Presence of Aldosterone-stimulating Hormone in Urine

The secretion of aldosterone differs from that of other steroids of the adrenal cortex in that it is not directly under pituitary control. Current concepts favour a location in the diencephalon for the centre which controls aldosterone secretion[1]. The transmission of the stimulus from the centre to the effector gland cannot be nervous, since the isolated gland can respond to suitable provocations by an increased secretion of aldosterone[2]. It is therefore concluded that the stimulus is humoral.

Evidence for the presence in the urine of adrenalectomized rats deprived of sodium of a substance which modifies the secretion of an electrolyte-active steroid when injected into intact rats has been presented by Orti *et al.*[3]. This substance may be the hypothetical hormone responsible for the stimulation of aldosterone synthesis or release. The present investigation confirms the presence of this substance in urine under certain conditions, and evidence is presented that it is an 'aldosterone-stimulating hormone'.

Rats of Wistar strain, weighing about 150 gm., were bilaterally adrenalectomized and maintained on rat cake, with 1 per cent sodium chloride solution to drink. Groups of 20 of these animals were deprived of food and drink for 36 hr., and their urine collected over the last 12 hr. of this period. This urine was injected subcutaneously into groups of 5 intact rats, together with a sodium load of 1 ml. of 0·45 per cent sodium chloride solution per 50 gm. body-weight, administered intraperitoneally. 5 intact rats served as controls, receiving a similar sodium load intraperitoneally and a volume of urine from adrenalectomized rats receiving 1 per cent saline was given subcutaneously, equal in volume to that of the urine injected into the experimental group. Urine was collected individually from the 10 rats at intervals of 40 and 80 min. after the injection of the urine. Cross-over tests were made three days later. Statistical analysis of variance shows that the administration of urine from the salt-deprived animals resulted in a lowering of the sodium/potassium ratio in the urine of the intact animals compared with the control group, as shown in Table 1.

Table 1

No. of rats	Time (min.)	Mean sodium/potassium, experimental	Mean sodium/potassium, control	*P*
40	40	1·71	2·34	0·005
40	80	1·09	1·37	0·01

The urine from the experimental and control groups was collected in separate pools. This urine was hydrolysed for 48 hr., extracted with chloroform and run on the chromatographic system of Zaffaroni *et al.*[4] (toluene–propyleneglycol), or the '*B*5' system of Bush[5]. Insufficient material was present for a definite chemical identification of aldosterone to be made. The material running in the position of aldosterone was eluted from paper and bio-assayed in adrenalectomized rats. The urine from the experimental group possessed sodium-retaining activity.

Serial assay on a similar pool of urine showed that the substance was stable at 4° C. for seven days. It would also withstand boiling for 5 min. with little loss of activity.

These observations substantiate the presence in the urine of dehydrated adrenalectomized rats of a substance which, when injected into intact rats, causes a lowering of urinary sodium/potassium ratio, and circumstantial evidence is presented that this is due to the release of aldosterone. Corticotrophin, 1 U.S.P. unit per rat, did not possess this activity. It is concluded that the substance responsible for this effect is 'aldosterone-stimulating hormone'.

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Biological Effects of Aromatic Extracts from Urine of Schizophrenics

Wada and Gibson[1] have recently reported that aromatic material extracted from the urine of schizophrenic patients induces behavioural and electroencephalographic abnormalities when injected intracisternally and intraventricularly in cats and monkeys. They used the fraction extracted by eluting with phenol-saturated water the material adsorbed on activated charcoal, according to the method of McGeer *et al.*[2].

Similar observations were made in the course of a series of experiments including the study of biological effects produced in mice, rabbits and dogs by aromatic fractions extracted from the urines of normal and schizophrenic subjects. The extracts were obtained by Decker's method[3], which is based upon elution with a water–methanol–butanol–ammonia mixture of the material adsorbed on activated charcoal. Of the group of substances thus extracted only the methanol-soluble fraction was investigated. Previous paper chromatographic analysis[4] of this fraction has shown that a larger number of aromatic substances is present in the urine of schizophrenic subjects.

The biological studies of such extracts included the determination of (*a*) the acute toxicity following intraperitoneal injection in mice; (*b*) the chronic toxicity in mice with histological examination of the brain and other viscera; (*c*) the behavioural effects following intracerebral injections in the rabbit; (*d*) the effect on the electrical activity of the cerebral cortex following topical applications in rabbits and dogs.

Both the aromatic fraction extracted from the urine of schizophrenic subjects and that from urine of normals have toxic properties. However, the fraction from schizophrenic patients appears to possess a higher acute toxicity and produces at lower dose-levels, upon chronic administration, degenerative changes of nerve cells of brain-stem nuclei. Furthermore, the aromatic urinary fraction from schizophrenic subjects has definite convulsant properties but only when brought into direct contact with the central nervous system. Intracerebral injections of minute amounts of the diluted extracts are readily followed by prolonged and generalized convulsions.