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THE UPTAKE AND EXCRETION OF WATER IN RATS POISONED WITH LEAD

BY

A. URSULA PARDOE AND MILES WEATHERALL

From the Department of Pharmacology, London Hospital Medical College, London

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The main injury produced by lead in rats' kidneys affects particularly the loop of Henle and the distal tubules (Pardoe, 1952). As there are grounds for associating these regions with the site of antidiuretic action of postpituitary extracts (cf. Pitts and Sartorius, 1950, for discussion), the excretion of water and the effect of vasopressin have been examined in rats poisoned with lead. Observations have been made also on the absorption of water from the alimentary canal and on its distribution in the body. This aspect of lead poisoning does not appear to have been investigated systematically previously, apart from a paper by Masciotta (1942), to which we have been unable to obtain access.

METHODS

Male albino rats from the local colony were used throughout. Some observations were made on the chronically poisoned rats described in the previous paper (Pardoe, 1952), and this set of rats will be referred to here as series I. Another chronic series (II) and two acute series (III and IV) of experiments were performed. Details of the doses used are shown in Table I. The rats weighed initially about 250 g. (series I and II) or 200 g.

TABLE I

DETAILS OF DOSAGE OF RATS WITH LEAD ACETATE OR SODIUM ACETATE

Exp. series No.	Cation	Single dose M-eq./kg.	No. of doses	Duration of dosing days	Route of administration	Comment
I^1	Pb	3.3	27	63	Oral	Dosing three times weekly throughout
		followed by				
I^2	Na	13.3	31	72		
II^1	Pb	13.3	29	98	Oral	Dosing three times weekly for first 18 doses, next three doses in four weeks, then twice weekly
II^2	Na					
III^1	Pb	13.3	2	7	Oral	
III^2	Pb	0.67	1	—	i.v.	
III^3	Pb	6.7	2	7	Oral	Intravenous dose given at same time as first oral dose
		0.33	1	—	i.v.	
III^4	Na	6.7	2	7	Oral	
		0.33	1	—	i.v.	
III^5	—	—	—	—	—	No treatment
IV^1	Pb	0.67	1	—	i.v.	
IV^2	Na					

(series III and IV) with standard deviations of about ± 10 per cent. The doses used generally were near to those expected to cause death, and dosing was stopped at certain times because the rats were weak or lethargic. The doses given intravenously were injected over periods of 40–60 sec. for each rat. Immediate fatalities were usual if this rate was exceeded, but no deaths occurred at the rate adopted. In all the experiments described no deaths occurred which could be attributed to the quantity of lead given to the animals.

Water absorption experiments.—The method of Heller and Smirk (1932) was followed. Rats were given lukewarm tap water (5 ml. per 100 g. body weight) by stomach tube, and were killed in groups of three at various times afterwards. Some rats were killed without giving them any water. The alimentary canal, from the lower end of the oesophagus to the rectum, was removed, with care not to lose any of its contents, and was weighed at once. All weights were expressed per 100 g. body weight and the mean amount of water absorbed was calculated from the mean weight of the stomach and intestines of the unhydrated animals plus five minus the mean weight of the stomach and intestines of the hydrated animals at a given time.

Tissue water content.—The water content of tissues was determined from their fresh weight and their weight after heating to 110° C. for eighteen to twenty-four hours in watch glasses or platinum crucibles.

Water diuresis experiments.—Food was withheld overnight, but access to water was allowed. In the morning each rat was given lukewarm tap water (5 ml. per 100 g. body weight) by stomach tube and placed either in a metabolism cage constructed on a glass funnel of 15 cm. maximum diameter or in a group of six rats in larger galvanized metal cages. Vasopressin ("Pitressin," Parke Davis: 0.004 or 0.0005 unit per 100 g. body weight) or nicotine hydrogen tartrate (0.25 mg. of nicotine base per 100 g. body weight) was injected subcutaneously immediately after the water if either of these drugs were being studied. The total urinary output was observed approximately every quarter of an hour until the rate of excretion had risen and become low again. The results were plotted graphically, and as a rule the time until 50 per cent of the dose of water had been excreted was determined from these graphs. The amount excreted at different times was similarly obtained graphically, and mean values were obtained from replicate estimates. The passing of a small volume of urine shortly after hydration and before the onset of diuresis, as described by Burn (1931), did not appear to us sufficiently important to render the use of the 50 per cent time unreliable. In some experiments an unusually gradual diuresis occurred in which there was no definite peak in the rate of excretion: the time to the peak rate was therefore not a feasible measure of the delay in diuresis for our purposes.

