REVIEW ARTICLE

THE ACTIVE PRINCIPLES OF THE NEUROHYPOPHYSIS

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Work on several aspects of neurohypophysial physiology and biochemistry has been progressing so rapidly during the last three years that a short review of these advances appears justifiable. The most important and most decisive achievements are undoubtedly due to du Vigneaud and his school who succeeded in isolating active principles from posterior pituitary extract in the form of pure polypeptides, to elucidate their structure and to confirm it by synthesis. Much effort has also been spent in attempts to define the site of origin of the activities found in neurohypophysial extracts. In particular the new concept that active principles are elaborated by hypothalamic nuclei and only stored or modified in the neural lobe, has been the subject of numerous investigations. But, the results of all these enquiries only emphasise the fact that the form or forms in which the neurohypophysial activities occur in the body are still unknown. However, it may be useful to survey the present position and to discuss some recent results which touch on this problem.

It must be stressed that even in the limited field reviewed the abundance of published material is such that only a selection of papers would be considered.

The Active Polypeptides (Oxytocin* and Vasopressin*)

It was recognised quite early that the pressor-antidiuretic and oxytocic activities of posterior pituitary gland extracts can be differentially extracted and precipitated[1,2,3] or fractionally adsorbed[2]. Later both approaches were used successfully to achieve almost complete separation and to obtain preparations of very high activity[4–10]. Du Vigneaud and his co-workers[6,11,12] were the first to apply separation by electrophoresis. Waring and Landgrebe[9] point out that the procedures used give a very low yield; their own method of separation gave a much higher yield but not a high degree of purity. The more recent method of Maier-Hüser *et al*[10] is reported to give high yields of relatively pure oxytocic material. However, it is now known that none of these methods of separation and purification led to the isolation of pure compounds. That was only achieved when Craig's method of counter-current distribution[13] became available to the workers at Cornell University[14].

Chemistry of Vasopressin and Oxytocin

A certain amount of useful knowledge was obtained from the analysis of not fully purified material. All investigators agreed for instance that

* It is proposed to allocate these names to the pure polypeptides only. When discussing extracts of the gland and of the hypothalamus, or the biological potency of body fluids, the terms pressor, antidiuretic and oxytocic activity will be used which do not prejudice the issue of the chemical composition of the unpurified principles.

even in partly purified extracts no substances other than amino-acids could be detected. Du Vigneaud and his associates[15], using a hydrolysate of an oxytocin preparation of 500 U/mg. found approximately 9 per cent. of cystine by Sullivan's method[16]; Stehle and Fraser[5] arrived at much the same estimate using the same method on a less pure preparation (250 U/mg.). Potts and Gallagher[17] on the other hand, reported that oxytocic material with a potency of 700 U/mg. contained 18·3 per cent. cystine, again as determined by the Sullivan method. Similar discrepancies were noted when the tyrosine content was estimated in preparations obtained by different workers: the figures were 14·3 per cent.[15], 10·7 per cent.[5] and 14·2 per cent.[17]. The oxytocic preparation of Stehle and Fraser also contained 6·1 per cent. of arginine but Potts and Gallagher reported that their material which was nearly three times as potent, contained less than 0·8 per cent. of arginine. Other amino-acids were also searched for by Stehle and Trister[18] and Freudenberg, Weiss and Biller[19] in hydrolysates of oxytocic and pressor preparations but in view of the lack of purity of their material, their results are by now of historical interest only.

However, beyond establishing it as likely that the active compounds were polypeptides, one further important fact could be ascertained from these preliminary investigations: It occurred to Sealock and du Vigneaud[20] that if oxytocin and vasopressin contained sulphur as the disulphide cystine, it would be interesting to see whether reduction with a mild agent like cysteine at room temperature and neutral *p*H would render these compounds biologically inactive. They found that—in contrast to insulin—the posterior pituitary principles were not inactivated by this treatment. Also after reoxidation, the potency was again almost completely recoverable. The investigators proceeded, in a characteristically thorough fashion, to show that reduction by cysteine had really been obtained. If the disulphide linkage had been reduced to sulphhydryl it also would be capable of benzylation. They showed accordingly that before reduction the potency of the pressor and oxytocic fraction was not affected by benzyl chloride but that biological activity disappeared when benzyl chloride was added after the treatment with cysteine. Figure 1 shows these reactions. It was thus proved that the active polypeptides, as in contrast to contaminating protein or protein derivatives, contain sulphur in organic linkage and that activity depended on the free sulphydryl grouping.

