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SOME OBSERVATIONS ON THE ANTIDIURETIC ACTIVITY OF RAT SERUM

BY

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In a recent paper, Birnie, Jenkins, Eversole, and Gaunt (1949) have described an antidiuretic substance (or substances) which they assume circulates in the blood of normal rats, whereas earlier workers (Walker, 1939; Hare, Hickey, and Hare, 1941) had failed to find such properties in the blood of rabbits and dogs. Birnie *et al.* (1949) also suggest that this substance may be of posterior pituitary origin, though Heller and Urban (1935) and Melville (1937) have shown that injected posterior pituitary antidiuretic hormone disappears very rapidly from the circulation.

In view of these contradictions, the presence of antidiuretic substances in the blood has been re-examined.

METHODS

Experimental animals.—Adult male albino rats weighing approximately 200 g. were used. They were kept on a commercially prepared diet (Dicker, 1949).

Antidiuresis test.—The method used was based on that of Birnie *et al.* (1949). Groups of 18 or 12 rats, deprived of food for 18 hours but denied free access to water for the last hour only, were placed individually in small metabolism cages (Dicker and Heller, 1945); urine was collected into graduated cylinders. Each rat was given two doses of tepid tap water by stomach tube (each dose = 5 ml./100 g. body weight), with an interval of one hour between doses. One hour after the second administration of water the total volume of urine excreted in the preceding two hours was measured (V_2). At the same time a third dose of water (5 ml./100 g.) was administered and the animals injected subcutaneously with the test material. The urine output of each animal was measured thereafter at intervals of 30 minutes for two hours. In order to ensure complete emptying of the bladder the animals were stimulated by prodding and handling before each administration of water and each measurement of urine volume. In all experiments the room temperature was adjusted to 18–22° C.

The volume of urine, V_2, was expressed as a percentage of the amount of water administered in the first two doses. Those rats for which this value deviated from the mean for the whole group by more than 50 per cent of the mean were rejected. Since the rats were injected subcutaneously, the method of calculation adopted allowed 30 minutes for the complete absorption of the injected materials. The following formula was used:

$$\text{Percentage water excretion} = \frac{V_t - V_{30}}{A + {}^{2}A) - {}^{1}A\varepsilon_{30})} \times \frac{100}{1}$$

where V_1 = the volume of water administered in each dose.
V_2 = the volume of urine excreted in the two hours before injection.
V_{30} = the volume of urine excreted in the first 30 minutes after injection.
V_t = the volume of urine excreted during the period of "t" minutes after injection (t = 60, 90, or 120 minutes).

Rats become conditioned to water administration—i.e., the diuresis of rats given water frequently is generally more rapid than that of unconditioned rats. For this reason rats were used for the antidiuresis test once only.

Preparation of test material.—(i) *Serum.*—Blood was collected from rats under ether anaesthesia by section of the carotid and jugular vessels, allowed to coagulate, and the serum separated by centrifugation.

(ii) *Plasma.*—Blood was collected into chilled centrifuge tubes containing heparin (10 units per ml. of blood). In some experiments tubes which had been treated with silicone (Fluid Dc 200, Dow Corning) were used. The tubes were centrifuged while surrounded with freezing mixture and the plasma separated.

(iii) *Heat coagulated serum.*—Serum diluted with an equal volume of 0.6 per cent (w/v) sodium chloride solution was treated with glacial acetic acid to give a *p*H of 5.0–5.2 and heated in a boiling water bath for 30 minutes. The coagulum was separated by centrifugation and the supernatant fluid was collected and neutralized before injection.

In order to allow comparison with plasma, heparin (10 units per ml.) was added to all injections other than plasma. In all experiments serum or plasma was obtained from at least 12 animals and pooled before injection.

Estimation of chloride.—Chloride in the urine was estimated by Volhard's method as modified by Harvey (1910).

Statistical treatment.—Each experiment was planned as a comparison between the effects of two or more treatments. A method of random sampling was used to select the rats for each treatment.