Inactivation of vasopressin by liver.—The method used was based on that described by Birnie, Blackmore, and Heller (1952). A normal or a lead-poisoned rat was killed and bled from the carotid artery and its liver was removed; 1 g. of liver was transferred to an ice-cold homogenizer and ground for 5 minutes with 2 ml. of ice-cold water; 3 ml. ice-cold water were added to the homogenate and ground further, after which the material was made up to 10 ml. with distilled water; 1 ml. aliquots were added to a series of centrifuge tubes. To these centrifuge tubes were then added 3 ml. of ice-cold M/15 phosphate buffer of *p*H 6.5 and 1 ml. vasopressin in different concentrations. The tubes were shaken and incubated at 37° C. for 30 minutes, and the presence or absence of antidiuretic activity in their contents was then demonstrated by injecting 0.3 ml. of the incubated solution intraperitoneally into each of four rats, which had just been hydrated with 5 ml. water per 100 g. by stomach tube, and observing their rate of urinary secretion.

Action of vasopressin on the weight of frogs.—The effect of vasopressin (0.5 unit per 20 g. frog) on the weight of frogs was determined as described by Heller (1942). Some of the frogs tested were previously exposed to lead by keeping them for two to three weeks

in water which contained 1 mM. lead chloride per litre and which was changed three times a week. One frog out of twenty-four died under these conditions, compared with none out of twelve which were kept in distilled water in an adjacent tank.

Error of experimental results.—In the experiments on the absorption of water, on the quantity of water in tissues, and on the effect of vasopressin on the weight of frogs, the variances of the different groups in a single experiment appeared to be independent of the means of the groups. Pooled estimates of the variance due to experimental error were therefore made by the usual methods of the analysis of variance, and were used to test the significance of observed differences when the analysis showed that the effects of treatment were larger than those attributable to chance variation. In the diuresis experiments the variance between replicate observations was clearly related to the value of the mean, although the relation was not a simple one. The variance and standard error were therefore calculated separately for each mean.

RESULTS

Absorption of water from the alimentary canal

The absorption of water from the alimentary canal was studied in the rats of series II and III. The results with series III are shown in Fig. 1. Essentially

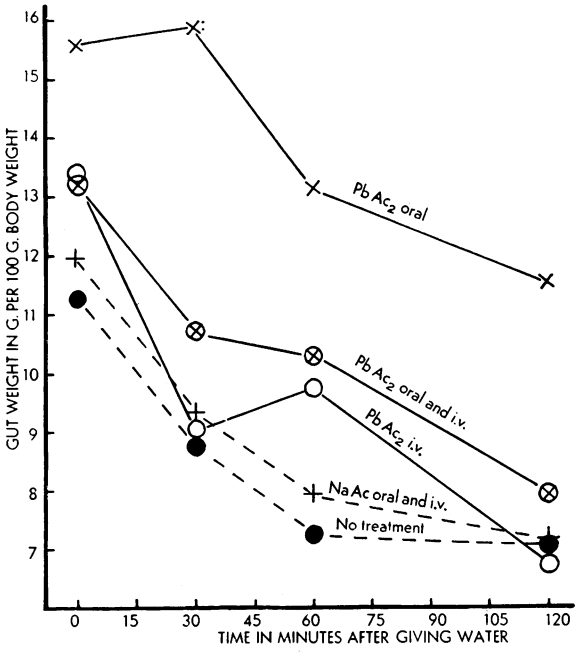

FIG. 1.—The absorption of water from the alimentary canal. Ordinates: mean weight of the alimentary canal and contents in g. per 100 g. body weight for groups of three rats. Abscissae: time in minutes after giving water, 5 ml. per 100 g. body weight, by stomach tube. ×——× After lead acetate orally. ⊕——⊕ After lead acetate orally and intravenously. ○——○ After lead acetate intravenously. +– – –+ After sodium acetate orally and intravenously. ●– – –● No previous treatment. Details of treatment are given in Table I. The values at 0 minutes were obtained by adding 5 g. per 100 g. to the mean weight of the alimentary canal when no water was given. The standard error of each of the points, estimated by the analysis of variance, is ±0.53 g. per 100 g. body weight.