Oxytocin. It has already been mentioned that further purification of polypeptide fractions resulted from the application of the counter-current distribution principle of Craig[13]. The oxytocic fraction was investigated first[14]. A preliminary purification of the starting material (20 U/mg.) was effected by extracting it with 2-butanol. This procedure yielded material with an activity of about 220 oxytocic units per mg. Subjected to distribution between 2-butanol and 0·05 per cent. acetic acid, the most potent material assayed at 575 U/mg. Repeated distribution of solutions of comparable potency yielded ultimately a preparation of about 850 U/mg. The characteristics of the distribution curve suggested that this material was very nearly pure. Oxytocic fractions of this degree of purity were

then used[21,22] to study the amino-acid content after hydrolysis with hydrochloric acid. The chromatographic method of Moore and Stein[23,24,25] was employed which, by the use of three starch columns, allows for the separation of most of the common constituents of protein hydrolysates. The oxytocic substance was found to contain the following eight amino-acids in molar ratios of 1 : 1, leucine, *iso*leucine, tyrosine, proline, glutamic acid, aspartic acid, glycine and cystine. It also contained ammonia in

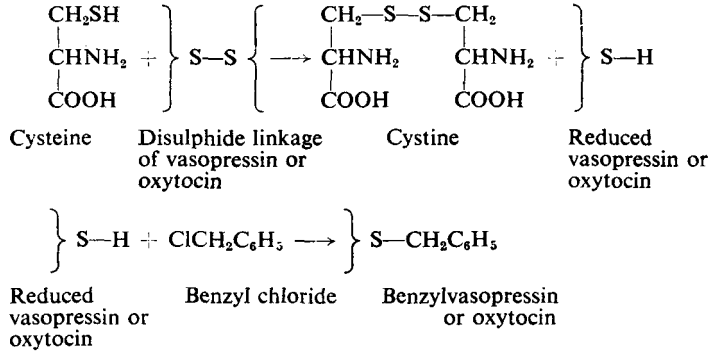

FIG. 1. The reaction of cysteine with vasopressin or oxytocin followed by benzylation.

the molar ratio 3 : 1 to any of the amino-acids. In terms of amino-acid residues and ammonia, these results accounted for 6·5 per cent. of the nitrogen of the unhydrolysed compound and the cystine content accounted for all the sulphur. Privat de Garilha, Maier-Hüser and Fromageot[26] working with a highly purified preparation of oxytocin prepared by the method of Maier-Hüser[10] confirmed these results. It seemed therefore likely that oxytocin was a complex of polypeptide nature composed of eight amino-acids and ammonia. Thermoelectric osmometer[27] measurements indicated a molecular weight in the range of a monomer compound with the amino-acids in peptide linkage and the ammonia in amide linkage. But the arrangement of the amino-acids had still to be determined. Du Vigneaud and his colleagues[28] showed first that oxidation of high potency oxytocin preparations with performic acid yielded a product which upon hydrolysis gave cysteic acid in place of the cystine present in the hydrolysate of unoxydised oxytocin preparation. Applying the dinitrophenyl technique of Sanger[29,30,31] and the *N*-dithiocarboxy method of Levy[32] for determining free amino-acids groups in peptides, they demonstrated further that only tyrosine and cystine were affected. In continuation of the work on performic acid-oxydised oxytocin[28] it was then found that treatment of either oxytocin itself or of the oxidised preparation with bromine water resulted in two fragments[33]. Upon hydrolysis the smaller of these fragments yielded 3 : 5-dibromotyrosine and cysteic acid, the other larger one gave cysteic acid together with leucine, *iso*leucine, proline, glutamic acid, aspartic acid, glycine and ammonia. The small fragment was subsequently[34] identified as β-sulphoalanyldibromotyrosine which suggested β-sulphoalanyltyrosyl as the sequence at the amino end of the

