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THE MECHANISM OF DRINKING INDUCED BY PARENTERAL HYPERONCOTIC SOLUTIONS IN THE PIGEON AND IN THE RAT

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SUMMARY

1. In pigeons, the I.V. injection of 0·3–1·3 ml. of 50% (w/w) solutions of either polyethylene glycol (mol.wt., 20,000) or dextran (mol.wt. 40,000) induced reliable, rapid dose-dependent drinking responses. The amount of water drunk in response to I.V. polyethylene glycol was greater than that in response to I.P. polyethylene glycol and twice that in response to I.V. dextran. I.V. polyethylene glycol induced a diuresis following the onset of drinking.

2. The dipsogenic effect of I.P. polyethylene glycol solutions in the pigeon was depressed by I.V. isotonic NaCl solution 1 hr before offering water but was increased by simultaneous I.V. hyperoncotic polyethylene glycol solution.

3. In contrast, in rats, the subcutaneous injection of 25% (w/w) polyethylene glycol (mol.wt. 20,000; 1·25–10·0 ml./kg body wt.) induced reliable drinking responses, while the same doses of polyethylene glycol, injected I.V., did not induce drinking consistently. The volume of water drunk by rats in response to subcutaneous polyethylene glycol was smaller per unit dose than that drunk by I.P. injected pigeons.

4. The results suggest that receptors for drinking induced by extracellular dehydration in the pigeon could be situated in the extravascular interstitial section of the extracellular compartment.

INTRODUCTION

When hyperoncotic solutions, i.e. solutions with a colloid osmotic pressure greater than that of blood plasma but with the same crystalloid osmotic pressure as plasma, are injected into tissues or body cavities, they draw isotonic fluid from the circulating blood and thereby induce hypovolaemia. Simultaneously, there is an increase in the packed cell volume and of the plasma protein concentration, i.e. the oncotic pressure of plasma.

The latter change, in turn, draws isotonic fluid from the extravascular, interstitial compartment into the blood vessels. The final result of the extravascular injection of hyperoncotic solutions, therefore, is a reduction in the functional extracellular fluid volume affecting both the blood volume and the extravascular, interstitial fluid

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volume. This occurs in the absence of any change in the osmolarity of the extracellular fluid. Extracellular dehydration induced by injecting hyperoncotic solutions of high molecular weight polyethylene glycol either subcutaneously (Stricker, 1966, 1973) or intraperitoneally (Fitzsimons, 1961), has been shown to induce copious drinking in rats, as well as in the iguana (Fitzsimons & Kaufman, 1977), a reptile, and the pigeon (Kaufman & Peters, 1980). Drinking in response to hypovolaemia is thought to be due to a reduction in inhibitory sensory impulses from stretch receptors situated in the thoracic capacitance vessels. These receptors, when stimulated in the normovolaemic animal, inhibit both the secretion of vasopressin and, it is postulated, drinking (Fitzsimons, 1975). The renin–angiotensin system does not appear to be involved in drinking induced by extravascular injections of hyperoncotic colloid solutions since, in the rat, nephrectomy does not interfere with drinking induced by extravascular polyethylene glycol injections (Fitzsimons, 1961; Lehr, Goldman & Casner, 1975).

In contrast to the extravascular injection, the intravenous injection of hyperoncotic solutions results in *hypervolaemia* and interstitial dehydration as a consequence of the entry of interstitial extravascular fluid into the blood vessels. In the rat or the dog, hypervolaemia induces diuresis and natriuresis (Gilmore & Weisfeldt, 1965; Lichardus & Pearce, 1966; Pearce, 1968; Bonjour & Peters, 1970; Knox, Schneider, Dresser & Lynch, 1970), but has never been reported to induce drinking. We were therefore surprised to discover that intravenous injections of hyperoncotic solutions of high molecular colloids, such as polyethylene glycol, with a molecular weight of 20,000 (PEG 20M) or dextran with a molecular weight of 40,000 (dextran 40) induced copious drinking in pigeons. The present experiments were undertaken in order to investigate this phenomenon.

