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MATURATION OF THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM

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New-born animals (Heller, 1949) and infants (Hansen & Smith, 1952; Barnett & Vesterdal, 1953) are unable to concentrate their urine to the same extent as adults. The following hypotheses or their combinations have been considered (Heller, 1958) to explain this difference: (1) too little of the antidiuretic hormone (vasopressin) is produced, (2) the osmoreceptors do not respond or do not respond adequately to the stimulus of dehydration, (3) the renal tubules do not react to vasopressin in the adult manner, either because of morphological or functional immaturity, or because they are under the influence of other hormones which antagonize vasopressin.

The validity of the first hypothesis could be tested by comparing blood levels of vasopressin in hydropenic new-born and adult mammals, but the difficulties of estimating vasopressin in the blood of new-born animals have so far been unsurmountable. Several investigators have therefore approached this problem by estimating the hormone content of the neurohypophysis. In relation to body weight Heller (1947) found considerably less antidiuretic activity in the glands of rats aged 18–42 hr than in the pituitaries of adults. Dicker & Tyler (1953), who referred hormone content to body surface, reported that the glands of dogs, cats and guinea-pigs at birth contained about half the activity of adult glands. However, since formulae for the calculation of the body surface of the new-born of these species were not available, they based their results on Lee's (1929) formula for the body surface of adult rats. This may have influenced their conclusions. The neurohypophysis of new-born infants has also been found (Heller & Zaimis, 1949) to contain less antidiuretic and pressor activity than that of adults.

The results presented in this paper extend previous findings in new-born rats (Heller, 1947) by reporting on the hormone content of rat neurohypophyses throughout the first month after birth. Both pressor and oxytocic activities were determined. This seemed important since vasopressor:oxytocic ratios

have been reported in infant animals (Dicker & Tyler, 1953; Acher, Chauvet & Olivry, 1956) which differ substantially from those of adults. A few guinea-pig and human glands were also investigated.

METHODS

Animals

Rats of both sexes aged from 1 to 28 days were used. Since the hormone content per gland appears to be related to body weight as well as to age, and body weight is known to be influenced by the number of young in the litter, litter size was uniformly restricted to six. Adult male rats of the same strain (Wistar) weighing 180–250 g served as controls.

Guinea-pigs aged about 24 hr and male adults of the same strain were used. Human pituitary glands were removed from 3 to 48 hr after death from bodies which had been kept in cold store until shortly before dissection.

Preparation of extracts

The rats were killed by decapitation. Whole pituitaries were extracted of animals aged up to 7 days, but neural lobes only of older animals. All glands were extracted immediately after the rats had been killed. Following the suggestion of Bentley & Dicker (1955) a number of neurohypophyses of infant rats of each age group were pooled for extraction (per extract, 18–24 glands were pooled of 1-day-old rats, 12–15 glands of 3–5-day-old rats, 6–9 glands of 7–14-day-old rats and 4–6 glands of 21–28-day-old rats). This allowed dilution of the acid extracts with the uterus suspension fluid (see below) to prevent decreases of pH which, as these authors have shown, may modify the response of the isolated uterus to oxytocin. Extracts for vasopressor assays were diluted with NaCl solution 0·9% (w/v).

Acetic acid extracts. The pooled glands, mixed with 0·4 ml. suspension fluid containing acetic acid 0·25% (v/v), were crushed in a small (3·5 ml.) glass homogenizer and transferred to a 15 ml. centrifuge tube, using a further 0·6 ml. of the acetic acid solution. The tube was then immersed in boiling water for 90 sec (control experiments having shown that boiling for 3 min did not increase the yield of hormones significantly) and centrifuged for 15 min at 3000 rev/min. The supernatant was decanted and filtered. Extracts of glands of adult rats were prepared in the same manner but the glands were extracted singly, a total of 2 ml. of the acetic acid solution being used.

Acetone extracts. The glands were either treated with acetone according to the British Pharmacopoeia (1958) or, in some instances, kept in 5–10 ml. of dry acetone in a refrigerator for 24 hr and then extracted with acetic acid as described above. To recover oxytocic activity from acetone, distilled water 0·5 ml. was added and the acetone evaporated at 40° C. The residual fluid was washed twice with ether (Ginsburg & Smith, unpublished). Guinea-pig and human glands were extracted with acetic acid only.