Owing to the elimination of rats because of exceptional diuresis in the first two hours, the treated groups did not always contain equal numbers of rats. In order to obtain a symmetrical structure for analysis of variance, rats had to be rejected from

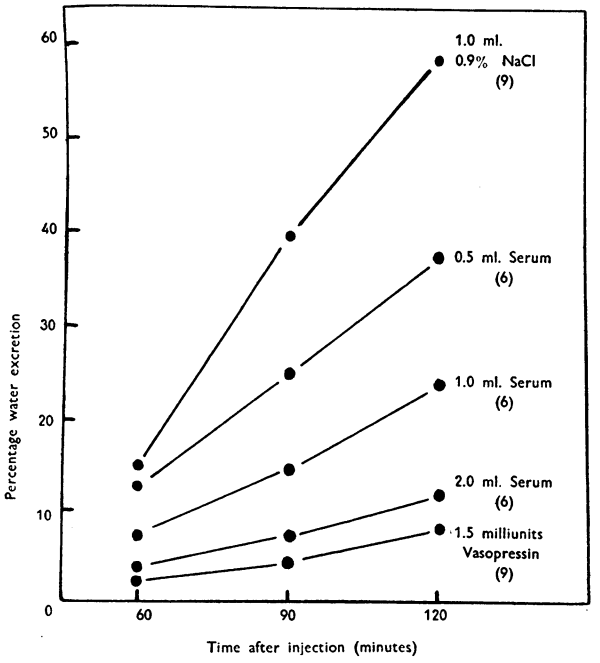

FIG. 1.—The antidiuretic effect of serum compared with that of vasopressin. The doses shown were given per 100 g. body weight. Number of rats injected in parentheses.

the larger groups. The rats rejected were those whose percentage water excretion after treatment was closest to the mean for their group. This would tend to increase the error and therefore to decrease the estimated significance of difference between treatments. Results are given as means and standard errors. The probability of P for t and F was obtained from the tables of Fisher and Yates (1943).

RESULTS

Effect of serum on water diuresis.—In four experiments (33 rats) the effect of subcutaneous injection of 1.0 ml. per 100 g. of serum freshly drawn from normal animals was compared with that of 1.0 ml. per 100 g. of 0.9 per cent (w/v) sodium chloride solution. The results are shown in Table I. There is a significant variation between the results of experiments performed on different days and with

TABLE I

THE EFFECT OF SUBCUTANEOUS INJECTION OF 1.0 ML. SERUM PER 100 G. ON THE WATER DIURESIS OF HYDRATED RATS, COMPARED WITH THE EFFECT OF 1.0 ML. SALINE PER 100 G., AND THE ANALYSIS OF VARIANCE OF THE PERCENTAGE WATER EXCRETION AT 90 MINUTES AFTER TREATMENT

Exp. No.	1.0 ml. Saline/100 g.				1.0 ml. Serum/100 g.			
	No. of rats	% Water excretion			No. of rats	% Water excretion		
		60 min.	90 min.	120 min.		60 min.	90 min.	120 min.
1 ..	4	22.2±1.6	37.9±3.0	—	4	18.3±1.0	36.2±3.2	—
2 ..	4	21.9±1.2	47.6±2.6	—	5	15.7±2.9	30.3±5.4	—
3 ..	4	13.6±3.4	27.9±3.8	42.5±4.2	4	2.1±0.7	7.7±0.5	18.8±3.6
11 ..	4	22.1±1.0	39.1±2.3	54.4±3.6	4	13.7±2.5	25.2±3.0	35.0±5.2
Mean..	—	19.9	38.1	—	—	12.6	25.2	—