METHODS

Pigeons of undetermined sex, obtained from a dealer, were kept in the laboratory in large cages (six to ten birds per cage) with natural light. The animals were allowed free access to food (mixed grain) and water except during the drinking tests. For measuring water intake and urine flow, the animals were transferred to cylindrical experimental cages, 50 cm in diameter, with mesh bottoms and sheet metal walls. The cages were seated in large plastic funnels for the collection of urine and faeces. Water was offered in a small cup, the contents of which were drained into a calibrated burette for measuring water intake to 0·1 ml.; after measurements, the water was returned to the cup by raising the burette. The animals were trained to drink in these cages, as described previously (Kaufman & Peters, 1980).

For I.V. injections and infusions long fine (PP 10) catheters were introduced into the brachial veins of the animals through a thin wall 21 G needle without anaesthesia. After catheterization, the birds were allowed to remain undisturbed in the experimental cages with water for at least 30 min. Injections were then made through the catheters from outside the cage, without the pigeon inside being aware of what was happening. For bleeding, the pigeons were prepared by inserting shorter, thicker catheters into the brachial vein through a thin wall 19 G needle. After catheterization, the animals were allowed to recover in the experimental cages for 30 min. They were then removed from the cage and restrained lightly in a towel; blood was withdrawn into a syringe and the birds were returned to the experimental cages. Water intake was measured at 30 min, 1 hr, and at each successive hour for a total of 6 hr. The birds were weighed before and at the conclusion of each experiment. Urine volumes were measured each hour and were not corrected for evaporation.

Male Wistar rats weighing about 230 g (Tierzucht-Institut der Universitat, Zurich, Switzerland) were kept in individual transparent plastic cages with permanent free access to food (pellets) and water (drinking bottles). Each rat was only used for one drinking experiment.

Before drinking tests, which were always started between 10.00 and 12.00 hr, each animal was removed from its cage, weighed and injected either intravenously or subcutaneously with a hyperoncotic solution or with 0·9% NaCl. Water intake was measured after 30 min. 1 hr and after each subsequent hour up to a total of 6 hr.

Polyethylene glycol (20 M for gas chromatography; Merck, Darmstadt, F.R.G.) was dissolved in 0·9% NaCl solution to yield a 50% (w/w) solution for experiments in pigeons and a 25% (w/w) solution for experiments in rats. The more dilute solution was selected for rats because of the difficulty of injecting a 50% solution through the thin needles used for I.V. injections in rats. Dextran 40 (Pharmacia, Upsala, Sweden) was similarly dissolved in 0·9% NaCl as a 50% (w/w) solution for experiments in pigeons.

All numerical data are given as means ± S.E. of means. The significance of differences between means was evaluated by *t* tests on paired or unpaired samples assuming a normal distribution of the values.

RESULTS

As shown in Fig. 1, I.V. injections of hyperoncotic solutions of both polyethylene glycol (mol.wt. 20,000) and dextran (mol.wt. 40,000) reliably induced drinking in pigeons so that the birds went into positive water balance. The smallest volume needed

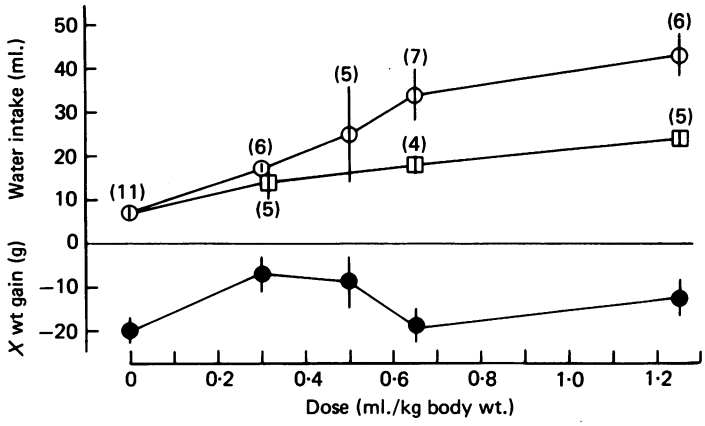

Fig. 1. Water intake of pigeons during 6 hr following I.V. injection of 50% (w/w) solutions of either polyethylene glycol (○) (PEG 20M) or dextran 40 (□) and changes of body weight after I.V. PEG 20M (●). The values are means ± S.E. of means. Numbers of experiments in brackets.