Calculation of body surface. Heller's (1952) formula for rats aged 12–36 hr was used for rats aged up to 7 days, and Lee's (1929) formula for adult rats, for rats aged 14 days and over. An error—which may be considerable—was thereby introduced into the calculation of the surface of rats in all age groups except in 1-day-old animals and adults. For example: if the body surface of a rat aged 14 days and weighing 20 g is calculated according to the formula for adult animals the results would be 75·6 cm^2; if the formula for new-born rats is used the surface would be 47·4 cm^2. The error does, however, decrease if Heller's formula is used for rats younger than 14 days and Lee's formula for older rats.

Calculation of kidney weight. Donaldson's (1915) figures were employed since they were shown (Heller, 1952) to be applicable to the strain of rats used.

Analyses

Water content of tissues. The animals were killed by decapitation and bled. All brain tissue rostral to the tentorial level was removed, stripped of the dura mater and dried at 100° C. Neural

lobes were removed with the help of a dissecting microscope, placed on tared pieces of platinum foil, weighed on a micro-torsion balance, placed in small weighing bottles and dried.

Vasopressor assay. Dekanski's (1952) method was used, but dibenyline was injected instead of dibenamine.

Antidiuretic assay. Ginsburg & Heller's (1953) procedure was used, with the modification that the bladders of the assay animals were cannulated and the readings were taken every 4 min.

Oxytocic assay. The method used was that recommended in the British Pharmacopoeia (1958), but oestrous rats were used and the suspension fluid was modified in that the calcium concentration was halved and the magnesium concentration doubled. The temperature of the fluid in the organ bath of 2·5 ml. capacity was slowly raised to 37° C, the uterus having first been subjected to one or two large doses (30–50 m-u.) of Pitocin (Parke, Davis & Co.) at 30° C. These modifications were found to suppress spontaneous contractions of the organ and to make it possible to use 0·2–1·0 m-u. of the hormone as the 'smaller dose' of the standard preparation. In an experiment in which eight groups of 4 doses were randomly applied to the same uterus (Holton, 1948) the limits for R were 0·966 and 1·059; the limit of error for the assay was 4·9%. Assays of extracts of unknown potency were of the standard (2 + 2) design and four groups of doses were used for each estimation. The milk-ejecting activity of some of the extracts was estimated by the method of Cross & Harris (1952).

Standard preparations. Since the gland extracts contained both neurohypophysial hormones, Pituitrin (pituitary extract; Parke, Davis and Co.) was used for pressor and oxytocic assays in preference to the fractional preparations. Each batch of Pituitrin was standardized against an extract of the international standard powder. Pitressin (Parke, Davis and Co.) was used for antidiuretic assays for reasons which have been discussed by Ginsburg (1956). Synthetic oxytocin (Syntocinon, Sandoz) was used as reference substance in chromatograms.

Inactivation of neurohypophysial hormones with sodium thioglycollate. Vogt's (1953) modification of the procedure of Ames & van Dyke (1951) was used.

Chromatography. Extracts of the glands of infant and adult rats were chromatographed on paper and stained or eluted as described by Heller & Lederis (1958).

Statistical treatment. Where appropriate, results are given as weighted means and their standard errors. The standard deviation of a weighted mean (σ_W) was calculated as follows: $\sigma_W = \sqrt{\left(\frac{S\Delta^2 N}{N-n}\right)}$, where $S\Delta^2$ = sum of squares of deviations from mean, N = number of pooled glands and n = number of assays. Student's t test for small samples as described by Fisher (1944) was used for estimating the significance of difference of means. The probability P for t was obtained from Fisher & Yates's (1943) tables.

RESULTS

Pressor activity in the neurohypophysis of infant rats

Table 1 shows the mean vasopressin content per neural lobe during the first month of extra-uterine life. The figures shown are based on pressor assays; some antidiuretic assays gave similar results. The two methods of extraction used (see Methods) yielded estimates of mean pressor activity which did not differ significantly for each age group.

In order to test whether the neurohypophyses of new-born and infant rats contained not only absolutely but also relatively less activity, the mean glandular hormone content at each age was related to three parameters of comparison, namely, to body weight, body surface and kidney weight. Figure 1 shows that—irrespective of the criterion used—the relative hormone levels were lower for some weeks after birth than those in adults. Pressor and

antidiuretic activity per unit weight of wet neurohypophysial tissue was also low in infant animals. For instance, the neurohypophyses of rats aged 7 days (the earliest post-natal date at which the posterior lobe could be reliably dissected) contained a mean of $59 \pm 7{\cdot}5$ m-u. pressor activity per milligram, as compared with $564 \pm 85{\cdot}5$ m-u./mg for adult neurohypophysial tissue.