similar results were obtained with the chronically orally poisoned rats of series II. In both series, the alimentary canals of the rats which had received lead were considerably heavier than those of the control rats. This occurred particularly when lead acetate had been given by stomach tube, but it was definite also when it had been given entirely intravenously. The increased weight was partly due to an increase in the weight of the actual tissues and partly to an increase in the contents (Table II). The increase in contents was particularly prominent when the lead had

TABLE II

THE WEIGHTS OF VARIOUS ORGANS IN RATS AFTER ADMINISTRATION OF LEAD ACETATE OR SODIUM ACETATE

Details of dosage are given in Table I. The rats of series III were killed eight days after beginning dosing and the rats of series II were killed 100–104 days after beginning dosing. All values are the means for groups of three rats, except that the values for series II[1] and II[2] normals are for groups of two rats. Standard errors of the means in each group have been obtained from the analysis of variance

Exp. series No.	Mode of dosing	Organ weights, g./100 g. body weight						Water content % of organ wt.	
		Stomach	Small intestine	Gut contents	Adrenals	Kidney	Liver	Kidney	Liver
III[1]	Acute oral $PbAc_2$	0.64	3.07	6.0	0.021	0.89	3.21	79.7	70.2
III[2]	Acute i.v. $PbAc_2$	0.62	3.32	3.4	0.025	1.08	4.57	80.1	72.3
III[3]	Acute oral and i.v. $PbAc_2$	0.65	3.26	3.8	0.022	1.00	3.89	79.2	71.8
III[4]	Acute oral and i.v. NaAc	0.51	2.41	3.0	0.018	0.75	3.08	78.4	70.1
III[5]	No treatment	0.53	2.75	2.6	0.021	0.73	3.12	77.8	70.5
	Standard errors of series III means	±0.03	±0.17	—	±0.001	±0.06	±0.21	±0.45	±0.25
II[1]	Chronic oral $PbAc_2$	0.82	2.60	—	0.015	1.24	3.52	81.1	70.1
II[1]	Chronic oral $PbAc_2$, water withheld for 24 hr. before death	0.80	2.38	—	0.021	1.13	3.91	75.0	70.3
II[2]	Chronic oral NaAc	0.51	1.45	—	0.014	0.70	—	76.5	—
II[2]	Chronic oral NaAc, water withheld for 24 hr. before death	0.57	2.02	—	0.015	0.65	2.38	75.9	68.8

been given by stomach tube. The control rats, which had received sodium acetate, had slightly more material in their alimentary canals both before and after water was given than had the rats which had had no previous treatment. The differences were trivial compared with those observed after lead, and they might have been due to chance ($t=1.33$, $P\simeq 0.2$). After water had been administered, the rate of absorption in the controls followed the expected course (Fig. 1). In the rats which had received lead acetate orally, the onset of absorption was delayed. Indeed, the mean weight of the gastro-intestinal tract half an hour after the administration of water was greater than could be accounted for by the original weight of the tract plus the administered water, both in series III (Fig. 1) and series II. The excess in series III could be due to the chances of sampling ($t=1.47$, $P\simeq 0.2$), and the weights of the alimentary canals of unhydrated rats in series II were obtained on a different day from those of the hydrated rats and so were not strictly comparable. The evidence is therefore only suggestive that there was an actual influx of material to the gastro-intestinal tract in these animals, but there is no doubt that absorption was delayed. Apart from this delay or influx, absorption proceeded normally or, in the rats which received lead acetate intravenously, if anything unusually quickly; though the results from these last rats were rather irregular.

The effect of nicotine hydrogen tartrate (0.25 mg. of nicotine base per 100 g. body weight) on the absorption of water was observed in some normal rats, in some

rats chronically poisoned with lead acetate by stomach tube, and in some acutely poisoned intravenously. Nicotine had no significant effect on the weight of the gastro-intestinal tract when no water was given. It tended to retard the absorption of water. For example, in the rats of series II, which had received sodium acetate, the mean weight one hour after giving water was 8.1 g. per 100 g. body weight when no nicotine was given and 10.1 g. per 100 g. when nicotine was given at the same time as the water ($t=2.32$, $0.1>P>0.05$). Similar differences were seen in the lead-poisoned rats of series II (13.9 and 16.4 g. per 100 g. body weight without and with nicotine; $t=2.90$, $0.05>P>0.02$). As these differences are in the same direction, they are very unlikely to be due entirely to chance. This point was not examined in more detail because the slowing of absorption was not very large and did not appear to be an important factor in the observed changes in the action of nicotine on water diuresis.

