HYPOTHALAMIC LESIONS AND HYPOVOLEMIA IN THE RAT

HYPOTHALAMIC LESIONS, HYPOVOLEMIA, AND THE REGULATION OF WATER BALANCE IN THE RAT

By

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SCOPE AND CONTENTS: Groups of rats were prepared with lesions of major hypothalamic areas, and their drinking responses and regulation of plasma sodium concentration during hypovolemia were observed. No evidence was found to suggest that the lesions of any group removed inhibitory control of drinking during hypovolemia, but evidence was obtained that lesions of the medial preoptic area interfered with the systems controlling water intake and the regulation of body water during hypovolemia. This observation was replicated in a second experiment, which also found that medial preoptic lesions induced a period of adipsia and aphagia, followed by recovery with residual deficits, including chronic hypernatremia. As a partial explanation of the observed results, it was suggested that the lesions altered the set-point for regulation of plasma sodium concentration.

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The usual common explanations for hypo- and hypernatremia should not be accepted, unless it is eminently clear that the criteria for that specific explanation are completely fulfilled. Should these be lacking, the qualities of the more unusual possibilities should be examined to provide evidence for or against their existence. If, in fact, they do exist, there are many more questions to be raised, the answers to which may provide insights into some of the most fundamental phenomena of biology.

Louis G. Welt, M.D. (1962)

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CHAPTER I

BACKGROUND

Basic Concepts

About 50-70% of the body weight is water. This fluid is divided by the membranes of cells into two compartments---the intracellular and the extracellular. The extracellular compartment contains approximately 1/3 of the body water, and is subdivided into the interstitial and intravascular compartments. The intravascular compartment contains only about 1/12 of the total body water (Pitts, 1968).

Fluid distribution among these compartments is determined by effective osmotic pressure; that is, by the osmotic pressure of substances which are unable to cross the membranes. An increase in effective osmotic pressure as a result of the introduction to one compartment of a substance which cannot cross a membrane will cause a shift of water and diffusible ions from the compartment of lower osmotic pressure to the compartment of higher osmotic pressure. This inflow will continue until osmotic equilibrium is reached or until it is opposed by hydrostatic pressure.

In the case of fluid shifts between the extracellular and intracellular compartments, effective osmotic pressure is determined by the

concentrations of the most abundant extracellular ions, sodium and chloride. These ions, although able to enter cells, are confined to the extracellular space as a result of the outward movement from cells of sodium (by an active process) and chloride (by a passive process) (Pitts, 1968). Thus these ions exert an effective osmotic pressure as though they were unable to penetrate cell membranes. An increase in sodium concentration of the extracellular fluid constitutes an increase in effective osmotic pressure and therefore water is withdrawn from cells; similarly, a decrease in sodium concentration causes water to enter cells. This inverse relationship allows the concentration of extracellular sodium (in practice, plasma sodium) to be used as an index of relative cellular hydration or dehydration (Welt, 1970).

Since sodium and chloride readily cross the capillary membrane, they do not exert an effective osmotic pressure on it. Instead, effective osmotic pressure is determined by the concentration of protein within the vascular system. According to Starling's Law, the effective osmotic pressure exerted by the plasma proteins tends to shift fluid from the interstitial space into the vascular system; this movement is opposed by the hydrostatic pressure developed by the heart pumping blood through the capillaries. Since blood pressure is highest at the arteriole end of the capillary and lowest on entering the venules, the net result is a dynamic balance in which there is a bulk flow of fluid leaving the capillary at the arteriole end and returning at the venule end, thus perfusing the tissues. In addition, diffusion of water and electrolytes occurs all along the capillary membrane. Because protein is

virtually confined to the vascular system, a decrease in protein concentration of the plasma usually indicates an increase in vascular volume due to the influx ofinterstitial fluid, whereas an increase in plasma protein concentration usually indicates a decrease in vascular volume due to the loss of plasma fluid. When total circulating protein can be assumed to remain unchanged, this inverse relationship allows the use of the plasma protein concentration as an index of vascular volume (Stricker, 1968).

Intracellular Dehydration and Regulation of Water Balance

Experimental techniques. Intracellular dehydration may be produced experimentally by injecting any substance which increases the effective osmotic pressure of the extracellular fluid (a "hypertonic" solution). Usually saline of concentration greater than 0.15 M is used, but other substances, such as sorbitol (Holmes and Gregersen, 1950) are also effective. When hypertonic saline is injected, plasma sodium concentration increases, but plasma protein concentration decreases as a result of the shift of water from cells to the extracellular space. When a substance other than sodium is used, plasma sodium as well as plasma protein concentration decreases (Holmes and Gregersen, 1950). Under these circumstances a decrease in plasma sodium concentration does not indicate cellular over-hydration.

Renal response. The renal response of a well-hydrated animal to acute cellular dehydration induced by hypertonic saline is a decrease

in urine volume (Hare, Hare, and Phillips, 1943) and an increase in urine osmolality (Bridges and Thorn, 1970). Both effects are attributable to the release of antidiuretic hormone (Gilman and Goodman, 1936). However, when urine volume is low and urine osmolality is high at the start of the test, the above responses may not be observed. Instead, the increased solute load may induce an osmotic diuresis, causing an increased urine flow and a decreased osmolality of the urine (West and Rapoport, 1950).

Drinking response. Cellular dehydration induced by the injection of hypertonic saline causes drinking (Gilman, 1937; Heyer, 1951; Adolph, Barker and Hoy, 1954). The two associated variables---an increase in total osmotic pressure and an increase in plasma sodium concentration---cannot be responsible for the observed drinking since (i) injected urea, in equi-osmolar concentration to hypertonic saline, causes little drinking because it enters cells freely (Gilman, 1937); and (ii) hypertonic sorbitol does cause drinking although it lowers plasma sodium concentration (Holmes and Gregersen, 1950).

Once initiated, drinking following hypertonic saline is proportional to the amount of injected salt (Wayner and Reimanis, 1958), but may (by itself) be insufficient to dilute body fluids to isotonicity (Adolph, Barker and Hoy, 1954). Fitzsimons (1961b) has resolved this paradox by demonstrating that when the kidneys are removed so that salt excretion is impossible, the amount drunk is exactly enough to dilute body fluids to isotonicity. In the same experiment he demonstrated that expansion of the extracellular space does not inhibit drinking caused

by cellular dehydration. Finally, Wolf (1950) has reported a threshold for drinking to cellular dehydration of 1-2%.

A remaining question concerns the basis of the small increase in water intake sometimes observed following injection of hypertonic urea (Adolph, Barker and Hoy, 1954; Fitzsimons, 1961b). As noted above, no drinking at all should be expected after treatment with urea, because urea rapidly penetrates cells. However, a blood-brain barrier to urea is known to exist (Coxon, 1964), and therefore the drinking following injection of urea may be explained as a result of the dehydrating effect of urea on brain tissue, despite hydration elsewhere in the body (Black and Stricker, 1970). It follows that the characteristics of the blood-brain barrier may be an important variable in control of thirst.

Hypovolemia and the Regulation of Water Balance

Experimental techniques. The direct method of inducing hypovolemia (loss of blood volume) through hemorrhage has two disadvantages (Stricker, 1968). First, if blood loss is sufficiently extensive, the experimental subjects may be too weak to show behavioural responses to the stimulus. Secondly, if a less severe blood loss is produced, the intravascular fluid volume may be rapidly restored by movement of fluid from the interstitium into the vascular system (Adolph et al, 1933). A more effective method of producing acute hypovolemia is to inject a large molecular weight compound, such as polyethylene glycol (PG), either

intraperitoneally (Fitzsimons, 1961a) or subcutaneously (Stricker, 1966). An injection of this type causes an increase in effective osmotic pressure of a localized region of the interstitium and therefore alters the Starling balance of hydrostatic pressure and osmotic pressure of the plasma proteins. Consequently, protein-free plasma is withdrawn from the vascular system and collects within an edema at the injection site. Unlike hemorrhage, this technique does not cause the removal of red blood cells from the vascular system. Moreover, the movement of fluid from the interstitium into the vascular system, in response to the increased concentration of plasma protein, does not restore plasma volume; instead, this fluid is also withdrawn into the edema (Stricker, 1968). Stricker (1966) has verified that the subcutaneous injection of PG produces an iso-osmotic withdrawal of fluid, and has further reported that (i) the plasma loss is proportional to the concentration of PG solution injected; and (ii) the plasma volume decreases linearly with time up to 8 hr after injection, and may remain decreased for more than 8 hr afterwards (Stricker, 1968; Stricker and Jalowiec, 1970). In contrast, it appears that the intraperitoneal injection of PG causes only a transient decrease in plasma volume, with values returning to normal within 8 hr (Hanson, 1970). Thus the latter method is inappropriate for experiments requiring prolonged hypovolemia.

Renal response. Hypovolemia as a result of hemorrhage in the rat has been shown to cause the secretion of both antidiuretic hormone (Ginsburg and Brown, 1956) and aldosterone (Solyom et al, 1963). Antidiuretic hormone promotes renal conservation of water by increasing the

permeability of the distal tubules and collecting ducts to water, allowing reabsorption of water to take place; aldosterone causes tubular reabsorption of sodium and excretion of potassium by an active process (Pitts, 1968). As noted earlier, a measure of the release of antidiuretic hormone is an increase in osmolality of the urine (Bridges and Thorn, 1970); a measure of aldosterone secretion is the ratio of potassium to sodium in the urine (Johnson, 1954). Retention of water and sodium may be further aided by the decrease in glomerular filtration rate that accompanies hypovolemia (Cort, 1952).

As expected, similar conservation of water and sodium occurs following hypovolemia induced by the subcutaneous injection of PG. The decrease in urine volume, the retention of sodium, and the excretion of potassium that are observed under these conditions (Stricker, 1966) strongly suggest the participation of antidiuretic hormone and aldosterone in the response. Such a response is, of course, highly appropriate for an animal sustaining an acute loss of sodium and water during hypovolemia.

Drinking response to hypovolemia. An extended period of controversy concerning the role of hypovolemia in elicitation of drinking (see Stricker, 1968; Stricker and Wolf, 1969) has been clearly resolved by the experiments of Fitzsimons (1961a) and Stricker (1966). These studies have demonstrated that an acute decrease in plasma volume is an effective stimulus for drinking in the rat.

In further work, Stricker (1968) has established that the drinking response reflects a true motivational state of need rather than reflexive drinking, by demonstrating that hypovolemic rats will

learn to press a bar in order to obtain a water reward. The regulatory nature of the response is also shown by the observations that a decrease in plasma volume as small as 4% is capable of eliciting drinking, and that the volume of water ingested by hypovolemic rats is directly proportional to their plasma loss (Stricker, 1968). Stricker and Wolf (1966) have further observed the development of an appetite for normally aversive sodium chloride solutions following hypovolemia, but since this response does not occur until 6-9 hr following induction of hypovolemia, while water intake begins within two hrs (Stricker and Jalowiec, 1970), the two responses are probably mediated through separate mechanisms.

The receptors responsible for drinking in response to hypovolemia are unknown. Stricker and Wolf (1969) have speculated that they may be the same as those thought to initiate physiological responses to decreases in blood volume (Gauer and Henry, 1963), and therefore may be localized within the cardiovascular system. It is less likely that the receptors are within the interstitium. Although subcutaneous injection of PG depletes the interstitium as well as the intravascular space (Stricker, 1966), drinking also occurs following treatment with formalin, which induces hypovolemia concurrently with expansion of the interstitium (Stricker, 1966). Thus interstitial dehydration is not a necessary condition for drinking during hypovolemia. The receptors are also unlikely to be found within the kidneys, since drinking induced by hypovolemia continues despite nephrectomy (Fitzsimons, 1961). The latter observation also rules out a renal hormone (see next section) as an essential intermediary in hypovolemic thirst.

Inhibition of hypovolemic thirst. Despite the regulatory nature of the response of drinking water during hypovolemia, this behaviour does little to restore the plasma deficit that initiated the response (Stricker, 1969). This result is not surprising, since ingested water is distributed among body fluids in the same proportions already present in the body. As a result, only 1/12 of the ingested water remains intravascular, while approximately 2/3 enters cells and over-hydrates them. The remainder enters the interstitium. Because a plasma deficit implies a loss of both salt and water, the need can only be reduced (without extreme over-hydration of cells) by the ingestion of salt as well as water. Thus, Stricker and Wolf (1967a) have reported that preloads of isotonic saline reduce hypovolemic thirst more effectively than equal preloads of water. But what happens when a hypovolemic rat is allowed to drink water? Does it continue to drink until water intoxication limits intake? Instead, a hypovolemic rat stops drinking before symptoms of water intoxication appear, yet before the plasma deficit is restored (Stricker, 1969). This observation indicates inhibition of hypovolemic thirst, because the drinking stops while need is still present. It appears that the critical stimulus for inhibition is cellular over-hydration and associated dilutional hyponatremia, not hypo-osmolarity, since a further experiment has demonstrated inhibition of drinking in the presence of hyperosmolarity and hyponatremia (Stricker, 1971).

Central control of hypovolemic thirst. How the brain controls water intake in response to hypovolemia is poorly understood. It is

known that after destruction of the lateral hypothalamus (at the level of the ventromedial nucleus) rats will not drink water during hypovolemia induced by subcutaneous PG (Stricker and Wolf, 1967b). This result suggests the participation of the lateral hypothalamic area in the regulation of drinking during hypovolemia. The contribution of other brain areas is less clear.[1]

According to Blass (1968) the "frontal pole" region is not essential for drinking to occur during hypovolemia, although it is necessary for drinking caused by cellular dehydration. However, the conclusion regarding intake during hypovolemia may be unwarranted: although the lesioned rats of this experiment drank the same volume of water as sham-operated rats following injection, no evidence was presented that they drank more after injection than after a control test. In a further study, Blass (1969) reported that lesions of the lateral preoptic area in rats did not disrupt drinking following hypovolemia but did impair drinking in response to cellular dehydration. In this case, the small intakes reported during "hypovolemic" stimulation (approximately 2 ml) suggest that the intakes would have been the same without the injection of PG.

1 In each of the studies to be cited, the evidence must be interpreted cautiously because the experimenters assumed hypovolemia to occur as a result of the intraperitoneal injection of PG without measuring plasma loss. In one experiment (Blass and Hanson, 1970) plasma loss was verified not for the group of primary interest but for an additional group which received a larger dose of PG than the first group. Because, as noted previously, the technique of intraperitoneal injection does not produce a reliable decrease in plasma volume, it is not clear that any of these studies were successful in producing a sustained hypovolemia throughout the test periods.

With regard to inhibitory control of hypovolemic thirst, Blass and Hanson (1970) reported that lesions of the septal area in rats caused over-drinking following intraperitoneal injection of PG but not of hypertonic saline. However, their results are confounded by the finding that the lesioned rats had a higher baseline intake of water than control rats, and thus would have drunk more water whether or not stimulated by hypovolemia. Since values of plasma sodium concentration reported for two further groups (one injected with PG and the other maintained ad libitum) did not differ, it is likely that both groups drank to the same level of body fluid dilution; that is, both groups exhibited comparable inhibition of drinking. Therefore it is not surprising that Tegart (cited by Wishart and Mogenson, 1971) failed to confirm their finding that rats with septal lesions overdrink during hypovolemia.

Finally, Rolls (1970) has reported that lesions of the median eminence causing diabetes insipidus do not remove inhibitory control of drinking during hypovolemia, because both lesioned and control groups were found to have similar values of intake minus urine volumes. Unfortunately, plasma sodium concentration was not measured either before or after testing. As a result, the final state of body fluid hydration, a more exact criterion of inhibitory control, remains unknown for each group.

Hormonal Control of Drinking

A series of experiments by Fitzsimons (1969) has provided convincing evidence that drinking may be elicited in the rat by a substance released from the kidney in response to procedures causing a fall in renal arterial blood pressure. He has concluded that the substance is probably the renal hormone, renin. This hormone is known to mediate the synthesis of another hormone, angiotensin (Pitts, 1968). When injected intravenously, both renin and angiotensin are able to elicit drinking in the rat (Fitzsimons and Simons, 1969; Fitzsimons, 1969). Moreover, the injection of the beta-adrenergic drug, isoproterenol, which decreases blood pressure (Lehr, Mallow and Krukowski, 1967), induces drinking only when the kidneys are present and therefore must act by releasing renin (Houpt and Epstein, 1969). Thus it appears that activation of the renin-angiotensin system is a reliable stimulus for drinking in the rat. Stimulation of this system may cause drinking through a direct effect on the brain, because the injection of angiotensin intracranially causes drinking (Epstein, Fitzsimons and Rolls, 1970). The alternative hypothesis that renin and angiotensin may initiate drinking through inducing hypovolemia has been suggested but remains controversial (Fitzsimons and Simons, 1969; Haefeli and Peters, 1971). However, drinking during stimulation of the renin-angiotensin system is subject to inhibition by cellular over-hydration in the same manner as drinking during hypovolemia (Stricker, 1971).

CHAPTER II

EXPERIMENT I

Introduction

The preceding discussion (see Chapter I, Hypovolemia and the Regulation of Water Balance) suggests that the only brain area clearly implicated in control of drinking during hypovolemia is the lateral hypothalamic area at the level of the ventromedial nucleus. However, the evidence presently available is insufficient to rule out the involvement of other central structures in mediation of the response. The aim of the present study will be to provide this information by carrying out a comprehensive survey of one region of the brain---the hypothalamus---for evidence of excitatory or inhibitory control of drinking during hypovolemia. As in previous studies, evidence of central control of hypovolemic thirst or its inhibition will consist of observations of changes in drinking response to hypovolemia following destruction of specific brain structures. However, unlike previous studies, the primary measure of response will not be water intake. If drinking in response to hypovolemia is a regulatory behaviour, then the volume of water ingested is only important when considered in relation to need.

Factors such as chronic dehydration, or increased renal or extra-renal loss of water may well occur following hypothalamic damage, and these factors could alter the total water intake of an animal during hypovolemia without changing its regulatory behaviour in relation to its state of body fluid hydration. For these reasons, the primary measure of "response to hypovolemia" will be the plasma sodium concentration, an index of cellular hydration (see Chapter I, Basic Concepts). Thus, interference with excitatory control of hypovolemic thirst will be indicated by a relatively high concentration of plasma sodium following access to water during hypovolemia; loss of inhibition will be indicated by a relatively low concentration of plasma sodium. Such measurements are necessary in order to demonstrate whether the lesions cause physiologically inappropriate drinking. Secondly, unlike previous studies which simply inferred the occurrence of hypovolemia, plasma volume (and therefore need state) will be directly assessed by measuring plasma protein concentration.