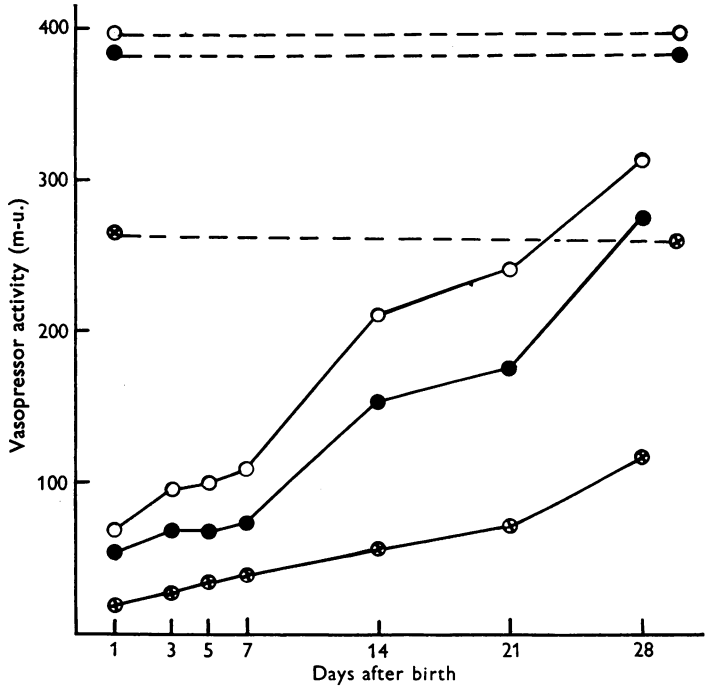

Fig. 1. Mean hormone content (vasopressor activity) in the neurohypophysis of infant and adult rats as related to three parameters of comparison: ○—○, m-u./100 g body weight; ●—●, m-u./g kidney weight; ⊗—⊗, m-u./100 cm² body surface. The horizontal lines indicate mean levels in adults.

Pressor activity in the neurohypophyses of new-born guinea-pigs and human infants

The hormone content of the neural lobe of infant and adult guinea-pigs was estimated, to obtain results in a species which is much more developed at birth than the rat. The large amounts of vasopressor activity found in the pituitaries of new-born guinea-pigs (Table 2) are in keeping with this difference in maturity. Human posterior lobes were investigated because it seemed advisable to repeat the observations of Heller & Zaimis (1949) with more recent methods of assay. The results (Table 2) were in good agreement with the older findings. In spite of great variations within each age group, mean hormone content per milligram of gland was again distinctly lower than in adults, but there was no difference when hormone content was referred to body weight.

TABLE 1. Vasopressor and oxytocic activity in the neurohypophysis of infant and adult rats. For details of the two methods of extraction used see text (p. 300)

Post-natal age (days)	Acetic acid extracts				Acetone extracts			
	Mean body weight (g)	Number of glands	Vasopressor activity* (m-u./gland)	Oxytocic activity* (m-u./gland)	Mean body weight (g)	Number of glands	Vasopressor activity* (m-u./gland)	Oxytocic activity* (m-u./gland)
1	5·9	46	3·7 ± 0·2	3·3 ± 0·8	6·5	67	4·6 ± 0·5	0·6 ± 0·1
5	10·8	52	11·4 ± 0·6	4·3 ± 0·5	11·2	68	11·0 ± 1·0	1·4 ± 0·2
7	14·3	19	13·5 ± 0·6	10·9 ± 2·0	11·6	18	14·6 ± 3·7	3·6 ± 1·5
14	20·5	20	36 ± 8·2	27 ± 3·4	19·8	19	49 ± 5·4	28 ± 4·9
21	29·9	22	71 ± 10·7	55 ± 12·7	37·5	28	91 ± 12·5	52 ± 6·2
28	53·1	17	139 ± 17·6	144 ± 6·3	49·7	16	155 ± 30·1	125 ± 8·8
Adult	216·0	12	854 ± 73·0	683 ± 94·2	211·0	4	839 ± 85·7	732 ± 96·8

* Weighted means ± S.E.

TABLE 2. Hormone content (vasopressor activity) in the neurohypophysis of infants and adults of different species. Means ± S.E.