performic acid-oxidised oxytocin molecule. Absorption in the ultraviolet region and other physical constants of synthetic β-sulpho-L-alanyldibromo-L-tyrosine agreed with determinations of the same constants of the smaller fragment[35]. The larger fragment was found to possess a free amino group on the *iso*leucine residue. Its amino-acid sequence was investigated by converting it into the *N*-dinitrophenyl derivative by treatment with dinitrofluorobenzene. The derivative was hydrolysed with hydrochloric acid and the hydrolysate fractioned on Amberlite into neutral and acidic components. The acidic fraction was further fractioned into weakly acidic and cysteic acid peptides. The latter yielded peptides of the following composition*: Asp. Cysteic, [Cysteic, Pro], [Cysteic, Pro, Leu], [Cysteic, Pro, Leu, Gly]. In peptide Asp. Cysteic the presence of a free amino group on the aspartic acid side could be assumed from the application of Sanger's[29] method for end-group analysis. Its composition was confirmed by the isolation of peptide [Asp, Ala] obtained by partial hydrolysis of desulphurised oxytocin. This, together with the three other peptides allowed the following sequence: Asp. Cysteic. Pro. Leu. Gly or (using the symbol CyS– for one half of a cystine residue in a sequence) Asp. CyS–. Pro. Leu. Gly (sequence I) in oxytocin. A number of other polypeptides were isolated from hydrolysates of unchanged oxytocin and from desulphurised oxytocin and their amino-acid composition determined by the same means. Since one of these was peptide [Cysteic, Asp. Glu], sequence I would become

CyS
|
Glu. Asp.CyS Pro. Leu. Gly.

Another peptide from desulphurised oxytocin contained only *iso*leucine and glutamic acid and therefore sequence

CyS
|
*i*Leu. Glu. Asp. CyS Pro. Leu. Gly.

could be derived. A further peptide, viz. [Tyr, CyS–SCy, Asp. Glu], led to sequence

[CyS, Tyr]
|
*i*Leu. Glu. Asp. CyS. Pro. Leu. Gly.

and this arrangement agreed with deductions from previous work[34] which had also suggested the sequence Cysteic. Tyr. *i*Leu. Application of the Edman[38] type of degradation applied to performic acid-oxidised oxytocin confirmed the linkage between tyrosine and *iso*leucine and gave the following sequence for oxytocin[39]:

CyS. Tyr. *i*Leu. Glu. Asp. CyS. Pro. Leu. Gly.

It is remarkable that a young Austrian chemist Tuppy[40] working independently, proposed simultaneously the same arrangement of amino-acids.

* The terminology used is that suggested by Brand and his co-workers[36,37].

Oxytocin would thus be an octapeptide formed of a cyclic pentapeptide containing cystine, with one-half of the cystine moiety possessing a free amino group and with the carboxyl group adjacent to the latter joined to the amino group of tyrosine. The other half of the cystine residue would be connected through its amino group to aspartic acid and would be linked through its carboxyl group to a side chain consisting of the tripeptide prolylleucylglycine.

Based on the evidence so far outlined and on the assumption that two of the three moles of ammonia in the hydrolysate of oxytocin derived from amide linkages involving the carboxyl group of glutamic and of aspartic acid, and the third from an amide grouping derived from glycine, du Vigneaud[41] proposed the following structure for oxytocin (Fig. 2).

