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THE FATE OF OXYTOCIN IN MALE AND FEMALE RATS

BY

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A method for quantitative extraction of oxytocin from blood is described. The disappearance of injected oxytocin from the circulation in rats was shown to depend upon its uptake in the kidneys and in organs of the splanchnic vascular area. In lactating rats there was uptake by an additional organ or tissue, probably the mammary gland. In animals without kidneys or lactating mammary glands and with no circulation in the splanchnic area oxytocin was distributed into a volume greater than the extracellular fluid volume, and after equilibration the concentration in plasma did not change significantly. During severe haemorrhage increased amounts of antidiuretic activity were detected in blood when there was no significant increase in oxytocic activity.

Many peptide hormones, for example, insulin, corticotrophin, and vasopressin, disappear rapidly from the circulation (Elgee and Williams, 1954; Richards and Sayers, 1951; Greenspan, Li, and Evans, 1950; Ginsburg and Heller, 1953a). In the case of vasopressin, this has been shown clearly to be due to the rapid uptake of the hormone from the blood by the kidneys and the liver, rather than to irreversible inactivation of the hormone in the circulating blood (Ginsburg and Heller, 1953a; Crawford and Pinkham, 1954; Dicker, 1954; Ginsburg, 1957).

Jones and Schlapp (1936) and more recently Chaudhury and Walker (1957) have shown that oxytocin, also, has a short lifetime in the circulation. These observations have been confirmed in the work described in the present paper and extended by an analysis of the parts played by specific organs and tissues in the removal of oxytocin from the circulation. A preliminary report of this work has been made (Ginsburg and Smith, 1958).

METHODS

Adult albino rats of both sexes (180 to 250 g.) were used.

Assay of Antidiuretic Activity.—The method of Ginsburg and Heller (1953b) was used except that the bladders of the rats were cannulated and measurements of urine volume were taken at 5 min. intervals. Where the amount of antidiuretic activity in the test samples permitted, (2 and 2) dose assay was used.

Assay of Oxytocic Activity.—Uteri from rats which were found by vaginal smear examination to be in pro-oestrus or early oestrus were suspended and superfused according to the method of Gaddum (1953). The constant temperature of the superfusing fluid was within the range 30 to 33°, depending on the reactivity of the uterus; the rate of flow was 3 to 4 ml./min. and the composition of the fluid was NaCl 4.0 g./l., KCl 0.42 g./l., $CaCl_2$ 0.06 g./l., $MgCl_2$ 0.005 g./l., glucose 0.25 g./l., $NaHCO_3$ 0.50 g./l., sucrose 58.6 g./l., and atropine sulphate 1 mg./l., saturated with 95% O_2 and 5% CO_2.

Extraction of Oxytocin from Whole Blood.—Blood was collected into chilled polyethylene tubes from a cannulated carotid artery in rats under ether anaesthesia. The rats had been given previously 100 U./100 g. body weight of heparin by intravenous injection. The blood was centrifuged at 3,500 rev./min. for 30 min. at 5°. The plasma was separated, placed in chilled glass centrifuge tubes and 10 vol. of dry acetone were added immediately. After centrifugation for 10 min. at 2,500 rev./min., the supernatant fluid was separated and the precipitate washed once with an acetone-water mixture (9 parts acetone to 1 part water). The acetone in the combined supernatant fluid and washings was evaporated in a stream of air at 40°. The cloudy aqueous residue was extracted twice with 7 vol. of diethyl ether to remove traces of acetone and other ether-soluble material. Ether, which had dissolved in the aqueous phase, was evaporated in a stream of air at 40° leaving a clear solution. In one series of experiments, the sodium and potassium concentrations in such extracts were estimated using a Lange flame photometer. The results of these experiments were used to determine the amounts of NaCl and KCl to be added to an extract to make its electrolyte composition similar to that of the superfusion fluid.

Inulin was estimated by the method of Schreiner (1950).

The preparations used were oxytocin (Pitocin, Parke, Davis), vasopressin (Pitressin, Parke, Davis), heparin (Pularin, Evans) and inulin (British Drug Houses).

