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EFFECT OF ANAESTHETICS AND HAEMORRHAGE ON THE RELEASE OF NEUROHYPOPHYSIAL ANTIDIURETIC HORMONE

BY

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The first demonstration of "physiological" or "pharmacological" activity in extracts from the posterior pituitary gland was made by Oliver and Schäfer (1895) when they discovered that these extracts raise the blood pressure. Since then, although the role of neurohypophysial principles in the physiological control of the renal excretion of water and of milk ejection has been well established, very little evidence has been adduced which indicates a physiological role for the pressor action of neurohypophysial antidiuretic hormone.

Rydin and Verney (1938) showed that removal of blood from arteries in dogs resulted in an antidiuretic response which they concluded "was humorally determined and that the humoral agent was not adrenaline." Ginsburg and Heller (1953b) found that the antidiuretic activity in blood increased during haemorrhage in anaesthetized rats, and they concluded that there was increased release of antidiuretic hormone from the neurohypophysis. The amounts of antidiuretic hormone found in blood were large enough to suggest that it may have had an effect on blood pressure in these conditions. The effect of haemorrhage on the neurohypophysis has therefore been more fully investigated, with particular reference to the anaesthetics used.

METHODS

Young adult rats (170–270 g.) of the albino Wistar strain were anaesthetized with ether, pentobarbitone sodium (65 mg./kg. intraperitoneally), urethane (2.0 g./kg. subcutaneously), chloralose (60–100 mg./kg. intravenously), or ethanol (3–5 ml./kg. intravenously).

Ethanol (30% v/v in 0.9% NaCl solution) and chloralose were injected into conscious animals through a polyethylene catheter which had been inserted in a femoral vein under ether anaesthesia 18 hr. previously.

The trachea, femoral vein, and the cephalic end of an external jugular vein were cannulated. Blood pressure was recorded from a femoral artery, rather than a carotid artery, in order to avoid disturbance of cerebral circulation.

"Slow" Haemorrhage.—In most experiments 0.5 ml. blood/100 g. body wt. was withdrawn from the external jugular vein at 4 min. intervals until 5–7 such withdrawals had been made. When blood samples (0.2–0.9 ml.) for biological assay were taken, an equal volume of rat blood was simultaneously returned into the animal through the femoral vein. The first samples for assay were taken two minutes before the first haemorrhage, and subsequent samples were taken in the middle of the interval between haemorrhages.

"Rapid" Haemorrhage.—In a few experiments the net loss of blood was achieved more quickly and remained more or less constant during the collection of the samples for assay. The barrel of a 10 ml. syringe was attached by polyethylene tubing to a cannula in a femoral artery. The syringe was held lower than the animal and blood was allowed to flow into it. When 5–6 ml. of blood had been withdrawn the syringe was raised so that the level of blood in it was 40–45 cm. above the rat's heart. In this way the blood pressure of the rat was maintained at 32–36 mm. Hg. Samples of blood for assay were withdrawn from an external jugular vein without net blood loss, as described above.

Both vagi were cut in the neck about 15 min. before blood withdrawal in some experiments. In others both carotid sinus areas were denervated. Since, after acute carotid sinus denervation, rats do not tolerate haemorrhage satisfactorily, this operation was performed under ether anaesthesia 18 hr. previously.

Extraction of Neurohypophysis.—Animals from which the neurohypophysis was removed for assay were killed by decapitation. In unanaesthetized rats this was done without excitement or even the need to hold them firmly. They were introduced into a cardboard tube open at both ends, about 2½ in. in diameter and 10 in. long. As the rat's head emerged from the opposite end of the tube it was decapitated by a single blow from an axe. The pituitary was dissected out at once and the neurohypophysis was separated from the adenohypophysis. After weighing, the neurohypophysis was homogenized in 0.5 ml. of 0.25% (w/v) acetic acid in a Potter–Elvejhem ground glass homogenizer. The homo-

genate and washings (3 times with 0.5 ml. of 0.25% acetic acid) were placed in a boiling water-bath for 5 min. and, after cooling, were filtered. The pressor and oxytocic potencies of the extracts were usually assayed on the same day as the preparation of the extract and on the day following. The extracts were stored in a deep-freeze cabinet at −20° C.

Assay Methods.—The method of Ginsburg and Heller (1953a) was used for the assay of antidiuretic activity by intravenous injection into unanaesthetized, water-loaded rats, with the modifications that the bladders of the animals were cannulated and that the urine volume was measured at 5 min. intervals. With blood samples of low antidiuretic activity, a (2+1) assay design was used. Otherwise a (2+2) procedure was adopted.