The hypothalamus has been chosen for study both because it is a region of primary importance in homeostasis of body fluids (Danowski, 1962), and because a specific hypothesis has been proposed suggesting that hypothalamic lesions may cause removal of inhibition of drinking during hypovolemia (Stricker, 1969). According to this hypothesis, the neural structures responsible for inhibition of drinking during hypovolemia may be localized either within the supraoptic nuclei or within the region through which supraoptico-neurohypophysial fibres pass (the median eminence) before terminating in the pituitary gland. This system

has been identified as the neurosecretory pathway for synthesis and storage of antidiuretic hormone, and its destruction results in diabetes insipidus, a disorder characterized by a high rate of water turnover due to an inability to concentrate urine (e.g. Bargmann, 1960). The evidence cited by Stricker in support of his hypothesis is of two types: (i) studies which suggest that the high water exchange associated with diabetes insipidus may be a result of primary polydipsia (i.e., continued drinking despite adequate hydration) in addition to an inability to concentrate urine; and (ii) studies which suggest that supraoptic neurons may be stimulated to fire by overhydration. However, a chronic increase in water exchange is not observed following lesions of the supraoptic nuclei alone (Fisher, Ingram and Ransom, 1935; Olivecrona, 1957; Parshkov and Molotkov, 1969), probably because of the difficulty in causing complete extirpation of these elongated nuclei. Therefore, a complete test of the hypothesis must consider both the effect of lesions that result in an increased water intake, and those that damage the supraoptic nuclei.

Methods

Subjects and maintenance. The subjects were male albino rats, initially weighing between 215 and 320 g (Hormone Assay, Chicago). They were housed individually in metabolism cages with wire-mesh floors in a constantly-illuminated room, and were maintained on water (presented in bottles with drinking spouts) and Purina food pellets which were

available ad libitum except during testing.

Surgical procedure and post-operative maintenance. The rats were anesthetized with sodium pentobarbital (Nembutal: 50 mg/Kg) preceded by atropine sulphate (0.6 mg). A local anesthetic (2% Carbocaine) was infiltrated around the ears and at the incision site. Bilateral brain lesions were produced under stereotaxic control using a 90% platinum-10% iridium electrode, insulated to within 0.5 mm of the tip. An electrode of this type destroys tissue with minimal deposition of irritative particles (Donovan, 1966). Direct current of 3.0 mA was passed between the anodal electrode inserted in the brain and a cathode in the rectum, and the size of the lesions was modified by allowing the current to flow for varying lengths of time. Sham operations consisted of all steps including insertion of the electrode a few mm into the brain, without passing current. Usually 5-10 operations were carried out on a single day, and each series included at least one sham-operated rat.

The rats were allowed to recover for 10-15 days post-operatively. This recovery period left sufficient time for the permanent phase of diabetes insipidus to develop in rats with lesions of the median eminence (Crawford and Frost, 1963). During recovery, water intakes were determined daily by weighing ($\pm$ 0.5 g) the water bottles attached to the cages.

Rats which were transiently adipsic and aphagic in the post-operative period were supplied with palatable mash, chocolate chip cookies, 10% sucrose solution and, when necessary, were tube-fed until

they began to eat. They were gradually weaned from the supplementary diet and sucrose solution, and were not tested until they were drinking only pure water and eating dry chow for 5 consecutive days. With only two exceptions, all rats met this criterion within the 15-day recovery period. The exceptional rats were #541 (POL group), which required 30 days, and #590 (SO group), which required 17 days.

Experimental treatments. Following recovery, each rat was anesthetized with ether, weighed, and injected subcutaneously in the middle of the back with 5.0 ml of 30% (w/w) PG (Carbowax, compound 20-M; Union Carbide Corp.) solution in isotonic saline warmed to body temperature. This procedure results in the withdrawal of protein-free plasma, as previously described (see Chapter I, Hypovolemia and the Regulation of Water Balance). The rats were replaced in their cages without food but with water present in graduated cylinders (± 0.2 ml). Urine was collected in graduated tubes (± 0.2 ml) placed beneath funnels attached to each metabolism cage. Cumulative water intake and urine excretion was recorded hourly for 9 hr.

The rats were then deeply anesthetized with Nembutal and blood samples were withdrawn from the abdominal aortas into heparinized containers. An additional group of non-treated intact rats (n = 6), maintained ad libitum, were bled to provide normal values. The plasma content of each blood sample was separated by centrifugation and was analyzed for plasma protein concentration by refractometry and for plasma sodium and potassium concentrations by flame photometry (Instrumentation Laboratory Inc.; lithium as the internal standard). Plasma

concentrations of sodium and potassium were corrected for the volume occupied by proteins, using tables from Wolf (1966).

The lesioned rats were perfused with isotonic saline followed by 10% formalin (phosphate buffered) and their brains were removed and stored in 10% formalin. Frozen sections of the brains were cut at 80 μ and stained according to the technique of Wolf and Yen (1968).

Values of daily water intake were calculated as the average intake for each rat for the three days immediately preceding testing. Statistical analysis was carried out by analysis of variance, and by the method of Dunnett (1964) for comparison of several treatments with a control. Dunnett's test requires the use of a single summary level of significance for the set of all comparisons between treatment groups and a control group (Winer, 1962). This level was taken as 0.05, 2-tailed.

Results

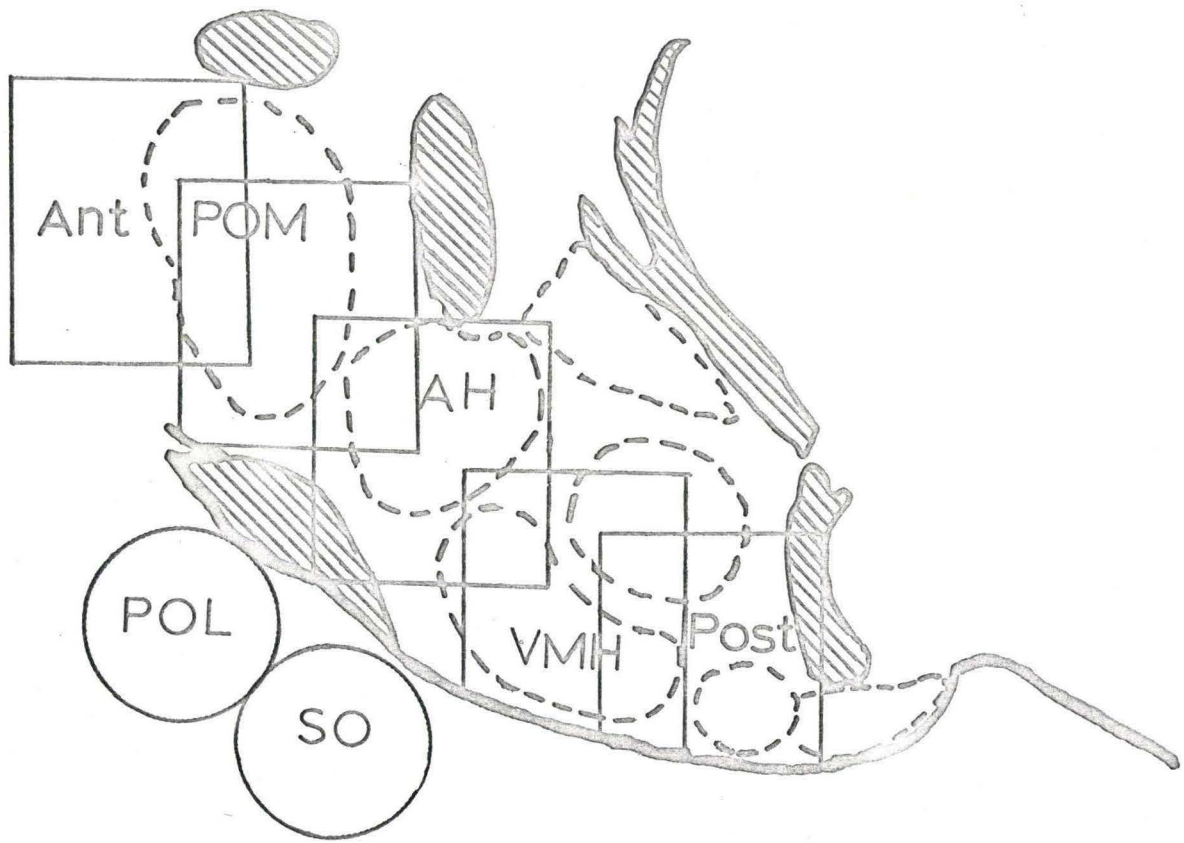
A total of 24 sham-operated and 150 lesioned rats survived the experiment. Data from 74 of the lesioned rats were discarded because they were found to have asymmetrical or poorly-placed lesions which did not correspond to the chosen categories. Data from the remaining 76 lesioned rats were placed into 8 categories, 7 categories according to lesion placement and an additional one consisting of all lesioned rats with daily water intakes (food present) greater than 50 ml/100 g of body weight (diabetes insipidus or DI group). A number of

rats within the latter category were also included in other categories.

Fig. 1 provides a schematic diagram of the hypothalamic areas that correspond to each category, and Appendix A presents photomicrographs of the actual brain damage sustained by each rat within each category. The lesion categories are: anterior (Ant; n=11); preoptic medial (POM; n=11); anterior hypothalamic (AH; n=13); ventromedial (VMH; n=7); posterior hypothalamic (Post; n=7); preoptic lateral (POL; n=12); supraoptic (SO; n=8); and the DI (n=11) category described above. These categories are self-explanatory, with the following exceptions: (a) the Post group consists of medial lesions at the posterior border of the hypothalamus; (b) the Ant group consists of medial lesions at the anterior border of the preoptic area; and (c) the lesions of the SO group, similar but somewhat posterior to those of the POL group, include considerable damage to both supraoptic nuclei. The structures destroyed in lesions assigned to each group are more fully described in Appendix A.

Mean values of water intake for 24 hr with food available are presented in Table 1. The water intakes of the VMH, DI and Post groups were each significantly higher than that of the sham-operated group; the other groups did not differ from the sham-operated group. Fig. 2 presents mean values of water intake and urine excretion for 9 hr following injection of 30% PG. In comparison with the sham-operated group, water intake was significantly higher for the Post and DI groups, and urine output was significantly higher for the VMH, Post and DI groups. The remaining groups did not differ from the sham-operated group in volumes of water ingested or urine excreted. The higher water exchange

Fig. 1. Sagittal section of hypothalamus, 1.1 mm lateral to the midline (from De Groot, 1959) with lesioned groups represented schematically. Circles indicate lesioned groups out of the plane of the diagram. Groups: Ant-anterior (n=11); POM-preoptic medial (n=11); AH-anterior hypothalamic (n=13); VMH-ventromedial (n=7); Post-posterior hypothalamic (n=7); POL-preoptic lateral (n=12); SO-supraoptic (n=8). An additional (DI) group, consisting of all lesioned rats with daily water intakes (food present) greater than 50 ml/100 g of body weight (n=11), was not anatomically homogeneous, and is not included in the diagram.


Ant
POM
AH
POL
SO
VMH
Post

of the VMH, Post and DI groups was probably a result of damage to the supraoptico-neurohypophysial tract, which presumably impaired secretion of antidiuretic hormone. Nevertheless, the hypovolemia was sufficient to produce an antidiuresis in these groups: little urine was excreted in the final few hours of the test period.

Table 2 presents mean values of plasma protein concentration from rats of each category, and from sham-operated rats, after injection of PG and access to water for 9 hr. Analysis of variance indicated that no differences existed in plasma protein concentration among the various groups ($p > 0.1$). Since plasma protein concentration from non-treated rats was found to be 5.6 ± 0.3 g/100 ml, the values reported in Table 2 are considerably above normal. This result indicates that the injection of PG was effective in causing a similar degree of hypovolemia in all groups, and that the hypovolemia persisted despite the ingestion of water for 9 hr. The decreases in plasma volume calculated from the increases in plasma protein concentrations were approximately 30% (see Appendix B for a sample calculation).

Mean values of plasma sodium concentration are presented in Table 2 for sham-operated and lesioned groups following injection of 30% PG and access to water for 9 hr.[1] The mean plasma sodium concentration for sham-operated rats was found to be 143.6 ± 0.6 mEq/L of plasma water. In comparison, the mean value for non-treated rats was 147.1 ± 0.4 mEq/L of plasma water. Therefore, the injection of PG caused sufficient drinking

[1] Individual data are presented in Appendix C.

TABLE 1

Mean Values (± SEM) of Water Intake for 24 Hr with Food Available

Group	n	Water Intake
		ml/100g body weight
Ant[1]	11	15.0 ± 1.2
POM	11	14.0 ± 1.4
AH	13	13.6 ± 1.4
POL	12	10.4 ± 0.4
SO	8	13.8 ± 1.1
VMH	7	32.0 ± 12.1 *
Post	7	56.2 ± 14.1 *
DI	11	76.2 ± 6.0 *
Sham-operated	24	10.6 ± 0.3

* $p < 0.05$ in comparison with sham-operated rats.

1 See Fig. 1 for abbreviations.

Fig. 2. Mean volumes of water ingested and urine excreted during the 9 hr period after injection of 30% PG. See Fig. 1 for abbreviations.

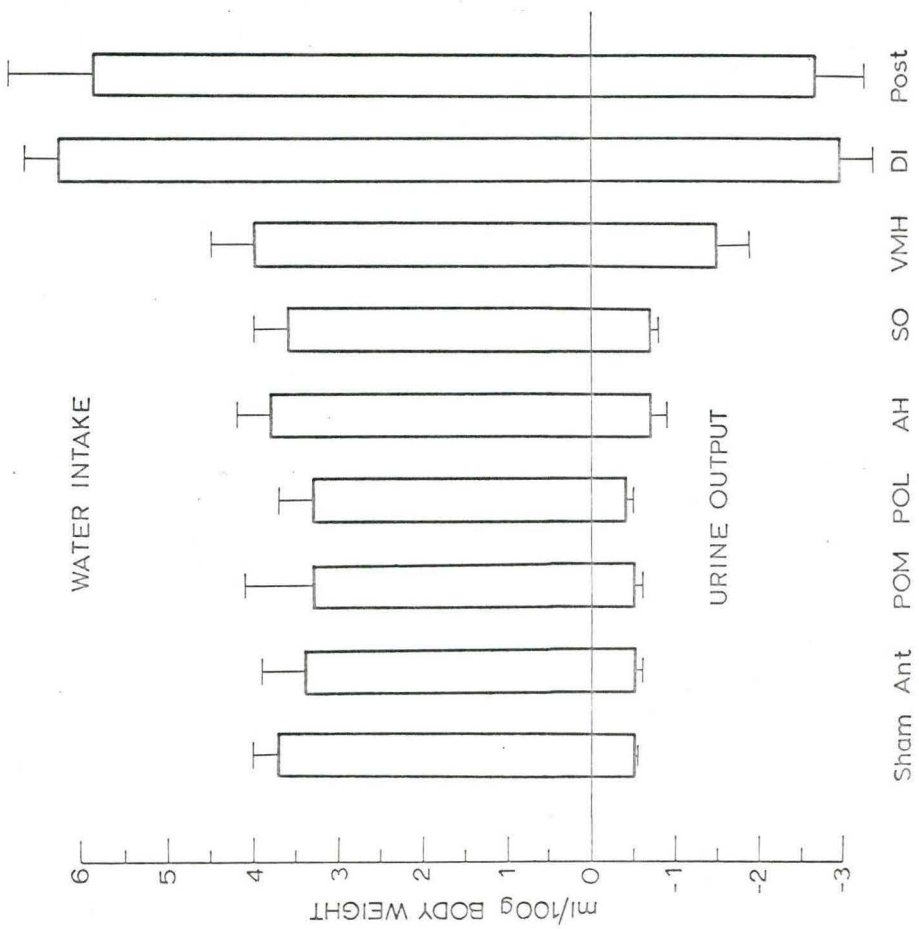

WATER INTAKE
URINE OUTPUT
ml/100g BODY WEIGHT
6
5
4
3
2
1
0
-1
-2
-3
Sham
Ant
POM
POL
AH
SO
VMH
DI
Post

TABLE 2

Mean Values (± SEM) of Plasma Protein and Sodium Concentrations After Injection of 30% PG and Access to Water for 9 Hr

Group	n	Plasma Protein g/100 ml	Plasma Sodium mEq/L plasma H_2O
Ant[1]	11	8.3 ± 0.3	146.7 ± 1.2
POM	11	8.6 ± 0.4	148.4 ± 2.1
AH	13	8.7 ± 0.3	145.3 ± 0.8
POL	12	8.2 ± 0.3	145.1 ± 0.9
SO	8	8.2 ± 0.3	143.4 ± 0.9
VMH	7	8.3 ± 0.2	142.8 ± 0.9
Post	7	8.6 ± 0.3	142.6 ± 1.2
DI	11	8.6 ± 0.2	142.9 ± 1.0
Sham-operated	24	7.9 ± 0.2	143.6 ± 0.6

1 See Fig. 1 for abbreviations.

and conservation of water to produce dilutional hyponatremia. None of the lesioned groups were found to have plasma sodium concentration significantly lower than this level of hyponatremia. However, the POM group was found to have values of plasma sodium concentration significantly higher than this level; in addition, the 3 mean values that are next highest are from groups with brain damage in areas adjacent to the medial preoptic area (Ant, AH, POL groups). These results indicate that, during hypovolemia, rats with lesions of the medial preoptic area did not drink sufficient water to reach the same level of dilution of body fluids as sham-operated rats.

Discussion

The results of this study do not provide evidence that hypothalamic lesions remove inhibitory control of drinking. In particular, they do not support the hypothesis (Stricker, 1969) that lesions destroying the supraoptic nuclei or resulting in a high water intake will cause rats to drink sufficient water during hypovolemia to excessively dilute body fluids.

This conclusion is based on two observations: (i) that all groups exhibited comparable degrees of hypovolemia despite ingestion of water during the preceding 9 hr, and therefore were in the same state of need; and (ii) no group exhibited a greater degree of over-hydration than sham-operated rats, as estimated by dilution of plasma sodium concentration. Note that had water intake alone been used as the basis

for determining the removal of inhibition, the opposite conclusion would have been reached for 2 groups (DI and Post); however, in each case an increased urinary loss balanced the increased intake.