Results

Reactivity of the Rat Uterus in Superfusion Fluid containing Different Concentrations of NaCl.—In preliminary experiments in which the uteri were superfused with Ringer-Locke solution (for rat uteri) described in the 1953 British Pharmacopoeia it was often necessary to apply as much as 1 mU. oxytocin/ml. to elicit a contraction of the uterus and also tachyphylaxis occurred. As Hughes, McDowall, and Soliman (1956) have shown that this tachyphylaxis may be prevented or retarded by reducing the concentration of sodium in the fluid bathing an isolated smooth muscle, the effect of using a superfusion fluid with reduced sodium concentration was investigated.

Fig. 1 shows the effect of changing the concentration of sodium chloride from 0.9 g./100 ml. to 0.4 g./100 ml. upon the action of oxytocin on a uterus. The concentration of the other ingredients of the superfusion fluid was unaltered, but 58.5 g. of sucrose/l. was added to the solution containing less NaCl, to maintain isotonicity. During each application of oxytocin the flow of superfusion fluid was stopped, 0.2 ml. of the test solution was applied in 10 sec. and allowed to remain in contact with the uterus for a further 10 sec. before the flow was restarted. When the B.P. solution was used, the contractions produced by application of 0.1 mU. of oxytocin in 0.2 ml. of fluid decreased gradually, but while using the solution with less NaCl the effect of the oxytocin was enhanced and became satisfactorily uniform.

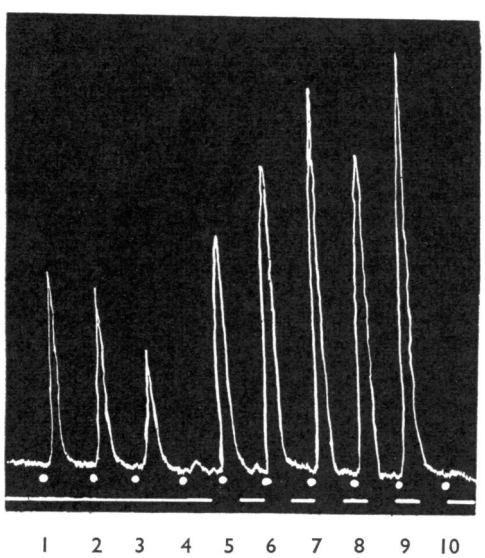

FIG. 1.—Effect of changing the concentration of sodium chloride on oxytocin induced contractions of a superfused rat uterus. Temperature of superfusion fluid, 33°; applications at 3 min. intervals. Solid line, 0.9 g./100 ml. of sodium chloride. Broken line, 0.4 g./100 ml. of sodium chloride. **1-3**: B.P. solution: 0.5 mU./ml. oxytocin. **4**: B.P. solution: no oxytocin. **5-9**: "Low sodium chloride" solution: 0.5 mU./ml. oxytocin. **10**: "Low sodium chloride" solution: no oxytocin.

Extraction of Oxytocin from Rat Blood.—Known amounts of oxytocin were added to rat plasma (4.0 and 8.0 mU./ml.) and extracted using the technique described above. In 5 experiments, the recovery of oxytocin was between 66 and 90% of the added amounts. The recovery of oxytocin added to whole rat blood in 8 experiments was between 78 and 104% of the amounts added. The mean recovery of oxytocin in all experiments with plasma and whole blood was $87 \pm 2.7\%$ (S.E. of means; $n=13$). In 2 of the experiments in which oxytocin was added to whole blood, the mixture was incubated for 10 min. at 37° before centrifugation and extraction. All the oxytocin was recovered from the plasma. This suggests that during the period of incubation oxytocin was not taken up by the blood cells. When precipitating plasma proteins, it was essential to add acetone to plasma, for with the reverse procedure less than 10% of the added oxytocin was recovered. Collection of the blood in chilled polyethylene tubes and strict adherence to the recommended time and speed for centrifugation were necessary, for, if the blood was centrifuged for less than 30 min. or collected in glass tubes, oxytocic activity which resisted treatment with sodium thioglycollate was present in the extracts. The most concentrated extracts of plasma were such that 1 ml. of the extract was equivalent to 1 ml. of plasma. The sensitivity of the uteri to oxytocin varied considerably and usually the lowest effective concentration was about 200 μU. oxytocin/ml. of standard solution, but occasionally very sensitive uteri were encountered with which as little as 20 μU./ml. of plasma could have been detected. Extracts of plasma from arterial blood from rats under ether anaesthesia seldom contained detectable oxytocic activity.