Oxytocic activity was assayed on the isolated virgin rat uterus as described by Holton (1948). The uterus was suspended in a 30 ml. bath in a modified Locke's solution at 30° C., and unknowns and standards were added at 3 min. intervals.

Dekanski's (1952) method was used for the assay of vasopressin on the rat's blood pressure. Rats weighing 230–300 g. were anaesthetized with urethane (0.8 ml./100 g. of a 25% w/v solution, subcutaneously). Blood pressure was recorded from a carotid artery and injections were given into a femoral vein. The rats were given dibenamine (10 mg./kg.) intravenously in three divided doses.

Pitressin (Parke Davis & Co.) was used for standards in pressor and antidiuretic assays and Pitocin (Parke Davis & Co.) was used in oxytocic assays.

Results

Antidiuretic Activity of Blood During Gradual Haemorrhage.—Figs. 1 and 2 show the changes in the antidiuretic activity of external jugular blood during the loss of successive aliquots of 0.5 ml. of blood/100 g. body weight at 4 min. intervals, in rats anaesthetized with ether, pentobarbitone, ethanol, urethane, and chloralose. Increases of more than 100-fold in the antidiuretic activity of the blood were found, and in order to illustrate such large changes on a single figure the antidiuretic activity of the blood has been plotted on a logarithmic scale.

The results of representative experiments with ether, pentobarbitone, and urethane are shown on Figs. 1a, 1b, and 1c respectively. Qualitatively and quantitatively, the results obtained are similar and they will therefore be discussed jointly. The samples of external jugular blood taken before haemorrhage had antidiuretic activity which was equivalent to 0.1–0.25 mU. vasopressin/ml. When five aliquots had been withdrawn from the rats, the antidiuretic activity of the blood was approximately one hundred times greater than that in the initial samples, and was in the range of 7.0 to 25 mU. vasopressin/ml. blood.

In some experiments the blood pressure did not fall by more than 30 mm. Hg until three aliquots had been removed (Fig. 1a); in other experiments the blood pressure fell by 40 mm. Hg or more after the withdrawal of only one aliquot (Figs. 1b and 1c). The first significant rise in antidiuretic activity of external jugular blood seemed to be associated with the first pronounced fall in blood pressure. For example, in a rat (Fig. 1a) anaesthetized with ether, after the removal of the first two aliquots, the blood pressure fell from 108 to 80 mm. Hg and the antidiuretic activity of the blood only changed from 0.12 to 0.18 mU. vasopressin/ml. After the withdrawal of the third aliquot the blood pressure fell to 35 mm. Hg and the antidiuretic

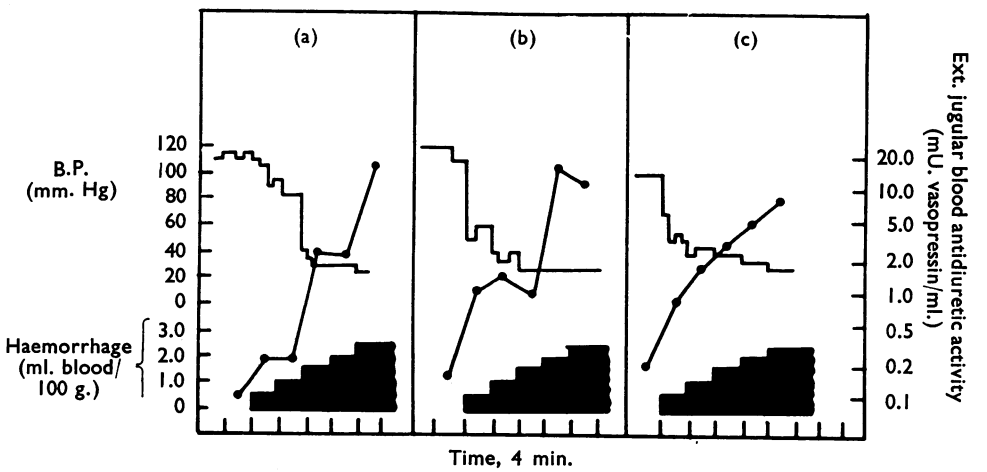

FIG. 1.—Effect of slow haemorrhage upon blood pressure, and antidiuretic activity in external jugular blood, in rats anaesthetized with (a) ether, (b) pentobarbitone, and (c) urethane. 0.5 ml. blood/100 g. was withdrawn at 4 min. intervals. ——— blood pressure, mm. Hg; •———• mU. vasopressin/ml. blood.

