THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE LEVEL OF PLASMA ANTIDIURETIC SUBSTANCES IN THE RAT

By KATHLEEN W. ROBINSON* and W. V. MACFARLANE*

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Summary

The level of plasma antidiuretic substances (ADS) was found to be doubled when rats were exposed continuously to an atmosphere of 95°F (35°C) and 30 mm Hg water vapour pressure for 28 days, and it was reduced to half with continuous exposure to cold (43°F (6·5°C)). When reduction in body weight accompanied heating, the antidiuretic potency of the blood was markedly increased.

Exposure to a hot atmosphere (104°F (40°C), 30 mm Hg water vapour pressure) for 2 hr, whether acutely or once daily for 28 days, failed to alter the concentration of ADS in the blood. A single exposure to these conditions for 4 hr produced a four-fold rise in ADS level.

The release of ADS appears to arise from dehydration induced by heat and not from the response of receptors to heat as such. Plasma protein concentration changed in the same direction as ADS concentration.

Acclimatization to heat or cold did not alter the sensitivity of the rat's kidney tubules to exogenous pitressin.

Adrenalectomy failed to produce any change in antidiuretic activity of the blood during short, acute, heat exposure.

I. INTRODUCTION

A homeotherm, relying largely on evaporative cooling to adjust body temperature in a hot environment, has adaptive mechanisms to prevent dehydration of the cells. These mechanisms are not fully understood. Water is retained by reduction of urine volume in man and animals during heat exposure (Weiner 1944; Hellmann and Weiner 1953). Peripheral vasodilatation, which accompanies heating, produces a compensatory fall in blood flow to the kidney (Radigan and Robinson 1949; Kenney 1952), but this blood shift is not alone responsible for reduced urine production. The release of antidiuretic hormone (ADH) from the neurohypophysis into the blood stream is a further possible means of conserving body water in a hot environment.

Several observers have noted a rise in antidiuretic substances (ADS) in human urine after heavy sweating (Hellman and Weiner 1953; Itoh and Kimura 1954). Recently, Itoh (1954) reported that the antidiuretic potency of rat serum, following a short exposure, showed a rise with heat and a fall with cold.

In an attempt to obtain further information on the effect of heat on blood levels of ADS, the ADS activity of rat's plasma was measured following (i) continued exposure over a period of 1 month to hot and to cold environments, and (ii) short term exposure to heat. The exposure time in (i) was considered sufficient to acclimatize the animals to the temperature conditions, since Sellers, Reichman, and Thomas (1951) have shown that acclimatization to cold is fully developed in rats after 4 or 5 weeks and there is evidence that acclimatization to heat is also rapid (Beattie and Chambers 1953). Changes in the amount of ADS produced in acclimatization could thus be studied.

* Sir William MacGregor School of Physiology, University of Queensland, Brisbane.

Birnie *et al.* (1949) and Birnie (1953) have claimed that the blood of adrenalectomized animals has an antidiuretic potency higher than that of intact rats because the liver destroys less ADH, while reduced renal clearance following adrenalectomy (Ginsburg 1954), or unknown causes (Mirsky, Stein, and Paulisch 1954) are considered by some workers to raise the ADS level. Others disagree with this concept (Ames and van Dyke 1952; Leaf *et al.* 1952). It was considered that, if adrenalectomy hinders the removal of ADS from the blood, the effect would be magnified in heated animals in which the production of these substances is increased. Therefore, a further experiment was designed to compare the levels of plasma ADS in adrenalectomized rats with normals during acute heat exposure.

II. Methods

Four series of experiments were conducted:

(*a*) *Continuous Exposure*

Male albino rats of similar weight (200 g) and age, from an inbred Wistar strain, were divided into three groups: one group was exposed continuously for 28 days in a hot-box to a temperature of 35°C; a second group was kept continuously over this period in a lighted refrigerator at 6·5°C; and a third group was exposed to external temperature conditions (av. monthly 3 p.m. temperature 22°C). The blood of all these animals was assayed for antidiuretic activity and for plasma protein concentration. This experiment took place in September-October. It was repeated (with omission of the cold exposure group) on other rats in March-April (av. monthly 3 p.m. temperature 27·2°C).

(*b*) *Repeated Heat Exposure*

A group of rats of the same sex, age, and weight as used in Section II (*a*) was exposed daily for 2 hr to an atmosphere of 40°C and 30 mm Hg vapour pressure for 28 days. The plasma ADS and protein levels were compared with a group of control rats maintained on the same level of nutrition at normal environmental temperatures.

