can be seen from the work on 6-mercaptopurine or 5-fluorouracil, and on the so-called mutagenic agents, to name only a few agents of promise. In the tumour problem, as with the viruses, however, we lack vital knowledge about the nucleic acids of the cell.

What are the significant differences between the nucleic acids of the tumour cell and those of the normal cell? Differences there must be, but I am sure they are not likely to be determined on the basis of gross analysis of cellular nucleic acid preparations as we now know them. For surely there must be many different nucleic acids in the nucleus of an animal cell, and the abnormality of the tumour cell is not *a priori* likely to be noticed in an analysis of the mixture for purine and pyrimidine bases. The vital difference may lie only in base sequence of a few of the nucleic acids present, although there is another possibility which should perhaps be borne in mind. In the last year or two there have been a number of discoveries of very small amounts of abnormal bases and nucleosides in hydrolysates of many natural nucleic acids. Since these preparations were doubtless heterogeneous (it is doubtful whether a homogeneous nucleic acid has yet been prepared), it seems to me that instead of these abnormal nucleosides being present as very widely spaced units in a long polynucleotide chain they may well represent residues from nucleic acids of quite unusual structure which are present as very minor components of the nucleic acid preparations examined. If this is so, is it possible that these minor components are important in the economy of the cell? Such questions—and they are important questions—we cannot answer until we can separate and study the nucleic acids as homogeneous individuals.

Conclusion

To sum up, then, it seems to me that the future progress of chemotherapeutic work based on nucleic acids in the tumour and virus field, if it is to be something other than a hit-and-miss procedure, depends on real physical and chemical advances being made in two main directions. Firstly, methods must be found—and they do not exist to-day—for separating into their individual components the nucleic acids of cells and of preparing pure individual nucleic acids: this may first be attained with the nucleic acids of some of the plant viruses. Secondly, we must perfect experimental methods which will allow us to study the structure of these pure nucleic acids in detail and clarify their relationship with one another. Given these advances, we would not have an immediate solution to the problems we have been discussing, but without them I find it hard to be hopeful about quick results in virus chemotherapy. For we are dealing here with a branch of chemotherapy which demands agents of a remarkable specificity which has so far been observed only in immunological studies.

It is this need for a very high degree of specificity, coupled with the need for complete and irreversible inactivation of certain polynucleotides, that makes the problem of virus and tumour disease so intractable. But the progress so far made in their treatment with chemical agents suggests that, with the further development of basic knowledge of nucleic acid structure and function, the combined work of the chemist and biologist will in the future yield a real solution of these vital problems.

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CONTROL OF ADRENAL SECRETION OF ELECTROLYTE-ACTIVE STEROIDS*

ADRENAL STIMULATION BY CROSS-CIRCULATION EXPERIMENTS IN CONSCIOUS SHEEP

BY

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PART II

In the light of the evidence that local changes of adrenal ionic environment were not sufficient to account for the changes of electrolyte-active steroid secretion which occur during change of Na^+ balance, two possibilities existed: (1) Other non-hormonal changes at the adrenal level were operative and, in combination with the ionic factors outlined above, were sufficient to cause alterations of adrenal secretion seen during change of sodium balance. (2) That a hormonal stimulus was acting upon the adrenal from a distance. In view of the fact that the transplanted adrenal gland is almost certainly denervated, the stimulus would be humoral, not neural.

It will be recognized readily, in relation to the many physiological factors which change in Na^+ depletion, that the way is open to induce many combinations of change at the adrenal level. Apart from infusions of

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pharmacologically active agents such as acetylcholine, noradrenaline, serotonin, and bradykinin, changes of blood flow can be induced by application of cuffs to the two carotid loops—for example, inflation of both cuffs to 200 mm. Hg reduces blood pressure in the head and increases it systemically as a result of the carotid sinus reflex. The cuff on the transplant loop may be placed either cranial or caudal to the adrenal so that the gland is included in either the high- or low-pressure section of the animal's systemic circulation. These blood-flow changes can be combined with concurrent ionic changes. Some experiments have been made, but the work has not progressed yet to a point permitting any conclusion. We wish to report here work on the second possibility.

Arterial Cross-circulation Experiments in Conscious Sheep

In endeavouring to find out whether such a hormonic agent existed, it seemed correct formally to try first *to demonstrate it in the circulating* blood rather than seek it in excreta or make an extract of a supposed site of origin. For the reasons stated already, the aim would be to make such experiments in conscious confident animals. To this end we have engaged in arterial cross-circulation experiments in conscious sheep. As a working hypothesis, and by analogy with corticotrophin, it was assumed that this supposed agent would be at greatest concentration in the blood stream of a Na^+-depleted adrenally insufficient sheep.

The *donor* sheep was prepared as follows. The daily D.C.A. supplement was increased from basal of 5 mg./day to 20 mg./day, and the daily Na^+ supplement was withheld. To maintain a Na^+-depleted adrenalectomized sheep in relatively normal condition it is necessary to give four times the basal amount of D.C.A. After three to four days the sheep had become depleted of 400–700 mEq of Na^+ and it was arranged that the last injection of 20 mg. of D.C.A. was given 30–36 hours before the intended cross-circulation experiment. This allowed time for the effect of the D.C.A. injection to wear off and the salivary Na^+/K^+ ratio to return to normal, as it invariably did in an adrenally insufficient animal (Goding and Denton, 1959). The plasma composition of this animal reflected adrenal insufficiency and severe Na^+ depletion.

It was ensured that the sheep with the adrenal transplant was in normal Na^+ balance by giving a large dose of Na^+ 18 and 5 hours before the experiment. Its salivary Na^+/K^+ was normal (20–40), and the plasma composition indicated normal balance. Venous drainage of the blood from the adrenal was arranged, and then the blood from the Na^+-depleted heparinized donor, which stood quietly in its cage, was led from the left carotid artery loop (Fig. 17) to the carotid artery supplying the adrenal transplant of the heparinized sheep in normal Na^+ balance. This loop was completely occluded above and below the transplant by 300 mm. Hg pressure, so that the cross-circulation line was the only source of blood supply. The recipient with a towel over its eyes was gently restrained on its side on a table covered with sponge rubber, about 30–50 cm. below the donor, and it lay quietly for three to five hours without change of cardiac rate. In any case, this animal's adrenal was removed from its circulation once the cross-circulation began, and hence if any disturbance had occurred after this it would have been without effect on the experiment.