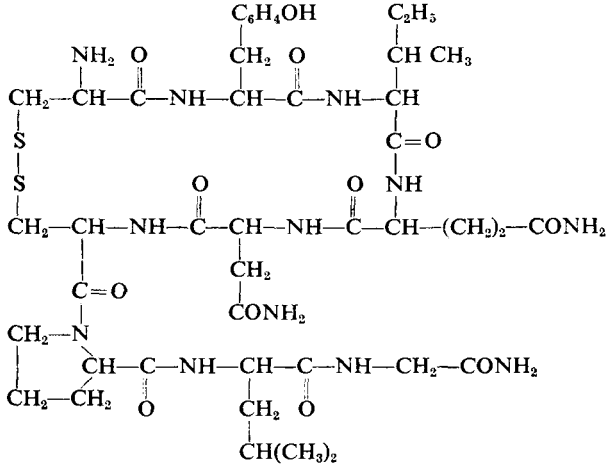

FIG. 2. Oxytocin.

Synthetic oxytocin. It has been mentioned previously (p. 226) that treatment of reduced oxytocin with benzyl chloride resulted in loss of activity[20]. Du Vigneaud and his colleagues[41,42] concluded therefore that, provided oxytocin could be regenerated from the benzylated compound and provided the proposed structure was correct, synthesis of the neurohypophysial peptide should follow from the preparation of the nonapeptide derivative *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosyl-L-*iso*leucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycine amide. Synthesis of this compound was accomplished by coupling *N*-carbobenzoxy-*S*-benzyl-L-cysteinyl-L-tyrosine with the heptapeptide amide, L-*iso*leucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycine amide. It was then treated with sodium in liquid ammonia, followed by aeration in dilute aqueous solution at *p*H 6·5. When subjected to counter-current distribution, the biological activity of the material thus obtained was found to be concentrated in a single peak. Its potency was not distinguishable from natural oxytocin when assayed by the chicken depressor method[43] and it had also the expected activity

when tested on the isolated rat uterus; it was effective on the human uterus *in situ* and produced milk-ejection in women when injected intravenously in a dose of 1 μg. Chemically also, no difference could be detected between the synthetic and the natural polypeptide: synthetic oxytocin showed the specific rotation $[\alpha]_D^{21\cdot5} - 26\cdot1 \pm 1\cdot0°$ compared with $[\alpha]_D^{22} - 26\cdot2$ for the natural product. Flavianates formed by the two substances were identical by all criteria tested. The distribution coefficients in both *s*-butanol-acetic acid and *s*-butanol-ammonia were the same, as was the electrophoretic mobility on paper and the infra-red pattern. The first synthesis of an active glandular peptide had therefore been successfully accomplished.

Vasopressin. Attempts to purify vasopressin prepared by the method of Kamm *et al.*[4] by chromatography were unsuccessful but countercurrent distribution in *n*-butanol-*p*-toluene sulphonic acid led to a product with the potency of about 400 U/mg.[44]. Analysis of this material by the technique of Moore and Stein[23,24,25] showed the presence of eight amino-acids, namely phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, arginine and cystine and of ammonia. The molar ratio of the amino-acids was approximately 1 : 1 and that of the ammonia was 3 : 1. Six of the amino-acids in vasopressin were also present in oxytocin. However, while oxytocin contains leucine and *iso*leucine, phenylalanine and arginine were found in vasopressin. Somewhat later[45] a vasopressin preparation was obtained which gave consistently a potency of about 600 U/mg. in both pressor and antidiuretic assays. It contained the eight amino-acids listed above but no leucine or *iso*leucine, indicating that it was entirely free from oxytocin. This amino-acid composition obtained when beef posterior pituitary glands were used as starting material but analysis of purified material from hog gland concentrates revealed[46] that hog vasopressin contained lysine instead of arginine. Fromageot, Achar, Clauser and Maier-Hüser[47] who purified vasopressin by procedures different from those employed by du Vigneaud and his colleagues confirmed the presence of arginine in ox vasopressin.

The difference in chemical composition of material with the same biological activity is obviously an interesting and unexpected finding though the possibility of differences in the molecular chemistry of the neurohypophysial hormones in different classes of vertebrates has been suggested on entirely different grounds as long ago as in 1941[48,49,50].