Effect of Haemorrhage on Oxytocic and Antidiuretic Activities in Blood.—As it has been shown that there is increased release of antidiuretic hormone from the neurohypophysis of anaesthetized rats during haemorrhage and that the concentration of the hormone in blood increases with the volume of blood withdrawn (Ginsburg and Heller, 1953c; Ginsburg and Brown, 1956), it seemed important to know whether, under similar conditions, the release of oxytocin was also increased.

The experiments were based on those described by Ginsburg and Brown (1956). Blood samples

containing the venous outflow from the cerebral circulation were withdrawn at 4 min. intervals from a cannulated external jugular vein in rats anaesthetized with ether. At the same time equal volumes of rat blood were returned to the animal by a femoral vein so that no change in blood volume should occur during collection of the sample. During each interval between the collection of such blood samples, more blood (0.5 ml./100 g.) was withdrawn without simultaneous return of blood, and thus the volume of blood lost by the animal increased regularly up to a total deficit of 3.0 ml./100 g. To obtain in each experiment sufficient material to determine both oxytocic and antidiuretic activities, blood samples were taken under similar conditions from two rats and the plasmas of corresponding samples were pooled.

The mean results of 4 experiments are shown in Fig. 2. No oxytocic activity could be detected in plasma, with blood deficits up to and including 1.5 ml./100 g., and in these instances the open rectangles in Fig. 2 give the mean concentrations at the limits of detectability. Antidiuretic activity in blood was raised as the blood loss increased. In blood taken when the deficit was 1.5 ml. blood/100 g. the antidiuretic activity was about 30 times that of control samples, while the oxytocic activity was still less than 0.4 mU./ml. plasma.

Inactivation of Oxytocin by Plasma in vitro.—Before investigating the disappearance of oxytocin from the circulation *in vivo*, it was necessary to determine whether there was enzymatic inactivation of oxytocin in rat plasma as in plasma of pregnant

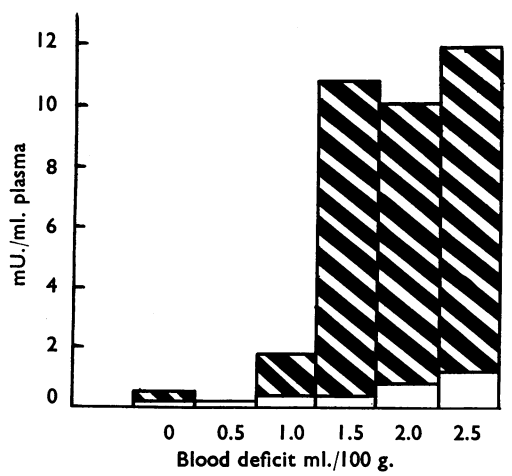

FIG. 2.—Oxytocic and antidiuretic activities in plasma of cerebral venous blood from the external jugular vein during progressive haemorrhage in rats anaesthetized with ether. Means of 4 expts. Open rectangles, oxytocic activity. Cross-hatched rectangles, antidiuretic activity.

TABLE I

INACTIVATION OF OXYTOCIN DURING INCUBATION WITH PLASMA AT 37° FOR 20 AND 40 MIN.

40 mU. of oxytocin was added to each ml. of plasma. Means±S.E. are given. The numerals in parentheses indicate the number of experiments made.

Plasma	% Inactivation	
	20 min. Incubation	40 min. Incubation
Rat: in oestrus	11·6±4·8 (6)	8·3±4·4 (6)
,, pregnant	13·5±3·4 (5)	10·0±8·8 (6)
,, lactating	12·9±4·7 (6)	9·2±3·1 (6)
Human: 1 day prepartum	90·6±3·3 (3)	96·6±1·1 (3)
0·9% NaCl soln. ..	—	5·9±3·7 (7)

women (Page, 1946; Hawker, 1955). Table I shows the effect of incubating oxytocin at 37° for 20 and 40 min. in the presence of plasma from normal female rats in oestrus, rats in the third week of pregnancy, lactating rats in the second week postpartum, and a human subject one day before delivery. After incubation in plasma from the pregnant woman, the oxytocin was almost completely inactivated, but the loss of activity after incubation with the rat plasmas was only 6.3 to 13%, an amount which can be reasonably attributed to losses during extraction.

The Disappearance of Injected Oxytocin from the Circulation in Rats.—Oxytocin (200 mU./100 g.) was injected intravenously into male rats anaesthetized with ether and given heparin. Blood samples were collected from a cannulated carotid artery at intervals after the injection. A control blood sample (1.0 ml.) was taken about 5 min. before injecting oxytocin. The total volume of all the blood samples from an animal was as much as 4.5 ml., and, to limit the blood deficit to 2 ml. or less, 2.5 ml. of blood from another rat was injected intravenously immediately after withdrawal of the control blood sample.