Species	Post-natal age (days)	Infants				Adults			
		Number of glands	(m-u./gland)	(m-u./mg wet gland)	(m-u./100 g body wt.)	Number of glands	(m-u./gland)	(m-u./mg wet gland)	(m-u./100 g body wt.)
Guinea-pig	1	5	398 ± 120	412 ± 65	496 ± 181	4	2,325 ± 546	639 ± 123	513 ± 114
Man	Up to 3	6	460 ± 107	32 ± 5·7	24 ± 4·4	5	15,230 ± 2,610	125 ± 42	23 ± 10·0
Rat	7	35	13·5* ± 1·7	59* ± 7·5	117* ± 49·5	16	850 ± 57	564 ± 86	400 ± 30

* Weighted means.

It is impossible to say whether the variations within age groups were 'genuine', due to terminal hormone release or hormone inactivation after death, or to a combination of these causes.

Oxytocic activity in the neurohypophysis of infant rats

While at all ages investigated much the same means for pressor activity per gland were obtained, irrespective of the method of extraction employed, considerably less oxytocic than pressor activity was found when the pituitaries of very young rats were treated with acetone before extraction with acetic

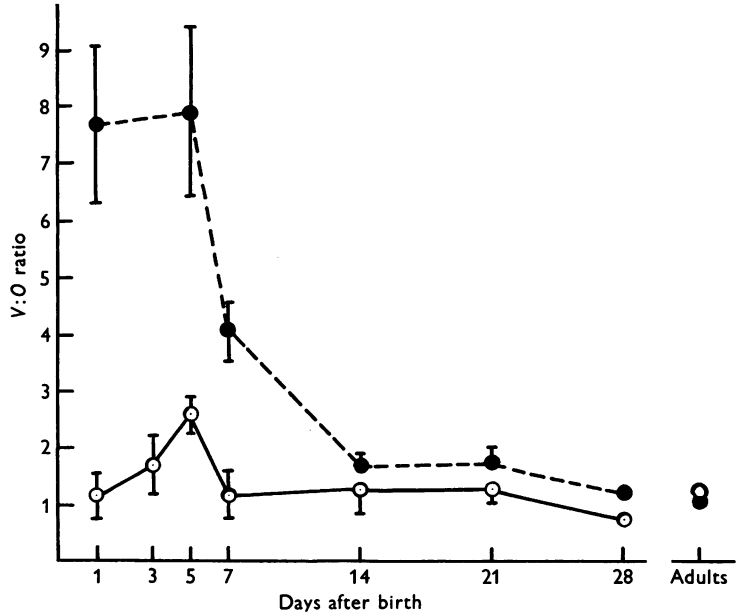

Fig. 2. The relation of vasopressor (*V*) to oxytocic (*O*) activity in extracts of infant and adult rat pituitaries as dependent on the method of extraction. ●---●, glands treated with acetone and subsequently extracted with dilute acetic acid (see Methods, p. 300); ○—○, glands extracted with acetic acid only: means and S.E. The numbers of glands in each age group were the same as in Table 1.

acid (Table 1). The vasopressor:oxytocic ratios (*V*:*O*) in acetone-treated glands of animals of less than 14 days of age were therefore much higher than those in glands of the same age groups which had been extracted with acetic acid only (Fig. 2).

These results raised the question whether the glands of infant rats contain an oxytocic substance, other than oxytocin, which is soluble in acetone. In favour of this possibility was the observation that frequently the isolated rat uterus, when in contact with extracts of pituitaries of rats aged less than 7 days, relaxed less readily than when exposed to extracts of the glands of older animals or to Syntocinon. Against it are the following findings: (*a*) The oxytocic activity

in 'acetic acid' extracts of infant pituitaries was completely inactivated by thioglycollate (8 experiments). The oxytocic activity in the acetone with which infant glands had been treated was likewise inactivated by thioglycollate (4 experiments). (*b*) When acetic acid extracts of infant rat pituitaries were chromatographed on paper according to the procedure of Heller & Lederis (1958) the proportion of oxytocic activity which could be eluted at the R_F of synthetic oxytocin was the same as that eluted from chromatograms