Organ weights and water content of tissues

As well as the stomach and small intestine, the liver, kidneys, and adrenals were always heavier in the rats which had received lead acetate (Table II). The greater weight of the livers and kidneys was due mainly to an increase in dry weight but partly also to an increase in water content. The increase in dry weight occurred surprisingly quickly. In a pilot experiment, 18 hours after lead or sodium acetate had been given by various routes, the dried livers of the animals to which lead had been given were on the average 21 per cent heavier and the dried kidneys 19 per cent heavier than those of the controls. At this time differences in water content were not significant. By eight days slight increases in the tissue water content were found consistently in the liver and kidney, though not definitely in the stomach and small intestine.

Water balance

When groups of three rats were placed in metabolism cages with or without access to water for twenty-four hours, the weight losses, urinary output, and fluid intake of the control rats and of the rats which had been receiving lead acetate for the previous three months did not differ greatly. However, the leaded rats, which were not allowed access to water, passed more urine than the controls (4.2 instead of 2.6 ml. per 100 g. body weight) and lost more weight (10.9 instead of 8.9 per cent) and had larger adrenals (Table II). The variation between the urine output of individual rats was not measured and the observations need to be repeated with more animals.

Excretion of water by the kidneys

When water was given to lead-treated rats, fairly normal diuresis occurred. Immediately after giving lead, the responses were irregular, possibly because the rats were excited after their recent handling (Table III, rats of series III). Later, the observed 50 per cent times were within normal limits (cf. Dicker, 1951, for references), except, rather surprisingly, for the control rats of series II. The rapidity of diuresis decreased in these rats after sodium acetate was administered, and they then always took over 120 minutes to excrete half their dose of water. This tardiness was not observed in the rats of series I, which also received sodium acetate, and it has no obvious explanation. It makes the much faster excretion by the lead-

treated rats of doubtful importance, especially as this faster rate was within the normal range and as the lead-treated rats of series I excreted water not significantly faster than their controls ($t=1.13$, $0.3>P>0.2$). Possibly lead causes some acceleration of normal diuresis, but the effects are not striking.

Effect of vasopressin on the excretion of water.—The results of experiments in which 0.004 unit of vasopressin (" Pitressin," Parke Davis) per 100 g. body weight was injected subcutaneously at the same time as water (5 ml. per 100 g.) was given by stomach tube are also shown in Table III and Fig. 4. In acutely poisoned rats (series III) the three groups which had received lead all started to show a diuresis sooner than the controls and excreted half the dose more quickly, but the difference between the control and treated groups was not larger than differences commonly observed between identically treated groups on the same day. In rats which were receiving repeated doses of lead or sodium acetate by stomach tube (series II) the mean delay in the sodium group was reasonably stable. In the lead group there was an initial period in which the sensitivity to vasopressin was much increased, after which the animals became abnormally insensitive. In these rats the hypersensitivity reappeared after a period between the sixth and ninth weeks in which

TABLE III

WATER DIURESIS IN RATS TREATED WITH LEAD ACETATE OR SODIUM ACETATE, AND THE EFFECT OF VASOPRESSIN AND NICOTINE

The figures given are the time in minutes from the administration of water (5 ml. per 100 g. body weight) by stomach tube to the time at which a volume of urine equal to half this dose had been passed. Details of dosage with lead acetate or sodium acetate are given in Table I. Vasopressin or nicotine was injected subcutaneously immediately after the water was administered. The results in series I were obtained from nine rats observed individually, in series II from eighteen rats observed in groups of six, in series III from six rats observed in a single group, and in series IV from five rats observed in a single group

Exp. series No.	Method of treatment	No. of doses given	Days from last dose	Time in minutes to half excretion					
				Plain water diuresis		Vasopressin 0.004 u. /100 g.		Nicotine 0.25 mg./100 g.	
				Pb	Na	Pb	Na	Pb	Na
III[1]	Oral	1	1	136	—	254	—	—	—
III[2]	Intravenous	1	1	136	—	230	—	—	—
III[3-4]	Oral and intravenous	1	1	96	95	250	310	—	—
III[5]	No treatment ..	—	—	—	97	—	284	—	—
IV	Intravenous	1	1	—	—	—	—	163	97
II	Oral, large doses ..	0	—	107		—	—	—	—
		10–13	1	82	—	254	196	—	—
		15–18	1	82	141	114	183	246	130
		19	3	79*	134*	—	—	—	—
	Oral, reduced doses ..	20	3	80	125	—	—	—	—
		20	8	97*	139*	—	—	141*	141*
	Oral, large doses ..	26–28	2	72	124	216	202	677	167
I	Oral (± S.E.) ..	58	1–2	75 ±3.8	82 ±5.4	94 ±8.8	133 ±9.1	—	—
	Oral (± S.E.) ..	58	75–83	—	—	104 ±6.7	135 ±4.6	—	—