Attempts to establish the amino-acid sequence in arginine-vasopressin by methods similar to those used for oxytocin suggested the arrangement[51,52,53]

CyS. Tyr. Phe. Glu. Asp. CyS. [Pro. Arg. Gly.]

with glycine amide in the terminal position[54]. Further work involving enzymatic cleavage of arginine-vasopressin lead du Vigneaud and his co-workers[54] to postulate the structure shown in Figure 3 which would also apply to lysine-vasopressin. These postulates are substantiated by the fact that synthesis parallel to that of oxytocin of the octapeptide structure proposed for lysine-vasopressin led to biologically active material[55].

More recently still du Vigneaud *et al.*[56] were able to report the preparation and partial purification of a product synthesised according to the structure proposed for arginine-vasopressin. The synthetic material after purification by counter-current distribution and electrophoresis, assayed at about 400 U/mg. in terms of both pressor and antidiuretic activity. The position of the activity of the synthetic product in counter-current distribution, electrophoresis and chromatography was the same as that of natural arginine-vasopressin.

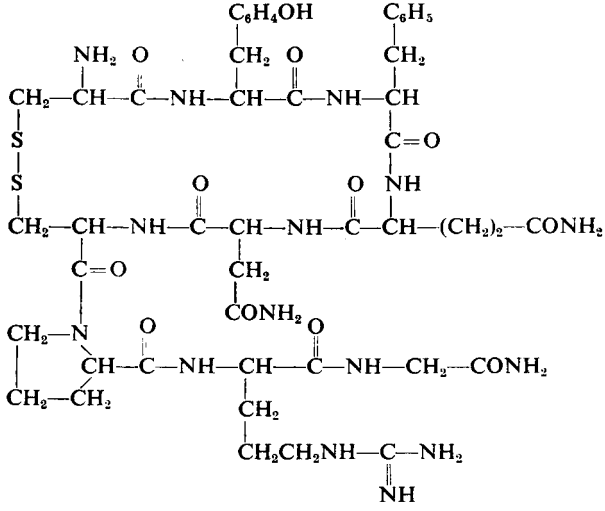

FIG. 3. Arginine vasopressin.

BIOLOGICAL ACTIVITIES OF OXYTOCIN AND VASOPRESSIN

The biological activities of the pure or almost pure polypeptides from beef and hog glands have been carefully examined. Similar assays on synthetic material are as yet incomplete.

Oxytocin. A preparation of oxytocin which, measured by the chicken blood pressure method[43] contained approximately 500 U/mg., showed no detectable pressor response when injected into cats anæsthetised with phenobarbitone[45]. The assay method used was a rather insensitive one. Even more convincing results could no doubt have been obtained by the technique of Landgrebe, Macaulay and Waring[57] since the anæsthetised rats employed in this method of assay respond to as little as about 1 mU. However, Popenoe *et al.*[45] could also demonstrate that their preparation when tested in dogs with experimental diabetes insipidus, contained less than 0·2 U/mg. in terms of antidiuretic potency. There is therefore little doubt that oxytocin possesses neither pressor nor antidiuretic activity. The milk-ejecting activity of an oxytocin preparation of a similar degree of purity was shown to be equivalent to its oxytocic potency[58,59]. The frog "water balance activity" of highly purified or pure oxytocin has not been examined.

Vasopressin. Chromatographic analysis of hydrolysates of highly purified vasopressin (600–650 U/mg.) failed to show the presence of leucine or *iso*leucine indicating that there was no contamination with oxytocin. Nevertheless, when assayed on the rat uterus the material had an oxytocic activity equivalent to about 30 U/mg. and when tested by the avian vasodepressor method an activity equivalent to 80–90 oxytocic units per mg. Coon[43] has pointed out that his chicken blood pressure method gives values 3 to 4 times higher than the uterus assay when pressor activity strongly predominates. The discrepancy between the two results is therefore only apparent and it can be concluded that arginine-vasopressin has an inherent oxytocic activity in the ratio of about 5 oxytocic units to 100 pressor units. Additional evidence for this inherent oxytocic activity of vasopressin was adduced by Taylor, du Vigneaud and Kunkel[52], who showed that the ratios of pressor to oxytocic activity in the pressor component isolated from Pitressin, from a crude vasopressin fraction and from a mixture of vasopressin and oxytocin were identical within the experimental error of the assays. Moreover, when oxytocin was deliberately added to vasopressin and the mixture was subjected to electrophoresis, it was found that oxytocic activity appeared in two peaks with the more basic peak in the vasopressin position. Synthetic arginine-vasopressin had the same ratio of pressor (or antidiuretic) to avian depressor potency as the peptide from beef glands[56].