It remains possible that an area mediating inhibitory control has been overlooked as a result of simultaneous damage to an inhibitory area and to an adjacent area concerned with excitatory control of water intake during hypovolemia. For example, bilateral removal of both the lateral hypothalamic area that mediates excitatory control of food intake and the ventromedial area controlling satiety initially causes aphagia, not hyperphagia (Teitelbaum and Epstein, 1962). This observation suggests that, in order to demonstrate removal of inhibition of hypovolemic thirst, a smaller series of lesions must be placed within the hypothalamus. On the other hand, removal of inhibition may require the destruction of larger areas of the hypothalamus than were carried out in the present study. An obvious problem with the latter approach is that it may be difficult to keep such animals alive. Unfortunately, it is impossible to demonstrate conclusively that no hypothalamic area exists with the postulated function. However, the present study has demonstrated that loss of inhibitory control does not occur given the size and distribution of lesions reported here.

Secondly, this study has reported the unexpected observation that rats with lesions of the medial preoptic area were hypernatremic relative to sham-operated rats after access to water for 9 hr during hypovolemia. This finding indicates that preoptic-lesioned rats do not drink to the same level of dilution as sham-operated rats. But

since these rats did drink similar amounts of water and excreted similar amounts of urine as sham-operated rats during hypovolemia, they may have lost water excessively through more dilute urine or greater extra-renal loss of water than sham-operated rats. If this suggestion is correct, it is surprising that, unlike DI rats, the preoptic-lesioned rats did not continue to drink until they reached the same level of hyponatremia as observed in sham-operated rats. Alternatively, the preoptic-lesioned rats may have begun the experiment in a state of hypernatremia, and thus their water intake during hypovolemia would have diluted their elevated plasma sodium concentration to the level observed in this study. In either case, these results suggest that lesions of the medial preoptic area cause abnormality of the systems controlling water intake and the regulation of body water during hypovolemia. The nature of this abnormality will be considered in Experiment II.

CHAPTER III

EXPERIMENT II

Introduction

In the preceding experiment it was found that rats with lesions of the medial preoptic area were hypernatremic relative to sham-operated rats when observed following access to water during hypovolemia. From this result it was concluded that the effect of such lesions is to impair the regulatory response of rats during hypovolemia.

The aims of the present experiment were: (i) to replicate this finding; (ii) to determine whether preoptic-lesioned rats are chronically hypernatremic or only become hypernatremic relative to control rats during hypovolemia; and (iii) to study the ability of these rats to regulate body fluids under various conditions. Since the observation of hypernatremia suggests an impaired ability to concentrate urine, preoptic-lesioned rats were compared with rats exhibiting diabetes insipidus as well as with sham-operated rats. This additional control group was used to determine the extent to which the results obtained for preoptic-lesioned rats could be attributed to a deficit in renal concentrating ability.

Methods

Rats of the same strain and from the same supplier as in Experiment I were used and were maintained as previously described. Room temperature was kept at 24°C. One group of rats received bilateral brain lesions in the medial preoptic area (n=47), while control rats received sham operations (n=12). Diabetes insipidus was produced in an additional group by lesioning the median eminence (n=12; DI group). Water intake was determined daily by weighing (± 0.5 g) the water bottles attached to the cages. Body weight was also determined daily (± 0.2 g). Rats which were adipsic and aphagic in the post-operative period were maintained as previously described until they recovered.

The lesioned rats were not tested until the criterion of 7 days post-operative recovery followed by 5 consecutive days of drinking only water had been satisfied. In addition, when occasional relapses to adipsia occurred during testing, further tests were discontinued until 5 consecutive days of drinking only water were again observed. Consequently, testing of each lesioned rat was initiated after recovery of water drinking for a fixed number of days as defined by the criterion rather than simply beginning after a fixed number of days following surgery. Initial testing of the sham-operated rats was arranged so that one usually was tested at the same time as a lesioned rat.

Fig.3 presents a flow chart of the testing procedure with the minimum time intervals between tests indicated. The surviving rats with preoptic lesions were randomly assigned to two groups, POM-test (n=13)

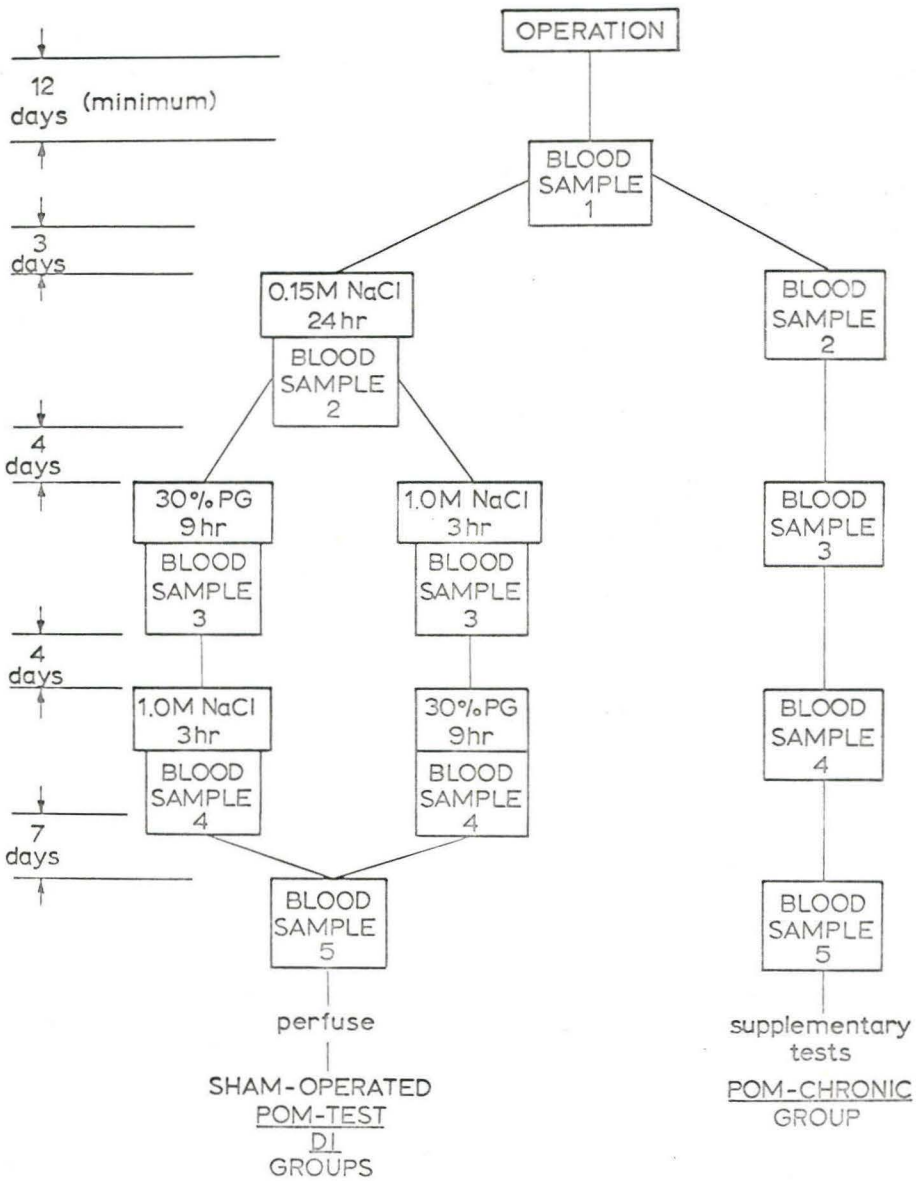
and POM-chronic (n=10). Sham-operated (n=12) and DI (n=12) groups received test injections whereas the POM-chronic group did not and was used as an untreated but lesioned control group.

At the start of each treatment period (9:00 A.M.) the rats were lightly anesthetized with ether, weighed (± 0.2 g), and injected with 5 ml/400 g body weight of the appropriate solution. Polyethylene glycol (30% w/w in 0.15 M NaCl) was injected subcutaneously but 0.15 M NaCl (used as a control injection) and 1.0 M NaCl solutions (used to induce cellular dehydration) were injected into the peritoneal cavity. Each rat was then replaced in its metabolism cage without food. Cumulative water intake was determined hourly for 9 hr after PG treatment, for 3 hr after 1.0 M NaCl treatment, and for 9 hr and at the 24th hr after 0.15 M NaCl. This final value at the 24th hr provided a measure of the daily water intake of the groups when deprived of food. Feces from the preceding 24 hr period were also collected for determination of dry fecal content (used in the calculation of extra-renal water loss).

At the conclusion of each test period the rats were anesthetized with ether and weighed, and the tips of their tails were severed for collection of blood in heparinized containers. After approximately 2 ml of blood had been collected, the tail of each rat was cauterized to prevent further bleeding and the rats were returned to their cages with food restored. In contrast, POM-chronic rats were periodically removed from their cages, anesthetized with ether, weighed, and returned to their cages following the collection of a tail blood sample.

Urine osmolalities were determined (by freezing-point depression;

Fig. 3. Flow chart of testing procedure for sham-operated, POM-test, DI, and POM-chronic groups.


OPERATION
12 days (minimum)
BLOOD SAMPLE 1
3 days
0.15M NaCl 24hr
BLOOD SAMPLE 2
BLOOD SAMPLE 2
4 days
30% PG 9hr
BLOOD SAMPLE 3
1.0M NaCl 3hr
BLOOD SAMPLE 3
BLOOD SAMPLE 3
4 days
1.0M NaCl 3hr
BLOOD SAMPLE 4
30% PG 9hr
BLOOD SAMPLE 4
BLOOD SAMPLE 4
7 days
BLOOD SAMPLE 5
BLOOD SAMPLE 5
perfuse
supplementary tests
SHAM-OPERATED
POM-TEST
DI
GROUPS
POM-CHRONIC
GROUP

Advanced Instrument osmometer) within 6 hr of collection to avoid spurious values due to bacterial decomposition of the samples. Values above 2000 mOsm/Kg, for which the osmometer was not calibrated, were assigned a value of 2000 mOsm/Kg. The samples were then frozen for later determination of urine sodium and potassium concentrations by flame photometry. Blood analysis and histological analysis of the lesioned brains were carried out as described in Experiment I. Fecal water loss was determined by drying the feces in an oven at 100°C for 48 hr. Extra-renal loss of water during food deprivation was calculated as: initial weight - (final weight + dry feces) + (intake - urine). An index of prandial drinking was obtained by the ratio: water intake during 24 hr with food present divided by water intake during next 24 hr with food absent (Teitelbaum, Cheng and Rozin, 1969).

The data have been analyzed and reported only for rats surviving the entire series of tests administered to each group. Statistical analysis was carried out by analysis of variance, and individual comparisons by t-test were made only when the overall analysis of variance indicated significance. Standard errors (SEM) are included with each mean value.

Results

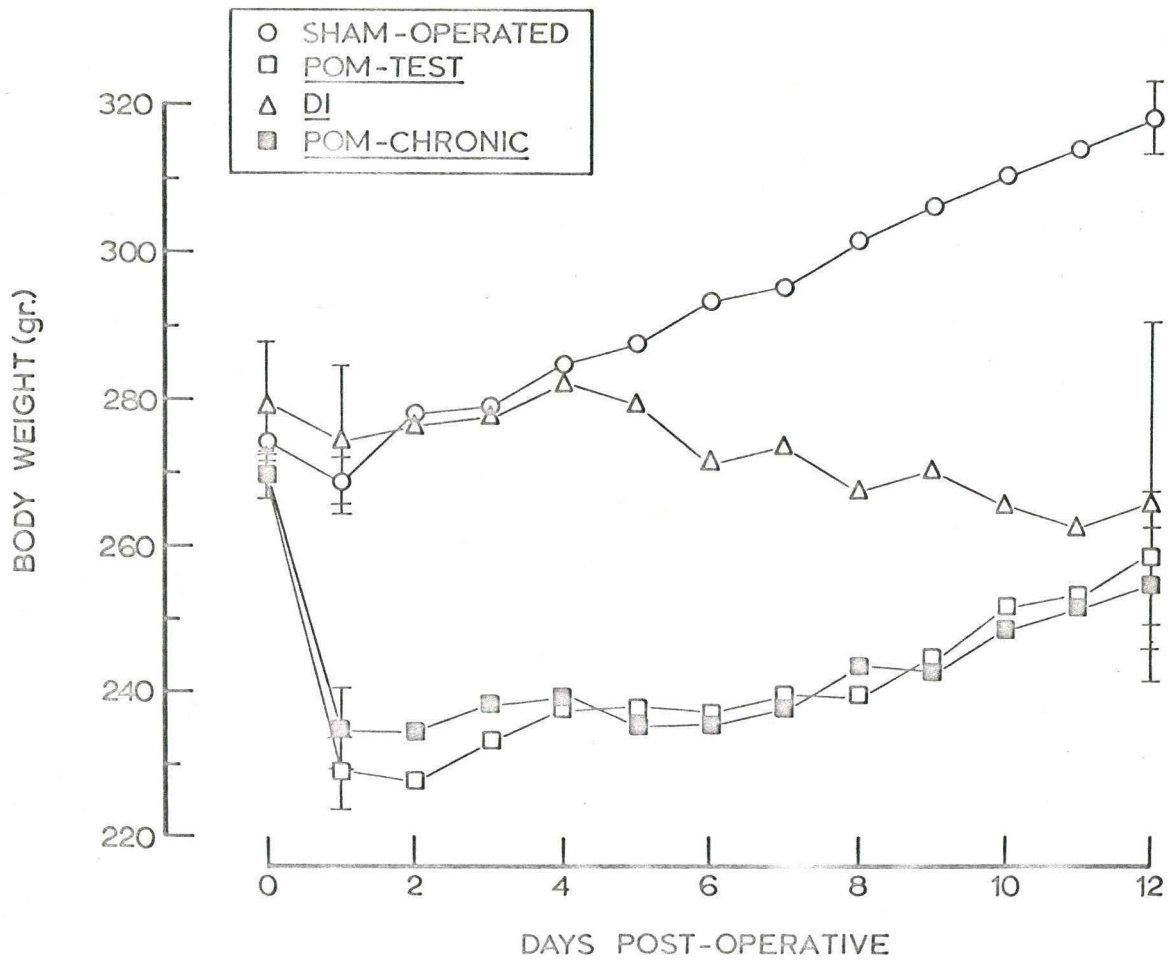
Post-operative recovery. Approximately 50% of the rats prepared with lesions of the medial preoptic area died. Since most deaths occurred in the immediate post-operative period (0-48 hr), the mortality

is likely due to the development of acute hyperthermia (Bartsch, Choinowski and Lindh, 1970) and pulmonary edema (Reynolds, 1963). A few animals died later, usually as a result of the accidental introduction of fluid into the lungs during tube-feeding.

Fig. 4 presents the means of the preoperative body weights of POM-test (n=10), POM-chronic (n=10), DI (n=5), and sham-operated groups (n=12), and their mean post-operative weights for 12 days after surgery. Unlike sham-operated and DI groups, rats with lesions of the medial preoptic area rapidly lost weight in the period immediately following surgery, a consequence of their failure to eat or drink. The weights recorded in Fig. 4 underestimate the severity of the disorder because they are based on surviving rats which were receiving highly palatable mash and sucrose solution, and in some cases were tube-fed. If the preoptic-lesioned rats had been maintained instead on water and dry chow, it is likely that they all would have starved to death. Following a period of adipsia and aphagia the rats slowly regained eating and drinking behaviours, recovering in a manner similar to that described by Teitelbaum and Epstein (1962) for rats lesioned in the lateral hypothalamus at the level of the ventromedial nucleus. The median time to recover water drinking (criterion: 5 consecutive days of drinking water without sucrose solution available) was 14 days, with an upper limit of 29 days. One rat which had not recovered by the 30th day was discarded.

The behaviour of preoptic-lesioned rats was similar to that of rats with lesions of the lateral hypothalamus. They smeared the wet

Fig. 4. Mean values of body weight for sham-operated (n=12) POM-test (n=10), POM-chronic (n=10) and DI (n=5) groups before operation and for the 12 day post-operative period.


SHAM-OPERATED
POM-TEST
DI
POM-CHRONIC
BODY WEIGHT (gr.)
320
300
280
260
240
220
0
2
4
6
8
10
12
DAYS POST-OPERATIVE

mash from the dishes onto the floor and walls of their cages and on themselves, and made no attempt at grooming: their toenails were caked with dried mash. A few lesioned rats periodically engaged in rapid circling movements while biting or attempting to bite the base of their tails, causing lasting teeth marks. After recovery of eating and drinking behaviours, the neatness of the rats improved (presumably due to the removal of wet mash from the cages and the resumption of grooming behaviour), but tail-biting continued to occur at infrequent intervals.

Treatment results.

1. Body weight. Table 3 presents the mean body weights of each group immediately before each test period. Rats with preoptic lesions maintained their body weights and therefore remained healthy, despite rigorous testing procedures which included frequent blood samples, and for POM-test rats, food deprivation, injection of hypertonic saline, and injection of PG. These treatments were apparently too stressful for the rats with diabetes insipidus, and they lost weight throughout the experiment. As a result, while all sham-operated and POM-chronic rats survived the entire series of tests, and only 3 of 13 POM-test rats died, 7 of 12 DI rats failed to complete the experiment.

2. Water intake with food available. Table 4 presents the mean intakes of water by POM-test, DI, sham-operated and POM-chronic groups for the 24 hr intervals preceding each test period. Analysis of variance of the three injected groups indicated significant interaction between groups and pre-treatment periods ($df = 8/92$; $F = 9.300$, $p < 0.001$). The mean intake of the DI group was considerably greater than the intake

TABLE 3

Mean Values (± SEM) of Body Weight (g) for POM-test, DI, Sham-operated and POM-chronic Groups Immediately Preceding Each Test Period

Group	n	Untreated (sample 1)	0.15 M NaCl	1.0 M NaCl	30% PG	Untreated (sample 5)
POM-test	10	270 ± 6	275 ± 7	279 ± 7	282 ± 7	286 ± 8
DI	5	272 ± 19	270 ± 20	245 ± 14	259 ± 20	225 ± 13
Sham-operated	12	324 ± 6	324 ± 7	322 ± 7	327 ± 7	331 ± 7
POM-chronic	10	266 ± 9	269 ± 9*	275 ± 11*	282 ± 10*	285 ± 11

* Untreated

of the POM-test group (df = 1/23; F = 27.40, $p < 0.001$) (more than 4 times as great when measured for the 24 hr period before the first blood sample) but declined so that by the final period this difference was no longer significant ($p > 0.05$). Despite consistently elevated mean values, the POM-test group did not differ significantly in intake from the sham-operated group (df = 1/23; F = 2.06, $p > 0.05$), probably as a result of the large variation in individual intakes of rats of the POM-test group. One rat of this group was clearly polydipsic (#7; range: 25.6 - 65.5 ml/100 g body weight). However, while there is some uncertainty whether or not the daily water intakes of POM-test rats can be considered elevated, there is no question that: (i) they are not hypodipsic; and (ii) with the single exception noted above, their intakes are much closer to those of sham-operated rats than to DI rats. This conclusion is also true for the POM-chronic rats.