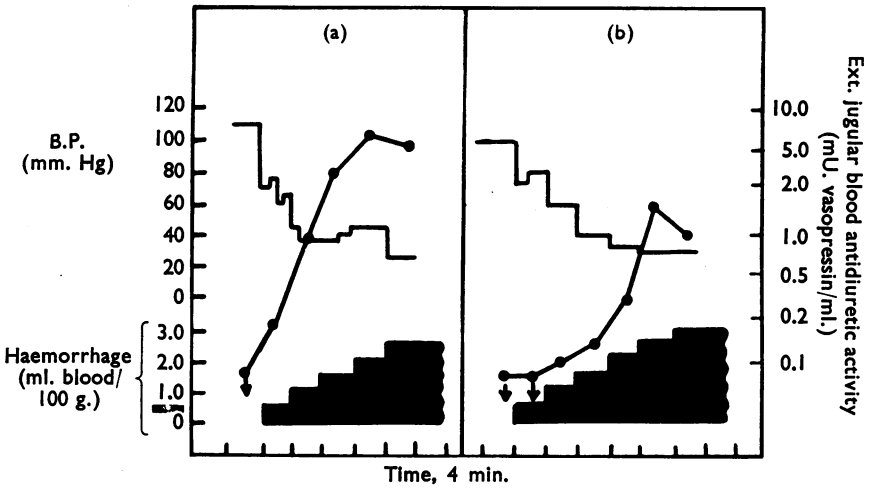

FIG. 2.—Effect of slow haemorrhage upon blood pressure, and antidiuretic activity in external jugular blood, in rats anaesthetized with (a) ethanol and (b) chloralose. 0.5 ml. blood/100 g. was withdrawn at 4 min. intervals. ——— blood pressure, mm. Hg; •———• mU. vasopressin/ml. blood.

activity of the blood increased to 2.0 mU./ml.; the antidiuretic activity was thus more than ten times greater than that of the previous sample and nearly 20 times more than in the initial sample of external jugular blood. In a rat (Fig. 1b), anaesthetized with pentobarbitone, after the removal of the first aliquot the blood pressure fell from 110 to 50 mm. Hg and the antidiuretic activity of the external jugular blood increased from 0.18 to 1.0 mU./ml.

The antidiuretic activity in the blood increased further as more blood was removed. In some experiments it rose gradually with each successive loss of blood. In other animals the antidiuretic potency remained for a while unchanged (within the probable limits of error of the assays), during the withdrawal of two or three samples, and then there was a second sudden and marked increase to levels between 15 and 25 mU./ml. With each of the three anaesthetics results of both types were obtained.

Three experiments were performed on rats anaesthetized with ethanol. Fig. 2a shows the results of a typical experiment. In the first sample

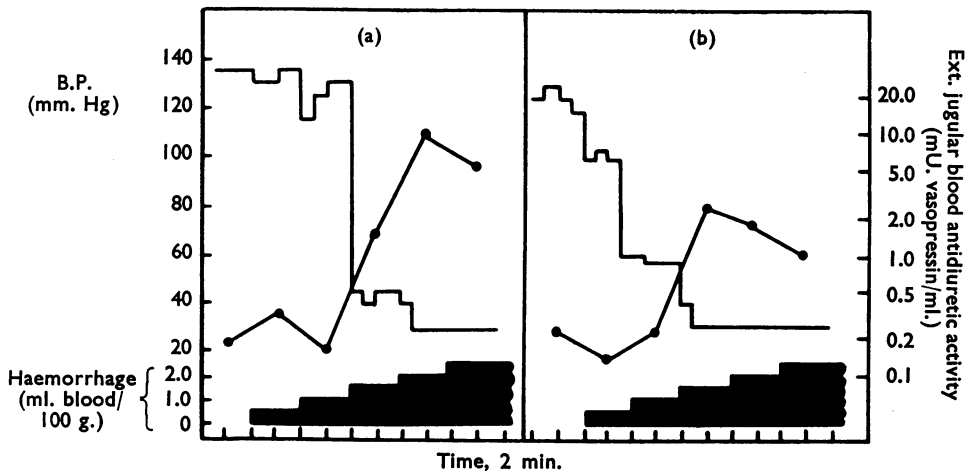

FIG. 3.—Effect of slow haemorrhage upon blood pressure, and antidiuretic activity in external jugular blood, in rats anaesthetized with pentobarbitone (a) after bilateral vagotomy and (b) after denervation of carotid sinuses and bilateral vagotomy. ——— blood pressure, mm. Hg; •———• mU. vasopressin/ml. blood.

of external jugular blood tested, before haemorrhage, no antidiuretic activity could be detected (i.e., less than 0.05 mU./ml.). When the blood pressure fell by 40 mm. Hg or more, the antidiuretic potency of the external jugular blood increased and was detectable. It continued to increase as more blood was removed, but without any obvious irregularities such as those shown on Figs. 1a and 1b. The greatest antidiuretic activities which were found in external jugular blood after the removal of five aliquots were of the order of 5 mU./ml. of blood, which is rather less than in comparable blood taken from rats anaesthetized with ether, pentobarbitone, or urethane.