Source of variance	Degrees of freedom	Sum of squares	Variance	F.	P.
Treatments	1	1,342.8	1,342.8	21.1	<0.001
Experiments	3	2,275.4	758.8	11.9	<0.001
Remainder	27	1,715.5	63.5	—	—
Total ..	31	5,333.7	—	—	—

different rats, but in all cases serum decreased the diuresis. The analysis shows that the antidiuresis produced by serum is highly significant ($P<0.001$). Table II shows the effect of serum which had been kept at 4° C. for 18 hours, compared with fresh serum and with 0.9 per cent sodium chloride solution. There was no loss of antidiuretic activity. Fig. 1 shows the effect of different doses of serum (0.5, 1.0, and 2.0 ml./100 g.), 1.5 milli-units of vasopressin (Pitressin, Parke Davis & Co.) per 100 g. and of 0.9 per cent sodium chloride solution. It shows that the antidiuretic activity of 2.0 ml. of serum is approximately equivalent to that of 1.5 milli-units of vasopressin.

TABLE II

THE EFFECT OF SUBCUTANEOUS INJECTION OF 1.0 ML. FRESH SERUM, 1.0 ML. OF SERUM KEPT FOR 18 HRS. AT 4° C., AND 1.0 ML. SALINE PER 100 G. ON THE WATER DIURESIS OF HYDRATED RATS

Exp. No.	Treatment	No. of rats	% Water excretion		
			60 min.	90 min.	120 min.
13	Saline	3	34.2±1.2	62.3±7.5	82.2±6.4
	18 hrs. old serum	3	22.7±4.2	38.9±4.2	54.5±3.7
14	Saline	3	33.9±4.3	69.3±11.0	80.7±9.6
	18 hrs. old serum	4	25.3±2.7	48.6± 4.8	65.2±6.5
	Fresh serum	4	30.1±2.2	52.6± 2.1	70.9±2.8

Effect of plasma on water diuresis.—In four experiments (42 rats) the effect of subcutaneous injection of 1.0 ml. per 100 g. of plasma was compared with that of 1.0 ml. per 100 g. of 0.9 per cent (w/v) sodium chloride solution. The results are shown in Table III. As in the experiments with serum there is some variation between experiments, but the diuresis of rats injected with plasma was always slower than that of the controls; analysis of variance, however, shows that this difference is not significant ($P>0.05$).

Effect of serum and plasma on the antidiuretic activity of vasopressin.—Vasopressin was added to serum to a concentration of 1.5 milli-units per ml. and kept

TABLE III

THE EFFECT OF SUBCUTANEOUS INJECTION OF 1.0 ML. PLASMA PER 100 G. ON THE WATER DIURESIS OF HYDRATED RATS COMPARED WITH THE EFFECT OF 1.0 ML. SALINE PER 100 G. AND THE ANALYSIS OF VARIANCE OF PERCENTAGE WATER EXCRETION AT 60, 90, AND 120 MINUTES AFTER TREATMENT

Exp. No.	1.0 ml. Saline/100 g.				1.0 ml. Plasma/100 g.			
	No. of rats	% Water excretion			No. of rats	% Water excretion		
		60 min.	90 min.	120 min.		60 min.	90 min.	120 min.
4 ..	6	25.5±1.3	47.9± 2.8	61.9±4.5	5	23.1±3.3	39.7±5.6	52.1±8.9
5 ..	5	26.1±2.5	49.1± 3.0	61.8±4.0	5	21.2±3.6	41.8±3.2	64.2±5.1
6 ..	6	25.9±2.6	46.7± 3.0	61.2±3.6	5	23.6±1.3	41.6±2.3	61.0±4.3
15 ..	5	36.1±7.6	62.7±10.3	80.1±8.3	5	33.3±2.1	57.1±4.6	75.0±7.3
Mean..	—	28.1	51.2	65.8	—	25.3	45.0	63.1

Source of variance	Degrees of freedom	Sum of squares	Variance	F.	P.
Experiments	3	4,746.3	1,582.1	12.5	<0.01
Times	2	28,947.5	14,471.9	114.2	<0.001
Treatments	1	435.0	435.0	3.43	>0.05
Remainder	113	14,318.9	126.7	—	—
Total ..	119	48,447.7	—	—	—

at 4° C. for 18 hours. The antidiuretic activity of this serum was compared with that of serum to which the same amount of vasopressin was added 15 to 30 minutes before injection: vasopressin was completely inactivated by serum in 18 hours and substantially inactivated in 15 to 30 minutes (Fig. 2). This confirms the findings of Heller and Urban (1935), who used defibrinated blood and serum (dog, rabbit, and human). Similar experiments were performed with plasma (Fig. 2). No inactivation of vasopressin or augmentation of its antidiuretic activity was observed.