3. Water exchange during food deprivation (0.15 M NaCl injection). Mean values of the volumes of water exchanged during food deprivation for 24 hr are presented in Table 5 for POM-test and sham-operated groups. No differences were observed between the two groups in volumes of water ingested, urine excreted, non-urinary loss of water, or in net exchange of water (all p's > 0.05). Each group was found to be in negative water balance at the 24th hr, a result of the voiding by the rats of excess fluid released from the alimentary canal during food deprivation (Morrison et al, 1967). These results indicate that the water exchange of preoptic-lesioned rats during food deprivation was apparently normal.

4. Prandial drinking. The mean ratio of water ingestion for

TABLE 4

Mean Values (± SEM) of Water Intake (ml/100 body weight) for POM-test, DI, Sham-operated and POM-chronic Groups for the 24 Hr Intervals[1] Immediately Preceding Each Test Period

Group	n	Untreated (sample 1)	0.15 μ NaCl	1.0 μ NaCl	30% PG	Untreated (sample 5)
POM-test	10	15.6±4.3	13.5±2.0	14.4±1.9	15.6±4.1	19.1±6.0
DI	5	66.6±10.2	53.0±9.0	50.1±5.0	50.5±12.2	31.2±11.4
Sham-operated	12	8.4±0.5	6.7±0.7	8.8±0.9	8.8±0.6	8.0±0.8
POM-chronic	10	14.3±2.0	14.0±2.2*	12.5±1.8*	14.8±1.8*	13.8±1.5

* Untreated

1 Food available in the cages.

TABLE 5

Mean Values (± SEM) of Water Exchange (ml/100 g body weight)
During Food Deprivation for 24 Hr
(0.15 M NaCl injection) for POM-test and Sham-operated Groups

Group	n	Water Intake	Urine Loss	Extra-renal Loss	Net Exchange
POM-test	10	8.3 ± 2.4	9.6 ± 2.3	6.9 ± 0.3	-8.2 ± 0.8
Sham-operated	12	4.9 ± 1.2	7.2 ± 1.1	6.2 ± 0.2	-8.5 ± 0.3

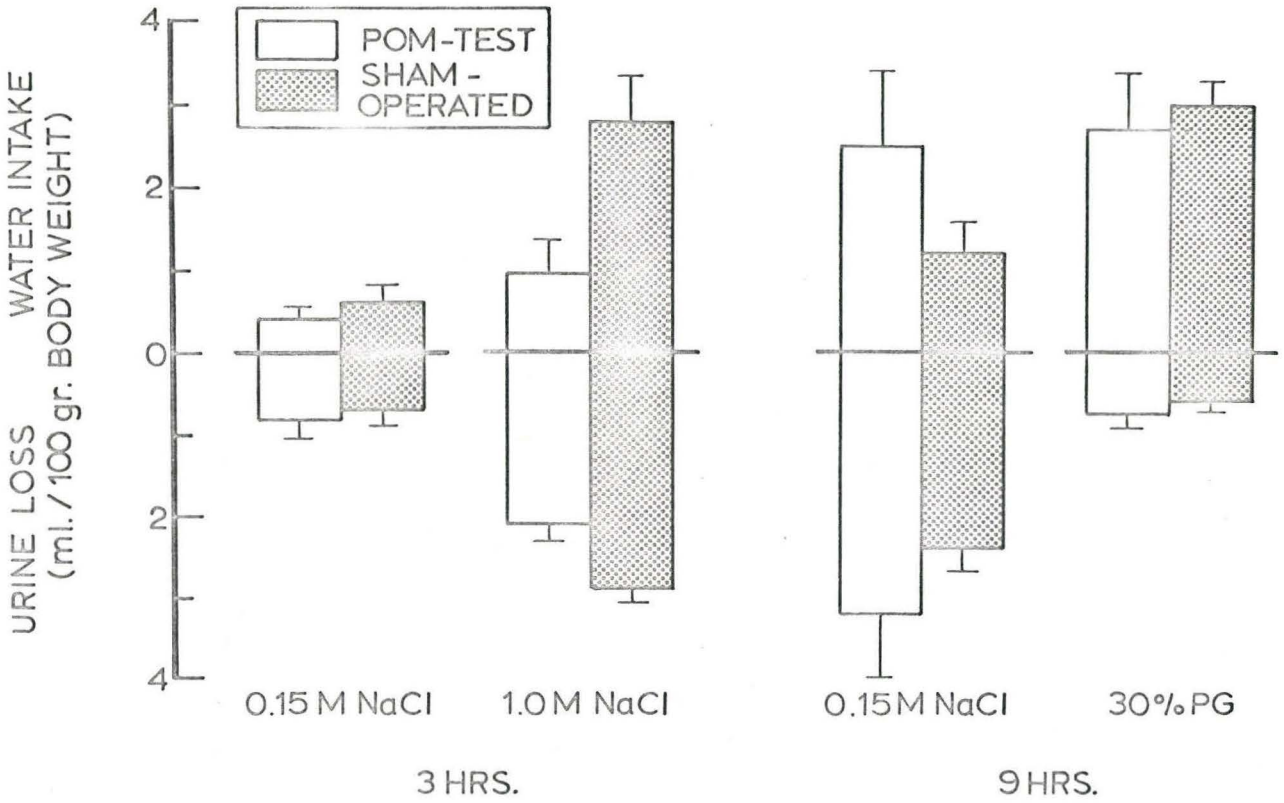
24 hr with food present compared with the next 24 hr period with food absent was found to be 3.9 ± 1.2 for POM-test rats and 3.7 ± 1.4 for sham-operated rats. This difference was not significant ($p > 0.05$). These results indicate that preoptic-lesioned rats were not prandial drinkers as defined in the study by Teitelbaum et al (1969).

5. Test water intake and urine excretion. Fig. 5 presents, for POM-test and sham-operated rats: (i) the mean volumes of water ingested and urine excreted in 3 hr after the injection of 1.0 M NaCl, together with intake and urine volumes for the same time period following the control (0.15 M NaCl) injection; and (ii) the mean volumes of water ingested and urine excreted in 9 hr after the injection of 30% PG, again with values for the corresponding control period for the same group. Two questions are of interest. First, within each group, how does the response to the experimental injection compare with the response to the control injection? Secondly, how does one group differ from the other in response to each experimental injection?

(a) Water intake. Injection of 1.0 M NaCl did not increase the water intake of POM-test rats above their intake during the previous control period ($p > 0.05$), although the injection did increase the drinking of sham-operated rats ($p < 0.01$). In across-group comparisons, POM-test rats did not drink as much as sham-operated rats to this stimulus ($p < 0.02$). Consequently, these results demonstrate an impairment of drinking in response to hypertonic saline by POM-test rats.

Similarly, POM-test rats did not drink more water following treatment with 30% PG than they did in the corresponding control test

Fig. 5. Mean values of water intake and urine loss for sham-operated and POM-test groups for the times indicated after injection of 0.15 M NaCl, 1.0 M NaCl or 30% PG.


URINE LOSS WATER INTAKE
(ml./100 gr. BODY WEIGHT)
4
2
0
2
4
POM-TEST
SHAM - OPERATED
0.15 M NaCl
1.0 M NaCl
0.15 M NaCl
30% PG
3 HRS.
9 HRS.

($p > 0.05$); in contrast, sham-operated rats did drink more ($p < 0.005$). However, when total intakes of the two groups are compared, no difference is evident between the intakes of POM-test rats and the intakes of sham-operated rats during treatment with 30% PG ($p > 0.05$). Since the within-group and across-group comparisons of the drinking response do not agree, it is not clear whether the response of the POM-test rats to this stimulus is impaired. The difficulty apparently lies with the higher (but not significantly higher: $p > 0.05$) intake of the POM-test rats compared with the sham-operated rats during the control period. As a result, the rats of the POM-test group did not have to greatly increase water intake above their usual intake in order to ingest a total volume of water equal to that of the sham-operated rats.

(b) Urine volume. Injection of 1.0 M NaCl caused both POM-test and sham-operated groups to increase urine volumes over control values (POM-test: $p < 0.01$; sham-operated: $p < 0.001$) but POM-test rats excreted less urine than sham-operated rats ($p < 0.01$). The latter result is likely due to the smaller intake of water by POM-test rats. The injection of 30% PG caused an antidiuresis: both groups decreased the total volumes of urine excreted compared with the values for the control period (p's < 0.01) and both groups excreted similar volumes of urine ($p > 0.05$). These results indicate that sham-operated and POM-test rats altered total urinary loss in similar fashion following 1.0 M NaCl and 30% PG treatments.

(c) Urine concentration. Table 6 presents various measures

of renal concentrating ability for POM-test, sham-operated and DI groups during the 3 test periods. After injection of 0.15 M NaCl, 1.0 M NaCl or 30% PG, the maximum osmolality of the urine excreted by POM-test rats did not differ from that of sham-operated rats (p's > 0.05), while the maximum osmolality of DI rats was far lower. Thus the ability of POM-test rats to conserve water renally was unimpaired under the conditions of the experimental treatments, in contrast to the clear impairment sustained by DI rats.

The maximum sodium concentration of the urine excreted by POM-test rats did not differ from that of sham-operated rats either during the control period (0.15 M NaCl) or after salt-loading by injection of 1.0 M NaCl (both p's > 0.05). Therefore the potential ability of POM-test rats to excrete sodium ions was unimpaired. However, the total amount of sodium excreted after injection of 1.0 M NaCl was significantly less for POM-test rats than for sham-operated rats ($p < 0.02$). It is likely that this result is due to the smaller volumes of urine excreted by POM-test rats in comparison with that excreted by sham-operated rats during this treatment period (which has been attributed to differences in water ingestion by the 2 groups).

POM-test rats did not differ from sham-operated rats in maximal K/Na ratio of the urine, a measure of aldosterone secretion (Johnson, 1954), after either 0.15 M NaCl, 1.0 M NaCl, or 30% PG treatments (all p's > 0.05). In addition, after 30% PG treatment (which induced sodium need) the minimum urine sodium concentrations of POM-test and sham-operated groups did not differ (both p's > 0.05). These results suggest

TABLE 6

Measures of Renal Concentrating Ability of POM-test, Sham-operated, and DI Groups (Means ± SEM) During the Test Periods After the Injection of 0.15 M NaCl, 1.0 M NaCl or 30% PG.

Treatment	Measure	POM-Test	Sham-Op	DI
0.15 M NaCl	Maximum osmolality (mOsm/Kg)	1399 ± 191	1410 ± 122	290 ± 72
	Maximum sodium (mEq/L)	163 ± 22	193 ± 17	51 ± 15
	Maximum K/Na	2.4 ± 0.5	2.3 ± 0.4	1.9 ± 0.8
1.0 M NaCl	Maximum osmolality (mOsm/Kg)	1066 ± 135	1179 ± 77	520 ± 51
	Maximum sodium (mEq/L)	324 ± 14	352 ± 27	216 ± 27
	Total sodium (mEq)	566 ± 71	874 ± 79	653 ± 123
	Maximum K/Na	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.1
30% PG	Maximum osmolality (mOsm/Kg)	1633 ± 134	1749 ± 100	509 ± 109
	Minimum sodium (mEq/L)	16.3 ± 7.3	13.3 ± 5.3	7.5 ± 5.4
	Maximum K/Na	37.7 ± 11.2	32.7 ± 8.1	11.0 ± 3.1

that the ability of POM-test rats to conserve sodium was unimpaired. Moreover, since the maximum K/Na ratios of the two groups were similar (during conditions representing minimal aldosterone secretion (1.0 M NaCl injection), control levels of aldosterone secretion (0.15 M NaCl injection), and high aldosterone secretion (35% PG injection), it can be argued that POM-test rats did not have an excessive secretion of aldosterone. Otherwise, the maximum K/Na ratios for this group probably would have been higher than the sham-operated group under at least one of the above conditions.

6. Plasma analysis.

(a) Plasma sodium concentration. Mean values of plasma sodium concentration are presented in Table 7 for POM-test, POM-chronic, DI, and sham-operated rats, for each of the 5 blood samples collected. Analysis of variance carried out on data from POM-test, DI, and sham-operated groups indicated significant interaction between groups and samples ($df = 8/96$, $F = 3.02$, $p < 0.01$). Considering across-group comparisons, plasma sodium concentrations of POM-test rats were strikingly higher than those of sham-operated rats for every sample ($df = 1/24$, $F = 31.75$, $p < 0.001$). For sample 1 (no treatment), for example, the difference between the means was greater than 10 mEq/L. Moreover, one rat (#75) lesioned in the medial preoptic area (and with water drinking completely recovered) had plasma sodium concentrations of 191 mEq/L of plasma water for sample 1 and 182 mEq/L of plasma water after the injection of 0.15 M NaCl. It subsequently died, and its data is not included in the mean for the group (see Appendix D).

In contrast to the obvious hypernatremia exhibited by POM-test

rats, DI rats were able to maintain values of plasma sodium concentration comparable to those of sham-operated rats ($df = 1/24$, $F = 1.01$, $p > 0.05$). Moreover, plasma sodium concentrations of DI rats were significantly lower than those of POM-test rats ($df = 1/24$, $F = 11.75$, $p < 0.005$). For sample 1, the difference between the means was greater than 8 mEq/L.

With regard to within-group changes in plasma sodium concentration from sample 1 to sample 5, the mean change in plasma sodium for POM-chronic rats was not significant ($p > 0.05$), indicating a lack of systematic change in level of hypernatremia over a time interval of 18-26 days. However, the mean change in these values for sham-operated and POM-test groups was significant: plasma sodium concentrations declined in both groups (sham-operated: $p < 0.02$; POM-test: $p < 0.05$). The hypothesis that POM-test rats showed a decline in plasma sodium values from sample 1 to sample 5 relative to POM-chronic rats was tested by computing the change in plasma sodium concentrations over this time period for each rat of the POM-chronic and POM-test groups. These difference scores were then compared statistically, and a significant difference was found between the two groups ($p < 0.05$). Since the only variable on which these two groups differed was in treatments, it appears that some aspect of experience with the experimental treatments caused the level of hypernatremia to decline in POM-test rats. The alternative suggestion that the result is due to the deaths of rats with high plasma sodium concentrations is unlikely for two reasons. First, only one of the three rats from the POM-test

TABLE 7

Mean Values (± SEM) of Plasma Sodium Concentration (mEq/L plasma H_2O) for Sham-operated, POM-test, POM-chronic, and DI groups Immediately Following Each Test Period

Group	n	Untreated (sample 1)	0.15 M NaCl	1.0 M NaCl	30% PG	Untreated (sample 5)
POM-test	10	157.6±2.4	152.6±1.2	159.3±2.3	150.8±1.7	151.0±1.6
DI	5	149.1±0.8	147.1±0.5	153.4±1.9	144.4±1.5	147.9±1.2
Sham-operated	12	147.0±0.4	148.5±0.5	149.0±0.9	143.3±0.5	145.6±0.3
POM-chronic	10	158.1±1.9	159.1±2.6*	159.9±3.3*	157.3±2.2*	157.7±3.0

* Untreated

group that died had a plasma sodium concentration above the mean for sample 1; second, the two groups did not differ in plasma sodium concentrations for sample 1 ($p > 0.05$). These data may be inspected in Appendix D.

The lack of a systematic trend in the plasma sodium values of POM-chronic rats suggests that the 5 blood samples may be used to compute a mean level of hypernatremia for each rat. Deviations from this set-point value of plasma sodium concentration provide an estimate of the precision with which each rat regulates its level of hypernatremia. The maximum deviation was found to be 4%, indicating a moderate degree of stability of the level of hypernatremia. A comparison of this type for POM-test rats would also be of interest, particularly since plasma sodium concentration was experimentally altered in this group. However, the decline in plasma sodium concentration between sample 1 and sample 5 invalidates such a comparison.

(b) Other plasma measures. Mean values of plasma potassium concentration of POM-test and sham-operated rats did not differ on sample 1 (POM-test: $\bar{x} = 5.6 \pm 0.3$ mEq/L plasma water; sham-operated: $\bar{x} = 5.1 \pm 0.2$ mEq/L plasma water; $p > 0.05$). Difficulty was experienced in obtaining blood on later trials, and hemolysis was occasionally observed. Since this caused contamination of the plasma samples by intracellular potassium, values of plasma potassium on further trials are not reported.

Mean values of plasma protein concentration are reported in Table 8 for all 5 trials for POM-test, POM-chronic, DI, and sham-

operated rats. Analysis of variance carried out on data from sham-operated and POM-test groups indicated no interaction ($df = 4/80$, $F = 2.38$, $p > 0.05$) and no differences between groups ($df = 1/20$, $F = 1.91$, $p > 0.05$). Differences across trials were significant ($df = 4/80$, $F = 115.91$, $p < 0.001$). The decrease following NaCl injection is due to expansion of the extracellular space as a result of the shift of water out of cells while the increase following injection of PG is attributable to the decrease in blood volume as fluid is withdrawn from the vascular system into a localized edema at the injection site. Both results suggest that the injections were effective in altering body fluids in the required direction to produce cellular dehydration or hypovolemia, respectively.

Supplementary Tests

After completion of the tests indicated in the flow chart, a series of supplementary tests were given to the POM-chronic group (n=10) and a group of intact rats (n=10). Statistical significance was determined by t-tests unless otherwise indicated.

Body temperature. This test was carried out to determine whether preoptic-lesioned rats were chronically hyperthermic. One month after blood sample 5 was obtained, body temperatures of POM-chronic and intact rats were measured by inserting a thermistor probe (Yellow Springs Instruments) 5 cm into the rectum. POM-chronic rats were found not to differ from intact rats in rectal body temperature (POM-chronic: $\bar{x} = 38.6^{\circ}C \pm 0.2^{\circ}C$; intact: $\bar{x} = 38.4^{\circ}C \pm 0.1^{\circ}C$; $p > 0.05$).