to induce a significant drinking response was 0·3 ml. 50% polyethylene glycol per kg body wt. and 0·6 ml. 50% solution dextran per kg body wt. As expected from the molecular weights, an equal dose of PEG 20M induced a drinking response approximately twice as large as that to dextran 40. In spite of the drinking, and in contrast to what has been observed after I.P. injections of polyethylene glycol 20M (Kaufman & Peters, 1980), the animals did not gain weight after I.V. polyethylene glycol and drinking; during the experimental period of 6 hr they lost the same weight as the controls (Fig. 1). In contrast to what happens after I.P. polyethylene glycol, the drinking response to I.V. PEG 20M occurred after injection of smaller volumes and, for a given dose, was larger (Table 1). The curve for drinking in response to I.V. polyethylene glycol, however, appeared to level off at smaller doses and at smaller water intakes than that to I.P. polyethylene glycol (Fig. 1, Table 1).

TABLE 1. Water intake of pigeons after I.P. or I.V. injections of increasing volumes of a 50% (w/w) solution of polyethylene glycol, mol.wt. 20,000 (PEG 20M). Values are means ±S.E. of means. Number of experiments in parentheses. The data for I.P. injections of PEG are taken from Kaufman & Peters (1980)

Dose of 50% PEG 20 (ml./kg body wt.)	Water intake of pigeons after I.P. injection (ml./kg body wt. per 6 hr)	Water intake of pigeons after I.V. injection (ml./kg body wt. per 6 hr)
0·3	—	17 ± 0·5 (6)
0·5	23 ± 1 (7)	25 ± 11 (5)
0·63		34 ± 5 (7)
1·0	22 ± 1 (7)	43 ± 8 (6)
1·25		
2·0	38 ± 5 (7)	—
5·0	60 ± 15 (6)	—
10·0	93 ± 21 (5)	—

In pigeons, drinking in response to I.V. polyethylene glycol began as rapidly as in response to I.V. hypertonic salt solutions (Fig. 2). Though its course was more protracted than that of drinking in response to hyperosmolar solutions, most of the drinking was terminated at the end of the third hour after I.V. polyethylene glycol. This contrasts with the drinking response to I.P. polyethylene glycol which reached its maximal rate 3–6 hr after injection (Fig. 2) (Kaufman & Peters, 1980).

The I.V. injection of polyethylene glycol induced drinking before diuresis (Fig. 3). The drinking response was thus not secondary to urinary water and salt losses. The delay in the onset of diuresis is not likely to have been due to dead-space error because pigeons have no urinary bladder and water-loaded pigeons were observed to void every few minutes.

In rats, subcutaneous injection of hyperoncotic (25%) polyethylene glycol (mol.wt. 20,000) induced a clear-cut dose-dependent drinking response (Fig. 4). In contrast, I.V. injection of the same hyperoncotic polyethylene glycol solution did not consistently induce drinking. A significant, albeit very small, drinking response was observed in one group of rats after I.V. injection of the 25% polyethylene glycol solution used in the present experiments at 5·0 ml./kg body wt. It was not established whether this response occurred before or after the expected diuresis.

The total water intake of pigeons within the 6 hr following an I.P. injection of a given dose of polyethylene glycol was the same when the animals were given access to water immediately (Fig. 5, column D) as when the animals were offered water only 4 hr after injection and when they thus had to drink the whole amount desired within 2 hr (Fig. 5, column A). The I.V. injection of isotonic saline 1 hr before offering water and 3 hr after I.P. polyethylene glycol resulted in a considerable depression of the ensuing water intake (Fig. 5, column B). Repletion of the extracellular space (i.e. mainly of the extravascular, interstitial space since isotonic saline rapidly leaves the blood stream after I.V. injection), therefore interfered with the drinking response to the I.P. polyethylene glycol. In contrast, the simultaneous injection of I.P. and of I.V. polyethylene glycol induced a water intake (Fig. 5, column D and E) larger than that induced by either I.P. (Fig. 5, column D) or I.V. polyethylene glycol (Fig. 5, column E)