In animals anaesthetized with chloralose, the antidiuretic activity of the blood was much less than when other anaesthetics were used. Even after the removal of five aliquots of blood, in two out of three animals, the antidiuretic activity of the blood was too low to be detected, i.e., <0.2 mU./ml. blood. In the third rat (see Fig. 2b) the highest concentration was 1.6 mU./ml., which is 3–4 times less than that observed with ethanol and 5–15 times less than that in rats anaesthetized with ether, pentobarbitone, or urethane.

The blood-pressure changes in rats anaesthetized with chloralose appeared to differ from those with other anaesthetics. The blood pressure fell in regular steps with each withdrawal of blood from 100 mm. Hg or more to about 35 mm. Hg after five or six aliquots had been removed, and a sudden collapse in blood pressure was not noted.

Effect of Bilateral Vagotomy and Denervation of the Carotid Sinuses.—Fig. 3a shows the results of an experiment on a rat under pentobarbitone anaesthesia after acute bilateral vagotomy. Although the initial blood pressure was higher than in rats with intact vagi, the changes in antidiuretic activity in external jugular blood during haemorrhage were essentially the same and are related to the changes in blood pressure. The greatest antidiuretic activity observed was 9.2 mU./ml. blood; in another similar experiment it was 6 mU./ml.

Fig. 3b shows the results of an experiment on a rat under pentobarbitone anaesthesia after denervation of both carotid sinuses 18 hr. previously and section of both vagi 15 min. before the first haemorrhage. The blood pressure fell with each haemorrhage, and the antidiuretic activity of the external jugular blood did not increase until the blood pressure reached 30–35 mm. Hg. The maximum activity was 3.3 mU./ml. blood in the experiment shown on Fig. 3b and less than 5.5 mU./ml. in another similar experiment; in rats under pentobarbitone anaesthesia, with vagi and the innervation of the carotid sinuses intact, the maximum activities were 15.0, 16.8, and 11.0 mU./ml. blood.

Effect of Neurohypophysectomy.—Blood was withdrawn from three rats under pentobarbitone anaesthesia which had been neurohypophysectomized 5–7 days previously. No antidiuretic activity (i.e., <0.1 mU./ml.) was detected in any samples of blood taken from the external jugular vein even after the loss of 6 aliquots of blood. The ability of these rats to maintain a high blood pressure in spite of the removal of up to 2 aliquots did not seem to be less than that of animals with the neurohypophysis intact. However, in the neurohypophysectomized rats the blood pressure fell to less than 20 mm. Hg when 4–5 aliquots had been removed, whereas in rats with neurohypophyses intact the blood pressure was usually above 30 mm. Hg.

Antidiuretic Activity of Blood after "Rapid" Haemorrhage.—The anaesthetic used in all experiments in which blood was removed rapidly from the animals was pentobarbitone. Fig. 4 shows the results of a typical experiment. Over a period of 2 min., 5.5 ml. of blood (approx. 2.5 ml./100 g. body weight) was withdrawn from a cannulated femoral artery. The cannula was attached to a reservoir of blood held with the blood level 44.5 cm. above the rat's heart, and during the subsequent 23 min., 0.75 ml. passed out of the animal into the reservoir. The changes in the antidiuretic activity of the external jugular blood showed two distinct phases. The first sample of external jugular blood, taken 2 min. after the blood pressure had been lowered, had an antidiuretic activity of 4.8 mU./ml. compared with 0.25 mU./ml. in the control sample, taken before any blood loss. The antidiuretic activity of the blood then fell and 12 min. later was only 0.5 mU./ml. The next two samples of external jugular blood showed another rise in antidiuretic potency, and the last sample tested (taken 23 min. after the blood pressure had been lowered) contained 3.0 mU./ml. In two other experiments, similar results showing two apparently separate increases in antidiuretic activity of external jugular blood were obtained.