The donor blood was reinfused into the carotid artery supplying the parotid so as to provide the maximum sensitivity for response by the biological indicator to any changes of steroid output induced. There are reasons for aiming to replace the cross-circulated blood by the intravenous route, but in order to obtain a significant result it might be necessary to be sure the cross-circulation would continue for at least

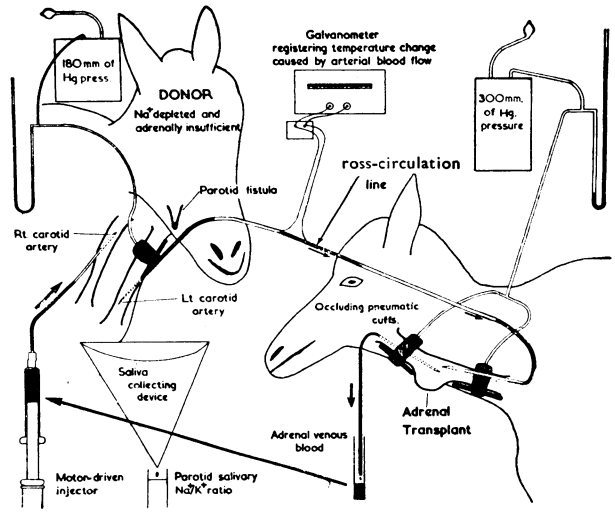

FIG. 17.—Method of cross-circulation in conscious animals. A wide-bore needle and polythene tube inserted into left carotid artery of Na^+-depleted adrenalectomized *donor* leads blood to carotid artery supplying adrenal transplant of *recipient*. This loop is occluded above and below transplant by cuffs at 300 mm. Hg Adrenal venous effluent is collected from indwelling jugular cannula, and blood is reinfused into right carotid artery loop of donor. Saliva from right parotid fistula of donor is collected and analysed at frequent intervals.

four to six hours. Because a long period of cross-circulation in conscious animals is technically difficult, we have in the first instance preferred this method of short-circuiting the systemic circulation, and have relied upon appropriate control experiments in interpreting the results.

It was found that this cross-circulation caused the parotid salivary Na^+/K^+ ratio to decrease over a maximum range. Fig. 18 shows the result of such an experiment. The intracarotid infusion of 82 ml. of adrenal venous blood of the transplant sheep in normal

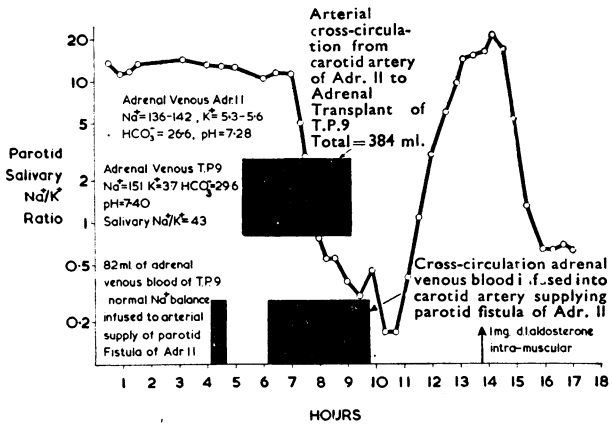

FIG. 18.—Adr. 11 (bilaterally adrenalectomized and Na depleted). Effect on parotid salivary Na^+/K^+ ratio of (*a*) ipsilateral intracarotid infusion of 82 ml. of adrenal venous blood from T.P.9 (normal Na^+ balance); (*b*) ipsilateral intracarotid infusion of 384 ml. of its own peripheral blood after cross-circulation through adrenal gland of T.P.9; (*c*) intramuscular injection of 1 mg. DL-aldosterone.

Na^+ balance had no effect on the Na^+/K^+ ratio of the adrenally insufficient Na^+-depleted donor sheep. However, after infusion of cross-circulated blood into the carotid artery of the donor sheep there was an early and precipitate fall in salivary Na^+/K^+ ratio. By 120 minutes the ratio had fallen from 194/17=11.4 to 63/114=0.55 (volume of adrenal venous blood infused =212 ml.), and after 264 minutes the ratio was 25/143=0.17. The ratio began to rise sharply 60 minutes after the carotid infusion ceased and was normal 120 minutes later. After it had returned to normal a test dose of 1 mg. of DL-aldosterone was given intramuscularly. About 60 minutes later the ratio fell steeply, but the change (196/12=16.3, to 63/99=0.64) was less than produced by the cross-circulation.

In these experiments three procedures were adopted to ensure that the cross-circulation was running, since, it was possible that, despite the pressure on the occluding cuffs of 300 mm. Hg, the "water-hammer" effect of the pulse would force blood past it if the cross-circulation line clotted. Hence (1) a Wheatstone bridge was constructed in which one arm consisted of a coil of fine wire wound around the cross-transfusion line—this enabled continuous observations to be made on blood flow through the line by virtue of the change in resistance with temperature; (2) the adrenal venous blood was analysed at intervals; and (3) after inflation of the cuffs the plasma of the recipient sheep was marked by intravenous injection of Evans blue, and it was confirmed at intervals that the adrenal blood contained none of this marker.

A second cross-circulation had the same result. It gave greater effect than the standard aldosterone injection.

Control Experiments

The absence of blood-incompatibility problems in these merino sheep carried the advantage that the two cross-circulation animals could be used for many control experiments involving transfer of blood. It was important to show that the procedure of "short-circuiting" the systemic circulation would not itself cause a parotid response. Thus the total adrenal venous blood of T.P.9 in normal Na^+ balance was withdrawn for 195 minutes, at the same rate as the blood flow of a cross-circulation, and infused into the carotid supplying the parotid of the Na^+-depleted, adrenally insufficient sheep. No effect at all occurred during 170 minutes (Fig. 19), whereas in a cross-transfusion a response was fully developed by 120–180 minutes. A response did begin at 170 minutes, but by this time approximately 15% of the circulating blood volume of T.P.9 had been removed via the adrenal vein. It is possible that by this stage this blood loss evoked adrenal-stimulating mechanisms. The standard dose of aldosterone had a larger effect. In a second experiment the total adrenal blood flow was withdrawn for 55 minutes (volume= 486 ml.) and infused without any effect on the salivary Na^+/K^+.