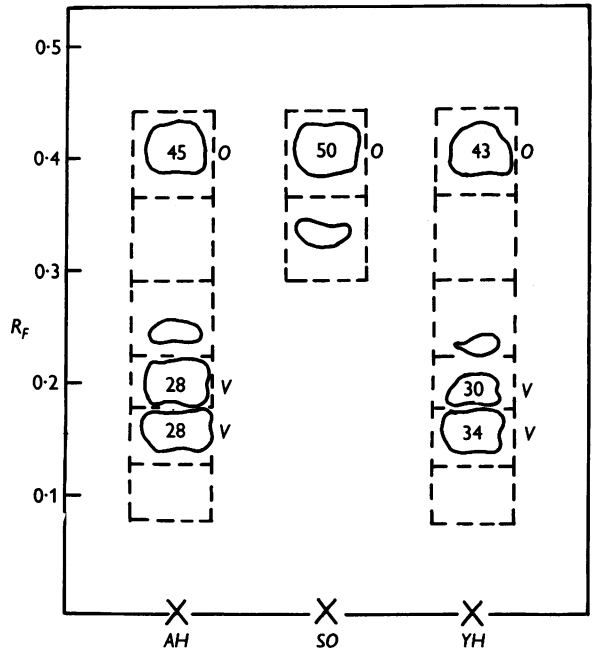

Fig. 3. Ascending paper chromatograms of acetic acid extracts of neurohypophyses of adult rats (*AH*) and of rats aged 7 days (*YH*); and synthetic oxytocin (*SO*). Two identical chromatograms were prepared but only one was stained. The other was cut into pieces indicated by the broken lines. The pieces were extracted, and oxytocic (*O*) and vasopressor (*V*) activities in the eluates were assayed. The figures within the 'spots' indicate percentage recovery of activities applied at the origin (X).

of adult rat glands (Fig. 3). (*c*) The milk-ejecting potency of 'acetic acid extracts' of infant rat pituitaries compared satisfactorily with their oxytocic activity (3 experiments).

These findings make it unlikely that more than traces of an oxytocic principle other than oxytocin occur in the gland of infant rats and they suggest that acetone extracts oxytocin preferentially to vasopressin. To investigate the matter further, the following experiments were done on neural lobes of adult animals. Ox posterior pituitary glands, obtained immediately after killing, were treated with acetone according to the pharmacopoeial procedure

(British Pharmacopoeia, 1958), i.e. the glands were placed in acetone (4 ml. per gland), removed after 3 hr and placed in a similar volume of acetone where they remained during the night. The two portions of acetone were pooled, diluted with distilled water and the acetone removed. A part of this residual fluid was taken up in Ringer–Locke solution for oxytocic and pressor assay, another part was used for paper chromatography. All such extracts (4 experiments) contained oxytocic activity which on chromatograms moved at the

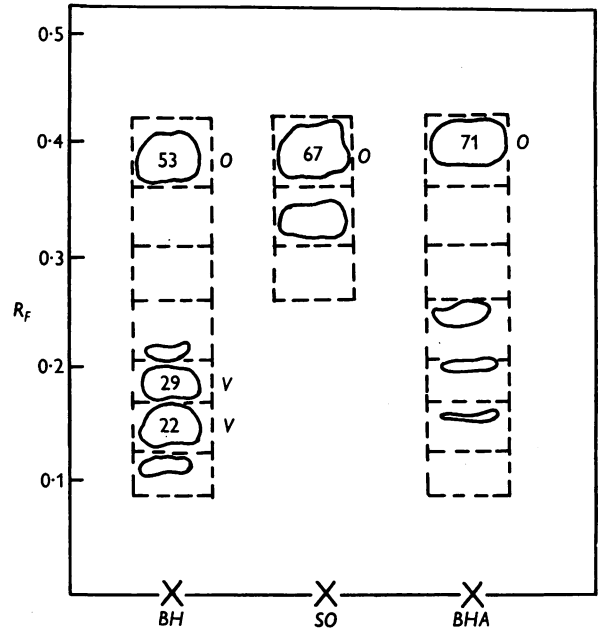

Fig. 4. Ascending paper chromatograms of an acetic acid extract of an ox neurohypophysis previously treated with acetone (*BH*), of the oxytocic activity in the acetone with which the bovine gland had been treated (*BHA*) and of synthetic oxytocin (*SO*). The figures within the 'spots' indicate percentage recovery of activities applied at the origin (X). *O*, oxytocic activity; *V*, vasopressor activity. No activity could be found in the eluates of spots for which no figures are given. For further details see legend to Fig. 3.

same rate as synthetic oxytocin (Fig. 4) and which was completely inactivated by thioglycollate. The amounts of oxytocin extracted by acetone from these ox glands were small (Table 3), in fact so small that the $V:O$ ratios were not much affected. No vasopressor activity could be demonstrated in the acetone residue.

Experiments with extracts of neurohypophyses of adult rats gave similar results; i.e. only about 2% of the total oxytocic activity could be recovered from acetone. However, when the glands of infant rats were subjected to the same procedures this percentage rose to about 60 in the case of 5-day-old animals (Table 3).