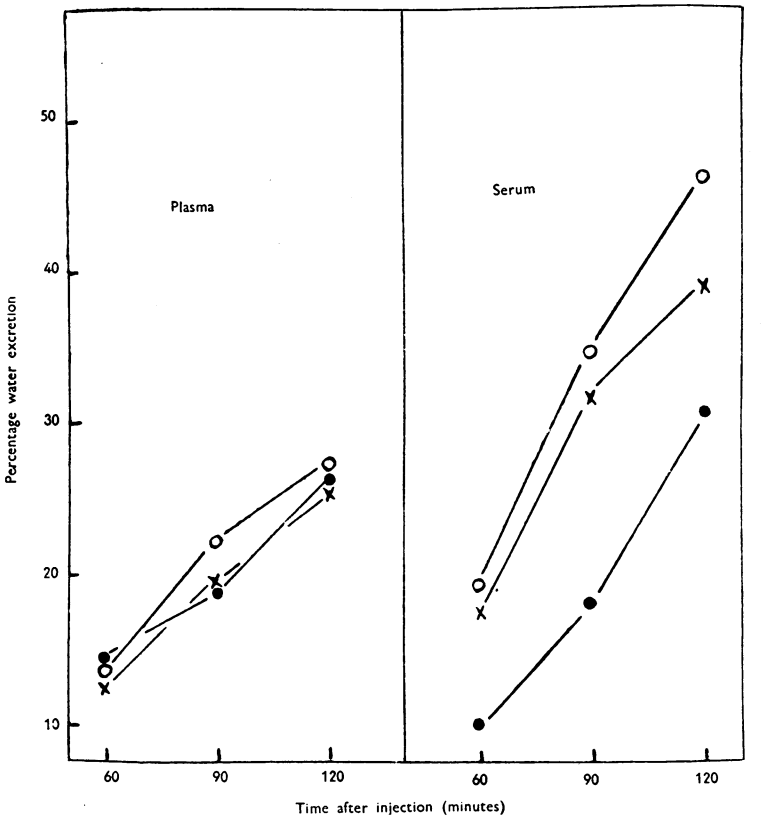

FIG. 2.—The antidiuretic effect of mixtures of vasopressin and plasma, and of vasopressin and serum. ●———● 1.5 milli-units of vasopressin per 100 g. ×———× 1.5 milli-units of vasopressin in 1.0 ml. of serum or plasma per 100 g., 15–30 minutes after mixing. O——O 1.5 milli-units of vasopressin in 1.0 ml. of serum or plasma per 100 g., 18 hours after mixing. Each point showing the effect of serum is the mean of results from 16 animals. Each point showing the effect of plasma is the mean of results from 10 animals.

In another series of experiments vasopressin was added to serum to give a concentration of 1.5 milli-units per ml. After 30 minutes, when considerable inactivation would have occurred (see Fig. 2), the serum was diluted and coagulated by heat. The antidiuretic activity of the supernatant fluid was compared with that from similarly treated serum to which no vasopressin had been added, and to 3.0 and 1.5 milli-