(*c*) *Acute Heat Exposure*

Male rats were subjected to one acute exposure at 40°C and 30 mm Hg vapour pressure. The plasma antidiuretic activity and the plasma protein concentration was measured after 2 and 4 hr in the heat.

(*d*) *Adrenalectomy*

The plasma ADS of a group of pair-fed controls and of a group of rats, adrenalectomized 10 days previously and fed no salt supplement, were compared after 2 hr exposure to 40°C and 30 mm Hg vapour pressure.

In all these experiments drinking water was allowed until 3 hr before blood assays were made.

Antidiuretic activity of plasma was determined by a modification of the method of Dicker (1953) in which intravenous injections are made into an assay rat under ethanol anaesthesia with a constant water load. The method is sensitive to injections

of 10 μU of pitressin. Male rats weighing *c.* 200 g were starved for 16 hr prior to the test, and then hydrated with an oral dose of alcohol-water (12 per cent. ethanol solution) equivalent to 5 per cent. of body weight. Thirty minutes later a similar dose of water at body temperature was administered. The bladder was catheterized, the external jugular vein cannulated with 1 mm diameter polythene tube, and an indwelling stomach tube inserted. Urine samples were collected in a 5-ml measuring cylinder graduated to 0·05 ml and measurements were made at 10-min intervals. The animal was kept constantly hydrated with 2 per cent. ethanol solution by stomach tube, the amount administered from a microburette at 10-min intervals being equal in volume to the urine voided. When a steady rate of urine flow had been obtained (0·15-0·2 ml/min), intravenous injections of plasma from the experimental animal were made into the jugular cannula (volume = 0·4 ml plasma washed through

TABLE 1

PLASMA ADS AND PROTEIN CONCENTRATION OF RATS EXPOSED CONTINUOUSLY FOR 28 DAYS TO HOT AND COLD ATMOSPHERES

	Cold Exposure 43°F (6·5°C)	Heat Exposure 95°F (35°C)	Control	Season
No. of animals	8	6*	8	Sept.–Oct.
Plasma ADS (μU/ml)	42±35	182±24	80±41	
t†	$P = 0·05$	$P < 0·001$		
No. of animals	—	10	10	March
Plasma ADS (μU/ml)	—	52±15	25±12	
t†	—	$P < 0·001$		

* Loss of body weight over the period averaged 16 per cent. of the initial value. Body weight remained constant in all other groups.

† Probability of difference of ADS level from controls.

with 0·1 ml of 0·85 per cent. saline). The antidiuretic response was measured by comparing the volume of urine excreted in the 20 min following injection with that for the same period just prior to injection. The antidiuretic effect was then compared with that obtained from known amounts of pitressin (Parke, Davis and Co., No. 161) injected into the same assay rat.

Blood was collected in a heparinized tube from the neck vessels of experimental rats by rapid decapitation. It was quickly centrifuged, and the plasma was injected into the assay animal within 15 min of being withdrawn. The plasma protein concentration of the blood was measured by an Abbé refractometer.

III. RESULTS

(*a*) *Continuous Exposure to Heat or Cold*

Plasma from rats kept continuously in a hot atmosphere for 28 days during the months September-October had an antidiuretic potency more than twice that of control rats, while rats kept in the cold for this same period had about half the value found in controls. This is illustrated in Table 1 and Figure 1. Statistical examination

of the results by the *t*-test, showed that values for heat-exposed rats were highly significantly different from the controls ($P<0{\cdot}001$), while those exposed to cold were probably significantly different ($P = 0{\cdot}05$).

As judged by plasma protein values, blood of the rats exposed to heat showed 4·9 per cent. concentration relative to the controls, while in those exposed to the cold there was 6·6 per cent. dilution of plasma proteins. The protein concentration was recorded as an indirect measure of plasma water.

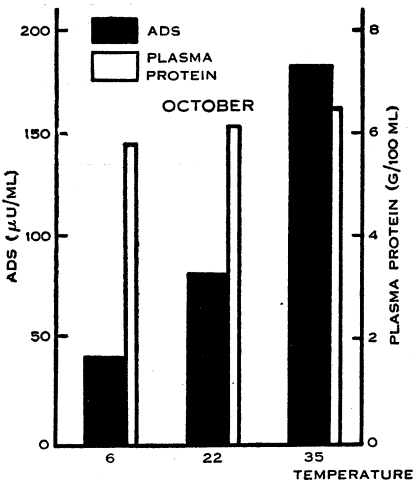

Fig. 1.