TABLE 3. Oxytocic activity recovered from acetone with which pituitary glands had been extracted

Post-natal age (days)	Oxytocic activity in acetone* (m-u./gland)	Activity extracted with acetic acid after treatment with acetone: Oxytocic activity (*O*) (m-u./gland)	Activity extracted with acetic acid after treatment with acetone: Vasopressor activity (*V*) (m-u./gland)	*V*:*O*	*V*:*O*† corrected	Percentage of total oxytocin content recovered from acetone
		Ox glands				
Adult	2370	78,900	89,200	1·13	1·09	2·9
Adult	1700	46,800	68,500	1·46	1·41	3·6
Adult	1320	73,100	83,400	1·19	1·12	1·8
Adult	1820	50,800	65,800	1·28	1·24	3·6
		Rat glands				
5	2·7	1·5	9·5	6·3	2·5	59·2
10	2·4	6·9	—	—	—	34·8
28	14·0	130·0	200·0	1·5	1·4	10·8
Adult	10·7	555·0	—	—	—	1·9

* No vasopressor activity could be found in the acetone. † The oxytocic activity recovered from acetone was added to that extracted with acetic acid and the sum assumed to be the total activity initially present in the gland. This assumption introduces an error, since recovery from acetone was not complete.

The water content of the acetone in which the posterior pituitary glands are placed seems to be one of the factors which determine the amount of oxytocin which is extracted. This is suggested by the results of the following experiments: A number of neurohypophyses of adult male rats were dried over phosphorus pentoxide, powdered, mixed and the powder divided into several portions which were weighed to 0·1 mg. One portion was extracted with acetic acid only, another was first treated with analytically pure acetone (which—according to the British Drug Houses Laboratory Chemicals Catalogue, 1958—may contain up to 0·7% (v/v) water) and a third with acetone to which 1% (v/v) of distilled water had been added. The mean $V:O$ ratio in three experiments with 8, 20 and 22 glands was 1·2 when the powder had been extracted with acetic acid only; the mean $V:O$ ratio after extraction with 'dry' acetone was 1·6 and that obtained after treatment with watery acetone was 2·1.

In several experiments with rat pituitaries the weight of wet neurohypophysial tissue per unit volume of acetone used for extraction (6·5 ml./mg) was the same for adult and infant glands, but in view of the foregoing results it seemed advisable to determine their water content. The brains of infant rats were found to contain considerably more water than those of adult animals: the brain of 1-day-old rats contained $88{\cdot}0 \pm 0{\cdot}15\%$ (6) water, that of 8-day-old rats $88{\cdot}0 \pm 0{\cdot}10\%$ (6) and that of adult rats $78{\cdot}6 \pm 0{\cdot}20\%$ (6). However, the water content of the neural lobe of 8-day-old rats was $74{\cdot}6 \pm 1{\cdot}30\%$ (7) as against $75{\cdot}5 \pm 1{\cdot}24\%$ (8) in adult rats: that is to say it was much the same ($P > 0{\cdot}6$). Similarly, small differences in the water content of the posterior pituitaries of human infants and adults had already been noted by Heller & Zaimis (1949).

Effect of dehydration on the hormone content of the neurohypophysis of infant rats

Figures 1 and 2 show that, according to the parameter of comparison chosen, the pituitaries of new-born rats contained 6–14 times less pressor and oxytocic activity than those of adult controls. Morphological evidence—which will be discussed later—suggests that this is due to immaturity of the hypothalamo-neurohypophysial system in this species at birth and for some time after. It may therefore be asked whether, at this stage of development, this complex neuro-endocrine system can be made to respond to a 'physiological' stimulus like dehydration which is known to increase the secretion of vasopressin in adults. When infant rats were separated from the mother for 24 hr (but were protected from cold) it could be shown that their neural lobe contained less pressor activity than that of litter-mates which had remained with her. The mean decrease in hormone content in dehydrated (and starving) rats aged 1–2 days was 27·1% (17), that in rats aged 4–5 days 23·3% (5) and that in

rats aged 7–8 days 20·6% (6). Vasopressor–antidiuretic activity in the neural lobe of male adult rats deprived of water and food for 24 hr was 1332 ± 135 (6) m-u. as compared with 1036 ± 135 (6) m-u. per gland in controls with free access to food and water. The difference is not significant, but it becomes so when hormone contents per 100 g body weight are compared (dehydrated rats: 604 ± 67 m-u./100 g, controls 408 ± 64 m-u./100 g; $P = 0{\cdot}05$). A slight rise in hormone content after 24 hr seems likely, considering that a pronounced increase in hormone storage has been shown to occur in rats when drinking water is withdrawn for 48–72 hr (Ames & van Dyke, 1950; Dicker & Nunn, 1957). It seems that adult rats have to be dehydrated for over 96 hr before the hormone content begins to fall (Simon, 1934; Dicker & Nunn, 1957).