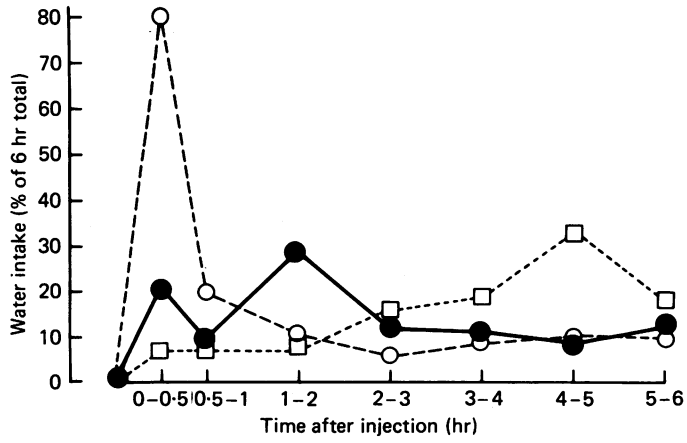

Fig. 2. Rate of drinking in pigeons at various time intervals after I.V. polyethylene glycol (PEG 20M) injected as a 50% (w/w) solution. The rates of drinking, expressed as per cent fractions of the total (6 hr) water intake were calculated as mean values from twenty-four experiments depicted in Fig. 1. The rates of drinking after I.V. PEG 20M (●) are compared to these after I.P. PEG 20M (□), or after I.V. hyperosmolar NaCl*(○).

* Data from Kaufman & Peters, 1980.

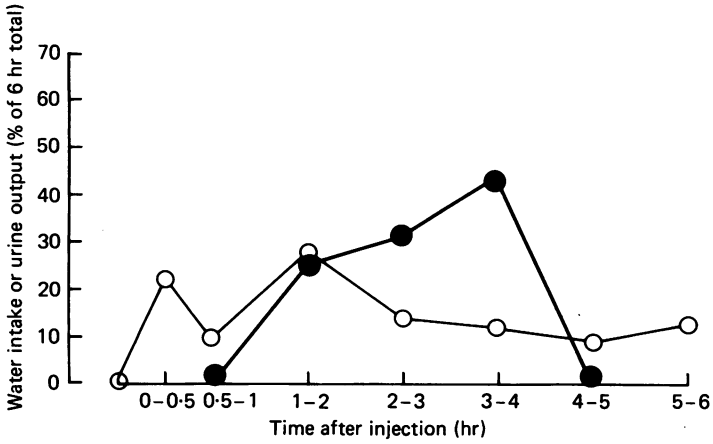

Fig. 3. Time and rate of diuresis (●) as compared to drinking (○) after various doses of 50% (w/w) PEG 20M solution injected I.V. into pigeons. Explanations as for Fig. 2.

alone, but smaller than the sum of both these effects. I.V. polyethylene glycol which reduced or even prevented the hypovolaemia induced by I.P. polyethylene glycol therefore did not depress drinking in response to the latter.

DISCUSSION

In the rat, the absence of a drinking response to I.V. injection of polyethylene glycol and the presence of a reliable dose-dependent response to subcutaneous poly-

ethylene glycol are compatible with the interpretation, outlined in the Introduction, that extravascular hyperoncotic solutions induce drinking through a decrease in the volume of the circulating blood (hypovolaemia). Neither the present results, nor other observations reported in the literature (Fitzsimons, 1961; Stricker 1966, 1973; Fitzsimons 1975; Fitzsimons & Kaufman, 1977), however, exclude the possibility

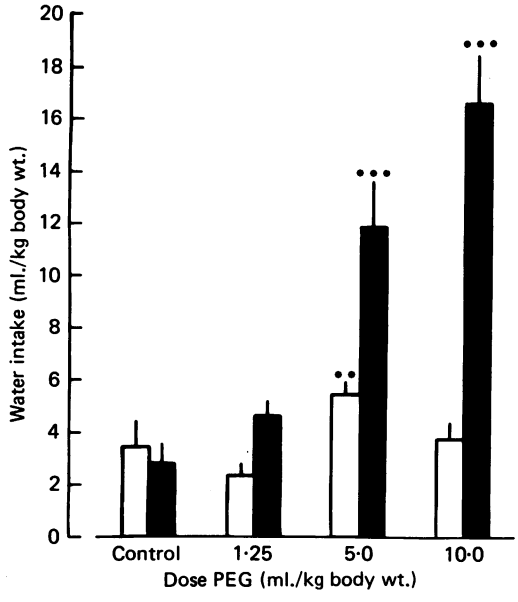

Fig. 4. Drinking responses to I.V. (open columns) or to subcutaneous injections (black columns) of a 25% (w/w) hyperoncotic solution of polyethylene glycol in rats. Columns are means ± S.E. of means (bars). Six rats per group. The significance of differences between animals injected with PEG 20M and the corresponding controls is shown by ** = $P < 0.01$, and *** = $P < 0.001$, the absence of asterisks indicating that an apparent difference failed to reach statistical significance. ($P > 0.05$.)