A further condition for control was that in the cross-transfusion there was an abrupt fall of Na^+ concentration and rise of K^+ concentration of the adrenal arterial blood, and hence the above experiment was repeated twice during the course of an infusion of 4% glucose with 10 mEq of KCl/l. added into the adrenal arterial blood. Though Na^+ was reduced by 10 mEq/l. and K^+ increased by 0.5 mEq/l., the effect was very much less than a cross-circulation (Fig. 20). These control experiments showed that the adrenal venous blood of a sheep in normal Na^+ balance had only a small effect on the sensitive biological indicator, whereas the cross-circulation from a Na^+-depleted sheep had an effect similar to that of infusing the adrenal venous blood of a Na^+-depleted sheep (cf. Fig. 7).

A further important control experiment is recorded in Fig. 21. In this instance, the same donor sheep, though adrenally insufficient, was in *normal Na^+ balance*. The cross-circulation caused a decrease of parotid salivary

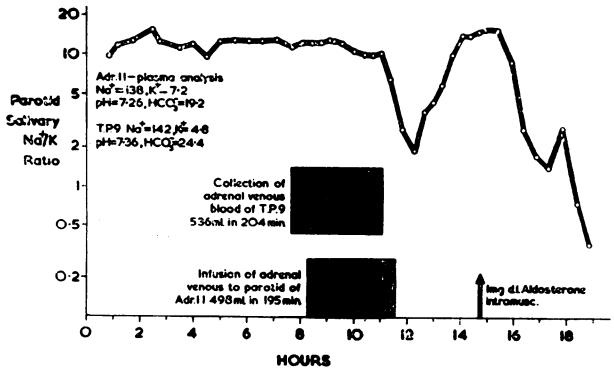

FIG. 19.—Adr. 11 (bilaterally adrenalectomized and Na^+-depleted), 17/4/58. Effect on parotid salivary Na^+/K^+ ratio of (*a*) ipsilateral intracarotid infusion of 498 ml. of adrenal venous blood of T.P.9 (normal Na^+ balance); (*b*) intramuscular injection of 1 mg. DL-aldosterone.

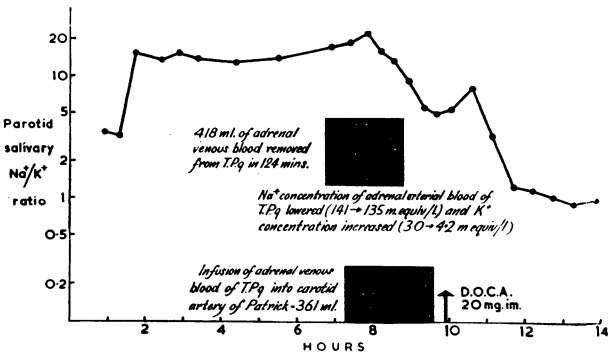

FIG. 20.—Patrick (bilaterally adrenalectomized and Na^+-deficient), 18/9/58. Effect on parotid salivary Na^+/K^+ ratio of (*a*) ipsilateral intracarotid infusion of 361 ml. of adrenal venous blood of T.P.9 (normal Na^+ balance) collected when adrenal arterial Na^+ concentration was lowered and K^+ concentration raised; (*b*) intramuscular injection of 20 mg. D.C.A.

Na^+/K^+, though it was much less than that caused by the standard dose of 1 mg. of DL-aldosterone. A further control procedure is shown in Fig. 21. Since there was variability of the rate of blood flow in the cross-circulation, and it was slower than the normal adrenal blood flow, the adrenal venous blood of T.P.9 was collected for 130 minutes under conditions of much more severe restriction of arterial inflow (rate=1.5–2.5 ml./minute) than occurred in any cross-circulation. There was an effect on adrenal secretion, but the response over the time interval was much less than a cross-circulation relative to the standard 1 mg. of DL-aldosterone injection.

Summary of Results

The results gave indication of a humoral stimulus other than ionic change affecting the adrenal. The principal question is whether or not the humoral agent in the blood stimulating the adrenal in this manner is corticotrophin. Certainly corticotrophin would be at maximal concentration in the blood of a Na^+-depleted adrenalectomized sheep which is the donor in a cross-

circulation. Formally, in considering the role of corticotrophin in adrenal secretion of aldosterone the following possibilities exist: (1) That the anterior pituitary, through corticotrophin, is the normal physiological regulator of adrenal aldosterone secretion, and secretion of corticotrophin occurring in Na^+ depletion stimulates the adrenal. (2) That the adrenal is stimulated to produce aldosterone by either a different humoral agent and/or local adrenal factors, but their action is enhanced by corticotrophin secretion caused by a non-specific stress reaction to the physiological consequence of Na^+ depletion—that is, corticotrophin is a contributory cause of adrenal aldosterone secretion. (3) That corticotrophin secretion, by virtue of ensuring a normal output of glucocorticoids, is a permissive condition of a normal aldosterone secretion (cf. Muller, 1958). (4) That the adrenal secretion of aldosterone is relatively independent of corticotrophin.

The evidence against direct anterior pituitary control of aldosterone secretion is (*a*) the relatively normal electrolyte metabolism in panhypopituitarism and hypophysectomy, and the fact that increased excretion of aldosterone in response to Na^+ depletion may occur in these conditions; (*b*) the failure of large doses of exogenous hydroxycorticosteroids to influence aldosterone secretion in the face of known suppression of anterior pituitary secretion (Ingle, 1938; Sayers and Sayers, 1949; Farrell, Banks, and Koletsky, 1956); (*c*) the much smaller effect of hypophysectomy on the zona glomerulosa, which, on the basis of histological (Deane and Greep, 1946; Deane, Shaw, and Greep, 1948) and isolated slice studies (Ayres, Gould, Simpson, and Tait, 1956; Giroud, Stachenko, and Piletta, 1958), appears to be the site of aldosterone production; (*d*) the relative inconstancy between batches of the effect of corticotrophin on aldosterone secretion and Na^+ metabolism.

