion of 4% glucose with 15 mEq of KCl /l. added into adrenal arterial circulation at 0.1–1.5 ml./minute for 360 minutes during rapid systemic correction of Na^+ deficiency. Rapid correction of Na^+ deficiency was made by infusion of 39 ml. of 4M NaCl = 156 mEq of Na^+ into right carotid artery. Effect of rapid correction of Na^+ deficiency on systemic arterial composition, and effect of adrenal arterial infusion on composition of adrenal venous blood are shown also. A control experiment on same animal is shown (Δ — Δ) in which same infusion was made into adrenal arterial circulation without concurrent rapid systemic correction of Na^+ deficiency.

the change of Na^+ balance began (Fig. 14). A control experiment is included also on Fig. 13 where exactly the same adrenal infusion was made but no change of systemic Na^+ balance was made concurrently (Δ — Δ). There was no effect. A further control experiment was made on T.P.12 under the same conditions except that a stepwise infusion of approximately isosmotic NaCl was made into the adrenal circulation. This would have had little or no effect on Na^+ and K^+ concentrations. A response to the rapid change of Na^+ balance induced at the same time occurred normally at 120 minutes.

Fig. 15 shows a typical experiment made on T.P.9. An intraruminal dose of 595 mEq of NaHCO_3 caused a rise of

FIG. 14.—T.P.12 (Na⁺-depleted), 21/5/58. Effect on corrected salivary Na⁺/K⁺ ratio of constant infusion of 4% glucose with 10 mEq of KCl/l. added at 1.5 ml./minute for 270 minutes during a rapid systemic correction of Na⁺ deficiency. Rapid correction of Na⁺ deficiency was made by infusion of 66 ml. of 3.8M NaCl=251 mEq of Na⁺ into right carotid artery. Effect of rapid correction of Na⁺ deficiency on systemic arterial composition and effect of adrenal arterial infusion on composition of adrenal venous blood are shown also.

9 mEq of plasma Na⁺/l. by 70 minutes, and the infusion of 4% glucose with 15 mEq of KCl/l. added at 1.5 ml./minute lowered the adrenal plasma Na⁺ to 123 mEq/l. and raised K⁺ by 2.9 mEq/l. to 6.8 mEq/l. The parotid response was blocked during the infusion, which lasted 230 minutes, but occurred 100–120 minutes after the infusion was stopped.

Summarizing, the stepwise infusion prevented the normal adrenal inhibition after rapid correction of Na⁺ deficiency in 9 out of 10 experiments, whereas in three out of four experiments with constant infusion the normal inhibition occurred.

Section IV. Local Increase of Adrenal Arterial Na⁺ Concentration with the Aim of Preventing Corticosteroid Secretion as Na⁺ Deficiency Began

In T.P.9 it was observed that the parotid salivary Na⁺/K⁺ ratio began to decrease from normal

FIG. 15.—T.P.9 (Na⁺-depleted), 24/4/58. Effect on corrected salivary Na⁺/K⁺ ratio of an infusion into adrenal arterial blood of 4% glucose with 15 mEq of KCl/l. added at 0.1–1.5 ml./minute for 230 minutes during rapid correction of Na⁺ deficiency by a dose of 595 mEq of NaHCO₃ into rumen. Effect of intraruminal dose on systemic arterial plasma composition, and effect of adrenal arterial infusion upon adrenal venous plasma composition is shown also.

B

approximately 11 to 15 hours after the last intraruminal dose of Na⁺ was given (×---×, ▲---▲). Two experiments were made under these conditions (○---○, ●---●) in which the adrenal arterial Na⁺ concentration was progressively increased over seven to nine hours (Fig. 16). During the infusion there was no fall in salivary Na⁺/K⁺ ratio, but there was a significant rapid fall 90–120 minutes after the infusion stopped. The amount of Na⁺ infused to the adrenal would not have prevented a significant degree of Na⁺ deficiency developing during the day, and the fall of salivary Na⁺/K⁺ ratio following shortly upon cessation of the infusion could not be attributed to the sudden onset of Na⁺ deficiency. This appeared to

FIG. 16.—T.P.9. Experimental observations at beginning of period of Na⁺ deficiency. Control experiments (▲---▲, ×---×) show usual fall of salivary Na⁺/K⁺ ratio in animal at this time. Effect on salivary Na⁺/K⁺ ratio (○---○, ●---●) of infusion into adrenal arterial blood of 400, 600, and 800 mEq of NaCl/l. at 0.2 ml./minute for 430–550 minutes is shown.

indicate that the local increase of adrenal arterial Na⁺ prevented the development, at the usual time, of the fall in the salivary Na⁺/K⁺ ratio in the response to Na⁺ depletion.

Section V. Control Experiments

Control experiments demonstrating the normal parotid response to rapid correction of Na⁺ deficiency under conditions where the left carotid artery was occluded cranial to the transplant have been cited already. The principal questions requiring consideration in relation to the methods used to alter locally the ionic environment of the adrenal were the effect of the infusion on the adrenal temperature, the plasma protein osmotic pressure, and whether the glucose used to induce ionic changes at the adrenal level had effects itself. These questions were only likely to have significance in the experiments where the adrenal Na⁺ concentration was lowered by infusion at rates as great as one-fifth to one-tenth of the adrenal blood-flow rate. In the experiments involving local increase of Na⁺ concentration during Na⁺ deficiency, the infusion rate (usually 0.2 ml./minute) was a very small fraction of the blood-flow rate, and glucose was not used. Appropriate experiments, including the use of mannitol, provided evidence against any of the effects observed being attributable to these factors.