TABLE 8

Mean Values (± SEM) of Plasma Protein Concentration (g/100 ml) for Sham-operated, POM-test, POM-chronic, and DI Groups Immediately Following Each Test Period

Group	n	Untreated (sample 1)	0.15 μ NaCl	1.0 μ NaCl	30% PG	Untreated (sample 5)
POM-test	10	6.3 ± 0.1	6.1 ± 0.1	5.5 ± 0.2	7.9 ± 0.0	6.2 ± 0.2
DI	5	6.4 ± 0.2	6.3 ± 0.2	5.3 ± 0.1	8.1 ± 0.2	5.4 ± 0.2
Sham-operated	12	5.9 ± 0.1	6.2 ± 0.1	5.6 ± 0.1	7.6 ± 0.1	5.7 ± 0.1
POM-chronic	10	5.9 ± 0.1	6.1 ± 0.1*	6.2 ± 0.1*	6.0 ± 0.1*	6.3 ± 0.1

* Untreated

Thus POM-chronic rats were not hyperthermic at this point in the experiment. Note, however, that this result does not rule out the possibility of hyperthermia at an earlier time.

Blood sample 6. This test was carried out to observe whether the hypernatremia previously observed in POM-chronic rats persisted and, if so, whether it was associated with an increase in plasma osmolality. Immediately following the measurement of body temperature described above, both groups were anesthetized with ether and tail blood samples collected and analyzed as before. Osmolality of the plasma samples was determined by freezing point depression. The plasma sodium values obtained for POM-chronic rats indicated continued hypernatremia. These values did not differ from the values obtained on the first trial for this group, taken a minimum of 48 days earlier (this sample: $\bar{x} = 160.6 \pm 4.6$ mEq/L plasma water; first sample: $\bar{x} = 158.1 \pm 1.9$ mEq/L plasma water; $p > 0.05$). This result again suggests the absence of a systematic change over time of the level of hypernatremia. The maximum deviation from the mean value of plasma sodium concentration determined for each rat in the previous 5 trials was 4.3%, excluding one rat. The exceptional rat (#61) had an increased plasma sodium concentration for sample 6 of 12.4% above its mean level, to 193.8 mEq/L plasma water (see Appendix D).

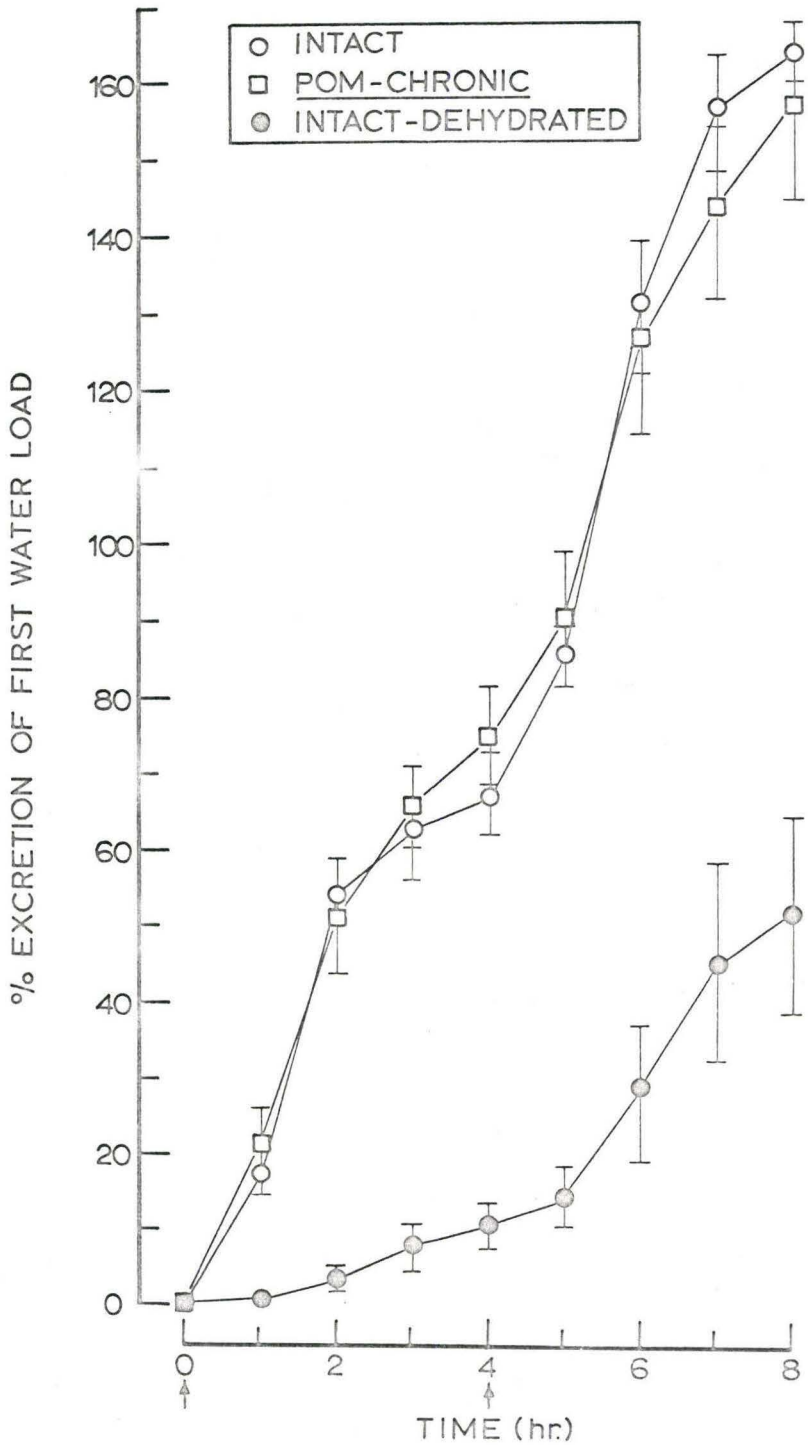
Values of plasma osmolality from POM-chronic rats were elevated in comparison with values from intact rats (POM-chronic: $\bar{x} = 325.3 \pm 8.8$ mOsm/Kg; intact: $\bar{x} = 305.0 \pm 1.0$). The Pearson product-moment correlation between values of plasma osmolality and plasma sodium con-

centration was found to be highly significant ($r = +0.97$; $p < 0.01$), with regression equation $y = 2x - 5.0$, where y = osmolality. This high correlation and the equation together suggest that the increase in osmolality can be completely accounted for by the increase in plasma sodium concentration and associated increase in plasma anion concentration.

Excretion of a water load. The purpose of this test was to determine whether the renal response of preoptic-lesioned rats to a water load resembled that of a hydrated rat or that of a dehydrated rat.

Approximately 4 days after the test described above, an intragastic load of demineralized water (5% of body weight, warmed to body temperature) was given to each rat of the POM-chronic and intact groups, and to an additional group of intact rats that had been deprived of water but not food for 24 hr (intact-dehydrated; $n = 6$). The rats were then replaced in metabolism cages without food or water. Four hours later an identical volume of water was given to each rat. Cumulative urine volumes were recorded hourly for 8 hr from the start of the test, and are presented in Fig.6. POM-chronic and intact rats excreted the same cumulative volumes of urine, whereas the dehydrated rats excreted less urine than either group. In order to estimate initial values of plasma sodium concentration of the intact-dehydrated group, blood samples were collected from an additional group of intact rats ($n=5$) deprived of water but not food for 24 hr, and not given a water load. Mean plasma sodium concentration of the dehydrated rats without water loading was 153.2 ± 0.8 mEq/L plasma water, a value considerably lower

Fig. 6. Mean cumulative volumes of urine excreted by intact (n=10) POM-chronic (n=10) or intact-dehydrated (n=6) groups after administration of 2 water loads at the times indicated by arrows. Each load was 5% of body weight. The intact-dehydrated group was deprived of water for 24 hr before testing.


INTACT
POM-CHRONIC
INTACT-DEHYDRATED
% EXCRETION OF FIRST WATER LOAD
0
20
40
60
80
100
120
140
160
0
2
4
6
8
TIME (hr.)

than that of POM-chronic rats. This result demonstrates that POM-chronic rats respond more like hydrated rats than dehydrated rats, despite a greater degree of dehydration as indicated by plasma sodium concentration.

Hypertonic saline replication. This test was carried out to replicate the important finding that preoptic-lesioned rats do not drink in response to cellular dehydration induced by hypertonic saline. A minimum of 11 days after the previous test, POM-chronic and intact groups were injected intraperitoneally with 5 ml/400 g body weight of 1.0 M NaCl solution. Unlike the initial test, the rats were not etherized before the injection. The rats were replaced in metabolism cages without food, and cumulative water intake was measured at half-hourly intervals for 4 hr, after which the rats were replaced in their cages with food restored. The results are presented in Fig.7(a) and confirm that the drinking response of preoptic-lesioned rats to hypertonic saline is impaired in comparison with that of intact rats (total intake: $p < 0.01$).

Isoproterenol test. The purpose of this test was to observe whether preoptic-lesioned rats would drink following stimulation of the renin-angiotensin system by isoproterenol (see Chapter II, Hormonal Control of Drinking). Nine days after injection of hypertonic saline, isoproterenol (0.165 mg/Kg body weight) was injected subcutaneously into POM-chronic rats (n=10) and a group of intact rats (n=12), and they were replaced in their cages without food. Cumulative water intake and excretion of urine were recorded at 15 min intervals. At the end of the second hr, each rat was deeply anesthetized with Nembutal

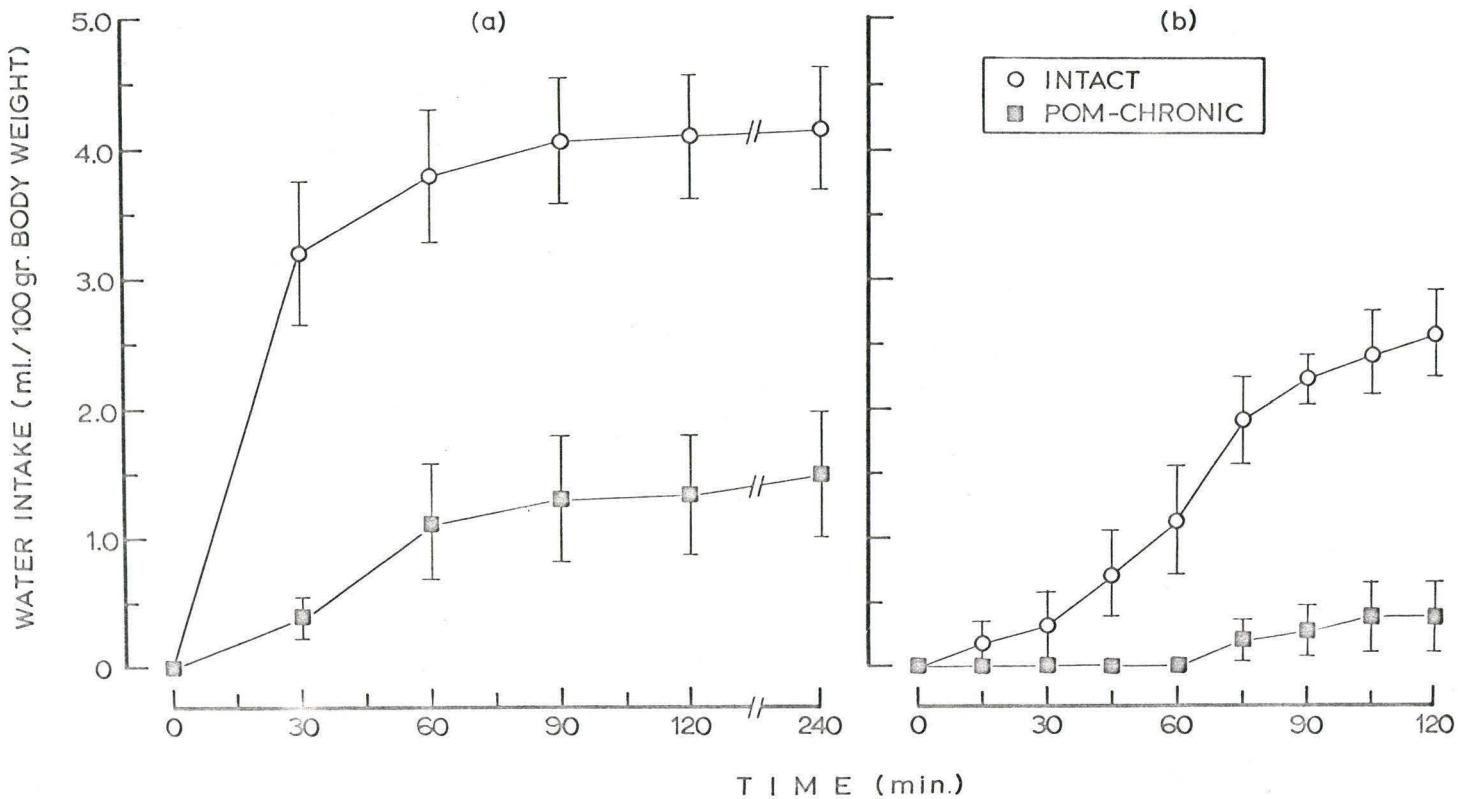
and a blood sample was withdrawn from the abdominal aorta into a heparinized container. The POM-chronic rats were then perfused with formalin, and histological analysis of their brains was carried out as described in Experiment I. Blood samples were also analyzed as previously described.

Mean values of water intake were calculated only for rats surviving the injection. One rat of the POM-chronic groups and 6 of the intact group died (this difference was not significant, $p > 0.1$; Fisher's exact test, 2-tailed (Siegel, 1956)), presumably of complications associated with acute hypotension. The results, presented in Fig.7(b), demonstrate that preoptic-lesioned rats drink significantly less water than intact rats (total intake: $p < 0.001$) after injection of isoproterenol. Moreover, plasma sodium concentration and osmolality measured from samples collected at the end of the test again confirmed that the impaired drinking was associated with concentration of body fluids (osmolality: $\bar{x} = 323.4 \pm 6.9$ mOsm/Kg vs 299.7 ± 0.7 mOsm/Kg; $p < 0.01$; plasma sodium: $\bar{x} = 155.4 \pm 3.0$ mEq/L plasma water vs 143.1 ± 1.1 mEq/L plasma water; $p < 0.01$). Therefore the drinking response of preoptic-lesioned rats to attempted stimulation of the renin-angiotensin system is also impaired.

Histological Results

For each rat of the POM-test and POM-chronic groups, 3 coronal brain sections are presented in Appendix E: through the anterior,

Fig. 7. Mean cumulative volumes of water ingested by: (a) POM-chronic (n=10) and intact (n=10) groups after the injection of 1.0 M NaCl; (b) surviving POM-chronic (n=9) and intact (n=6) rats after the injection of 0.165 mg/Kg of isoproterenol.


(a)
(b)
INTACT
POM-CHRONIC
WATER INTAKE (ml./100 gr. BODY WEIGHT)
5.0
4.0
3.0
2.0
1.0
0
0
30
60
90
120
240
0
30
60
90
120
TIME (min.)

central, and posterior parts of each lesions. The lesions were, in general, large and symmetrical about the third ventricle, and destroyed the medial preoptic area. The lesions were bounded in an anterior-posterior direction by the diagonal band of Broca and the anterior hypothalamus; ventrally by the optic chiasma and suprachiasmatic nuclei; and laterally by the lateral preoptic area. Partial damage was usually caused to each of these structures. The lesions were considerably removed from the lateral hypothalamic area (LHA) where lesions have been reported previously to cause adipsia and aphagia (Teitelbaum and Epstein, 1962).

Discussion

Lesions of the medial preoptic area in the dog and goat have been previously reported to cause disruption of drinking behaviour. In dogs, damage described as "anterior hypothalamectomy" (Witt, Keller and Batsel, 1952) caused "a complete absence of thirst" that was "temporary or permanent". A further study (Keller, Witt and Batsel, 1959) described the effective area as "prechiasmal" and reported that dogs with lesions of this area did not drink water "when salt-loaded or when drastically dehydrated" although liquid intake of food was not disrupted. Unfortunately, these important findings have been published only as abstracts, and the conclusions are not accompanied by quantitative data or comparisons with control groups. This work is supported by the observation of Andersson and Larsson (1961), who reported that a

goat in which the medial preoptic area had been destroyed by proton irradiation became "permanently adipsic" but would continue to eat hay.

However, similar observations have not been reported following medial preoptic lesions in the rat. In one study, Smith and McCann (1962) investigated the effect of medial preoptic lesions on water intake as part of a survey carried out by placing bilateral lesions at 1 mm intervals in the hypothalamus. In a later discussion of this work, McCann (1964) explicitly noted that they were unable to observe impairment of drinking following preoptic lesions. In contrast, the results of the experiment reported here demonstrate that bilateral lesions of the medial preoptic area of rats cause distinct alterations of both feeding and drinking. The syndrome was observed to consist of two phases.

Phase I. This phase began shortly after placement of the lesions. Its characteristics included severe disruption of feeding and drinking, loss of body weight, and eventual slow recovery of ingestive behaviour and weight gain. The syndrome did not appear to correspond to the "pure" adipsia without aphagia reported by Keller, Witt, and Batsel (1959) and by Andersson and Larsson (1961); instead, the rats progressed from adipsia and aphagia, to drinking of sucrose solution and eating of wet mash, and finally to complete recovery, recognized by increasing body weight of the rats when maintained on water and dry chow alone.

The recovery from adipsia and aphagia as outlined above was originally reported to occur only following lesions of the lateral hypothalamic area (LHA) (Teitelbaum and Epstein, 1962), and the LHA

was accordingly assigned a unique role in control of food and water intake. However, the results of Gold (1967) and those reported here suggest a wider distribution of anatomical sites for production of the syndrome. According to Gold, two areas in addition to the LHA, the "critical forebrain area" and the "critical midbrain area", cause adipsia and aphagia when lesioned. The present results demonstrate the medial preoptic area to be a further "critical area" anatomically distinct from the areas described by Gold and from the LHA. This finding provides support for the belief (Albert, Storlien, Woods, and Ehman, 1970) that the LHA can no longer be considered of special importance in control of food and water intake.