DISCUSSION

In the main the maturation of the hypothalamo-neurohypophysial system has been judged by three criteria: (*a*) the differentiation and development of the cell bodies of the nuclear neurones, (*b*) the abundance and microscopical appearance of neurosecretory material, and (*c*) the hormone content of the neural lobe. Concerning the first of these, it has been shown by Auer (1951) that in the golden hamster the hypothalamic nuclei appear as separate structures only after birth. The last nuclear mass to differentiate is the supraoptic nucleus, which is still difficult to identify on the fifth post-natal day. In the dog and in man, on the other hand, the hypothalamic nuclei are clearly discernible before birth (Papez, 1940). Cytological features of post-natal nuclear cell maturation have been described by Rodeck (1958) in the rat and other mammalian species including man.

The results of the numerous investigations on the first appearances of gomori-positive material in the hypothalamo-neurohypophysial system (Bargmann, 1949; Dawson, 1953; Benirschke & McKay, 1953; Scharrer, 1954; Diepen, Engelhardt & Smith-Agreda, 1954; Green & van Breemen, 1955; Rodeck & Caesar, 1956; Rodeck, 1958; Amoroso, Harrison, Harrison-Matthews, Rowlands, Bourne & Sloper, 1958) can be summarized as follows. At birth the neurosecretory material is scanty in those forms which are also immature by other criteria. Usually it appears first in the neural lobe, but in the rat even there only on the 4th to 6th post-natal day in the form of very fine dust-like granules which aggregate as the animals get older. It can therefore not be excluded that the hormones may occur in the hypothalamo-neurohypophysial system—perhaps at some developmental stages only— in a form which cannot be demonstrated microscopically or ultramicroscopically. This would limit the value of the histological approach considerably. It seems desirable, therefore, to relate microscopical findings to results of hormone estimations by biological assay. This has to some degree been attempted in the present investigation. Small amounts of pressor and oxytocic activity were

found in the neurohypophyses of rats killed during the first days after birth, that is at a stage of development when chrome-alum-haematoxyphile material cannot be demonstrated under the light microscope. Electron-microscopical investigations—now in progress—may show in what form the active material is stored in the pituitary at this stage of development.

The neurohypophyses of new-born guinea-pigs and human beings were considerably richer in hormone than those of rats of the same age. If, for example, vasopressor activity per milligram of gland is compared and the ratio adult: infant is calculated from the results shown on Table 2, the ratio for 7-day-old rats is 9·5, that for human infants aged up to 3 days is 3·9 and that for guinea-pigs aged 1 day 1·6. There appears thus to be a satisfactory parallelism between the ontogeny of the hypothalamo-neurohypophysial system, as gauged by pituitary hormone content and abundance of neurosecretory material, and over-all maturity at birth as judged by such criteria as the eruption of teeth, the development of locomotion and the opening of the eyes (Koldovský, Křeček, Křečková & Mikulaš, 1953). If these correlations apply to mammals generally, it can be expected, for instance, that the neurohypophysial hormone content in the new-born hamster (Auer, 1951) resembles that of new-born rats, but that the hormone levels in new-born seals (Amoroso *et al.* 1958) are more similar to those in new-born guinea-pigs.

Hormone assays unsupported by histological evidence cannot reveal whether the low amounts of active principles in an endocrine tissue are due to increased release or to slow rate of synthesis. But, in conjunction with the morphological evidence already discussed, it seems likely that the neurohypophysis of the new-born rat contains less hormone either because the rate of production is low or because the submicroscopical structures concerned in hormone storage (Palay, 1957; Bargmann, 1958) are as yet incompletely developed. There is also the possibility that the hormones found in the neurohypophysis of immature animals are peptides which differ chemically from the adult hormones and which are less potent. However, since it could be shown in the present paper (Fig. 3) that the active principles extracted from the neural lobe of infant rats behave chromatographically like arginine-vasopressin and oxytocin, this is unlikely. Whether the infant rat gland contains in addition traces of substances not present in adult neural lobes which may affect the isolated uterus or the rat's blood pressure needs further study.