Effect of Anaesthetics and of Haemorrhage on the Pressor and Oxytocic Activities of the Neurohypophysis

In view of the very high levels of antidiuretic activity found in blood during haemorrhage it seemed possible that the amounts of hormone liberated might cause significant changes in the

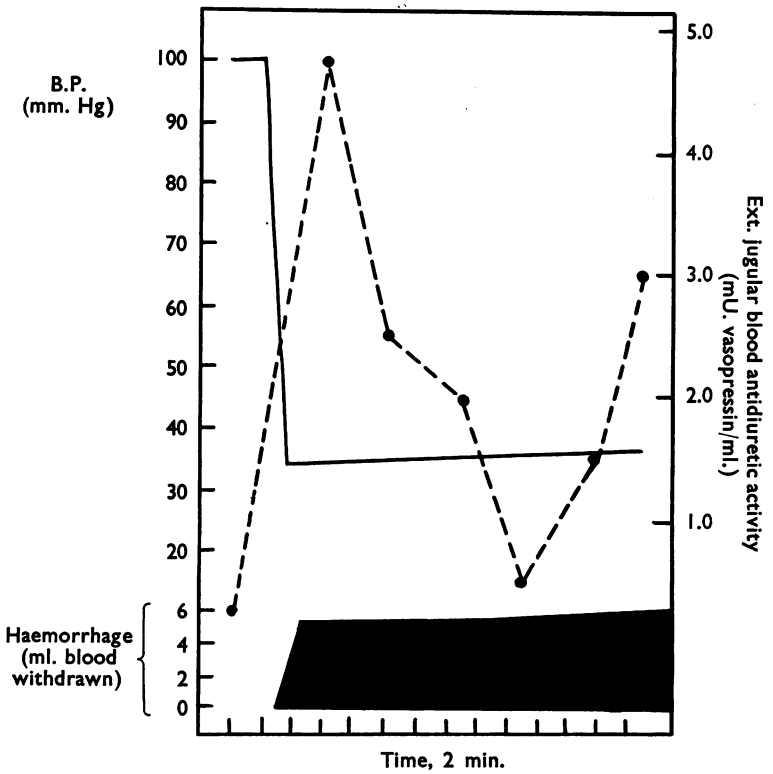

FIG. 4.—Effect of rapid haemorrhage on blood pressure, and antidiuretic activity in external jugular blood. 5.5 ml. blood was maintained at 33–37 mm. Hg. ——— blood pressure, mm. Hg; •— — —• mU. vasopressin/ml. blood.

active contents of the neurohypophysis. Table I shows the oxytocic and pressor activities of rat posterior pituitaries obtained from decapitated unanaesthetized rats and rats anaesthetized with pentobarbitone, chloralose, urethane, ether, or ethanol. Half the anaesthetized rats were bled under conditions similar to those of the "slow" haemorrhage experiments (7 haemorrhages, each of 0.5 ml. blood/100 g. body weight at 4 min. intervals); the remainder were anaesthetized for the same period as the rats which were bled, and the carotid and jugular vessels were exposed in the neck. Each gland was extracted separately, and the extracts (with a few exceptions) were assayed for oxytocic and pressor activity.

All the rats from which glands were taken for assay were male albino rats of the Wistar strain of similar weights and ages, but they were obtained from two sources. The supply of rats from source A (another laboratory) could not be repeated and the remainder came from a dealer (source B). The glands of the unanaesthetized rats from A had significantly greater oxytocic and pressor activities than those from B; the ratio of pressor to oxytocic activity in both groups was not significantly different from 1.0.

The activities of glands from animals under chloralose, ether, or ethanol anaesthesia were not significantly different from those from unanaesthetized rats of the same batch, and there was no significant change in the activities of the glands from rats which had been bled.

Rats from both groups, A and B, were anaesthetized with urethane, and the hormonal activity in the glands of rats in the former group was greater than in the latter, as in the unanaesthetized controls. In all rats anaesthetized with urethane, there was more oxytocic activity in the neurohypophysis relative to the pressor activity than in unanaesthetized rats of the same group. The mean ratio of pressor to oxytocic activity in all unanaesthetized rats was 1.12 ± 0.09 (S.E.; 14 observations) compared with 0.49 ± 0.10 (12) in all rats anaesthetized with urethane. This difference is highly significant ($t=3.25$, $P<0.01$). This change seems to be attributable to an increase in oxytocic activity and a decrease in pressor activity in the glands of rats anaesthetized with urethane, compared to those of unanaesthetized animals. Haemorrhage did not affect the hormonal activities of the neurohypophyses of rats anaesthetized with urethane.