In the light of this we made a cross-circulation in which the donor sheep was heavily loaded with hydroxycorticosteroids for 36 hours before the experiment with the object of suppressing endogenous corticotrophin production. The results are shown in Fig. 22. It received 100 mg. of cortisone per day for two days, and on the day preceding the experiment it received 100 mg. of cortisone and 20 mg. of D.C.A. at 3.45 a.m. It was given a further 100 mg. of cortisone at 5.25 p.m. on that day. At 6.55 a.m. on the day of the experiment (nine hours before the cross-circulation) a further 50 mg. of cortisone was given. As can be seen in Fig. 22, this had a small effect on the salivary Na^+/K^+ ratio. Six hours before the transfusion 17 mg. of hydrocortisone (as hemisuccinate) was given by intravenous infusion over a period of five minutes. This caused the ratio to fall again, but it rose over the next two and a half hours despite intravenous hydrocortisone infusion at the rate of 6 mg./hour. Allowing for the weight of the animal (about 30 kg.), this last dose was at least twice that employed by Bethune, Nelson, and Thorn (1957) to suppress corticotrophin production in patients with Addison's disease.

Three hours later the cross-circulation was begun, and this was our most successful experiment technically, in so far as 1.75 litres of blood flowed through the adrenal. The procedure stimulated the adrenal to cause a maximal parotid response, which was sustained for two hours after the infusion of adrenal venous blood was stopped. The donor sheep was then given an infusion of peripheral blood from a sheep in normal Na^+ balance to replace adrenal venous blood taken for analysis. After the parotid salivary Na^+/K^+ returned to normal the usual test dose of 1 mg. of aldosterone was given. The cross-circulation had a much larger effect. *Thus, despite this procedure aiming to suppress corticotrophin secretion, the response was maximal.* The adrenal blood flow increased progressively during the experiment from 4 to 7 ml./minute. This change, concurrent with the development of the response, was further evidence against the response being caused by adrenal haemodynamic effects.

Further suggestive evidence in sheep that corticotrophin was not the primary cause of secretion of aldosterone came from experiments in which Na^+-depleted sheep were given a very large single dose of

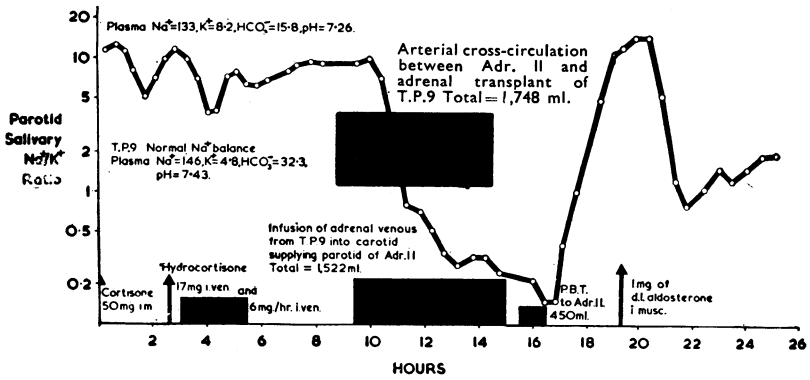

FIG. 21.—Adr. 11 (bilaterally adrenalectomized and in normal Na^+ balance), 7/3/58. Effect on parotid salivary Na^+/K^+ ratio of (*a*) ipsilateral intracarotid infusion of 205 ml. of its own peripheral blood after cross-circulation through adrenal gland of T.P.9 (normal Na^+ balance); (*b*) ipsilateral intracarotid infusion of 216 ml. of adrenal venous blood of T.P.9 collected during severe restriction of blood flow; (*c*) intramuscular injection of 1 mg. DL-aldosterone.

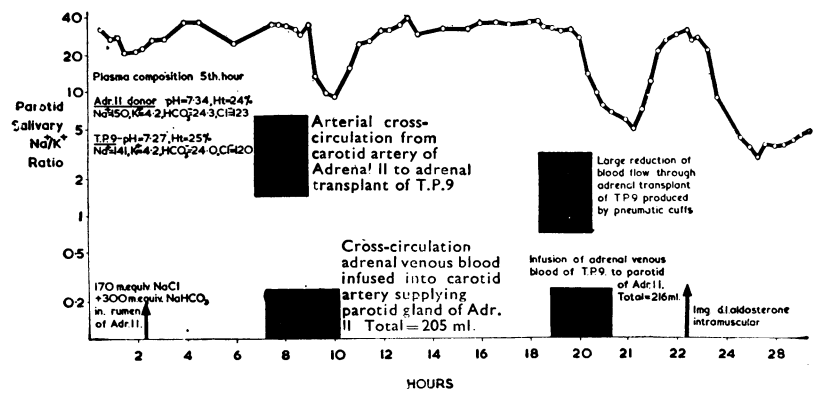

FIG. 22.—Adr. 11 (bilaterally adrenalectomized, Na^+-depleted, and receiving large doses of cortisone and cortisol), 29/5/58. Effect on parotid salivary Na^+/K^+ ratio of (*a*) intramuscular injection of 50 mg. cortisone; (*b*) intravenous injection of 17 mg. hydrocortisone hemisuccinate followed by intravenous infusion at 6 mg./hour of same material; (*c*) ipsilateral intracarotid infusion of 1.52 litres of its own peripheral blood after cross-transfusion through adrenal gland of T.P.9 (normal Na^+ balance); (*d*) intramuscular injection of 1 mg. DL-aldosterone.

cortisone (150 mg. intramuscularly) or hydrocortisone (20 mg. intravenously) followed by an intravenous infusion of hydrocortisone at 13.5 mg./hour for two and a half hours. This dose was again larger than that used by Bethune *et al.* (1957) to suppress corticotrophin secretion, but the salivary Na^+/K^+ remained at its low level during the next seven hours. There was no effect similar to a rapid change of Na^+ balance, a result which might have followed if it were supposed that the adrenal secretion of aldosterone in Na^+ depletion was caused by corticotrophin.

So far, the results of the cross-circulation have shown the stimulus to electrolyte-active secretion to be humoral, and are suggestive of the possibility of a hitherto unrecognized hormone. The results will be convincing in regard to this interpretation if cross-transfusion from a hypophysectomized adrenally insufficient Na^+-depleted donor gives this result. The findings with the biological indicator should at the same time be correlated with chromatographic analysis of the adrenal venous blood.