* Observations on one group of six rats.

the dosage of lead had been greatly reduced (Fig. 2) and the insensitivity did not reappear before they were killed. In the other long series (I), diuretic responses were not studied at all until lead acetate had been administered for eighteen weeks continuously. At this time the rats which had received lead were notably less sensitive to vasopressin than were the controls (Fig. 2); and in these rats the insensitivity persisted for at least ten weeks after the administration of lead acetate had been stopped. In these animals at the end of eighteen weeks' treatment, the difference in the mean time to excrete half the dose of water after vasopressin was highly

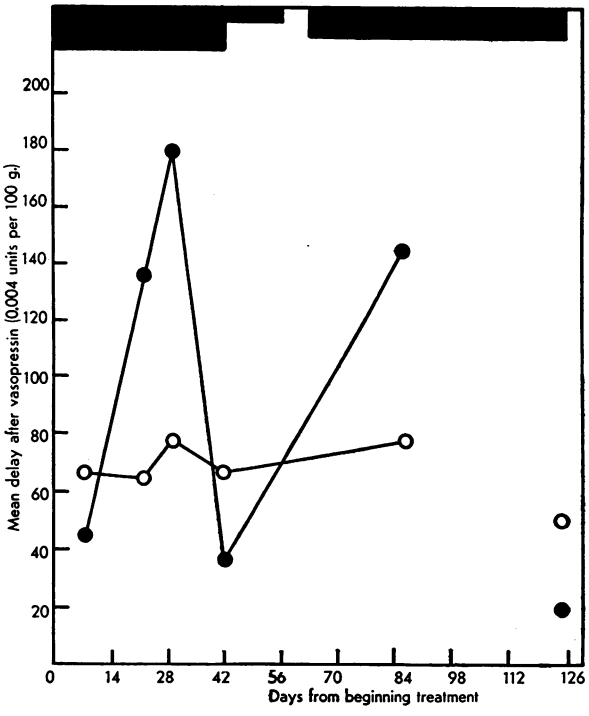

FIG. 2.—The effect of vasopressin at various times during the administration of lead acetate. Ordinates: Delay in time to fifty per cent excretion of dose of water given by stomach tube when vasopressin (0.004 unit per 100 g.) was given subcutaneously immediately after the water. Abscissae: Time in days from starting administration of lead acetate. ●——● Rats treated with lead acetate. ○——○ Rats treated with sodium acetate. The points joined by lines are for rats of series II. The isolated points at 124 days are for rats of series I. The blocked figure at the top of the graph represents the dose of lead acetate per week and applies to series II: dosage in series I was of the same order of magnitude (see Table I), but did not follow the detailed course indicated.

significant ($P<0.01$). As the rats to which lead had been given excreted water slightly more quickly than the controls, the difference between the delays produced by 0.004 unit vasopressin per 100 g. is less than the difference between the times to half excretion, but it is still substantial (18.6 instead of 50.5 minutes) and also highly significant ($P<0.01$). In terms of the dose-response curve obtained in this laboratory for the delay of diuresis by vasopressin in normal rats, this difference indicates that 0.004 unit of vasopressin per 100 g. body weight was having about as much effect in the leaded rats as 0.0014 unit of vasopressin per 100 g. body weight would be expected to have in the controls.

Effect of nicotine on the excretion of water.—The effect of nicotine hydrogen tartrate (0.25 mg. of nicotine base per 100 g. body weight) injected subcutaneously immediately after water had been given was examined in the rats of series II and IV. It caused a much greater delay in those rats which had been exposed to high doses of lead immediately previously than in control rats, or in rats which had not