Fig. 3 summarizes the results: each point is the mean concentration found in 3 to 7 experiments. When the oxytocin concentration in plasma is plotted on a linear scale (Fig. 3*a*), the points fall on an exponential curve; when the oxytocin concentration is plotted on a logarithmic scale (Fig. 3*b*) the fit to a straight line is very good apart from one point. The mean concentration (±S.E.) in plasma fell from 20.6±2.85 mU./ml. (n=7) 1 min. after injection to 1.0±0.09 mU./ml. (n=3) 6 min. later. On the assumption of an exponential relationship between oxytocin concentration and time after injection, the mean half-life was 1.65±0.13 min. (n=7).

The Disappearance of Oxytocin from the Circulation in Nephrectomized Rats.—Experiments

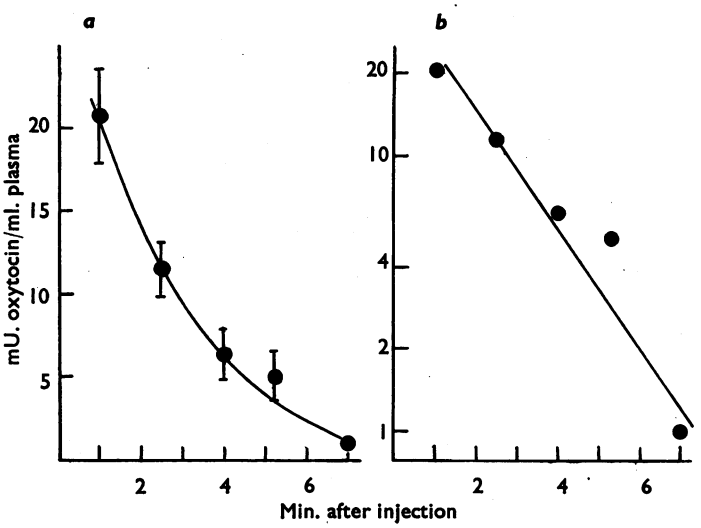

FIG. 3.—Concentrations of oxytocin in arterial plasma in intact male rats anaesthetized with ether following intravenous injection of 200 mU. of oxytocin/100 g. Each point is the mean of 3 to 7 experiments. *a*, linear; and *b*, log plots.

similar to those described in the previous section were performed on rats in which both kidneys had been removed, and in sham operated animals in which either the kidneys were exposed but not handled, or the kidneys were handled, ligatures were passed round the renal vessels and the perirenal fat was removed. Oxytocin (200 mU./100 g. of body weight) was injected intravenously 15 to 20 min. after completion of the operation.

The rate of disappearance of oxytocin from the circulation was not affected by sham operation when the kidneys had not been handled (half-life= 1.61 ± 0.11 min., n=4), but in animals subjected to the more severe sham operation the half-life was 2.73 ± 0.34 min. (n=6). It seems likely that the capacity of the kidneys to remove oxytocin from the circulation was not affected by their exposure but was depressed by handling, and in all subsequent operations in the abdomen care was taken to avoid handling the kidneys.

Nephrectomy retarded the disappearance of oxytocin from the circulation and the half-life was 2.95 ± 0.2 min. (n=6). The difference between this and the mean half-life in intact animals (1.65 min.) indicated the contribution of the kidneys to the disappearance of oxytocin from the circulation in intact animals.

Effect of Excluding the Splanchnic Vascular Area in Rats with and without Kidneys.—The circulation in the splanchnic vascular area was excluded by ligating the coeliac superior mesenteric arteries, dividing the ascending colon and left colic artery between ligatures, and ligating the portal vein. The satisfactory arrest of the circulation in the splanchnic vascular area was demonstrated at the end of each experiment by the bloodless appearance of the intestines despite the ligation of the portal vein, and by the absence of bleeding when the liver was cut.