The only rats in which haemorrhage had a significant effect on the activities of the neurohypophysis were those anaesthetized with pentobarbitone. The anaesthetic by itself reduced both the oxytocic and pressor activities to a significant extent ($t=12.0$, $P<0.001$ and $t=2.43$, $P<0.05$ respectively) with a greater loss of oxytocic potency so that the pressor/oxytocic ratio was 2.27 ± 0.37 (5) compared with 1.16 ± 0.25 (4) in the unanaesthetized controls from the same batch. The change of ratio is also significant ($t=4.9$, $P<0.001$). In the glands of rats which had been bled under pentobarbitone anaesthesia the ratio of pressor to oxytocic activity was not different from that in the anaesthe-

TABLE I

EFFECT OF ANAESTHETICS AND OF HAEMORRHAGE ON THE OXYTOCIN AND VASOPRESSIN CONTENTS OF RAT NEUROHYPOPHYSIS

Potencies in mU.±S.E. P/O=ratio of pressor to oxytocic activity. The numerals in parentheses give the number of glands assayed. A and B refer to two different sources from which rats were obtained.

Anaesthetic		A		B		All Rats
		Oxytocic Potency	Pressor Potency	Oxytocic Potency	Pressor Potency	P/O
None		778±34 (4)	902±74 (4)	436±50 (10)	451±43 (10)	1·12±0·09 (14)
Pentobarbitone sodium	Not bled	284±51 (5)	529±119 (8)	–	–	2·19±0·23 (10)
	Bled	432±19 (5)	901±110 (6)	–	–	
Chloralose	Not bled	734±105 (4)	703±87 (4)	–	–	1·11±0·12 (8)
	Bled	752±82 (4)	841±23 (4)	–	–	
Urethane	Not bled	1,470 (2)	465 (2)	685±40 (4)	366±25 (4)	0 49±0·10 (12)
	Bled	1,009 (2)	502 (2)	607±75 (4)	325±30 (4)	
Ethanol	Not bled	–	–	422±57 (4)	421±45 (4)	1·03±0·11 (8)
	Bled	–	–	415±46 (4)	440±100 (4)	
Ether	Not bled	–	–	419±93 (4)	569±63 (4)	1·38±0·17 (8)
	Bled	–	–	425±40 (4)	499±50 (4)	
None, "sham operated"				439±53 (5)	443±31 (6)	1·06±0·13 (5)

tized controls, but absolute activities in the bled rats were significantly higher. After haemorrhage the oxytocic and pressor activities of the glands were 432±19 mU. and 910±110 mU. respectively, compared with 284±51 mU. of oxytocic activity and 529±119 mU. of pressor activity in the rats which were only anaesthetized. These differences between the glands of bled rats and the anaesthetized controls are significant, $t=2.48$, $P<0.05$, and $t=4.0$, $P<0.01$, for oxytocic and pressor activities respectively.

Cavallero, Dova, and Rossi (1952) found that after "sham-adrenalectomy" in rats the antidiuretic potency of the neurohypophysis was reduced. Since the animals which were anaesthetized with ethanol and chloralose had been provided on the previous day with indwelling venous cannulae for the intravenous administration of the anaesthetic, a further series of controls was necessary. Conscious rats were decapitated 18 hr. after an operation under ether anaesthesia during which cannulae were placed in an external jugular vein and in the bladder. There was no difference between the hormonal activities in the glands of the operated rats and those of unoperated controls.

DISCUSSION

Although great increases in the concentration of antidiuretic hormone in the blood were observed during severe haemorrhage it does not necessarily follow that they were due to increased liberation of the hormone from the gland. During haemorrhage, the resistance of vascular beds alters to different extents so that blood flow in the brain, heart and thoracic muscles is reduced to a smaller extent than in the skin, splanchnic area and limb muscles (Franklin, 1951; Mott, 1953). The external jugular outflow in the rat, although largely cerebral (Greene, 1935), also contains effluent from skin, glands, and muscles, and the reduction of the latter during haemorrhage would increase the proportion of cerebral (and pituitary) outflow. However, it is improbable that the neurohypophysial contribution to external jugular blood flow could be increased 100-fold, and an increase of that order would be needed to explain the rise in the antidiuretic activity of blood by re-distribution of blood flow. Moreover, when rats were bled under chloralose anaesthesia, the increase in antidiuretic activity in blood was very much less than

when other anaesthetics were used, but presumably the changes in vascular resistance during haemorrhage were similar.

Reduction in the rate of disappearance of the hormone from the circulation could increase the concentration in blood. In rats, antidiuretic hormone is removed from the circulation by organs in the splanchnic area and the kidneys (Ginsburg and Heller, 1953b and c), and the persistence of the hormone in the circulation will depend, in the first place, on the proportion of the total blood volume perfusing these organs in unit time. This proportion may not be greatly altered by haemorrhage, for, although the hepatic and renal blood flow may be reduced, the total blood volume is also lowered. In "rapid" haemorrhage experiments, where the blood pressure was kept at approximately 35 mm. Hg, antidiuretic activity in blood increased at first and then disappeared, so that it was only 1/20th of its peak value within 12 min. (see Fig. 4). Thus, decreased clearance of antidiuretic hormone from the blood during haemorrhage does not account for the increased concentration of the hormone in the blood.