Antidiuretic assays of vasopressin preparations containing 600–650 and 450 pressor units on rats and diabetes insipidus dogs showed the same number of antidiuretic units. The ratio of pressor to antidiuretic activity of synthetic vasopressin was 1 : 1. The two activities were therefore due to the same chemical compound. Since, however, the site of the pressor and the antidiuretic action (in mammals at least) is a different one, the possibility cannot be excluded that chemically related peptides may show a different ratio of activities.

The Site of Formation of the Specific Activities Found in the Hypothalamico-Neurohypophysial System*

Earlier workers found it difficult to believe that the neural lobe of the pituitary which cytologically does not resemble a glandular structure, was the site of origin of endocrine principles. Later, this difficulty seemed to resolve itself due to two developments, one conceptual and the other factual. The conceptual advance consisted in the recognition that nerve cells may elaborate and release small-molecular substances without histological evidence for a secretory process. The liberation of the neurohumoral transmitters and perhaps that of substance P[60] which may be a polypeptide[61,62], are illustrations of this point. The factual development consisted in the description of a characteristic glia cell in the neurohypophysis of the ox[63] which received the name pituicyte and was regarded

* There is sufficient evidence to regard this system as a morphological and functional entity. It is therefore proposed to adopt the abbreviation HNS where H stands for the hypothalamic nuclei and the axons of the supraoptico-hypophysial tract and N for neurohypophysis (= the neural lobe or pars nervosa plus the median eminence).

as the secretory tissue element. Comparing the pituicytes with other neuroglia cells, Griffiths[64] and Shanklin[65] concluded that they resembled astrocytes and oligodendroglia in having finely granular non-fibrous cytoplasm, typical ovoid gliosomes and cytoplasmic processes but that they differed from these cells in the chick, pig and man in forming numerous interconnections. However, this differention does apparently not apply to the glia cells in the pituitary of the horse[66], the cat and the dog[67]. In the opinion of Vasquez-Lopez[66] and Collin and Stutinsky[68] the concept of "pituicytes" is therefore not justifiable. Gersh[69] and Gersh and Brooks[70] believed that they had shown a relationship between the state of animals and the presence of osmophilic lipoid inclusions in the "pituicytes" but their findings were not confirmed[71,72,73]. Moreover, there is the *a priori* objection that histological observations of this type cannot differentiate between changes in storage and changes in secretion.

Hypothalamic Neurosecretion

A new theory has lately come to the fore. Scharrer[74] had recognised as long ago as in 1928 that certain nerve cells in the hypothalamus of a teleost fish showed histologically demonstrable features indicative of a secretory process. Similar cells were later found in higher vertebrates[75] and in man[76,77] in the supraoptic and paraventricular nuclei. The "neurosecretory cells" resemble ordinary nerve cells in having Nissl substance, neurofibrils and axons but differ from them in showing cytoplasmic granules of various sizes. Larger droplets can be demonstrated with a variety of dyes but the most selective method of staining—as first shown by Bargmann[78]—is that with Gomori's chrome-hæmatoxylin-phloxin, which stains the neurosecretory material a deep blue.