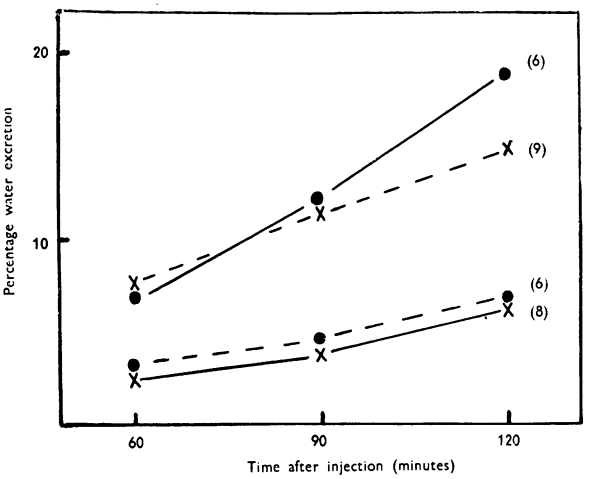

FIG. 3.—The antidiuretic activity of the supernatant fluid obtained from heat coagulated normal serum and from heat coagulated normal serum containing vasopressin. ●———● 1.5 milli-units of vasopressin per 100 g. ●- - -● 3.0 milli-units of vasopressin per 100 g. ×----× Supernatant fluid of the coagulum obtained by heating 1.0 ml. of serum per 100 g. ×———× Supernatant fluid of the coagulum obtained by heating 1.0 ml. of serum containing 1.5 milli-units per 100 g. Number of animals in parentheses.

units of vasopressin. The results in Fig. 3 show that the antidiuretic effect of 2.0 ml./100 g. of the "heat coagulated serum" supernatant fluid (2.0 ml. of supernatant fluid is equivalent to 1.0 ml. serum) is approximately the same as that of 1.5 milli-units of vasopressin per 100 g. The antidiuretic activity of 2.0 ml. of the "heat coagulated serum" to which vasopressin was added was approximately the same as that of 3.0 milli-units of vasopressin per 100 g. Thus the heat coagulation of serum enhances its antidiuretic activity (cf. Fig. 1) and reverses the inactivation of vasopressin.

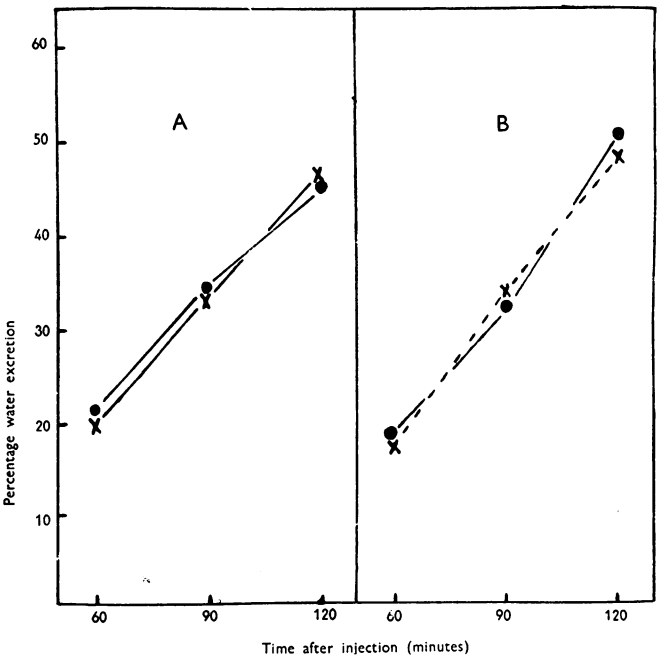

FIG. 4.—A. Comparison between the effect of plasma obtained from rats injected with 10 milli-units vasopressin per 100 g. (mean of 13 test rats) and that of plasma from control animals (mean of 11 test rats). B. Comparison between the effect of plasma of dehydrated rats (mean of 10 test rats) and that of normal animals (mean of 11 test rats). ●———● 1.0 ml. normal plasma per 100 g. ×———× 1.0 ml. plasma of rats injected with 10 milli-units vasopressin per 100 g. ×- - - -× 1.0 ml. of plasma of dehydrated rats per 100 g.