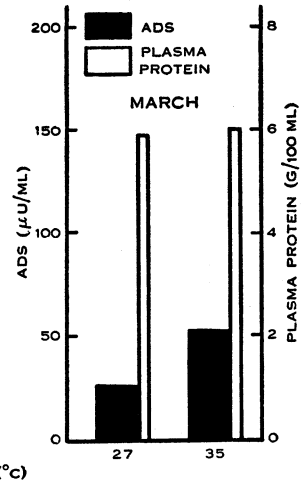

Fig. 2.

Fig. 1.—Histogram relating the mean ADS levels in the plasma of rats kept during summer at 6°, 22°, and 35°C, to the mean plasma protein concentrations simultaneously determined. Heat increased ADS and plasma concentration, cold decreased both quantities.

Fig. 2.—ADS and plasma protein in rats kept at 27° and 35°C during autumn. The ADS levels are lower than those recorded in Figure 1, but they vary similarly with plasma protein concentration.

Additional evidence of the high antidiuretic potency of the plasma of the heat-acclimatized rats was provided when attempts were made to produce a water diuresis in some of their number. The hydration was carried out under prevailing atmospheric conditions, and commenced immediately after the animals were removed from the hot environment. Oral administration of water in amounts which were diuretic in both cold-acclimatized and control rats failed to increase urine flow above 0·3 ml/hr in the heat-adapted animals (Table 2).

In this experiment, while both control and cold rats maintained body weight over the experimental period, that of rats exposed to heat fell an average of 16 per cent. during the 28 days.

In contrast, when the experiment was repeated in March-April, body weight was maintained at the initial value in both heat-exposed and control rats (Fig. 2). Results then showed that although the antidiuretic potency of the blood of hot rats was significantly above the controls ($P<0{\cdot}001$), the rise was much less than in the

earlier experiment. Plasma protein levels of both groups were comparable (6·0±0·3 and 5·9±0·4 respectively).

TABLE 2

RESPONSE OF HOT- AND COLD-ACCLIMATIZED RATS TO A WATER LOAD

Atmosphere	Water Load (ml)	Urinary Output (ml/hr)	Period of Time (hr)	Fluid Retained by Body (ml)
Hot*	29	0·31	7	27
	23	0·30	5·5	21
Cold†	22	3·3	6	2
	25	3·5	6	4
Control	36	3·8	7	9
	25	3·0	6	7

* 28 Days at 35°C. Body weight reduced by 16 per cent. over the period.
† 28 Days at 6·5°C.

The difference in ADS values for controls in the two experiments may possibly be explained as a seasonal fluctuation. This is suggested by data (unpublished) from other experiments at present in progress.

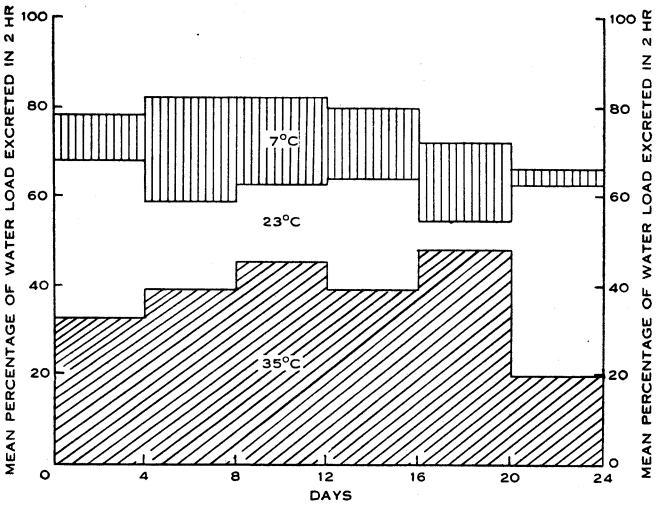

Fig. 3.—Water excretion during 2 hr after a water load of 5 per cent. of body weight, expressed as a percentage of the water administered, in a series of 180-g male Wistar rats. Cold-acclimatizing rats excreted a mean of 75 per cent., and heat-acclimatizing rats only 40 per cent. of the water load in 2 hr. Groups comprised 15 rats.

In Figure 3 is shown the mean percentage of water load excreted in 2 hr by 180-g male Wistar rats when tested periodically under three different atmospheric

conditions to which the animals were exposed continually for 24 days. Values are means for 15 rats in each group. Percentage of water load excreted bears an inverse relationship to the values found for ADS levels in these heat-adapted, cold-adapted, and control animals.