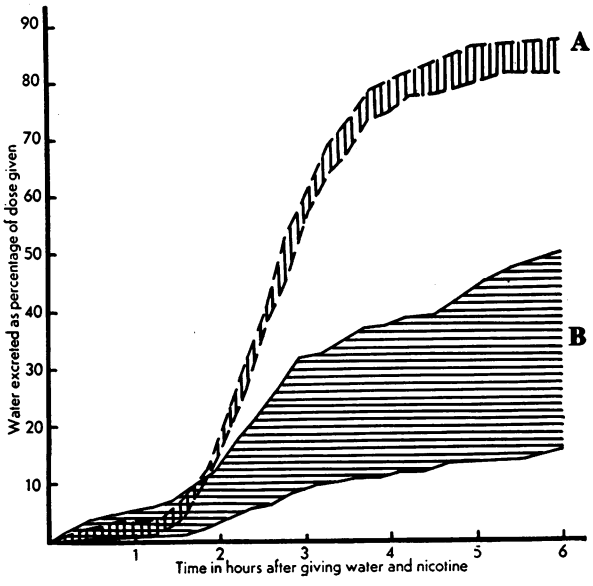

FIG. 3.—Water diuresis after nicotine in lead-poisoned and control animals. Ordinates: Percentage of dose of water (5 ml. per 100 g. body weight) excreted by groups of six rats. Abscissae: Time in minutes from administration of water by stomach tube. A, range of three groups of six control rats. B, range of three groups of six lead-poisoned rats. Nicotine hydrogen tartrate (0.25 mg. nicotine base per 100 g. body weight) was injected subcutaneously immediately after water was given. This experiment was performed on rats of series II 85 days after beginning to give lead acetate. Details of dosage are given in Table I.

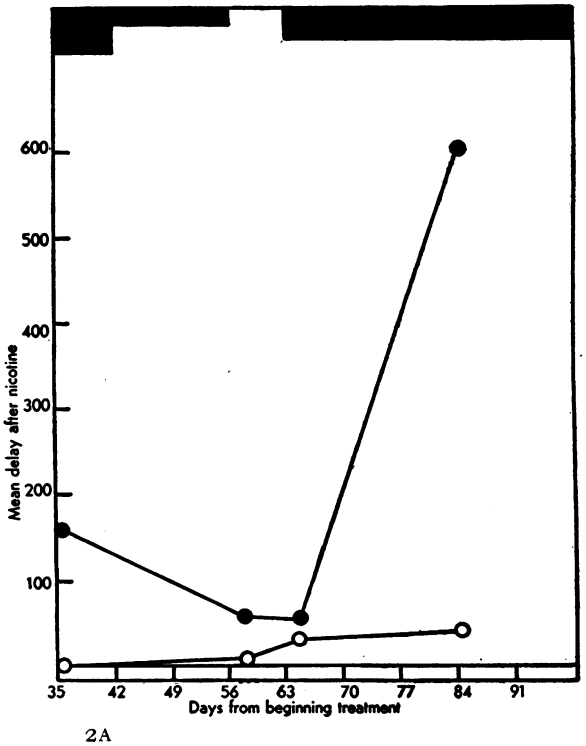

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FIG. 4.—The effect of nicotine at various times during the administration of lead acetate. Ordinates, abscissae, and symbols, but not scales, as in Fig. 2. All the points are for rats of series II.

received lead for some days (Table III and Figs. 3 and 4). The dose of nicotine used had much less effect than 0.004 unit of vasopressin per 100 g. in the control animals but much more effect in animals which had just received large doses of lead, and in the latter the effect of nicotine persisted for many hours (Fig. 4). Nicotine if anything delayed the absorption of water. However, nicotine exerted the same very prolonged antidiuretic action when it was injected fifty to sixty minutes after water had been given, at a time when at least three-quarters of the water had been absorbed and only one-quarter excreted. The prolongation is therefore not substantially dependent on changes in water absorption, though these may contribute to it. There was no obvious difference in the frequency of convulsions or the degree of prostration produced in the two groups of rats, so the lead-poisoned rats did not appear to be generally hypersensitive to nicotine.

Destruction of vasopressin by the liver.—Various quantities of vasopressin were incubated for thirty minutes at 37° C. with homogenates of liver taken from normal and from lead-poisoned rats of series II, and their antidiuretic activity was then compared. It was found that 1.0 g. of normal rat liver under these conditions

TABLE IV

INACTIVATION OF VASOPRESSIN ON INCUBATION WITH HOMOGENIZED LIVER

Exp.	Concentration of vasopressin in incubated suspension of liver	Dose of suspension injected into rats	Time of excretion of half of dose of water	
			Liver from normal rats	Liver from rats poisoned with lead
	unit/ml.	ml./100 g.	min.	min.
1	0.00	0.3	113	115
	0.002	0.3	100	136
	0.002*	0.3	145*	155*
2	0.00	0.3	91	106
	0.02	0.3	120	102
	0.16	0.3	163	147

* Vasopressin added *after* incubation.