Other procedures which aim to demonstrate the secretion of a hormone are (1) the loss of the effect after removal of the site of origin, (2) isolation of the substance from excreta, and (3) extraction of the material from the supposed site of origin.

Loss of Effect After Removal of Site of Origin

In considering the loss of the effect after removal of the site of origin, a possibility was that, alternative to the usual view that the adrenal controlled the kidney in certain respects, the kidney might be the site of a receptor sensitive to haemodynamic change or supply of Na^+ and, accordingly, secrete an adrenotrophic hormone. However, this hypothesis fell when a normal parotid response to Na^+ depletion was demonstrated in a nephrectomized sheep. There was, however, positive evidence suggesting that the site might be in the brain. The variation in Na^+-retaining activity between batches of corticotrophin (Selkurt, 1954), and the presence of material stimulating aldosterone synthesis by zona glomerulosa slices in some batches of p.p.e. "infundin" (Giroud *et al.*, 1958), and the stimulation of aldosterone secretion in hypopituitarism by monkey and human growth hormone (Beck, McGarry, Dyrenfurth, and Venning, 1957), raises the question of the presence of a contaminant in some preparations. Inspection of the bulk material used for extraction by the laboratory producing corticotrophin here in Australia showed that the material sometimes included a considerable amount of pituitary stalk with the hypothalamus often adherent.

There have been reports of salt-wasting syndrome in patients with some cerebral diseases (Peters, Welt, Sims, Orloff, and Needham, 1950; Welt, Seldin, Nelson, German, and Peters, 1952). Rauschkolb and Farrell (1956) have reported that, in dogs, decerebration or decapitation reduces the aldosterone content of adrenal venous blood collected during anaesthesia to 25%, whereas hypophysectomy reduced it to only 66% of the control group (Rauschkolb, Farrell, and Koletsky, 1956). These dogs, which were anaesthetized with pentobarbitone sodium, were presumably in normal Na^+ balance, and the procedure involved the collection of a considerable fraction of the animal's blood volume via the adrenal vein. In a study on the secretion of the acutely transplanted adrenal (Fleming and Farrell 1956), reference was made to the replacement of this blood by infusion of dog blood. However, in other studies (Rauschkolb *et al.*, 1956; Farrell *et al.*, 1956; Rauschkolb and Farrell, 1956; Farrell, Fleming, Rauschkolb, Yatsu, McCally, and Anderson, 1958) this does not appear to have been done. Hence the baseline conditions relative to which a putative agent was examined were the combined effects of trauma, anaesthesia, and blood loss.

The rates of secretion of aldosterone for the control groups in these experiments—20–30 μg./100 kg. body weight/hour—are of the order reported by Reich and McDonald (1958) for Na^+ deficiency in the conscious sheep. The result is clearly seen in the experiments on the effect of blood loss (Farrell, Rosnagle, and Rauschkolb). There were six groups, each of five to seven dogs, and consecutive adrenal venous collections were made at 20-minute intervals for 180 minutes. For the pooled specimen during the first 20 minutes, the mean aldosterone detection rates of the groups were 8.8, 23.2, 9.3, 15.1, 8.5, and 9.5 μg./100 kg. body weight/hour. The reason for the variation between groups was not immediately evident. The secretion rate doubled or more during the 180 minutes of collection of adrenal blood. The aldosterone detection rates during the first 20 minutes corresponded in some groups to the reduction from control values caused by decerebration (Rauschkolb and Farrell, 1956). Thus decerebration appeared to reduce the adrenal stimulus resulting from severe blood loss. It remains to be determined whether comparable removal of the nervous system dislocates the adrenal stimulation resulting from Na^+ deprivation. The possibility of variability of assay under conditions of this complexity was shown in the investigation of steroidogenic properties of purified corticotrophins (Farrell *et al.*, 1958), where a considerable difference was found between the effect of two specimens of corticotrophin from the same original lot.

The question arises whether, from a quantitative aspect as well as from the viewpoint of the supposed mechanism to be demonstrated, the decerebration evidence might be advanced by including observations on Na^+-deficient animals—that is, on those already secreting aldosterone at a convincing rate in response to the normal stimulus. Investigations in this laboratory have examined (*a*) whether removal of the brain rostral to the pons in a Na^+-deficient animal causes changes similar to rapid correction of Na^+ deficiency; (*b*) whether removal of the brain rostral to the pons interfered with the changes following rapid correction of Na^+ deficiency; and (*c*) whether the same extent of brain removal in a Na^+-replete animal alters the response to Na^+ deficiency.

The investigation is still in progress, and at this stage we can only report (*a*) that such decerebration does not reproduce the whole effect of rapid correction of Na^+ deficiency, though in some instances the salivary Na^+/K^+ did rise 120 minutes after the operation by one-third of the amount necessary to return it to normal; (*b*) a normal response occurred after rapid correction of Na^+ deficiency made eight hours after a considerably more rostral decerebration—that is, quadrigemina and portion of mammillary bodies intact; and (*c*) 36 hours after decerebration and hypophysectomy were carried out on a Na^+-replete animal it was depleted of about 500 mEq of Na^+ (bilateral parotid secretion and buccal saliva loss). The salivary Na^+/K^+ ratio had fallen from 11–12 to 3–4.

In these experiments the surgical procedures of vessel ligation preceding decerebration usually lowered the

salivary Na^+/K^+ ratio, as did the operation of decerebration itself in a Na^+-replete animal. Thus factors unrelated to change of Na^+ balance were acting, at least temporarily, under the conditions of these experiments, and this will have to be taken into account in any eventual attempt to interpret results.

Isolation of Substance from Excreta

Orti, Ralli, Laken, and Dumm (1957) reported that extracts of urine of Na^+-depleted adrenalectomized rats when injected into normal rats caused Na^+ retention. The effect, however, was not large. Dr. R. Bradley and Mr. J. Nelson used the same procedure to make an extract of 16 hours' output of urine in the Na^+-depleted adrenally insufficient sheep used in the cross-circulation. Infusion of this extract during 368 minutes direct into the adrenal arterial supply of a mildly Na^+-depleted sheep had no effect on the salivary Na^+/K^+ ratio.