It seems reasonable therefore to conclude that, during haemorrhage, neurohypophysial antidiuretic hormone is liberated into the circulation in increased amounts. Two distinct and separate phases in this liberation of the hormone could be discerned. The first phase, which was observed in all experiments (except those on neurohypophysectomized rats and in chloralose anaesthesia) was associated with the first considerable fall in blood pressure. This observation agrees with those of Brun, Knudsen, and Raaschou (1945a and b) and Noble and Taylor (1953), who found that persistent oliguria, and the appearance of an antidiuretic substance in the urine after fainting, was due to the brief circulatory collapse. Also, Andersson and Larson (1954) have suggested that the antidiuresis produced by vomiting can be attributed to a rapid fall in blood pressure causing release of antidiuretic hormone from the neurohypophysis. Stimulation of the central end of a divided vagus nerve causes liberation of posterior pituitary hormones (Chang, Chia, Huang, and Lim, 1939; Andersson, 1951) indicating the existence of vago-pituitary connexions. Brun *et al.* (1945b), Nobel and Taylor (1953) and Andersson and Larson (1954) suggest that, following a fall in blood pressure, afferent impulses by way of such connexions stimulate the pituitary. In the present experiments, bilateral vagotomy did not materially affect the increase in the antidiuretic hormone concentration in blood during haemorrhage. Thus, although they may take part, afferent impulses in the vagus nerves in rats do not play a decisive role in the mediation of this response to a fall in blood pressure. This finding may be peculiar to rats, since Blood, Kosman, and D'Amour (1955) have shown that, although pulmonary depressor reflexes can be elicited in rats, they are not abolished, as in other species, by vagal section. The results obtained after denervation of the carotid sinuses plus bilateral vagotomy are difficult to interpret. The antidiuretic activity in external jugular blood was lower than in rats with their nerves intact, but this difference might possibly be due to the altered pattern of blood-pressure changes during haemorrhage rather than the destruction of nervous connexions between baroreceptors and the pituitary.

The second increase in antidiuretic hormone concentration in blood usually took place when the blood pressure had been less than 50 mm. Hg for about 15 min. Ferritin and adenosine triphosphate liberate antidiuretic hormone from the neurohypophysis (Baez, Mazur, and Shorr, 1952; Dexter, Stoner, and Green, 1954), and it is tempting to suggest that increased secretion of antidiuretic hormone follows the release of these substances from anoxic liver and muscle.

Friedan and Kellar (1954) have found that to lower the blood pressure of unanaesthetized dogs to 70 mm. Hg it is necessary to withdraw more blood from normal animals than from dogs with experimental diabetes insipidus. In rats anaesthetized with pentobarbitone the withdrawal of blood usually produced a sudden fall in blood pressure to below 70 mm. Hg in both normal and neurohypophysectomized animals. However, it was noted that in rats with intact neurohypophyses the blood pressure, after its collapse, remained at about 35 mm. Hg, whereas in the neurohypophysectomized animals it fell to below 20 mm. Hg. This observation is similar to that of Friedan and Kellar (1954), and, together with our other findings, supports their suggestion that the neurohypophysial pressor principle serves a physiological function in maintaining blood pressure during haemorrhage.

The absence of detectable antidiuretic activity in external jugular blood in rats anaesthetized with ethanol or chloralose (before haemorrhage) agrees with observations of Ames and van Dyke (1952) and Dicker (1953), and with expectation based on the continuance of water diuresis during anaesthesia with these drugs. However, the description of ethanol anaesthesia as a state of "functional neurohypophysectomy" (Dicker, 1954) does not apply as far as the stimulation of the posterior pituitary by haemorrhage is concerned. In contrast to ethanol,

anaesthesia with chloralose did suppress the liberation of antidiuretic hormone during haemorrhage; how this was achieved is not clear at present.