TABLE 3

SENSITIVITY TO PITRESSIN OF CONTROL, HOT-, AND COLD-ACCLIMATIZED RATS

Pitressin Injection (μU)	Per Cent. Reduction in Urine Volume (mean of 2 animals in each group)		
	Control	Heat-acclimatized	Cold-acclimatized
20	30	28	26
40	50	46	47
60	62	60	61
80	73	72	70

Table 3 illustrates the reliability of the assay method and its independence of the prior treatment of the rats. The sensitivity to injections of graded doses of pitressin of cold-acclimatized, heat-acclimatized, and control rats used as assay animals on a constant 10 ml/100 g body weight load, is essentially the same in all groups. The greatest difference is 13 per cent. and the average is 5 per cent.

(*b*) *Repeated and Acute Heat Exposure*

Daily exposure of rats for a short period (2 hr) to a hot atmosphere (40°C, 30 mm Hg vapour pressure), although more severe heating than that used in the

TABLE 4

ANTIDIURETIC POTENCY AND PROTEIN CONTENT OF RAT PLASMA FOLLOWING SINGLE AND REPEATED EXPOSURE TO A HOT ATMOSPHERE (104°F (40°C), 30 MM HG VAPOUR PRESSURE)

Condition of Animals	Time of Exposure to Heat	No. of Animals	ADS (μU/ml)	Plasma Protein (g/100 ml)	Rectal Temperature (°C)
Normal	2 Hr daily for 28 days	7	28±15	5·9	—
,,	2 Hr single exposure	9	30±16	6·0	39·5
,,	4 Hr single exposure	5	133±29	6·9	40·9
,,	Control	6	27±18	5·9	36·8
Adrenalectomized	2 Hr single exposure	5	29±12	6·0	—

continuous exposure experiment, failed to produce a rise in level of blood ADS in 28 days. A single day's exposure, however, to this same hot temperature for a longer period (4 hr) was effective in increasing the ADS of the blood sample (Table 4 and Fig. 4). A *t*-test analysis indicated that this difference between the 2-hr and the 4-hr exposures was highly significant ($P<0{\cdot}001$).

Plasma protein levels were raised (to 6·9 g/100 ml) after 4 hr at 40°C but remained near the control level of 5·9 g/100 ml with only 2 hr of heating.

(c) *Adrenalectomy*

Adrenalectomy did not increase the concentration of ADS nor alter plasma protein levels during a 2 hr exposure to heat (Table 4 and Fig. 4).

IV. Discussion

Itoh (1954) suggested that the release of ADH from the posterior pituitary gland of rats, which is accelerated by exposure to heat and inhibited on exposure to cold, results not from dehydration induced by heat but rather from heat *per se* acting by some mechamism other than dehydration.

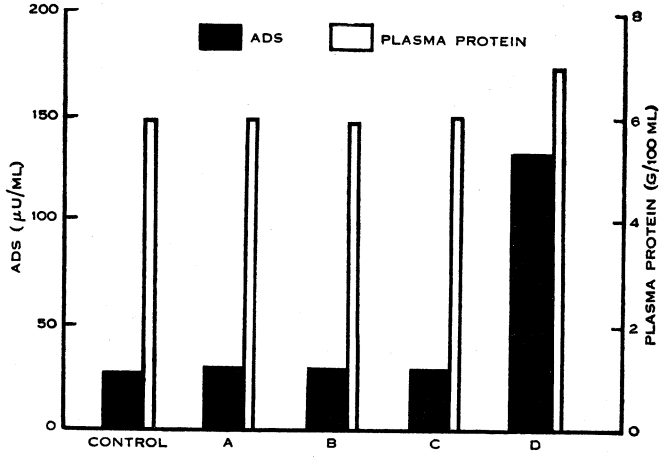

Fig. 4.—Plasma protein and ADS were unaffected by 2 hr at 40°C (*A*), by adrenalectomy and heating for 2 hr (*C*), and by daily exposure for 2 hr to 40°C for 28 days (*B*). Four hr at 40°C (*D*) tripled the ADS concentration and raised the plasma protein level from 6·0 to 6·9 g/100 ml.