15 to 20 min. after ligating the blood vessels, oxytocin (200 mU./100 g.) was injected intravenously and the concentrations of oxytocin were determined in plasma or blood withdrawn at intervals up to 7 min. after the injection. The rate of disappearance of oxytocin from the circulation was slightly less than in intact animals, the mean half-life for 4 experiments being 2.12 ± 0.20 min. compared with 1.65 min. in intact rats; this difference is not statistically significant ($0.1 > P > 0.05$). Also, exclusion of the splanchnic vascular area in acutely nephrectomized animals did not significantly alter the half-life compared with that found in animals nephrectomized only ($P > 0.05$). Thus, unlike the kidneys, the organs of the splanchnic vascular area do not affect the rate of disappearance of oxytocin from the circulation during the first 7 min. after injection.

In other experiments blood was collected during 26 min. after the injection of oxytocin. In animals which were only nephrectomized the concentration of oxytocin in plasma fell continuously; 14 min. after injection the mean concentration in plasma was less than 0.4 mU./ml. (Fig. 4). In animals in which the circulation of the splanchnic vascular area had been excluded in addition to nephrectomy, the concentration of oxytocin fell during the 7 min. immediately after injection as rapidly as in animals which were nephrectomized only, but thereafter there was little or no further change in oxytocin concentration in plasma.

This constant plasma oxytocic activity suggests that, after removal of the kidneys and exclusion of the circulation from the splanchnic vascular area, no organ or tissue remained in which oxytocin could be rapidly destroyed or inactivated. The continuous fall of the concentration of oxytocin in plasma in animals which were nephrectomized only must therefore be due to the uptake of oxytocin by the organs of the splanchnic vascular area. However, this is not rapid enough to affect materially the rate

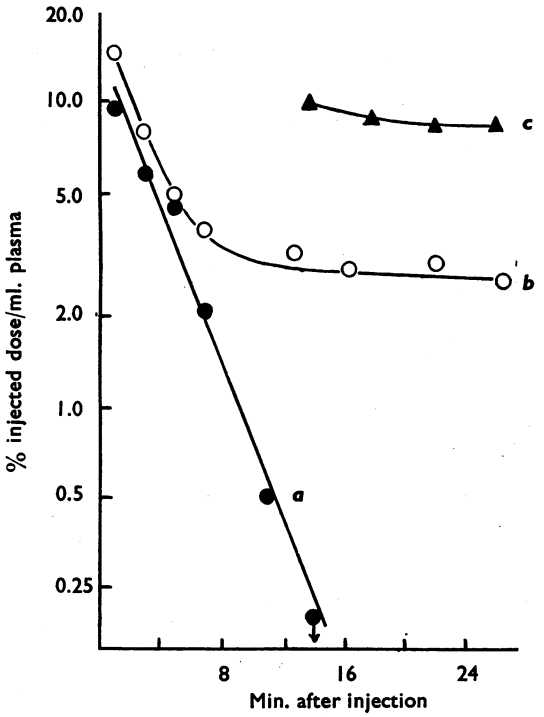

FIG. 4.—Concentrations of oxytocin in arterial plasma following intravenous injection of 200 mU./100 g. of oxytocin. *a*, nephrectomized rats; *b*, nephrectomized rats without splanchnic circulation; *c*, the concentration of inulin in arterial blood of nephrectomized rats without splanchnic circulation after intravenous injection of 40 mg. of inulin/100 g. Each point is the mean of 4 to 8 experiments.

at which oxytocin disappears from plasma during the first 7 min. after the injection.

Inulin Space in Nephrectomized Rats with Ligated Splanchnic Vascular Bed.—The inulin space was measured in nephrectomized rats with the vessels supplying the vascular splanchnic bed ligated. Inulin (40 mg./100 g. of body weight) was injected intravenously and blood samples were withdrawn at intervals up to 26 min. After injection the plasma concentration of inulin fell and became constant after 18 to 22 min. This concentration corresponded to a volume of distribution of 11.8 ± 0.45 ml./100 g. of body weight (n=7). The volume of distribution for oxytocin, obtained from the equilibrium oxytocin concentration in plasma in nephrectomized rats without splanchnic circulation, was 43.1 ±4.2 ml./100 g. (Fig. 4).