Extraction of Material from Supposed Site of Origin

Farrell (1957) has reported preparing a diencephalic extract which stimulated aldosterone output in adrenal venous blood of decerebrate dogs. In collaboration with Dr. Bradley and Mr. Nelson we have taken the heads off 450 sheep within three minutes of death, and removed the ventral hypothalamus and pituitary stalk. The pieces were collected into a beaker at $-60°$ C. The anterior pituitary was not included. So far it has been found that intravenous injection of a saline extract of the residue after acid–acetone extraction of the material has reduced or prevented the normal response to rapid correction of Na^+ deficiency (Fig. 23). The

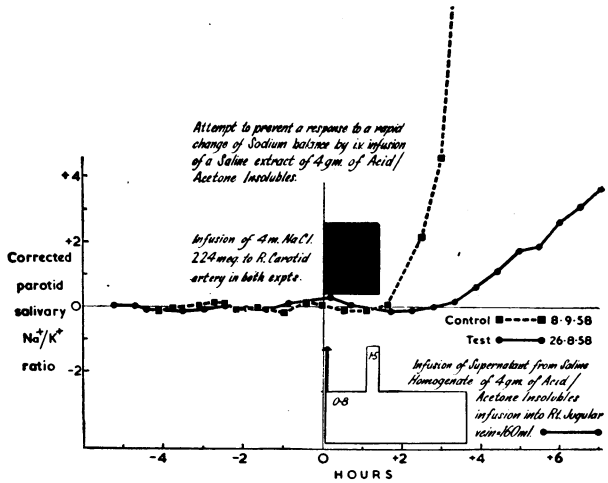

FIG. 23.—Ned (Na^+-depleted), 26/8/58. Effect on parotid salivary response to rapid correction of Na^+ deficiency of intravenous infusion of the supernatant of a saline homogenate of ventral hypothalamus (see text): ●——●. Control experiment: ■ - - - - ■.

effect has not yet been tested by infusion in hypophysectomized animals or at one-thousandth of this concentration direct into the adrenal circulation, nor have any experiments been made to determine to what extent the effect might be attributable to extraction of an area particularly rich in pharmacologically active materials. The assertion by Stack-Dunne and Young (1954) in relation to corticotrophin that chemical artifacts with biological activity can be produced by extraction procedure would be a further reason for not supposing at this stage that this effect is related to any agent which circulates in the blood under physiological conditions.

Nature of Stimulus to Receptor Controlling Aldosterone Secretion

If there is a hormonal factor contributing to regulation of aldosterone secretion the question arises of the stimulus to its release. The receptor may not be in the same place as the tissue secreting the humoral agent. and we have alluded already to the possibility that the effective stimulus or stimuli may be other than the ionic concentration changes shown to be active at the adrenal level. Two initial possibilities to be considered are whether the mechanism (*a*) is akin to a Na^+-accounting machine in that, for example, serially arranged transfer mechanisms in a specific local vascular field register the load of Na^+/unit time in the local blood supply; or (*b*) is responsive to some physiological consequence of change in the amount of fluid in the vascular or extracellular compartment of the body—for example, stimulation of a pressure or stretch receptor, or determining the area of surface available for a particular transfer process.

It has been shown in the sheep that there is a normal response to Na^+ depletion when concurrent water depletion caused a rise of plasma Na^+ during Na^+ deficiency instead of the usual fall (Denton, 1958). Furthermore, there is a normal response if Na^+ deficiency is corrected rapidly by the infusion of hypotonic NaCl (2 l. of 102 mEq/l. during 40 minutes) so that no rise of plasma Na^+ occurs. Gross water deficiency, which causes a considerable contraction of plasma volume, does not cause a decrease of salivary Na^+/K^+ ratio. If the sheep which is concurrently depleted of water and Na^+ for several days is allowed to drink 3–4 litres of water there is a rapid fall of plasma Na^+, and other evidence of expansion of plasma volume, but no effect on the salivary Na^+/K^+ ratio (Denton, 1958). These experiments do not, however, effectively decide between the alternatives. We prefer to defer discussion of these and other experiments involving alteration of compartment volumes and pressure of circulating fluids, and deal here with another general biological aspect of the problem.

In considering the stimulus to a putative diencephalic system controlling aldosterone, it would seem prudent to bear in mind at the outset the known complexity of other forms of chemical regulation. The control of respiration is a case in point where changes in the milieu with regard to pCO_2, *p*H, and pO_2 may have additive or antagonistic effects on respiration, and other afferent influences via the vagus, from the cortex, or from other centres, such as preoptic thermoreceptors, may be determinant (Pitts, 1946; Gray, 1950; Andersson and Persson, 1957). Similarly, the work of ethologists—for example, Lorenz (1950), Tinbergen (1951), and Thorpe (1958)—has shown that some of the hierarchically organized neural mechanisms influencing hormonal secretion in the course of determining behaviour are very complex indeed. In this vein, and consistent with the inquiry described by Richter (1958), we wish to describe the remarkable appetite for salt shown by Na^+-deficient animals.

A Na^+-depleted sheep will lick much more from a block of rock salt than a sheep in normal Na^+ balance (Denton, 1957a). When sheep with parotid fistulae were offered a choice of solutions—for example, 420 mEq of NaCl/l., 420 mEq of $NaHCO_3$/l., 140 mEq of KCl/l., and H_2O—it was found that they drank much more of the Na^+-containing solutions when the daily supplement of

$NaHCO_3$ was withdrawn (McDonald and Raschke, unpublished). Moreover, some animals first drank NaCl, but after six to eight days they drank predominantly $NaHCO_3$—a more appropriate choice in the face of the loss of $NaHCO_3$ from the parotid fistula. With subsequent episodes of Na^+ deficiency they showed a preference for $NaHCO_3$ at the outset.