The results of the present investigation do not, however, support such a concept. Although a 2-hr exposure of rats to 40°C raised body temperature from a mean value of 36·8 to 39·5°C, no increase in blood ADS was observed. It seems reasonable to expect that a 2·7°C rise in body temperature should provide sufficient stimulus for the activation of any heat-sensitive mechanism for ADH release. Only when some concentration was apparent (as indicated by plasma protein levels) following a longer exposure of 4 hr to this atmosphere did plasma ADS increase. Under these atmospheric conditions a 200-g rat loses water by evaporation at the rate of 5 g/hr (unpublished data). The loss of 10 g after 2 hr heating is evidently insufficient to produce a noticeable threat to the water reserves, but when it amounts to 20 g (approx. 1/6 of the body water), an adaptive water conservation release of ADS is initiated. Furthermore, when rats were subjected to continuous long-term exposure to heat and cold, the amount of ADS in the blood closely paralleled the plasma protein level. On the other hand, repeated daily 2-hr exposures to a high temperature

did not cause dehydration. Apparently compensation by water replacement during the periods at room temperature took place, since no evidence of haemoconcentration was observed. The parallel changes in plasma ADS and plasma protein in different thermal environments are illustrated in Figures 1, 2, and 4.

Evaporative water loss initially involves reduction of plasma volume, and this concentrates the plasma electrolytes as well as plasma proteins. Subsequent extrusion of water from the cells may reduce plasma dehydration. The situation is made more complex by migration of sodium into, and potassium out of, cells during long heating (unpublished data). There is, however, concentration of plasma proteins when the plasma electrolyte osmotic pressure is raised. Plasma proteins seem to be an index of plasma water, though there is no evidence that the protein osmotic pressure is the effective stimulus to ADH release.

It is interesting to compare the high values for ADS in the September-October experiment, involving continuous exposure to heat, when 16 per cent. reduction of body weight occurred, with the smaller rise that followed heating when the experiment was repeated at the same temperature without loss of weight. Other workers have reported higher than normal ADS blood levels in animals and men fed protein deficient rations (Leslie and Ralli 1947; Schweppe and Freeman 1951; Guggenheim and Hegstead 1953), and *in vitro* studies would suggest (Birnie, Blackmore, and Heller 1952) that the protein-deficient liver destroys ADH less rapidly than the normal liver. Guggenheim has demonstrated that rats maintained on a thiamine-deficient diet exhibit a delayed diuretic response to a water load with impairment of ability of the liver to inactivate pitressin (Guggenheim 1954*a*), although the derangement of water metabolism in pyridoxine- and pantothenic acid-deficient rats appears to be related to interference with adrenocorticotrophic hormone production rather than to ADH accumulation in the body (Guggenheim 1954*b*).

To explain the difference in our results between the two continuous heat-exposure experiments it is suggested that, in the earlier series, two factors were operating to raise the ADS level of the plasma: (i) heat acting through the stimulus of haemoconcentration to increase the output of ADS, and (ii) malnutrition resulting in an impairment of normal ADS destruction. In the latter series, factor (ii) was removed and the antidiuretic potency of the blood fell correspondingly.

The sensitivity of the kidney to pitressin, and it is presumed, to ADH, in heat- and cold-acclimatized rats appears to be the same as that of rats living in thermally neutral environments. Adaptation of an animal to heat does not involve change in kidney response to ADH, but rather in the synthesis or release of the hormone as regulated by environmental factors. Hydration tests would indicate that differences in ADS levels of heat-adapted, cold-adapted, and control rats are related to differences in their respective rates of excretion of a water load.

No delay in removal of ADS from the blood of adrenalectomized rats was apparent under the conditions of these experiments. Animals, from which both adrenals had been removed 10 days previously, exhibited the same ADS response to 2 hr acute heat exposure as the controls. This is in agreement with the findings of Ames and van Dyke (1952) and it seems to be the result obtained when intravenous rather than intraperitoneal injection is used in the assay procedure.

The findings of the present investigation, indicate that ADS are released into the blood of rats following heat exposure. This occurs only after the amount of water lost in evaporative cooling is sufficient to produce a significant, although small, change in water reserves. Plasma protein concentration gives an indirect measure of this change. The ADS appears in both long-term and acute heat exposure, in amounts depending on the immediate extracellular water concentration. There is a possibility that the intracellular water reserve may be involved in seasonal ADS release. It would be expected that in sweating mammals, which rely on evaporative cooling to maintain body temperature in hot climates, the release of ADS would play an even more important role in heat adaptation than is seen in the rat. Experiments on man in this context are being carried out.

V. Acknowledgment

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