It is curious that these observations of adipsia and aphagia following medial preoptic lesions in the rat have not been reported previously, despite at least two studies (Han, 1962; Smith and McCann, 1962) specifically concerned with this question. The failures to observe this syndrome may be a result of the difficulty commonly experienced in preparing rats with large bilateral lesions of the medial preoptic area. Such rats frequently develop fatal pulmonary edema in the immediate post-operative period (Maire and Patton, 1956); they may also die through development of acute hyperthermia (McCann, 1964). According to Hamilton (1963): "We believe that with perfect bilateral lesions [in the anterior hypothalamus], the survival rate is so poor that these Ss do not live to be tested.....".

The successful preparation of an adequate number of preoptic-lesioned rats in the present study may be due to the use of a platinum-

iridium electrode to produce the lesions. This technique does not deposit the metallic particles (Donovan, 1966) which may be responsible for the development of pulmonary edema (Reynolds, 1963). Nevertheless, it was found necessary to prepare a large number of rats in order to obtain two groups of reasonable size, since approximately 50% of all rats operated upon died.

Phase II. The next phase of the syndrome followed recovery of feeding and drinking but was marked by residual abnormality in regulation of plasma sodium concentration: many of the lesioned rats were found to be severely and chronically hypernatremic. This hypernatremia was stable in rats maintained without testing, persisting for more than two months following placement of the lesions. In tested rats it persisted despite food deprivation, cellular dehydration, hypovolemia, and attempted stimulation of the renin-angiotensin system. Curiously, experience with these procedures appeared to cause a decrease in the level of hypernatremia, a finding which must be considered tentative until replicated. The syndrome was also marked by impairment of drinking in response to the injection of hypertonic saline and isoproterenol. Moreover, in confirmation of Experiment I, the syndrome was found to be associated with abnormal regulation of plasma sodium concentration after hypovolemia induced by 30% PG injection.

Hypernatremia following hypothalamic damage has been occasionally reported in the experimental literature. Lewy and Gassman (1935) found that the blood chlorides (and therefore the blood sodium) increased following unilateral stimulation and destruction of the "paroptic"

(preoptic?) nucleus in cats, and in some cases the chlorides remained elevated for several days. Andersson and McCann (1955) observed hypernatremia coincident with a period of adipsia in dogs following lesions of the perifornical region of the hypothalamus. In a similar study, Ganong et al (1961) reported the presence of transient hypernatremia lasting no longer than three or four weeks after lesions of the perifornical region or infundibulum. They noted that the dogs were not adipsic, but did not measure water intake or urine excretion. Finally, Stevenson, Welt and Orloff (1950) reported that rats with long-established hyperphagia caused by destruction of the ventromedial nucleus had a decreased ratio of water intake to food intake, and were mildly hypernatremic in the fed and watered state, although not when deprived of food. They concluded that the hypernatremia was due to "hypothalamic hypodipsia".

Hypernatremia has been more frequently reported in the clinical literature. In some of these cases, the cause of the disorder appeared to be dehydration of debilitated or unconscious patients as a result of excessive water loss or inappropriate administration of sodium chloride solution (Zierler, 1958). However, a number of more recent studies (Killefer and Stern, 1970; Avioli, Early, and Kashima, 1962; Weitzmann and Triedman, 1960) have reported hypernatremia in circumstances where these complications can be ruled out. The disorder in these cases was likely related to the specific presence of hypothalamic damage.

The problem of continued drinking. Hypernatremia of the degree

observed in this study implies considerable loss of intracellular water (Welt, 1970; Leaf, 1962). In intact rats, such loss is associated with a powerful stimulus to drink. In the rats of this experiment with diabetes insipidus, the tendency towards a similar loss of water was observed to cause a greatly augmented water intake, thus reducing the hypernatremia of this group to a moderate level. But hypernatremic rats with preoptic lesions did not increase their intakes in the same manner as DI rats: consequently, the chronic hypernatremia of the preoptic-lesioned rats must be related to a lasting abnormality of osmoregulatory thirst. This conclusion is supported by the observation (replicated as a "supplementary test") that preoptic-lesioned rats drink little water to the injection of hypertonic saline, which induces cellular dehydration. However, if control of thirst in response to cellular dehydration is damaged in preoptic-lesioned rats, why do they drink at all? Which stimuli continue to be effective in initiating drinking behaviour if control by cellular dehydration is absent?

Hypovolemia and stimulation of the renin-angiotensin system have been discussed previously as stimuli for drinking (see Hypovolemia and Regulation of Water Balance; Hormonal Control of Thirst). Two further suggestions are: dryness of the mouth (Epstein, 1967) or other cues associated with food intake (Fitzsimons and Le Magnen, 1969), and hyperthermia (although the role of hyperthermia as a specific control of thirst is still controversial; Hainsworth et al, 1968; Lund et al, 1969). Each of these possibilities will be discussed in turn.

The results are ambiguous with respect to whether hypovolemia

is an effective stimulus for drinking in preoptic-lesioned rats. Although these rats did not increase their intake during hypovolemia over that ingested during a control period, they did drink the same amount of water as sham-operated rats. And, while their elevated plasma sodium concentration following drinking during hypovolemia indicates an abnormality of some type, this result may be more related to dysfunction of the osmoregulatory system than of the system for regulation of volume. Thus, no conclusion can be drawn concerning the role of hypovolemia in the mediation of the continued drinking observed in preoptic-lesioned rats.

It is unlikely that the renin-angiotensin system is an important stimulus for the continued drinking of preoptic-lesioned rats, because the drinking response of these rats to isoproterenol (which acts by releasing renin; see Hormonal Control of Thirst) was found to be clearly impaired. It is not known whether this result is due to an inability of preoptic-lesioned rats to secrete renin, or whether it is due to interference with central control of drinking in response to this stimulus.

The contribution of factors associated with the ingestion of food, such as dryness of the mouth, to the continued drinking of preoptic-lesioned rats cannot be evaluated in this experiment. If the water intake during food deprivation or the "prandial ratio" of these rats had differed in the appropriate direction from that of sham-operated rats, it would have been possible to conclude that food-associated cues were an important control of drinking in preoptic-lesioned rats, as has been concluded for rats with LHA lesions (Teitelbaum and Epstein,

1962). But the failure to observe impairment of drinking does not imply the converse conclusion. If food associated cues were the only factors maintaining water intake in preoptic-lesioned rats, food deprivation would not necessarily result in impairment of drinking, because this procedure reduces but does not eliminate such cues. For example, dryness of the mouth may occur during food deprivation, and factors related to food intake may continue to operate for some time after removal of food. Secondly, a reduction in food-related cues by food deprivation may allow other controls of drinking to become relatively more prominent, and it may be these unspecified stimuli which maintain drinking during food deprivation in both preoptic-lesioned and sham-operated rats. But this possibility does not rule out food-associated cues as effective stimuli for thirst when food is present in the cage.

Finally, the results do not suggest that the drinking observed in preoptic-lesioned rats was a result of a chronic increase in body temperature, despite the fact that hyperthermia has been reported to follow preoptic lesions in rats (Bartsch, Choinowski and Lindh, 1970). When measured approximately two months after surgery, the preoptic-lesioned rats of this study were no longer hyperthermic, although they were still hypernatremic, still did not drink when injected with hypertonic saline, and still continued to drink water daily.

In summary, this discussion does not provide a solution to the problem of why preoptic-lesioned rats continue to drink if their ingestive response to cellular dehydration is impaired. Despite the additional impairment of the renin-angiotensin system as a control of

drinking, the possibility remains that other controls of thirst are still functional in preoptic-lesioned rats, and are sufficient to induce the daily water intake observed.

Etiology of the hypernatremia. A second question concerns the etiology of the hypernatremia observed in preoptic-lesioned rats. Because these rats continue to drink and, in fact, are not hypodipsic, it might be expected that their intake would be sufficient to reduce their high plasma sodium concentration to the level of plasma sodium concentration in intact rats. Since it clearly does not, there must be an additional factor that helps to maintain the hypernatremia.

The results do not suggest that excessive loss of water contributed to the hypernatremia. Rats with preoptic lesions were found not to have an increased urine loss compared with sham-operated rats during food deprivation, induced cellular dehydration, or hypovolemia. Their maximum ability to concentrate urine during the above tests appeared unimpaired; moreover, their extra-renal loss of water during food deprivation was found to be the same as that of sham-operated rats. In contrast, rats with diabetes insipidus, which were only slightly hypernatremic, exhibited severe impairment of renal conservation of water under all of the above conditions. These striking differences between preoptic-lesioned and diabetic rats indicate that the hypernatremia of preoptic-lesioned rats is not attributable to an inability to concentrate urine.

Secondly, the lack of hyperphagia (as assessed by changes in body weight) observed in preoptic-lesioned rats suggests that the hypernatremia

of these rats, unlike the hypernatremia of the rats described by Stevenson, Welt and Orloff (1950) cannot be due to a relative lack of water intake in comparison with food intake. This conclusion is supported by the observation that preoptic-lesioned rats, again unlike hyperphagic rats, remained hypernatremic despite food deprivation for 24 hr.

Finally, the hypernatremia is unlikely to be caused by hypertension, hyperaldosteronism, or Cushing's disease. In these disorders, the elevation of the plasma sodium concentration is usually within the range of normal values, and is never observed to increase as much as in the present study (Fregly, Yates, and Landis, 1955; Mills, 1964). In addition, preoptic-lesioned rats do not exhibit the decrease in plasma protein concentration that would be expected if the extracellular fluid were expanded as a result of sodium retention; they do not have an elevated ratio of potassium to sodium concentration of the urine, which would indicate excessive secretion of aldosterone (Johnson, 1954); and they do not exhibit hypokalemia, a symptom associated clinically with hyperaldosteronism and Cushing's disease (Mills, 1964; Fanestil, 1969; Leaf, 1962).

From this discussion it can be concluded that none of the above suggestions is likely to be the additional factor required to explain the development of hypernatremia despite an apparently adequate intake of water. An alternative hypothesis is that the hypernatremia is caused by an elevation of set-point of regulation of plasma sodium concentration. According to this hypothesis, the hypernatremia may initially develop from a deficit in body water incurred during the

period of post-operative adipsia. Although drinking behaviour recovers, the deficit in water balance is never restored; instead, preoptic-lesioned rats maintain the hypernatremia by ingestive and renal responses appropriate to a new level of plasma sodium concentration. Once a steady-state is reached, these responses need be no different from that of sham-operated rats in order to achieve regulation at a new set-point. Thus, to the question: why do preoptic-lesioned rats continue to drink?--- the set-point hypothesis provides the answer that all stimuli that normally cause drinking in intact rats should also cause drinking in rats with an elevated set-point. To the question: why is the hypernatremia maintained despite an apparently adequate intake of water?--- the reply is that, although the ability of preoptic-lesioned rats to conserve water may be unimpaired as assessed by specific test stimuli, these rats may otherwise concentrate urine inappropriately in relation to their state of extreme dehydration. Evidence in support of this statement is provided by the observation that preoptic-lesioned rats excrete a water load rather than retain it and thereby reduce their hypernatremia. Their renal responses can be considered appropriate only if the set-point of regulation has been increased to a higher level.

The hypothesis of an altered set-point is also supported by the observation that the level of hypernatremia of POM-chronic rats showed only moderate variation and did not change systematically during the experimental period of observation (minimum: 48 days). This result suggests but does not prove the existence of regulation at a new set-point. For example, a rat with diabetes insipidus may conceivably

maintain slight hypernatremia as a result of an unavoidable water loss, but this constancy does not indicate a change of set-point. However, the argument for an altered set-point is somewhat stronger in the case of preoptic-lesioned rats because of the impressive degree of hypernatremia observed in these rats, and the corresponding absence of an increase in water intake.

Moreover, the hypothesis is successful when applied to the drinking response of preoptic-lesioned rats after injection of 30% PG. According to the model, the intake of rats regulating at a new set-point should be similar to that of intact rats. This was found to be true for the response of preoptic-lesioned rats drinking during hypovolemia induced by 30% PG injection. The elevation of plasma sodium concentration that persisted during this test, in contrast to the hyponatremia of the sham-operated rats, is further evidence in favour of a change in set-point. If the hypothesis were true, inhibition of drinking would be initiated at a higher level of plasma sodium concentration than in sham-operated rats.

The hypothesis is less successful when applied to the drinking response of preoptic-lesioned rats to the injection of hypertonic saline and isoproterenol, because these rats drank considerably less water under these conditions than control rats. Therefore it is likely that the effect of the preoptic lesions was to cause further abnormality than simply to alter the set-point of regulation of plasma sodium concentration. One suggestion is that the lesions have also reduced the sensitivity of the regulatory system. But if the response of preoptic-

lesioned rats to hypertonic saline is impaired for this reason, then why were they still sufficiently sensitive to changes in plasma sodium concentration (as required by the set-point hypothesis) to drink as much water daily as sham-operated rats? Perhaps, the answer is either (i) they drink in response to "physiological stimuli" which are slower in onset than hypertonic saline or isoproterenol injection; or (ii) their response to these stimuli is not absent, but delayed.

The proposal that the disorder sustained by preoptic-lesioned rats is an alteration of set-point of the regulatory system for plasma sodium concentration is best studied by observing how well these rats defend their plasma sodium concentration against experimental alterations of its level. The data on plasma sodium concentration presented in Table 7 are not incompatible with the above hypothesis, but cannot provide a clear test because the use of the same rats for repeated trials apparently caused the level of plasma sodium concentration to decline, leaving the baseline level of regulation unknown. Nevertheless, although the hypothesis is not without difficulties, at present it provides the most complete description of the experimental results.

CHAPTER IV

CONCLUDING DISCUSSION

The initial aim of the investigation reported here was to obtain evidence for the presence of inhibitory or excitatory hypothalamic areas mediating drinking behaviour during hypovolemia. The study was carried out in Experiment I by preparing groups of rats, each with lesions of major hypothalamic nuclei, and observing their drinking responses and levels of plasma sodium concentration during hypovolemia. The experiment was designed to specifically test the hypothesis of Stricker (1969) that lesions of the supraoptic nuclei or those that result in the high water turnover of diabetes insipidus may cause removal of inhibition of hypovolemic thirst. The study failed to find evidence that lesions within the hypothalamus result in removal of inhibition, since no group was found to have a mean level of plasma sodium concentration lower than that of the sham-operated group. The study did provide evidence, however, that lesions of the medial preoptic area resulted in insufficient drinking to reduce the level of plasma sodium concentration to that of the other groups, suggesting that the lesions interfered with the neural control of drinking during hypovolemia.

The conclusion that inhibitory control of drinking cannot be disrupted by lesions within the hypothalamus does not necessarily refute the proposal that the inhibitory mechanism is located in the brain,

although the proposal is weakened because of the strong expectation that an inhibitory area would be located within the hypothalamus, if anywhere. However, it suggests that such control is either localized in brain areas outside the hypothalamus, or is not organized in a manner that it is susceptible to disruption by discrete brain lesions. As reviewed in the Introduction, there is little evidence at present to suggest that extra-hypothalamic areas participate in mediation of inhibition of drinking during hypovolemia. The study of Blass and Hanson (1970), which proposed the septum as the locus of inhibitory control, is methodologically unconvincing and one study (Tegart, quoted by Wishart and Mogenson, 1971) has failed to replicate it. The view repeatedly presented here is that before such claims can be accepted it is necessary to use physiological measures to demonstrate overhydration of body fluids.

The negative findings also suggest that alternative hypotheses to specific central inhibition of hypovolemic thirst be considered. One way is to postulate a central integrating mechanism (CIM), which sums the inputs from peripheral receptors responsive either to cell size or plasma volume. The CIM initiates drinking when it reaches a threshold level of excitation from the combined input of the receptors. When cell size and plasma volume are within normal limits the CIM receives some excitation but remains below threshold. Cellular dehydration will

increase excitation and cellular over-hydration will decrease excitation of the CIM; plasma loss will increase excitation.[2] Then "inhibition" of hypovolemic thirst will occur when cellular over-hydration reduces the amount of excitation available to cause the CIM to reach threshold. It then becomes necessary to use a stronger hypovolemic stimulus to elicit drinking than when over-hydration is not occurring. A feature of this model is that lesions of the CIM will always result in decreased drinking, never over-drinking. But what would happen during hypovolemia if the receptors monitoring cell size were selectively inactivated or if their pathway to the CIM was selectively destroyed? In this case, hypovolemia alone would never result in sufficient excitation to allow the CIM to reach threshold for drinking. For if "inhibition" of hypovolemic thirst occurs when a decrease in input from receptors monitoring cell size reduces excitation of the CIM below threshold, then the total removal of this input would be expected to profoundly depress the activity of the CIM. In other words, the model implies that selective loss of thirst due to cellular dehydration without loss of hypovolemic thirst is impossible.

Alternatively, the mechanism regulating inhibition of drinking may be due entirely to peripheral events. The continued ingestion of water may lead to an aversive internal state that discourages further ingestion of water, while saline intake will continue because it is motivated by need reduction and by the palatability of the fluid. But

2 But it is unnecessary to postulate that plasma expansion decreases excitation. Fitzsimons (1961b) has shown that this alteration of body fluids does not reduce drinking behaviour.

the hypothesis is contrary to the observation that a water preload will discourage drinking in the same manner as ingested water does (Stricker, 1969), although there is little chance for the aversive state to become associated with the response. Therefore the unlikely assumption must be made that the association between intake and the aversive state develops quite rapidly (within a few licks) in order for the hypothesis to be valid.

Experiment I also provided unexpected evidence that lesions of the medial preoptic area interfered with neural control of drinking during hypovolemia. This result was replicated and extended in Experiment II. Medial preoptic lesions were found to cause a well-defined syndrome occurring in two phases. The first phase consisted of a period of adipsia and aphagia, followed by slow recovery of drinking and eating. The second phase consisted of apparent recovery, with residual deficits in the drinking response to specific stimuli, and in regulation of plasma sodium concentration. These results have a number of implications for an understanding of central neural control of appetitive behaviour.

First, the results demonstrate that "severe and persistent deficits" in appetitive behaviour may occur after lesions in an area outside the LHA. In agreement with a number of other studies (Gold, 1967; Albert et al, 1970) these results argue against a unique role for the LHA in the regulation of food and water intake.

Secondly, the observation that disruption of food intake occurs following lesions in an area traditionally believed to be concerned

with temperature regulation (Myers, 1969) suggests that there may be a relationship between the two regulatory systems. The data provides support for the thermostatic theory of food intake proposed by Brobeck (1960) and, in particular, for his hypothesis that, because of the importance of the rostral hypothalamus in temperature regulation, this brain area may have an important influence on food intake.