Recent investigators (Dicker & Tyler, 1953; Acher *et al.* 1956) interested in the hormone content of immature posterior pituitary glands have reported that the ratio of vasopressor to oxytocic activity ($V:O$ ratio) in the rat pituitary is very high shortly after birth, reaching approximate unity in the third post-natal week. The high ratios in very young animals have been confirmed, but only when—as recommended by the British Pharmacopoeia (1958)—the glands were treated with acetone before extracting with dilute acetic acid.

When the acetone treatment was omitted the mean $V:O$ ratios in the glands of rats aged 1–5 days varied irregularly from 1·2 to 2·7 and were near unity in older animals. That oxytocin is preferentially extracted by acetone is not surprising, since it is known (Acher & Fromageot, 1955) to be generally more soluble in organic solvents. It is, in fact, owing to this property that the two peptides can be separated by counter-current distribution. The higher solubility of oxytocin could also have been deduced from the work of Dudley (1923), who at one stage of his attempts to separate the neurohypophysial hormones obtained a powder of high oxytocic potency a twentieth of which could be extracted with 'dry' acetone. Similar results were obtained by us when ox or adult rat pituitaries were extracted. Moreover, it could be shown that the oxytocic material extracted by acetone was inactivated by thioglycollate and that on paper chromatograms it moved at the same rate as synthetic oxytocin.

We cannot say at present why relatively more oxytocin can be removed by acetone from infant than from adult rat glands, but this is apparently not the only physiological situation in which this phenomenon occurs. It can also be shown in the neural lobes of lactating rats in which again $V:O$ ratios are high when acetone is used (Dicker & Tyler, 1953; Acher *et al.* 1956), but in which the ratios do not differ significantly from those in non-lactating controls when simple acetic acid extraction is used (Rennels, 1958; Heller & Lederis, 1959). It remains to be seen whether these disparities in solubility are due to modifications of the peptide-carrier complex or whether perhaps they are produced by cytological differences in storage. The difference between the reactions to stains of neurosecretory material in the pituitaries of infant and adult rats (Dawson, 1953) may be worth noting in this connexion.

While the amount of hormones stored in the neural lobe of infant rats was very low as compared with adults, it seems possible that the mechanism for release of the vasopressor-antidiuretic factor by dehydration operates already shortly after birth. Our finding, that the pituitaries of infant rats deprived of milk for 24 hr contained 20–30% less vasopressor-antidiuretic activity than those of litter-mate controls, could be so interpreted. It would be remarkable if this applied, for it would mean that antidiuretic hormone is released at an age when the kidneys are unable to respond to it (Heller, 1949; Adolph, 1957). The alternative explanation could be that the neuro-hormonal reflex is not activated, but that withdrawal of food and fluid at so early an age inhibits hormone synthesis or transport. In adult rats withdrawal of food and water for the same period stimulates both the release and—as is shown by the concomitant increase in hormone storage—the rate of synthesis of the antidiuretic principle.

SUMMARY

1. Vasopressor and oxytocic activities were estimated in the pituitary glands of rats from the post-natal age of 1 to 28 days. When compared with the neurohypophyses of adults, the pituitaries of infant rats contained substantially less vasopressor and oxytocic activity. This applied whether hormone content was expressed per milligram of gland or whether it was related to body weight, body surface or kidney weight.

2. The neural lobe of new-born guinea-pigs was considerably richer in activity than that of new-born rats. The hormone content of the neurohypophysis of human infants aged up to 3 days was intermediate.

3. The ratio vasopressor:oxytocic activity in the glands of rats aged 1–5 days varied irregularly from 1·2 to 2·7 and was near unity in older animals.

4. Watery acetone was shown to extract small amounts of oxytocic activity from ox and adult rat neurohypophyses. When chromatographed on paper the oxytocic material extracted was found at the same R_F as that of synthetic oxytocin and was inactivated by thioglycollate. No pressor activity could be detected in the acetone.

5. Acetone extracted relatively larger amounts of oxytocic material from the pituitaries of rats aged up to about 7 days than from the glands of adult animals.

6. After withdrawal of milk for 24 hr the neurohypophyses of rats aged 1–8 days contained less vasopressor activity than the glands of litter-mate controls. In contrast, the neural lobes of adult rats which had been deprived of food and water for the same period contained slightly higher amounts of activity than those of animals with free access to food and water.

7. The relation of these results to morphological findings in the neurosecretory system of infant animals and to over-all maturity at birth is discussed.

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