Summary of Experiments I–V

They aimed to establish whether changes of Na⁺ and K⁺ concentration of adrenal arterial blood had a direct effect on the adrenal secretion of electrolyte-active

steroid. First, and particularly important, a large local rise of Na^+ concentration, up to or above normal in a Na^+ -depleted sheep, did not produce an effect similar to a rapid correction of Na^+ deficiency, though the change was maintained for an adequate time. In raising the question of whether any misleading factor could have inadvertently entered into these experiments, the principal issue is that a normal response to rapid correction of Na^+ deficiency is prevented if an animal is disturbed or frightened. Now, these animals were trained and confident, and there was no evidence from demeanour, saliva secretion rate, or cardiac rate that they were in any way disturbed. In fact, it was in the control experiments where the 4 M NaCl raised systemic Na^+ rapidly by 20 mEq that there was some restlessness and evidence of thirst, yet the response occurred normally. It seemed legitimate to suppose that if a simple rise of Na^+ concentration or Na^+/K^+ ratio of the adrenal arterial blood caused this response, we would have reproduced it by these experiments.

The experiments involving decreased Na^+ and increased K^+ to the adrenal indicated that this change could stimulate electrolyte-active steroid production if continued long enough, and that progressive reduction of Na^+ and increase of K^+ could prevent inhibition of it during rapid change of Na^+ balance. In this latter series, the fact that the normal response did occur about 90–100 minutes after stopping the adrenal infusion was strong evidence that the local ionic change at the adrenal level had prevented adrenal inhibition. Conversely, in the experiments where the normal response to commencing Na^+ deficiency was prevented by gradually increasing adrenal arterial Na^+ concentration (Section IV), there would have been an abrupt fall of adrenal arterial Na^+ concentration when the infusion stopped. A response occurred shortly afterwards, though the procedure of abruptly lowering adrenal Na^+ concentration (Section I) of itself did not stimulate the adrenal.

These findings, along with those of Section II, suggested the possibility that alteration of a standing state at the adrenal level involved concurrent action of at least two components of a causal system. That is, the results recorded led to a conclusion that appropriate local change of the Na^+ and K^+ concentrations of adrenal arterial blood did not cause the same extent and time relationship of change of adrenal secretion which occurred during alteration of Na^+ balance. However, there was evidence that such local ionic changes affected the adrenal secretory responses which occur in these circumstances.

The local ionic changes may be a contributory condition in a set of jointly sufficient and severally necessary conditions which operate at the adrenal level to cause the physiological response to change of Na^+ balance. Thus in relation to the question of whether the adrenal responds directly to local changes in its ionic environment or whether it responds to a stimulus from a distance, it is possible that there is a causal system involving both, and, moreover, that stimuli to the receptor controlling the humoral stimulus may be physiological effects of Na^+ deficiency other than change of systemic ionic concentrations. In the experiments cited above we may have dissociated to some extent the two stimuli, which usually work concurrently and synergistically.

[Part II, together with a list of references, will appear in our next issue.]

STEROID THERAPY IN HIRSUTISM AND VIRILISM

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The effects of steroid therapy in hirsute women with increased excretion of 17-ketosteroids have been reported by Spence *et al.* (1956), who described the use of cortisone acetate by the oral and intramuscular routes, hydrocortisone acetate by intramuscular injection, and oral prednisone and prednisolone. Further observations are reported here on the use of oral prednisone and prednisolone, and of intramuscular hydrocortisone acetate, and on a trial of a new preparation, prednisolone trimethylacetate, which consists of a microcrystalline suspension of 200 mg. of the hormone in 2 ml. of an aqueous isotonic buffered solution.

Prednisolone Trimethylacetate

The effects of prednisolone trimethylacetate on the urinary excretion of 17-ketosteroids were studied in five patients. One of these was a female pseudo-hermaphrodite aged 15 years (Case 5); the other four were hirsute women with oligomenorrhoea (Cases 1–4). Each patient was given one or more intramuscular injections of 200 or 400 mg. of prednisolone trimethylacetate. The urinary 17-ketosteroid output was estimated by the standard M.R.C. method over at least two 24-hour periods before injection and thereafter on successive days or at intervals of up to one week.

The resting 17-ketosteroid excretion varied between 35.6 and 62.3 mg. (mean 46.8 mg.) per 24 hours in Case 5, and the mean output in Cases 1–4 ranged from 17 to 25 mg. per 24 hours.

Prednisolone trimethylacetate was originally given in a single intramuscular dose of 200 mg. to two hirsute women (Cases 1 and 2). This produced no significant

FIG. 1.—Effect of prednisolone trimethylacetate (P.T.M.A.) injected intramuscularly and of oral prednisone on urinary 17-ketosteroids. (Case 1.)