Third, the results suggest the question: Do the medial preoptic area and the lateral hypothalamic area have distinguishable differences in function? This study has demonstrated that preoptic-lesioned rats, like rats with LHA lesions, have a well-defined period of recovery from adipsia and aphagia. Moreover, neither drink to the injection of hypertonic saline. But unlike rats with lesions of the LHA, preoptic-lesioned rats continue to drink when food is removed. Further comparisons, for which data is not available, may help define the contributions of each of these brain areas to regulation of appetitive behaviours. For example, do preoptic-lesioned rats fail to eat following insulin injection as has been determined for rats with lesions of the LHA (Epstein and Teitelbaum, 1967). Conversely, is the "altered set-point" hypothesis applicable to rats with LHA lesions? At present, it is not known whether rats with LHA lesions and recovered from adipsia and aphagia are hypernatremic, whether they respond to isoproterenol, whether their ability to concentrate urine is impaired, or whether they excrete a water load (although Dorn and Rothballer (1968) have reported that during adipsia they fail to show an antidiuresis to a water load). And although it appears that both types of

lesions cause abnormality of regulation during hypovolemia (Stricker and Wolf, 1967b), the abnormality was defined by different criteria and assessed by different tests in the two studies. Thus whether lesions of the LHA and medial preoptic area result in the same alterations of food and water regulation remains a provocative question.

Finally, the disorder observed in preoptic-lesioned rats after recovery from adipsia and aphagia may be of clinical significance. This phase of the syndrome was characterized by elevation of plasma sodium concentration, impaired response to stimuli known to cause drinking in intact rats, absence of a renal concentrating deficit, and intake and excretion of water corresponding to a state of hydration rather than to the state of dehydration indicated by the elevated plasma sodium concentration. A similar syndrome, known as "essential hypernatremia" has been described in humans (Weitzman and Triedman, 1960; Avioli et al, 1962; De Rubertis et al, 1971). Welt (1962) states with regard to this syndrome that it "....raises the exciting questions whether there exist clinical expression of ' new settings' of the osmoreceptors so that the usual operations of the systems.....might function around different mean osmolalities...." An answer to this question may be facilitated by the study of an experimental model of "essential hypernatremia" in rats with lesions of the medial preoptic area.

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APPENDIX A

Histological Analysis of the Lesioned Brains of Experiment I

This appendix provides: (i) a description of the boundaries of each lesion category, and of the neural structures destroyed within each category; and (ii) on the page following each description, a set of three photomicrographs of coronal sections through the anterior, central, and posterior part of each lesioned brain (read from left to right)[1] that had been included in the category described on the preceding page.

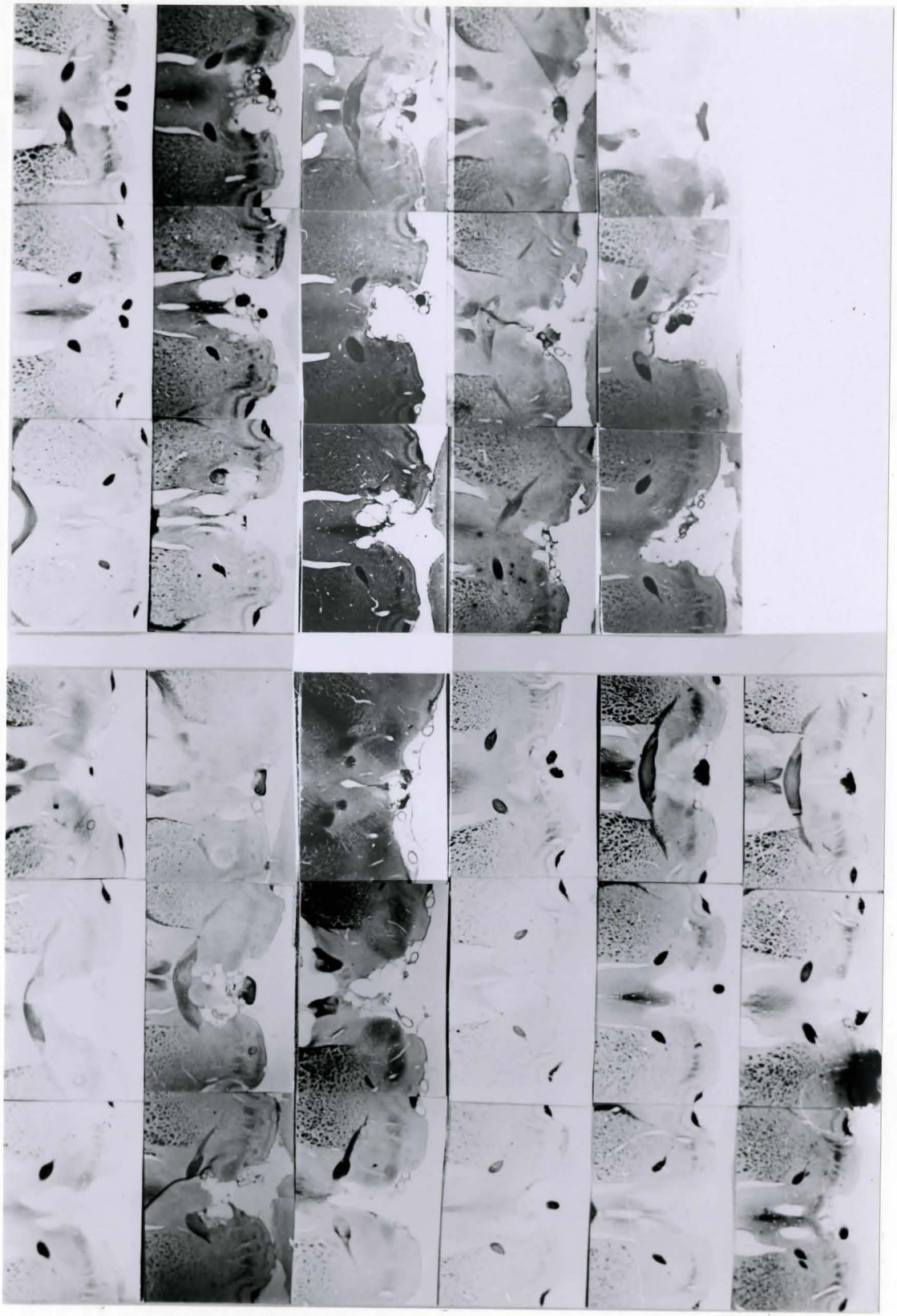
1 When viewed by turning the page 90° in a clockwise direction.

Anterior (Ant) Group (n=11)

The lesions of this group were medial, and were centred on the anterior border of the medial preoptic area. They extended in a posterior direction into the medial preoptic area as far as the suprachiasmatic nuclei. In an anterior direction, damage was caused to the diagonal band of Broca and to the medial septum. The lesions were bounded dorsally by the anterior commissure (but this structure received little damage), and ventrally by the optic nerves and optic chiasma (which also received little damage). In approximately half of the rats, a small strip of tissue was left intact, extending along the midline down from the septum. In the remaining cases, the damage extended fully across the midline.

Photomicrograph Legend

Rat No. (Left Side)	Rat No. (Right Side)
511	505
459	504
514	502
506	441
499	443
501	

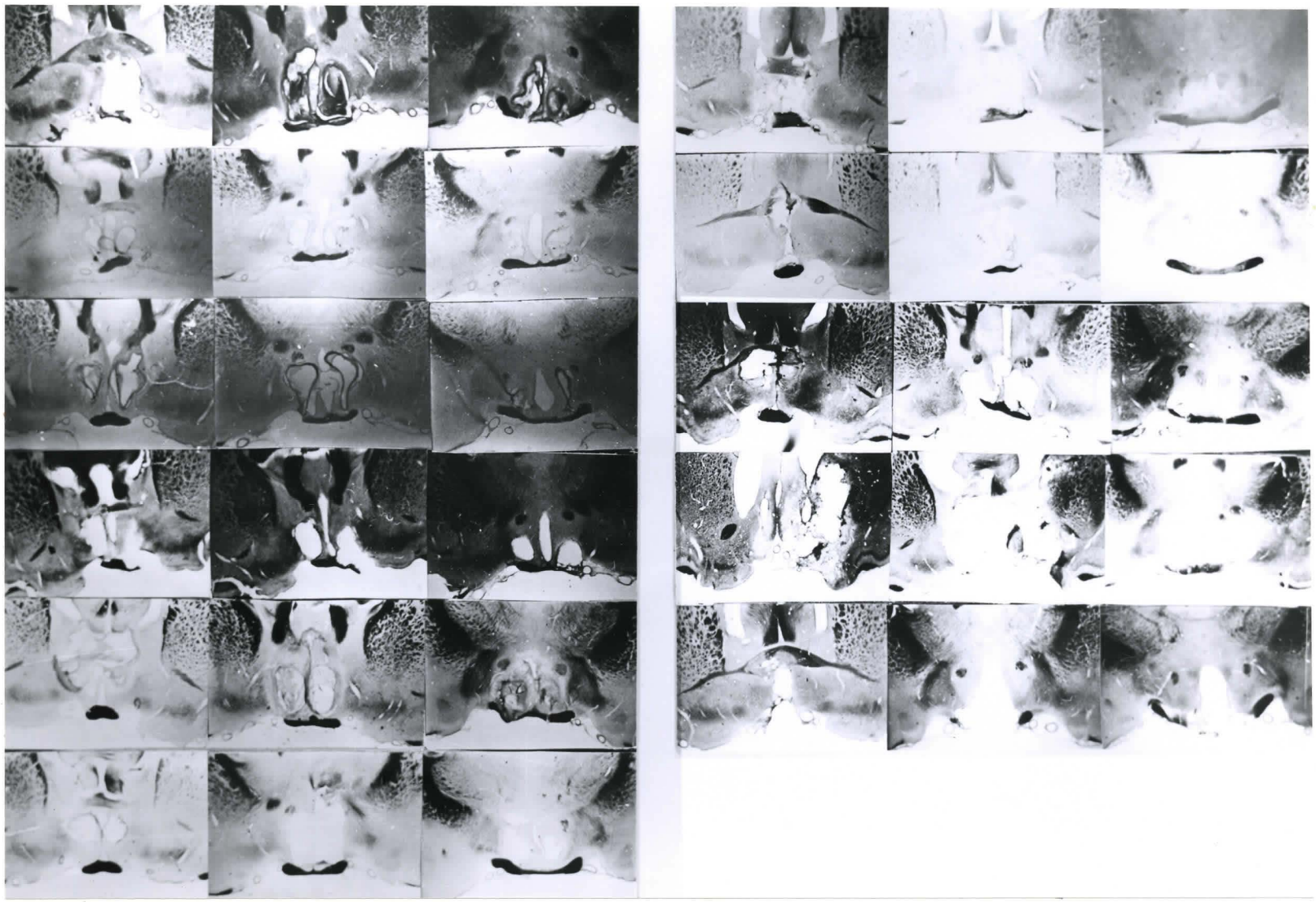


Medial Preoptic (POM) Group (n=11)

The lesions of this group were centred within the medial preoptic area at the level of the suprachiasmatic nuclei. In a few cases the damage did not fully extend across the midline and left a narrow strip of tissue along the third ventricle; in the remaining cases the damage was complete. The anterior border of the lesions overlapped slightly the posterior border of the Ant group, with the dividing line approximately in the plane of the beginning of the optic chiasma. The lesions were bounded ventrally by the optic chiasma, which usually received slight damage. The suprachiasmatic nuclei were destroyed in about half of the cases. Dorsally, the lesions extended to the plane of the anterior commissure, which was occasionally slightly damaged. In a posterior direction, the damage extended into the anterior hypothalamic area. Laterally, the lesions extended about 1½ mm from the midline.

Photomicrograph Legend

Rat No. (Left Side)	Rat No. (Right Side)
521	345
376	440
447	473
405	520
523	513
410	

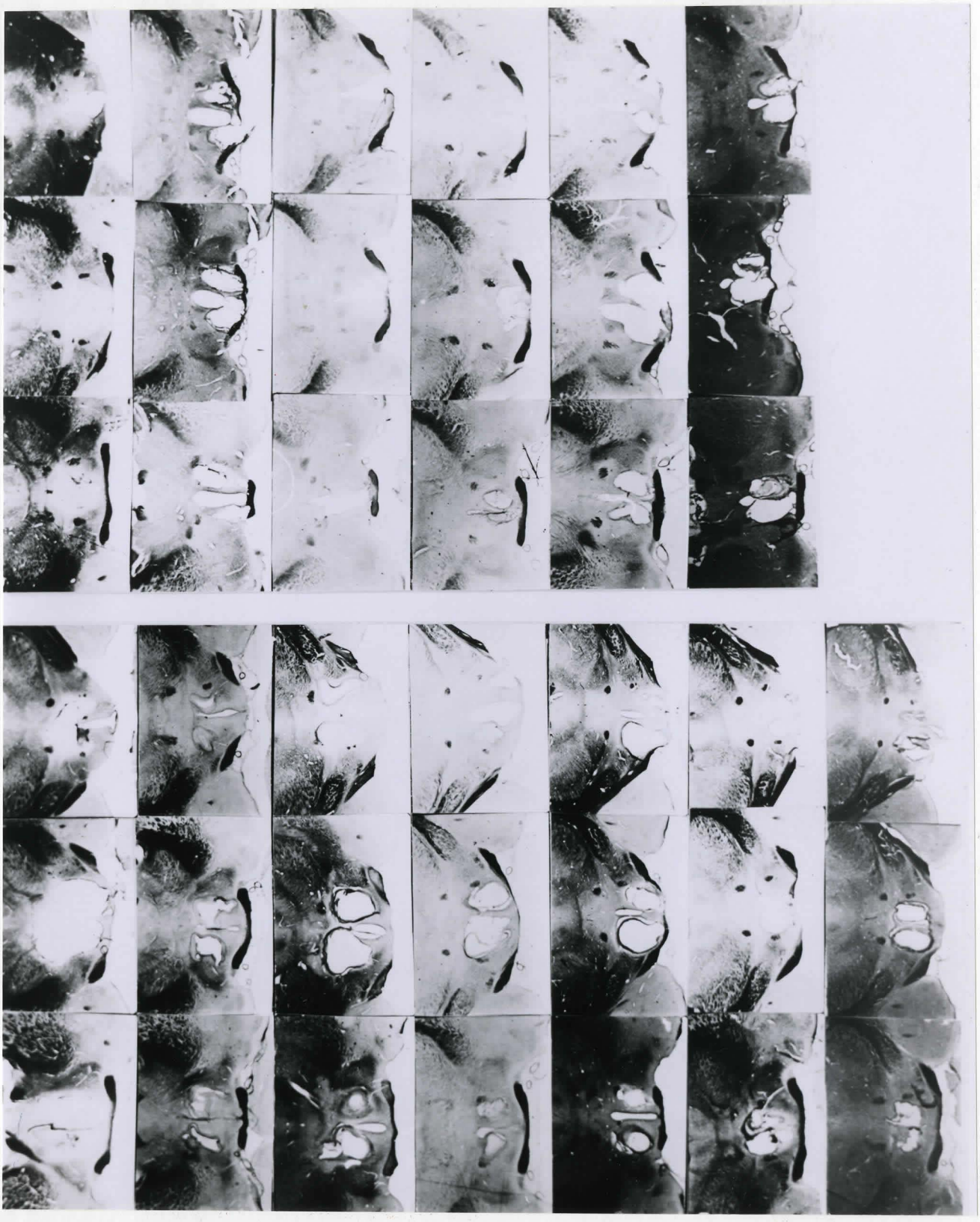


Anterior Hypothalamic (AH) Group (n=13)

The lesions of this group were medial and were centred within the anterior hypothalamic area. In an anterior direction, they extended into the medial preoptic area (the division between the preoptic and anterior hypothalamic area is not clearly defined histologically), thus overlapping the POM group; in a posterior direction, they extended until the beginning of the ventromedial nuclei, which were generally undamaged. The lesions did not extend laterally farther than the fornices. Ventrally, the lesions were bounded by the optic chiasma, which received slight damage in a few rats. In most cases, the anterior portion of the periventricular nuclei was damaged.

Photomicrograph Legend

Rat No. (Left Side)	Rat No. (Right Side)
546	556
397	462
249	460
438	449
247	435
554	478
515	

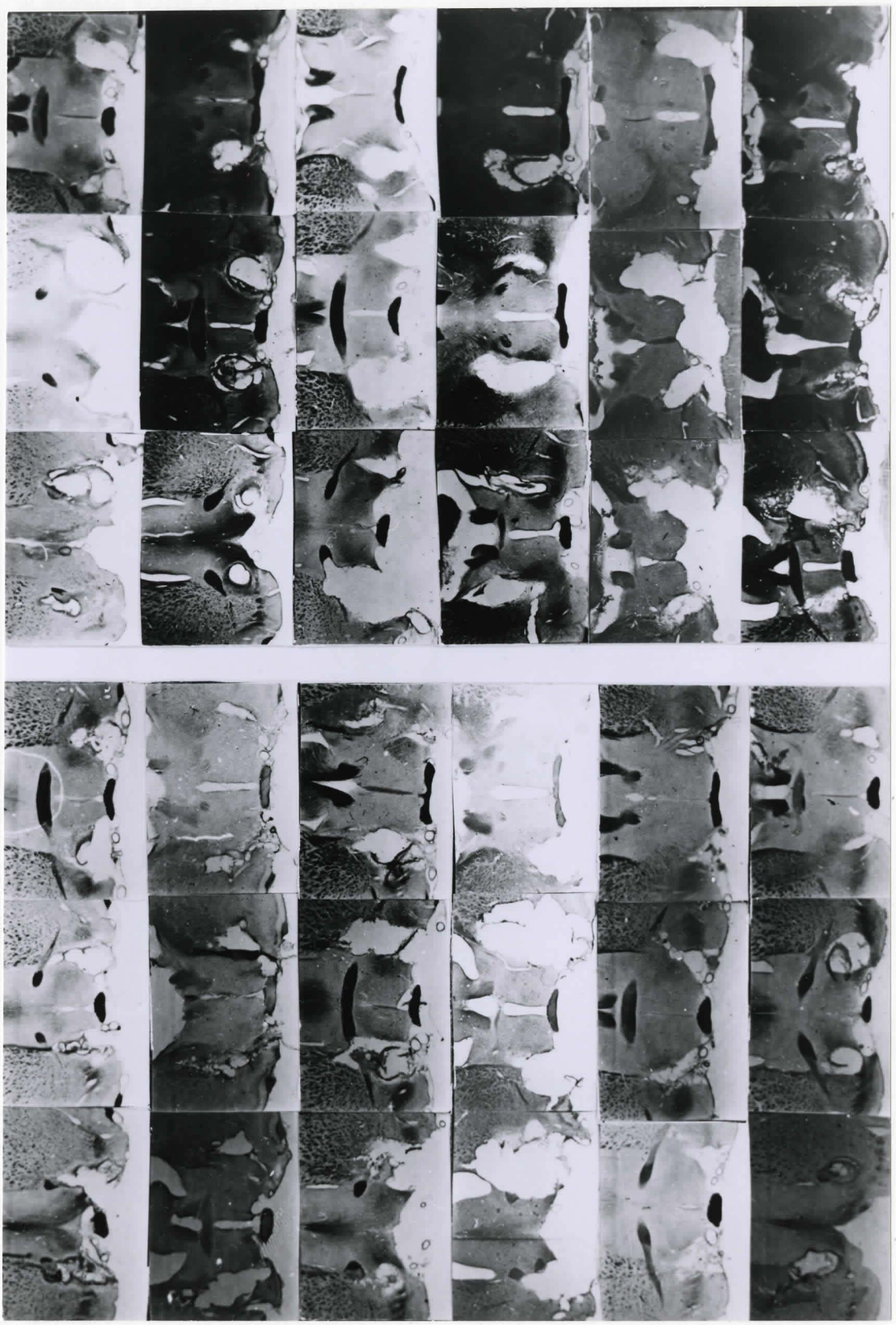


Preoptic Lateral (POL) Group (n=12)

The lesions of this group primarily damaged the lateral preoptic area. The region of destruction extended in an anterior direction until the plane of exit of the optic nerve, and included slight damage to the diagonal band of Broca. In a posterior direction the lesions extended as far as the suprachiasmatic nuclei. In a dorsal-ventral direction, damage occurred from above the anterior commissure down to the base of the brain. The medial forebrain bundle received considerable damage, while the supraoptic nucleus was occasionally damaged unilaterally.