Effect of plasma from dehydrated rats.—Fig. 4 shows that the subcutaneous injection of 1.0 ml./100 g. of plasma from rats which had been deprived of food and water for 36 hours did not have a greater antidiuretic effect than that from normal rats.

Effect of plasma from rats injected with vasopressin.—Ten milli-units of vasopressin per 100 g. were injected subcutaneously into rats and blood withdrawn after 30 to 40 minutes—i.e., when the antidiuretic effect would be at its maximum. Fig. 4 shows that the plasma from these rats was not more antidiuretic than the plasma from normal rats.

Chloruretic effect of serum.—Chloride was estimated in the urine of rats injected with 1.0 ml. serum per 100 g. and with 1.0 ml. 0.9 per cent (w/v) sodium chloride solution per 100 g. In seven rats treated with serum the mean chloride excretion 120 minutes after injection was 2.2 ± 0.64 (S.E.) mg. compared with 2.9 ± 0.3 (S.E.) mg. for nine rats treated with saline, which shows that serum had no chloruretic action.

Discussion

In confirmation of the findings of Birnie *et al.* (1949), it was found that the subcutaneous injection of rat serum into hydrated rats has a significant antidiuretic effect. It should be noted that in the experiments of Birnie *et al.* the serum was injected within five minutes of collection, whereas in the experiments reported here the serum had been collected at least 30 minutes before injection. The antidiuretic effect of 2.0 ml. serum/100 g. was approximately equal to that produced by 1.5 milli-units of vasopressin.

Birnie *et al.* (1949) assumed that the antidiuretic substance was present in the circulation, and suggested for several reasons that it might be of posterior pituitary origin. It is difficult to accept the view that the antidiuretic substance of serum is circulating because it could be shown that the subcutaneous injection of 1.0 ml. plasma/100 g. into hydrated rats had no significant antidiuretic effect. Against the suggestion that the antidiuretic substance of serum is of posterior pituitary origin are the following arguments: (*a*) Serum inactivates vasopressin, in accordance with Heller and Urban's (1935) findings; plasma on the other hand does not inactivate vasopressin. (*b*) Serum from dehydrated rats had the same antidiuretic activity as that from normal animals. It seems likely, therefore, that the antidiuretic property of normal rat's serum is produced during the coagulation of blood. From recent investigations by Ginsburg and Heller (unpublished observations), it cannot be identified with serotonin.

Birnie *et al.* (1949) found that the antidiuretic substance in serum disappears rapidly on standing: this could not be confirmed. It is quite clear from the results presented that serum injected subcutaneously had the same antidiuretic titre whether fresh or kept for 18 hours at 4° C.; also the finding of Birnie *et al.* (1949) that serum was chloruretic could not be confirmed. However, since the chloruretic action of posterior pituitary extracts, for example, is an inconstant effect, this discrepancy may be due to factors such as diet (Heller and Stephenson, 1950).

It has been generally assumed that the assay of antidiuretic activity by subcutaneous injection may entail considerable error, as it has been stated (Noble,

Rinderknecht, and Williams, 1939) that blood extracts have augmentor effects. No such augmentor effects were found in our experiments: the subcutaneous injection of plasma to which vasopressin had been added had an antidiuretic effect comparable to that of an equal amount of vasopressin in saline; furthermore, the antidiuretic activity of vasopressin in the supernatant fluid obtained from heat-coagulated serum was approximately equal to the sum of the separate activities.

SUMMARY

1. No significant antidiuretic activity could be demonstrated in heparinized plasma from normal and dehydrated rats.

2. Serum from normal rats had a marked antidiuretic effect when injected subcutaneously into hydrated rats.

3. The amount of antidiuretic activity in 2.0 ml. of serum was comparable to that of about 1.5 milli-units of vasopressin (Fig. 1). Serum inactivated vasopressin, but plasma did not. The inactivation of vasopressin by serum was reversed by heat coagulation.

4. Injections of plasma or serum had no augmentor effect on the antidiuretic effect of vasopressin.

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