Disappearance of Injected Oxytocin from the Circulation in Female Rats.—The rate of disappearance of oxytocin after intravenous injection into normal female rats in oestrus was similar to that found in male rats (mean half-life=1.73 ± 0.10 min.; n=5). In pregnant rats 14 to 20 days after mating, the mean half-life for oxytocin was 2.01 ±0.08 min. (n=5) which is not significantly different from that found in oestrus animals ($P>0.05$). In lactating rats 3 to 14 days after parturition, the disappearance of injected oxytocin was significantly faster than in non-lactating animals, the mean half-life being 1.19 ±0.06 min. (n=6, $P<0.001$). Non-lactating females after

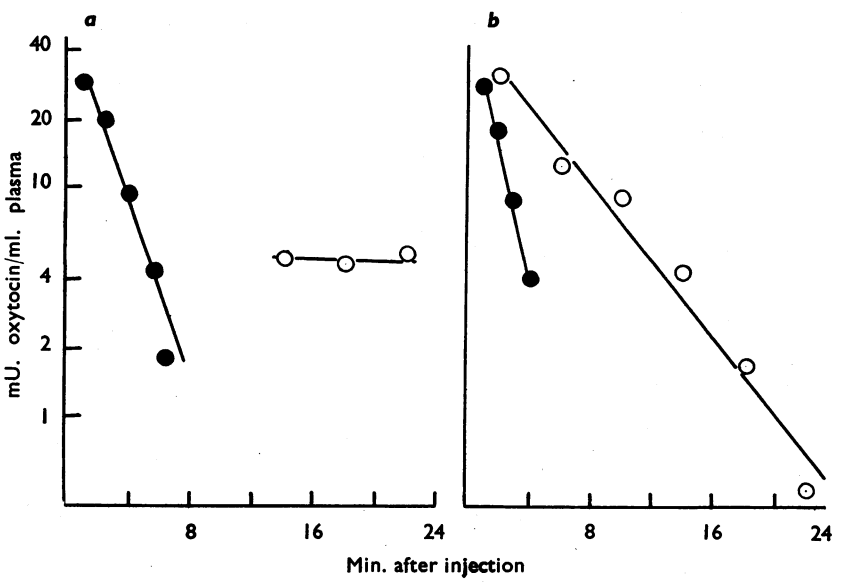

FIG. 5.—Effect of nephrectomy and exclusion of the splanchnic circulation on the disappearance of injected oxytocin from the circulation in: *a*, oestrus non-lactating rats; *b*, lactating rats. Each point is the mean of 3 to 6 experiments. ●——●: intact animals. O——O: nephrectomized and without splanchnic circulation.

bilateral nephrectomy with exclusion of the circulation in the splanchnic vascular area gave similar results to male rats (Fig. 5), there being no significant change in the concentration of oxytocin in plasma from 14 to 26 min. after the injection. On the other hand, after bilateral nephrectomy and exclusion of the splanchnic vascular bed in lactating animals, a constant concentration was not established and the oxytocin in plasma fell continuously and the plasma contained less than 0.2 mU./ml. 26 min. after the injection. In non-lactating females, the concentration was 5.2 ± 0.5 mU./ml. at this time.

Discussion

In male and non-lactating female rats the rapid rate of disappearance of injected oxytocin from the circulation is the resultant of three components and in lactating females there are four. Oxytocin disappeared most rapidly in rats with both kidneys and circulation to the splanchnic vascular area intact and with lactating mammary glands. When these organs or tissues were absent or excluded from the circulation, the concentration of oxytocin in plasma fell during the first 10 min. after injection and thereafter did not change greatly during the experiment. From this it may be concluded that no organ or tissue remained in which oxytocin was bound, destroyed, excreted, or in any other way irreversibly withdrawn from the exchangeable pool of oxytocin.

The volume of distribution for oxytocin under these conditions, calculated from the concentration of oxytocin in plasma when equilibrium was established, was three to four times greater than that found for inulin under the same conditions. Thus during the first 10 min. after injection in animals without kidneys and circulation in the splanchnic vascular bed, the fall in plasma concentration of oxytocin was due to equilibration of oxytocin in extra- and intra-cellular fluids. The fall in concentration was exponential during the first 7 min. with a mean half-life of 2.81 min. compared with 1.19 min. in intact lactating animals and 1.65 min. in intact males. Confirmation of the extensive penetration of oxytocin into body fluids was sought in experiments where oxytocic activity was assayed in extracts of skeletal muscle taken from animals after injection of oxytocin. No conclusion could be drawn from these experiments because, even when no oxytocin was injected, the extracts contained an unidentified oxytocic substance which, like oxytocin, was inactivated by treatment with sodium thioglycollate.