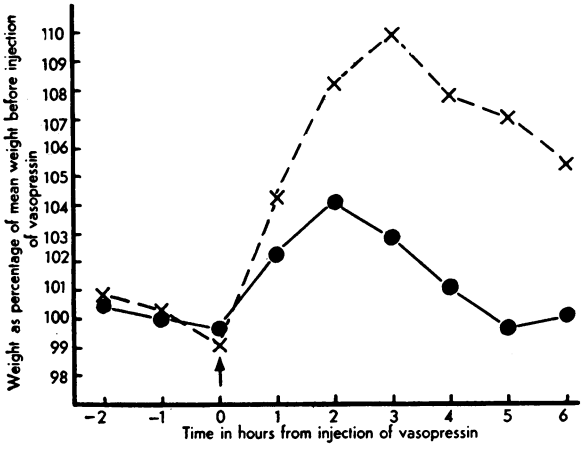

FIG. 5.—The effect of vasopressin on the weight of normal and lead-poisoned frogs. Ordinates: Mean weight of a group of four frogs, as a percentage of the mean weight in three successive hourly observations. Abscissae: Time in hours from injection of vasopressin (0.5 unit per 20 g.). × - - - × Normal frogs. ● —— ● Frogs kept in lead chloride (1 mM./litre) for 21 days before experiment.

completely inactivated 0.27 unit of vasopressin, and partially inactivated 0.81 unit. As Table IV shows, there was no appreciable difference in the antidiuretic activity of vasopressin which had been incubated with normal liver and that which had been incubated with the same weight of liver from rats poisoned with lead.

Effect of vasopressin on the weight of frogs.—The effect of vasopressin in doses of 0.5 unit per 20 g. on the weight of normal and lead-poisoned frogs was observed in two sets of experiments, each on four pairs of frogs. The mean results of one experiment are shown in Fig. 5. Vasopressin caused a much larger increase in the weight of the normal frogs than the lead-poisoned frogs, and an analysis of variance showed that in each experiment the difference would have occurred by chance less than once in a thousand experiments.

Discussion

The primary object of these experiments was to demonstrate whether the histologically demonstrable injury produced by lead salts in the renal tubules was associated with a reduced sensitivity to posterior pituitary extracts. The evidence on the whole suggested that this was so, but it was clear that other changes in the absorption and excretion of water took place in lead poisoning and complicated any interpretation of the observations. Most of these changes need to be investigated further, and our object is mainly to draw attention to their occurrence.

The alimentary canals of rats to which lead had been administered were dilated and contained more material than usual. This was not surprising when large doses of concentrated solutions of lead salts had been given by stomach tube, but it was observed also after intravenous administration when direct astringent and osmotic effects on the mucosa were avoided. Lead appears in the stomach wall and in its contents after intravenous injection of lead salts in rabbits (Ginsburg and Weatherall, 1948), and it is reasonable to expect a similar finding in rats: nevertheless the quantity of lead so reaching the contents of the alimentary canal in our rats would be unlikely to be more than about one-thousandth of the dose given by stomach tube. After dosing by stomach tube, absorption of water was delayed; after intravenous injection of lead there appeared to be no slowing of absorption and so the increase in the contents is more likely to be due either to stimulation of secretion or to interference with the musculature of the alimentary canal than to primary failure of absorption of material taken by mouth. As the effect was seen after intravenous injection of doses much smaller than those used by stomach tube, it is more probably due to a central or reflex action of lead than to a purely local action in the alimentary canal itself.

In addition to the increased contents, the stomach and intestines themselves were heavier in the animals to which lead had been given, and this was true also of the liver and kidneys. This increase was due partly to an increase in dry matter and partly to an increase in water content. We have no evidence about how the increments in dry weight occurred. The increase in water content was not great. For example, in the rats of series III the mean water content of the kidneys for all the control animals was 78.1 per cent and for all the leaded animals was 79.7 per cent. The corresponding figures for the liver were 70.3 and 71.4 per cent. However, during water diuresis, the water load of the tissues, calculated as the difference between the water absorbed from the gut and the water excreted by the kidneys,

is of the order of 2 per cent of the body weight. This water is probably not distributed uniformly, and we have not found comparable differences, for example, in skeletal muscle. However, differences of 1.1 to 1.6 per cent in the water content of some tissues before hydration may well affect the response of the body to water. From preliminary observations not reported here it appears that the water content of at least the kidneys in leaded animals rises less than in normal animals during water diuresis; and this appears to be of considerable importance in interpreting the results.