that there are receptors sensitive to the loss of fluid situated in the extravascular, interstitial part of the extracellular fluid compartment as well as within the blood vessels themselves. The failure of I.V. polyethylene glycol (mol.wt. 20,000) to induce drinking in the rat, could be attributable to renal excretion of the colloid, since glomerular capillaries filter substances of molecular weight around 20,000. Experiments with dextran of higher molecular weight were not carried out in rats, because dextran solutions are known to provoke an anaphylactoid reaction in this species (Morrison, Richardson & Bloom, 1951; West 1977). The smallness of the drinking response to subcutaneous polyethylene glycol in the rat as compared with the pigeon might also be explained by partial entry into the circulating blood and renal excretion of the injected colloid, although a number of other explanations are possible.

In the pigeon, rapid and large drinking responses to I.V. polyethylene glycol or dextran were certainly not caused by the primary diuretic response since diuresis occurred after and not before drinking. Furthermore, the weight loss of pigeons in the 6 hr following an I.V. polyethylene glycol injection was not greater than in controls, as would have been expected had the primary response been diuretic.

Drinking in response to I.V. hyperoncotic solutions cannot possibly be ascribed to hypovolaemia since such solutions are known to increase rather than to decrease the total plasma and blood volume. Furthermore, if drinking in response to I.P. hyperoncotic solutions was due to secondary hypovolaemia, it should have been abolished by repleting the plasma volume by I.V. polyethylene glycol, but this was not the case.

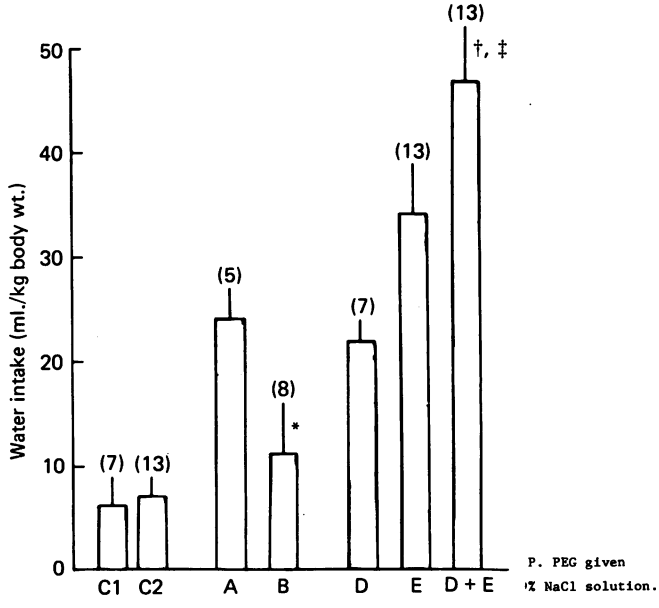

Fig. 5. Water intake of pigeons during 6 hr following I.V. or I.P. PEG 20M given alone or in combination with each other or with I.V. 0·9% NaCl solution. C_1: control. Injected I.P. with isotonic saline 5·0 ml./kg body wt. C_2: control. Injected I.V. with isotonic saline 2·0 ml./kg body wt. A: injected I.P. PEG 1·25 ml./kg body wt. Offered water at 4 hr. B: injected I.P. PEG 1·25 ml./kg body wt. plus I.V. isotonic saline 22 ml./kg body wt. at 3 hr. Offered water at 4 hrs. D: injected I.P. PEG 1·25 ml./kg body wt. Offered water immediately. E: injected I.V. PEG 0·625 ml./kg body wt. Offered water immediately. D+E: injected I.P. 1·25 ml./kg body wt. plus I.V. PEG 0·625 ml./kg simultaneously. Offered water immediately.

* Significantly different from A ($P \leq 0{\cdot}05$).
† Significantly different from D ($P < 0{\cdot}01$).
‡ Significantly different from E ($P < 0{\cdot}05$).

The assumption that hyperoncotic solutions induce drinking by inducing hypovolaemia, which in turn decreases the stimulation to stretch receptors situated in intrathoracic blood vessels, resulting in an urge to drink, therefore appears inapplicable to pigeons.