Photomicrograph Legend

Rat No. (Left Side)	Rat No. (Right Side)
525	529
340	240
526	530
545	221
446	541
445	426



Supraoptic (SO) Group (n=8)

The lesions of this category were specifically chosen because they included extensive damage to both supraoptic nuclei. The lesions were similar but somewhat posterior in placement to those of the POL group, extending from just anterior to the start of the supraoptic nuclei to the termination of the supraoptic nuclei. Extensive damage was also caused to the lateral preoptic area, the anterior part of the lateral hypothalamic area, and the medial forebrain bundle. The optic tracts were usually slightly damaged.

Photomicrograph Legend

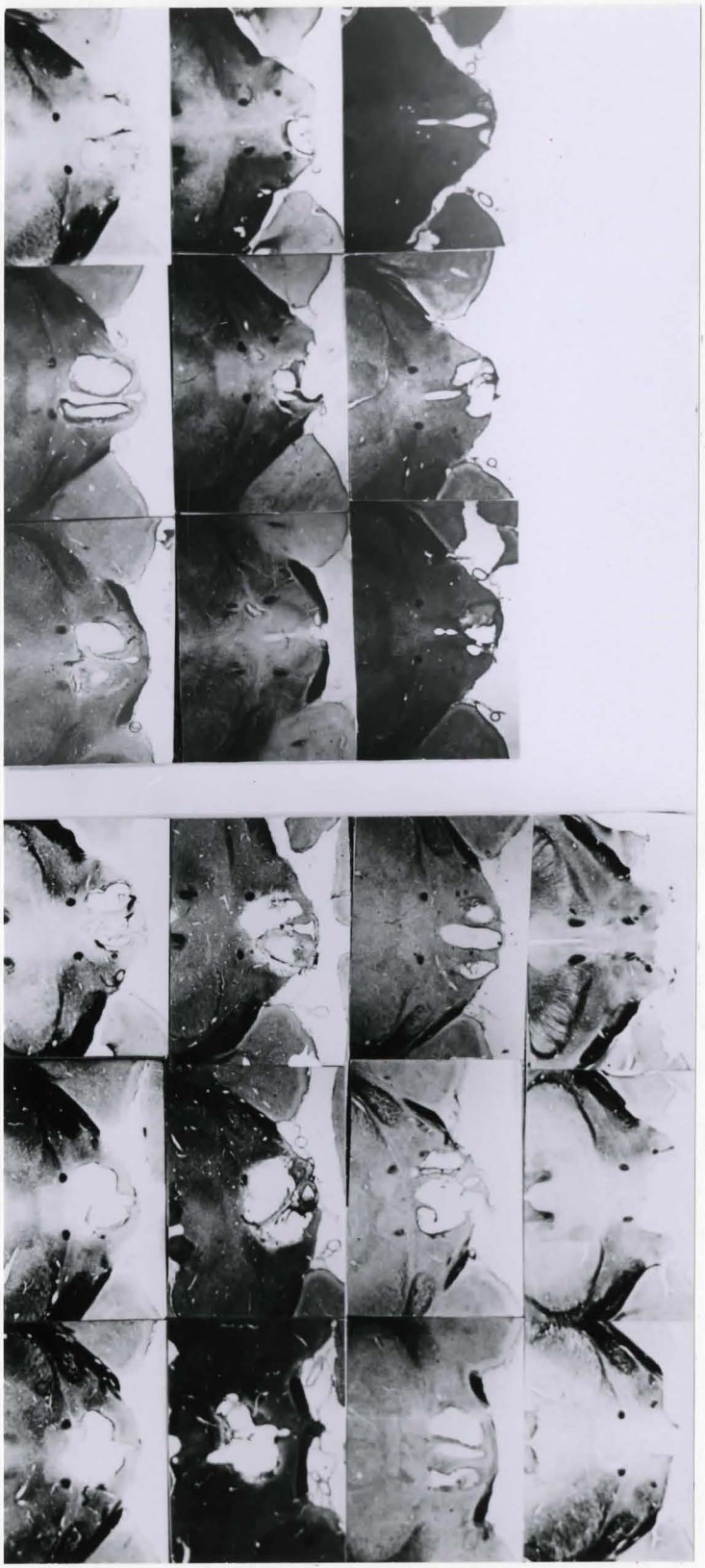
Rat No. (Left Side)	Rat No. (Right Side)
222	367
581	531
351	218
590	527

Ventromedial (VMH) Group (n=7)

The lesions of this group were centred within the ventromedial nuclei. In an anterior direction, they extended into the anterior hypothalamic area and, in a posterior direction, into the dorsal premammillary nuclei. The lesions were bounded laterally by the fornices, and extended across the midline, damaging the arcuate nucleus in a few cases. The lesions also caused basal damage which probably partially disrupted the supraoptico-neurohypophysial tract.

Photomicrograph Legend

Rat No. (Left Side)	Rat No. (Right Side)
516	519
579	A3
437	492
595	

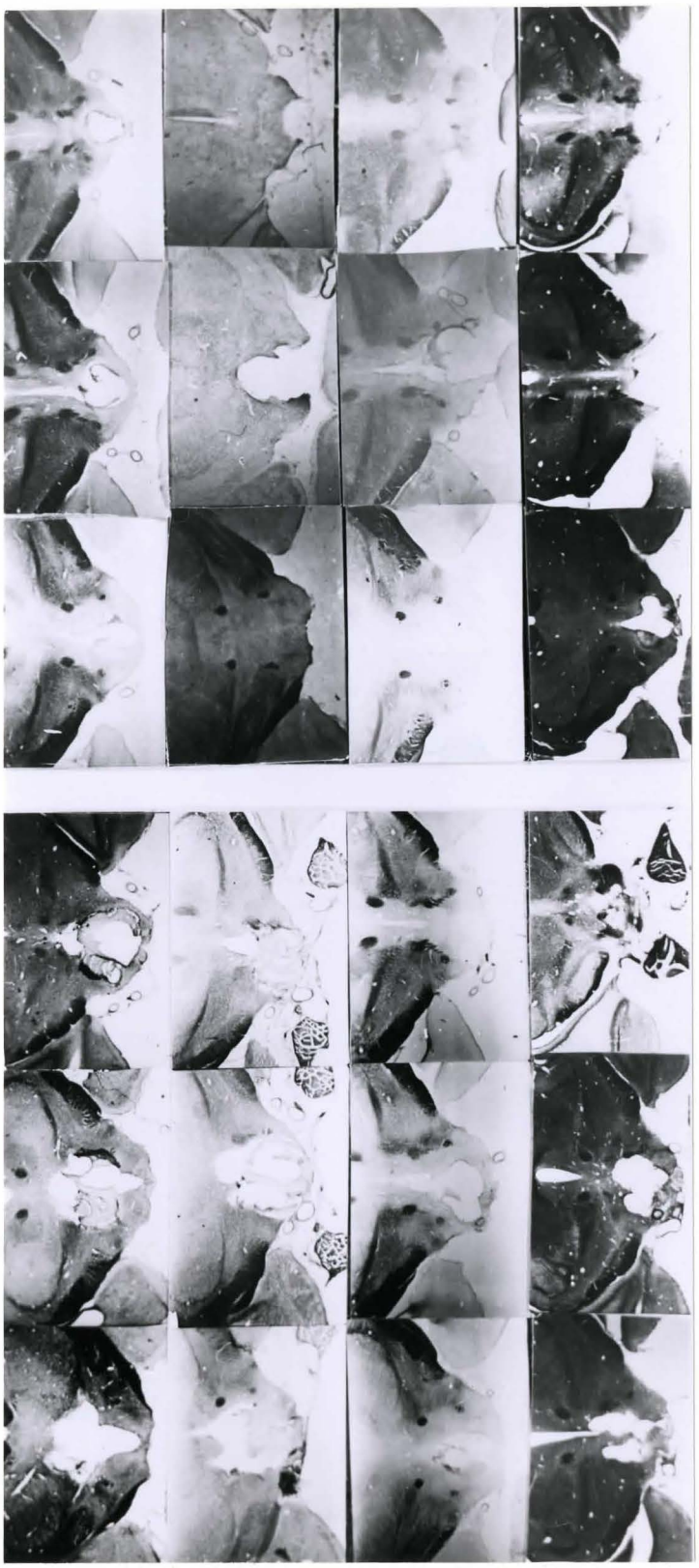


Posterior (Post) Group (n=7)

The rats of this group sustained lesions within the medial mammillary nuclei. The lesions extended in an anterior direction into the premammalliary hypothalamic nuclei and, in a posterior direction, into the posterior mammillary nuclei. The posterior portion of the arcuate nucleus and the ventral surface of the brain were usually damaged.

Photomicrograph Legend

Rat No. (Left Side)	Rat No. (Right Side)
592	167
548	391
392	493
394	



Diabetes Insipidus (DI) Group (n=11)

The lesions of this group were not selected on the basis of histological analysis, but because their daily water intake with food available was greater than 50 ml/100 g of body weight. The high water intake of these rats undoubtedly resulted from damage to the supra-optico-neurohypophysial tract, but histological examination of the lesioned brains did not show this clearly. In a few rats, the median eminence was damaged. In the remaining rats, the damage was farther posterior, within the mammillary and premammillary nuclei. Presumably, in the latter rats the supraoptico-neurohypophysial tract was interrupted within the stalk region of the neurohypophysis. This region is not retained through the staining and mounting procedure used in this experiment, and therefore this explanation could not be directly verified.

(Photomicrographs not presented)

APPENDIX B

Sample Calculation of Plasma Loss after 30% PG Treatment (Experiment I)

From Stricker (1968):

$$x/C = \frac{PP_2 - PP_1}{PP_2}$$

where:

x = volume of protein-free fluid withdrawn from vascular system

C = initial plasma volume

x/C = relative reduction in plasma volume

PP_2 = final concentration of plasma protein

PP_1 = initial concentration of plasma protein

Using mean values of plasma protein concentration from sham-operated rats:

PP_2 = 7.9 g/100 ml

PP_1 = 5.6 g/100 ml

Therefore:

x/C = 0.29

i.e. a 29% reduction in plasma volume

APPENDIX C

Individual Values of Plasma Sodium Concentration
After Injection of 30% PG and Access to Water for 9 Hr

Ant[1]		POM		AH		POL		SO	
Rat#	Plasma Sodium mEq/L H_2O	Rat#	Plasma Sodium mEq/L H_2O	Rat#	Plasma Sodium mEq/L H_2O	Rat#	Plasma Sodium mEq/L H_2O	Rat#	Plasma Sodium mEq/L H_2O
511	151.0	521	155.0	546	146.8	525	145.9	222	138.6
459	142.1	376	133.4	397	142.1	340	144.2	581	141.1
514	151.5	447	145.4	249	144.7	526	148.3	351	145.0
506	144.7	405	146.4	438	143.4	545	140.6	590	143.8
499	140.9	523	149.3	247	147.6	446	148.6	367	145.7
501	145.3	410	141.9	554	139.9	445	144.0	531	145.5
505	143.3	345	146.5	515	143.8	529	147.0	218	142.2
504	144.6	440	153.5	556	142.1	240	142.6	527	145.6
502	151.6	473	149.5	462	148.6	530	147.2		
441	149.5	520	154.3	460	144.6	221	141.5		
443	148.8	513	157.1	449	150.5	541	149.4		
				435	148.2	426	142.3		
				478	146.6				

VMH		Post		DI		Sham-operated	
Rat#	Plasma Sodium mEq/L H_2O	Rat#	Plasma Sodium mEq/L H_2O	Rat#	Plasma Sodium mEq/L H_2O	Rat#	Plasma Sodium mEq/L H_2O
516	144.0	592	141.9	392	147.0	436	145.7
579	140.6	548	139.8	394	140.0	518	145.8
437	140.8	392	147.0	167	146.5	450	145.1
595	140.6	394	140.0	492	146.6	475	147.4
519	141.6	167	146.5	400	142.8	503	140.6
A3	145.1	391	140.0	160	145.8	455	147.4
492	146.6	493	142.8	350	140.1	490	146.2
				399	143.4	508	142.8
				163	141.6	528	145.6
				170	139.3	549	142.7
				594	138.5	444	144.4
						544	136.6
						466	142.9
						463	146.5
						421	143.8
						512	147.1
						431	143.2
						32	139.9
						555	140.0
						578	136.1
						591	142.3
						584	145.7
						596	144.0
						524	144.7

[1] See Fig. 1 for abbreviations.

Note that #376 of the POM group has a very low value of plasma sodium concentration, in opposition to the general trend of values for this group. However, the brain damage sustained by this rat cannot be easily distinguished from that of other POM rats. In particular, the lesions of #405 and #447 appear identical to that of #376, although the plasma sodium concentrations of these other two rats are not unusual. The following points should be kept in mind when evaluating this result: (i) the low value for #376 is the only one (of all experimental groups) to fall outside the range of values for sham-operated rats; (ii) 6 of the remaining 10 values of the POM group are above the range of values for sham-operated rats; (iii) the data from #376 was included in the calculations and the mean value of the POM group was still significantly higher than that of the sham-operated group; and (iv) in Experiment II, only high values were found after similar lesions of the medial preoptic area.

Since this anomalous result could not be replicated, its significance is uncertain. The possibility that an error occurred in labeling of the brain slide in this case cannot be discounted.

APPENDIX D

Individual Values of Plasma Sodium Concentration (mEq/L plasma water) of POM-test, POM-chronic, DI and Sham-Operated Groups Immediately Following Each Test Period

Group	Rat#	Untreated (sample 1)	0.15 M NaCl	1.0 M NaCl	30% PG	Untreated (sample 5)
	3	172.7	159.4	173.6	163.5	152.8
	7	155.5	149.0	167.6	142.7	145.4
	9	149.0	150.5	150.6	150.7	148.7
	41	148.6	147.8	152.3	145.3	149.9
POM-test	53	153.2	151.5	156.3	149.9	148.3
	55	153.9	150.8	154.9	149.3	150.8
	63	164.9	150.7	162.0	150.4	150.4
	69	163.1	158.4	164.9	151.2	164.5
	81	161.4	155.2	155.7	153.7	151.4
	85	154.0	152.5	154.9	151.6	147.3
	13	156.3	153.8	157.6	149.5	--
Incomplete	75	191.1	182.3	--	--	--
(died)	77	156.3	151.6	--	151.4	--
		149.8	150.6	156.1	145.6	145.3
		148.1	150.9	146.7	144.5	146.7
		145.6	149.5	150.6	141.1	144.6
		145.3	151.0	147.4	143.5	146.3
		145.3	148.0	151.0	143.4	145.6
		147.4	149.1	149.3	142.0	143.9
Sham-		145.9	147.6	152.3	145.6	144.7
operated		145.6	147.3	145.2	142.9	147.2
		147.6	148.0	149.1	143.3	145.1
		149.3	146.3	148.2	145.5	146.8
		146.8	146.9	145.8	141.5	145.7
		147.0	147.2	146.7	141.2	144.9
		147.8	146.8	150.9	146.8	151.7
		152.0	148.6	152.7	142.5	145.1
DI		148.2	147.5	154.7	148.1	145.7
		148.3	146.8	148.7	139.5	147.6
		149.1	145.7	160.0	145.1	149.3
			Untreated	Untreated	Untreated		Untreated (sample 6)
	37	153.5	151.9	155.3	153.1	153.4	158.8
	45	155.3	153.7	155.3	153.2	150.1	151.0
	49	165.0	168.5	175.0	167.6	165.2	170.7
	57	163.1	161.2	164.4	162.0	159.9	159.0
	59	164.3	163.7	164.5	163.1	169.9	172.2
	61	166.7	175.7	178.2	166.5	175.1	193.8
	67	152.5	154.3	151.0	152.5	154.0	149.5
	73	154.4	157.3	156.5	157.6	155.7	152.9
	79	154.8	153.6	148.7	149.9	147.2	150.8
	83	151.4	151.1	150.4	147.6	147.0	147.2

APPENDIX E

Photomicrographs of the Lesioned Brains of Experiment II

This appendix provides a set of three photomicrographs of coronal sections through the anterior, central and posterior part of each lesioned brain (read from left to right)[1] of the POM-test group (page 112) and of the POM-chronic group (page 113).

Photomicrograph Legend

Rat No. (Left Side)	Rat No. (Right Side)
POM-test Group (n=10)	
3	55
7	63
9	69
41	81
53	85
POM-chronic Group (n=10)	
37	61
45	67
49	73
57	79
59	83

[1] When viewed by turning the page 90° in a clockwise direction.