The part played by the adrenals also requires investigation, since they have commonly been enlarged in lead-poisoned animals and it is at least possible that this enlargement is associated with changes in functional activity.

Differences in the diuretic responses of normal and lead-treated rats have, therefore, to be considered against a background of differences in initial tissue hydration and possibly in the rate of water absorption. Plain water diuresis was not greatly altered. There was perhaps a little acceleration, but the evidence, discussed above, is equivocal. The antidiuretic action of vasopressin was at first enhanced and later diminished. A considerable reduction in sensitivity to vasopressin was seen also in frogs poisoned with lead. The inhibition of water diuresis by nicotine was much augmented four to six weeks after exposure to lead had begun, and it appeared that this augmentation depended upon the maintenance of a high dosage of lead. It disappeared when dosing was reduced and reappeared when it was increased again. The responses to nicotine were clearly distinct from those to vasopressin at the same time.

The insensitivity to vasopressin developed at about the same time as histological changes appeared in the renal tubules (Pardoe, 1952), and was presumably due to the injury to these cells. There was no visible evidence of earlier injury and it is difficult to account for the hypersensitivity to vasopressin observed about the fourth to sixth weeks of poisoning. Possibly lead initially increased the sensitivity of the tubular cells before visibly damaging and desensitizing them. The tubular injury involved both the loop of Henle and at least the distal convoluted tubules, but exact identification of the affected parts of the tubules was difficult in the presence of the structural damage caused by lead. The present observations do not, therefore, provide much useful additional evidence about the site of the antidiuretic action of posterior pituitary extracts.

The hypersensitivity to the antidiuretic action of nicotine has to be accounted for separately, since it was conspicuous in animals which responded more or less normally to vasopressin, and in animals which were relatively insensitive to vasopressin. Some evidence was obtained of delayed absorption of water under the influence of nicotine both in normal and in lead-poisoned animals, but the characteristic prolonged reduction in the rate of urine flow was produced in lead-poisoned animals when the nicotine was injected an hour after giving water, at a time when absorption was nearly complete. Unless nicotine has antidiuretic actions by some other pathway than stimulating the pituitary-hypothalamic mechanism, it is simplest to assume that this mechanism is rendered more sensitive by lead, so that a single stimulus has a much larger or more prolonged effect than normal. We have already argued that the observed effects of lead given orally and intravenously in producing dilatation of the stomach are probably of central origin, and it seems

plausible that both these changes indicate actions of lead on vegetative centres in the central nervous system. However, this is only a speculative interpretation and further experiments are needed to support or refute it.

SUMMARY

1. Some functions of the kidneys and the amount of water in certain organs under various conditions have been studied in rats poisoned with lead acetate. Rats which had been treated with equivalent amounts of sodium acetate were used as controls.

2. The onset of absorption of water from the alimentary canal was delayed in rats which had received lead acetate by stomach tube, but then proceeded normally. It was little affected when lead had been given intravenously. Absorption was possibly also somewhat delayed both in normal and in leaded rats by nicotine.

3. In the rats which had received lead acetate, the stomach, small intestine, liver, kidneys, and adrenals were heavier than in the controls. In at least the liver and kidneys there was both an increase in the dry weight and an increase in the water content of the organs. There was also an increase in the contents of the alimentary canal, particularly but not only in the rats which had received lead by stomach tube.

4. Water diuresis was slightly but not significantly accelerated in rats to which lead acetate had been given repeatedly by stomach tube.

5. The inhibitory effect of vasopressin on water diuresis in rats poisoned with lead was variable. In the first few weeks of administering lead, vasopressin had more effect than in normal rats. Later the animals became abnormally insensitive, at first reversibly and later apparently irreversibly.

6. In rats which were receiving large doses of lead acetate frequently, the anti-diuretic effect of nicotine was greatly increased. This increase was not related to an increased sensitivity to vasopressin. It disappeared within a few days of reducing the dose of lead and reappeared when intensive dosing was resumed.

7. Vasopressin in very large doses had highly significantly more effect on the weight of normal frogs than it had on the weight of frogs which had been kept in 0.001 M lead chloride in the previous two to three weeks.

8. Possible causes of these changes are discussed.

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