Further studies (Sabine and Denton, unpublished) have shown that intake is related to need. If $NaHCO_3$ solution and water were available to the animal, and the concentration of the $NaHCO_3$ solution was varied, the volume drunk varied inversely with the concentration (Fig. 24). Thus the average daily intake for the period remained nearly constant and was remarkably close to the Na^+ loss from the fistula. If the Na^+-containing solutions were made available for only 15–60 minutes each day, the animals nevertheless drank adequate Na^+ to maintain balance. When a sheep was

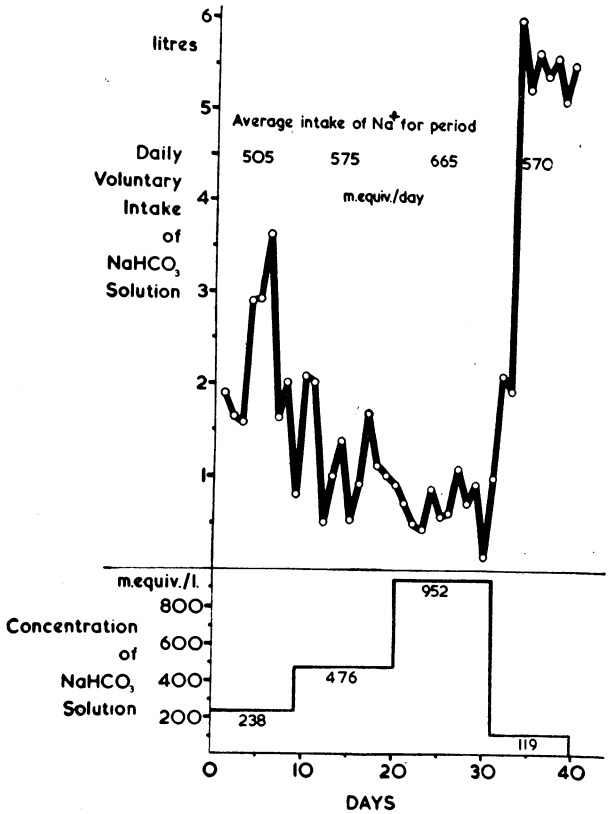

FIG. 24.—P.F.33, May/June, 1957. Intake in litres/day of $NaHCO_3$ solution, concentration of which was changed every 10–11 days. Animal had access to $NaHCO_3$ solution and H_2O during whole day. Daily loss from its fistula was 3–4.5 litres (from Sabine and Denton, unpublished).

in normal Na^+ balance it paid little attention to the usual observer preparing the Na^+ solutions and placing them in position. However, an animal with experience of the procedure would, when Na^+-depleted, bleat and kick the side of its cage, and there was a large psychic secretion of saliva (cf. Denton, 1957c) when it saw the preparatory moves. The finding that the animals would take adequate Na^+ when only a short period of access was permitted opened the way for detailed analysis of the behaviour entailed in the satisfaction of the Na^+ appetite.

Observation of the sampling behaviour showed that when the animal was given access to the series of solutions, NaCl, $NaHCO_3$, KCl, and H_2O, the number of sampling episodes/hour—for example, smelling, tasting, or drinking—increased greatly during Na^+ deficiency (Fig. 25). The evidence on rejection or acceptance of a solution, according as they were

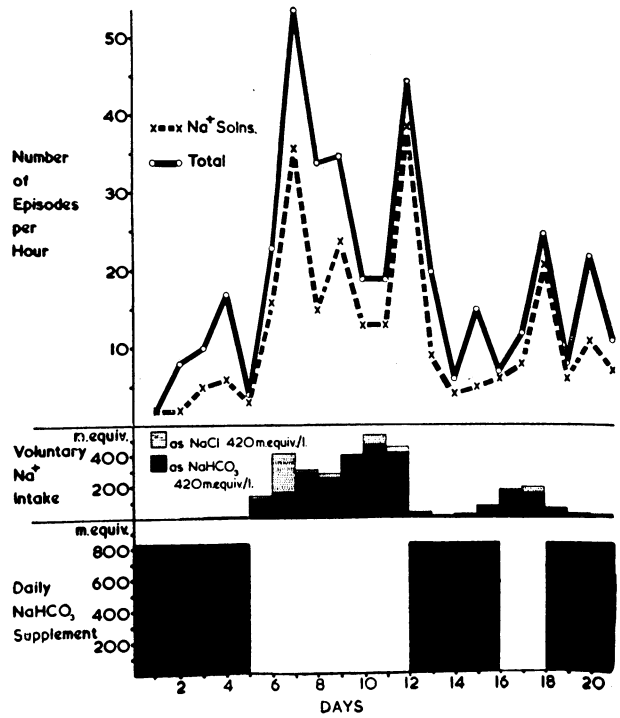

FIG. 25.—P.F.33, Aug./Sept., 1957. Effect of withholding daily supplement of 800 mEq $NaHCO_3$ on voluntary intake of solutions of $NaHCO_3$ (420 mEq/l. and NaCl (420 mEq/l. which were available for one hour/day only. Number of episodes of sampling (smelling, tasting, drinking) all solutions ($NaHCO_3$, NaCl, KCl, and H_2O) O——O, and number of episodes involving Na^+ solutions × - - - - × are shown in upper section (from Sabine and Denton, unpublished).

encountered in the initial survey by the animal, also corresponded to the preference shown in the overall intake. Preliminary experiments have been made in which one carotid artery loop was occluded and an infusion of 4 M NaCl made into the other at 1.6 ml./minute. This raised the Na^+ concentration of the blood on both sides of the head by 20 mEq/l. during the 15-minute period of choice, but the infusion itself repaired the animal's Na^+ deficiency by a small degree only. During normal Na^+ balance this caused the sheep to drink water. In Na^+ deficiency this did not happen, but the procedure significantly reduced the animal's intake of Na^+. Moreover, following a number of such episodes on alternative days of depletion, the animal was permitted access to the solution without an infusion. There was residual modification of behaviour in that Na^+ intake was still reduced despite deficiency, though there was great interest in the Na^+ solutions as evidenced by greater than usual frequency of tasting. Subsequent to a period of normal Na^+ balance without testing, the animal manifested its usual ability to drink a large and adequate amount of Na^+ solution when deficient.