The highest antidiuretic activities found in 1 ml. of blood were equivalent to 2–3% of the vasopressin content of the neurohypophysis. However, the neurohypophyses of rats bled under ether, ethanol, and urethane anaesthesia did not have lower hormone contents than those of the control anaesthetized rats; and in rats anaesthetized with pentobarbitone the glands had significantly greater oxytocic and vasopressor activities after haemorrhage. Since haemorrhage under these conditions undoubtedly leads to the liberation of antidiuretic hormones into the blood, this finding can only be explained by increased repletion of the hormones stored in the gland. It may be necessary therefore to distinguish between *tropic* stimuli which affect the posterior lobe so that the hormones are transferred in increased amounts from the gland into the blood, and *trophic* stimuli which increase the amounts of biologically active material in the gland. Ames and van Dyke (1950) found increased antidiuretic activity in the posterior pituitaries of rats which had been deprived of fluid for 3 days, but in most investigations the hormone content of the neurohypophysis has been found to decrease under conditions or after treatments which would increase liberation of hormones from the gland. However, close examination of some of these results leads to the conclusion that trophic stimulation of the neurohypophysial-hypothalamic system also occurred. The normal rate of antidiuretic hormone secretion has been estimated in dogs to be of the order of 1–5 mU./hr. (Shannon, 1942) and 0.3–1.2 mU./hr. in rats (Dicker, 1954); assuming that the vasopressin content of the posterior lobe does not vary greatly from day to day, this rate must be approximately equal to that at which the hormone store is replenished. In Hild and Zetler's (1953) experiments, after restoration of access to water to dogs which had been thirsted for 14 days, the vasopressor potency of neurohypophyses increased at a rate of 2.8 units/day, i.e. the rate of repletion was about 20–100 times greater than normal. More striking perhaps are the results of Dexter *et al.* (1954), who showed that, following depletion by ATP injection, the antidiuretic activity of rat posterior pituitaries was restored by over 200 mU. (one third of the content of a normal gland) in 50 min., i.e. 330–1,300 times faster than the normal rate. Similarly, Rothballer (1953) found that painful stimuli in rats caused almost complete depletion of neurosecretory substance in the neurohypophysis within a few minutes; after 2 hr. the normal content of neurosecretory material was restored. Since the activity in the hypothalamus is small compared with that in the pituitary, repletion at such rates cannot be explained solely on the basis of increased rate of flow of the axoplasm current (Scharrer and Scharrer, 1954). Such stimuli must also increase the rate at which active material is formed in hypothalamic nuclei. The amounts of active substances in the neurohypophysis are determined by the rate of repletion as well as by the rate of hormone release, and the turnover of hormones by the gland may be independent of the level at which they are stored. Changes in the hormone content of the gland are therefore not reliable as an index of secretion.

The ratio of pressor to oxytocic activity in the neurohypophysis was not affected by haemorrhage, but it was altered by some anaesthetics. In round figures the relative proportion of pressor to oxytocic activity (expressed in terms of the appropriate international units) changed from 1 : 1 in unanaesthetized rats to 2 : 1 in rats anaesthetized with pentobarbitone, and to 1 : 2 in rats anaesthetized with urethane. The latter result is interesting, since Macaulay (1950) and van Dyke, Adamson, and Engel (1955) have stated that no instance of a vasopressin-oxytocin ratio of less than unity can be discovered.

Summary

1. In rats anaesthetized with ether, pentobarbitone, or urethane, the antidiuretic activity in external jugular blood before haemorrhage was 0.1–0.25 mU. vasopressin/ml.; it rose to 7–25 mU./ml. after the withdrawal of 2.5 ml. blood/100 g. body weight over a period of 20 min.

2. In rats anaesthetized with ethanol, the initial antidiuretic activity in external jugular blood was less than 0.1 mU./ml.; it rose to 5 mU./ml. or more, after withdrawal of 2.5 ml. blood/100 g. body weight.

3. In rats anaesthetized with chloralose the antidiuretic activity in external jugular blood was too low for detection both before and after haemorrhage, except in one animal where 1.6 mU./ml. was found after withdrawal of 2.5 ml./100 g. body weight.

4. Section of the vagi or denervation of the carotid sinuses did not prevent the increase in antidiuretic activity in the blood during haemorrhage.

5. No antidiuretic activity was found in the blood of neurohypophysectomized rats even after withdrawal of 3 ml. blood/100 g. body weight.

6. The results suggest that during haemorrhage the stimulation of the neurohypophysis to release antidiuretic hormone in increased amounts coincided with a big fall in blood pressure. After the blood pressure had been in the region of 35 mm. Hg for 20 min., a second increase in the antidiuretic activity in blood was observed.

7. Haemorrhage did not change the amounts of oxytocic or vasopressor activities in the neurohypophysis of rats anaesthetized with ether, ethanol, urethane, or chloralose. In rats under pentobarbitone anaesthesia the vasopressor and oxytocic activities in the neurohypophysis were greater in those which had been bled.

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