Although generalized penetration of oxytocin into extra- and intra-cellular fluids is possible and may be rapid, preferential uptake by the kidneys, organs of the splanchnic vascular area and the lactating mammary gland determines the fate of oxytocin in intact animals. The experiments of Heller and Lederis (1957) suggest that oxytocin may be bound to plasma proteins, but this is not incompatible with rapid diffusion into extravascular spaces if the formation of the complex is reversible.

Of the three special sites of uptake, the least important is the splanchnic vascular area. The best evidence for the participation of the splanchnic vascular area is the effect of excluding this area from the circulation of nephrectomized animals. In animals nephrectomized only, oxytocin disappeared continuously and no oxytocic activity was detected in blood 14 min. after injection. After excluding the splanchnic vascular area in nephrectomized animals there was no appreciable fall in oxytocin concentration between 10 and 26 min. after the injection. The mean concentration of oxytocin in plasma when equilibrium was reached was at least ten times greater than that found in plasma of rats with the splanchnic circulation intact 14 min. after injection. However, the rate of disappearance of oxytocin was not significantly affected by exclusion of the splanchnic circulation in animals with kidneys.

The kidneys play a more significant role in the removal of oxytocin from the circulation than do the organs of the splanchnic vascular area. Nephrectomy slowed the disappearance of oxytocin, the mean half-life increasing from 1.65 to 2.85 min. Chaudhury and Walker (1957) have obtained a similar result in rabbits. The present experiments give no indication of the ultimate fate of oxytocin taken up by the kidneys, but Larson (1939) has shown that up to 30% of oxytocin injected into cats and dogs can be recovered from urine within 2 hr. of injection.

In lactating animals the disappearance of oxytocin from the circulation was significantly faster than in males or non-lactating females. When the kidneys were removed and organs of the splanchnic vascular area were excluded from the circulation equilibration did not occur, and hence it was concluded that in lactating animals there is an additional organ or tissue in which preferential uptake of oxytocin takes place. This cannot be attributed to the appearance in blood of a system which inactivates oxytocin. It is most probable that the mammary gland is involved. The uterus, which is in a hypertrophied state, could also be implicated, but in non-lactating rats in oestrus

preferential uptake was restricted to the kidneys and the splanchnic vascular area, as in intact males. Sawyer (1954) found increased inactivation of oxytocin by ground-up uteri taken from pregnant rats after 14 to 21 days' gestation. In our experiments using rats at the same stage of pregnancy, the rate of disappearance of oxytocin from the circulation was similar to that in non-pregnant animals. More critical tests could have been devised to seek preferential uptake of oxytocin by the pregnant uterus, but these would have involved extensive operations in the abdomen which might have affected the uterus.

The half-life of oxytocin in the circulation has been determined in rabbits (Chaudhury and Walker, 1957), and can be calculated for cats from the results of Jones and Schlapp (1936). The values are: rat, 1.65 min., rabbit, 3.3 min., and cat, 8.5 min. The inverse relationship between the half-life and the size of the animal is not surprising, since circulation time will be a determinant of the rate of disappearance with a substance which disappears rapidly.

Although the hormones of the neurohypophysis occur in the gland in roughly equal proportions in terms of international units, there is evidence of a considerable preponderance of oxytocin over vasopressin in the secretion from the stimulated gland. The experimental evidence has usually been obtained by recording simultaneously the effects of stimuli on diuresis and uterine motility (Harris, 1948; Cross, 1951; Abrahams and Pickford, 1954), but it has been confirmed by estimation of oxytocic and antidiuretic potencies in blood (Chaudhury and Walker, 1958; Bisset, Lee, and Bromwich, 1956). Our results (preponderance of antidiuretic over oxytocic activity in cerebral venous blood collected during stimulation of the neurohypophysis by progressive haemorrhage) confirm that the hormones are not secreted in the same proportions as they occur in the gland, but unlike previous findings the results show that oxytocin is not always in excess.

The method described in this paper for extracting oxytocin from blood has the merits of simplicity and consistently high recoveries of added oxytocin. The use of acetone for preparing posterior pituitary powder does not immediately suggest that the oxytocin would remain in solution after acetone precipitation of plasma proteins, although Dudley (1923) and Heller and Lederis (1959) observed that some oxytocic activity was present in acetone extracts of posterior pituitary glands. When the extraction method was applied to plasma containing added vasopressin, recoveries were not satisfactory (never being greater than 60%) as might be expected since vasopressin is generally less soluble in organic solvents than oxytocin (Acher and Fromageot, 1955).

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