This leaves us with three possible explanations. In the pigeons, drinking in response to I.V. hyperoncotic solutions could be due to the increase of the colloid osmotic pressure of the blood plasma. This finding is compatible with the occurrence of a similar, albeit smaller, drinking response to I.P. hyperoncotic polyethylene glycol solution, since the passage of colloid-free fluid from the circulating blood to the peritoneal cavity would result in an increase in the protein concentration and of the colloid osmotic pressure of the blood. The combined I.V. and I.P. injection of poly-

ethylene glycol could have additive effects on the plasma colloid osmotic pressure. An increase in the concentration in blood plasma of macromolecular compounds unable to diffuse through capillary walls could in turn induce drinking by stimulating receptors in the walls of the blood vessel. Such receptors could be cells with walls having permeability characteristics similar to those of the walls of blood capillaries. These cells would shrink as a consequence of an increase of the oncotic pressure of the blood plasma. Information from receptors in the walls of blood vessels or elsewhere would reach the brain to arouse thirst directly or else would induce release of renin from kidneys which in turn would stimulate thirst. It has been shown recently (Hall & Guyton, 1976) that in the mammal a rise in plasma oncotic pressure in the absence of a change in blood volume may induce renin release from the kidneys.

Alternatively, I.V. polyethylene glycol or dextran, and I.P. injections of similar solutions, could induce drinking in the pigeon by acting on volume receptors situated in the extravascular, interstitial (extracellular) fluid space. Such receptors could be in any tissue including the brain. The present results are compatible with the hypothesis that drinking in response to I.V. as well as to I.P. hyperoncotic solutions is triggered off by a decrease in the extravascular interstitial fluid volume. In particular the depression of drinking in response to I.P. polyethylene glycol 1 hr after an I.V. injection of isotonic saline, i.e. at a time when most of the saline must have left the circulating blood, argues in favour of a primary role for extravascular receptors. Finally, the fact that pigeons do drink in response to a very small blood loss of 5 ml. blood/kg body wt. (Kaufman & Peters, 1980), and that this response does not occur until several hours have elapsed since haemorrhage, suggests that drinking occurs at a time when the blood volume has been restored to normal but the extravascular extracellular fluid volume is still depressed. We found that the plasma volume, measured as the distribution volume of I.V. Evan's blue, was the same 4 hr after haemorrhage ($10{\cdot}2 \pm 0{\cdot}4\,\%$ of total body wt., $n = 6$) as in normal pigeons ($10{\cdot}2 \pm 0{\cdot}6\,\%$ of total body wt., $n = 7$). The hematocrit of the bled pigeons, at this time, had fallen from 52 % to 43 %, indicating the entry of fluid from the extravascular interstitial space into the circulating blood and thus a fall in the concentration of plasma proteins.

The third possibility is that drinking occurred secondarily to an anaphylactoid reaction induced by the injected colloid. Such reactions are well documented in rats injected with dextran, mannan, ovomucoid and carrageenan (West, 1979, Anderson, Warne & West, 1976, Morrison *et al.* 1951). However, Morrison *et al.* (1951) reports that pigeons do not show an anaphylactoid response to dextran, and it is our experience that pigeons injected with polyethylene glycol or dextran never showed the oedema of the head and feet that is characteristic of these anaphylactoid reactions. Furthermore there are no reports in the literature of polyethylene glycol having anaphylactoid activity. Since the anaphylactoid reactions which have been reported in rats followed I.P. or subcutaneous injection of the colloids, the suggestion that water intake might be secondary to anaphylaxis would put in question not just these present experiments involving I.V. injections but also all previous experiments on thirst induced by I.P. or subcutaneously injected colloid.

Thus the more rapid and larger drinking responses to I.V. compared with I.P. administration of hyperoncotic solutions is compatible with the conclusion that

drinking in response to these colloids is induced by a primary decrease of the extravascular interstitial fluid volume. The results also suggest that, in the pigeon, drinking induced by extracellular dehydration may not be inhibited by osmotic dilution of the body fluids consequent to the ingestion of pure water to the same extent as in mammals. This may explain in part the larger water intakes of I.P.-injected pigeons compared with subcutaneously injected rats. Additionally it may well be that the renal clearance of these colloids is more rapid in the rat than in the pigeon.

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