Sheep have been concurrently depleted of both Na^+ and water so that there was a rise of plasma Na^+ concentration during deficiency instead of the usual fall, and the behaviour was compared with that seen in the same animal during either uncomplicated water deficiency or Na^+ deficiency. When offered water and

Na^+ solution the animal's brain appeared quite able to differentiate the situations. With uncomplicated depletions the animal rejected one solution but avidly drank the appropriate one. With the concurrent depletion it would accept either initially. Having drunk a large amount of one it would refuse more but immediately drink a large amount of the other if offered. If both were offered simultaneously the animal would satisfy itself with one and immediately pass to the other and drink it. In these studies, measurements of plasma volume were made as well as comprehensive plasma analyses, and it is interesting to note that in one group the plasma volume was reduced a comparable amount by each type of depletion. The type of fluid drunk, however, was specifically related to the deficiency. On the other hand, in many studies of the series, there was little evidence of relation between readiness to drink Na^+ solutions and the concentration of Na^+ in the plasma.

Central Integration of Individual Systems Regulating Na^+ Balance

This brief account may serve to indicate that because of the magnitude of the electrolyte losses, and because the size of the animal permits staged surgical procedures and complicated manipulations, there is a promising field for investigating the mechanism of appetite. The question arises whether the provoking stimuli are the same as those determining aldosterone secretion in the animal. It is possible that, just as the function of other diencephalic and brain-stem regulatory centres may determine or be determined by cerebral cortical events, so may this remarkable selective appetite for Na^+ be the cerebral behaviour determining projection of a Na^+ regulatory centre which also influences a hormonic stimulus to aldosterone secretion. We have referred already to the finding that the normal inhibition of adrenal secretion following rapid correction of a Na^+ deficiency may be prevented or modified if the significance of distance receptor information induces a state of apprehension in the animal. Further, Andersson, Jewell, and Larsson (1958) have shown that stimulation in the hypothalamus between the fornix and mammilo-thalamic tracts, but lateral to the drinking area, induces vigorous licking of a salt block in the goat.

There is a parallelism between this group of findings and the established facts on antidiuretic hormone secretion, osmoregulation, thirst, the inhibition of an established water diuresis by fear or disturbance (Verney, 1947), and the demonstration of compulsive drinking upon localized hypothalamic stimulation. If a neural-organization-regulating Na^+ does exist, it appears to be separate from the osmoregulating system in that intracarotid injection of 2.7M glucose, an adequate osmotic stimulus, does not appear to affect it, and the selective appetite during concurrent water and Na^+ deprivation shows that the animal's brain readily distinguishes the two situations. The evocative stimulus of such a Na^+-regulating neural centre may be, as with osmoregulation, predominantly the result of change of a single property of the *milieu intérieur*, or it may be that, analogous to respiratory control, a number of factors may be effective, and the eventual demonstration that one factor is effective need not rule out the efficacy of other stimuli any more than the demonstrable response of the respiratory mechanism to pCO_2 excludes the fact of its response to pO_2, *p*H, and many other influences.

Similarly, the quantitative effect of a trophic hormone at the adrenal level might be the resultant of an interplay of many factors including the direct effect of ionic changes in adrenal blood as well as, for example, the effect of corticotrophin in a permissive or contributory causal role. Of course, it may be that the two systems contributing to Na^+ regulation—namely, aldosterone secretion and selective appetite for Na^+—are only loosely functionally linked if at all, and the evoking stimuli to the two systems may be different. However, the complex integration of behaviour-determining mechanisms with hormonal components in simpler species—for example, the three-spined stickleback—could encourage the view that integration of comparable complexity could be involved in the evolution of a mechanism as important for survival of higher species as sodium control. A unifying hypothesis of a brain centre is provocative of useful experiments aiming to elucidate the issue, and has the coincident virtue of encouraging consideration of the clinical investigation of aldosterone regulation in the general biological context.

Summary

The paper describes experiments aiming to solve the clinically important question of how the adrenal secretion of aldosterone is controlled. To investigate the control of secretion of a hormone it is desirable that the experiments be made on conscious confident animals and it be contrived that there is easy access to the arterial supply and venous drainage of the endocrine gland under these conditions. The neck adrenal transplant preparation permits, in conscious sheep, experiments aiming to determine whether the gland responds directly to changes of arterial sodium and potassium concentration or whether it responds to a hormonal stimulus.

The results indicate that changes of adrenal ionic environment may be a contributory cause of changes of electrolyte-active steroid secretion occurring in sodium deficiency, but they do not account for the whole range of function which the gland shows.

Cross-circulation experiments in conscious animals have shown that there is a large stimulation of adrenal secretion of electrolyte-active steroid when the blood of a sodium-depleted adrenally insufficient donor animal is passed through the adrenal transplant of another in normal sodium balance. As this stimulation does not appear to be due to corticotrophin, further experiments have been based on a working hypothesis that a hitherto unrecognized hormonic stimulus originates in the neuraxis. With such a working hypothesis, a fact for concurrent consideration is the remarkably accurate appetite for sodium solutions shown by sodium-deficient sheep.

A possible view of the evidence discussed is that the evolution of control of such an important regulation as sodium balance involves central integration of a number of mechanisms, and that the selective appetite for sodium and the stimulation of aldosterone secretion are functionally linked together.

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RESPONSES OF 6- AND 9-MONTHS-OLD INFANTS TO TWO AND THREE DOSES OF POLIOMYELITIS VACCINE

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The earliest time to start immunization against poliomyelitis is when satisfactory response will follow in all subjects. We have already shown that the response to active immunization in infants with high levels of maternally transmitted antibody is unsatisfactory (Perkins, Yetts, and Gaisford, 1958, 1959a). It was not possible to overcome the inhibitory effect of these antibodies in 1-week-old infants with current vaccines, even though the volume and number of the antigenic stimuli are increased, but we were able to show that in 16-weeks-old infants a more satisfactory basal immunity followed primary immunization with three doses of vaccine given at monthly intervals, though the maternal antibody still present in some of these infants inhibited their response to the most important component, type 1 (Perkins, Yetts, and Gaisford, 1959b). It seems clear, therefore, that satisfactory response will not be obtained in all infants until maternal antibody has fallen to non-inhibitory levels, and it is estimated that this will not be until after the age of 6 months.

An investigation was therefore made of the responses of 6- and 9-months-old infants both to two and to three doses of vaccine given as a primary course of immunization.

Procedures

Immunization of Infants.—Thirty-two infants were given intramuscularly three doses of vaccine, each of 1 ml., at intervals of four weeks. Post-immunization