Pathway of secretion. Assuming that the Gomori-positive substance represents secretory products, how does this material reach the circulation? There seem to be three possibilities: (*a*) Direct secretion into the blood. There is no morphological evidence for such a process. It is also unlikely for functional reasons (see p. 238). (*b*) Secretion into the third ventricle. No histological observations seem to be known which would suggest that this secretory pathway is important in higher vertebrates. Moreover, if, as now seems likely, the neurosecretory cells in the hypothalamic nuclei produce substances whose activities resemble those of neurohypophysial extracts, such activities should be detectable in the cerebrospinal fluid. However, cerebrospinal fluid produces neither oxytocic nor vasopressor effects which can safely be attributed to endocrine principles[79]. Vogt[80], for example, who used the most recent methods of assay found no activity ($<2{\cdot}5$ mU/ml.) in dog's cerebrospinal fluid. (*c*) Transport along the supraopticohypophysial tract. Secretory material seen in the nerve cells has also been traced along the axons into the infundibulum[78,81,82,83]. Experimental evidence suggesting a flow from the nuclei to the gland was adduced by Hild[84] who cut the pituitary stalk in frogs and observed the accumulation of Gomori-positive material rostral to the cut. Similar results in mammals were obtained by Scharrer and Wittenstein[85] and Hild and Zetler[86] in the dog and by Stutinsky[87] in the rat.

Mode of transport along axons. It has been suggested[88,89] that the secretory material in the hypothalamic nuclei is carried by the axoplasm current. The rate of plasmatic flow has been determined to be about 3 mm. per day which would agree with the speed with which neurosecretory material has been seen to move along crustacean axons[90,91]. But experimental evidence which will be discussed later (p. 241) raises the question whether this slow rate would account for the relatively high speed with which the mammalian neurohypophysis can be repleted (see also Rothballer[98]).

Storage in the neural lobe. Smith[92] has estimated that the mass of neurosecretory material in the p. nervosa of rats killed with pentobarbitone amounts to approximately 25–30 per cent. of the total bulk of the gland. Within the neurohypophysis no Gomori-stainable substance has been seen in the "pituicytes." The Herring-bodies are now regarded as nerve fibres bulging with neurosecretory products[78,93,94,95]. The Gomori-positive substance seems to be spatially related to the intraglandular blood vessels: it surrounds the vessels in the human[96] and the monkey neurohypophysis[89]. In the opossum[95] it is associated with the nerve terminals of the supraopticohypophysial tract which approach the blood vessels at an acute angle.

Release from neurohypophysis. Material staining with Gomori's method or chrome hæmatoxylin has been found in the neurohypophysial vessels of the giraffe[97], the rat[98] and the dog[89].

Relationship Between Neurosecretory Material and the Posterior Pituitary-like Activities in the Hypothalamico-Neurohypophysial System

The biological activity of hypothalamic extracts. Their cytological observations on the secretory activity in the hypothalamus led Bargmann and Scharrer[98a] to the concept that the "neurohypophysial hormones" originate not, as hitherto assumed, in the posterior pituitary but in the supraoptic and paraventricular nuclei. To make this theory acceptable it had to be shown that there is a quantitative relationship between the neurosecretory material in any part of the HNS and the biological activity that can be extracted.

Table I shows that pressor, antidiuretic and oxytocic effects have been obtained with hypothalamic extracts of several mammalian species. Melville and Hare[103] showed in dogs that the antidiuretic activity in hypothalamic extracts came mainly from the region of the supraoptic nuclei. Hild and Zetler[106] in the same species dissected the region of the supraoptic and paraventricular nuclei separately and, using half of the hypothalamus for histological controls, estimated the hormone content in the other half and compared it with extracts of the tuber cinereum and of the posterior lobe.

An association of posterior pituitary-like activities with the Gomori-positive material in the HNS has thus been substantiated but the quantitative interpretation of the results shown on Table I is difficult: simple extracts of hypothalamic tissues such as those used by Hild and Zetler[106] and Dicker and Tyler[107] contain biologically active substances which must be differentiated from those in posterior lobe preparations. Vogt[80] has

pointed out that noradrenaline may occur in the hypothalamus in concentrations as high as 1–2 μg./g. fresh tissue and Dicker and Tyler[107] reported that they were unable to do pressor assays in rats with extracts of dog hypothalamus because a fall of blood pressure was produced almost invariably. Histamine and acetylcholine were ruled out but they may have been dealing with substance P the concentration of which in the hypothalamus is high[62,109]. Moreover, 5-hydroxytryptamine is also found

TABLE I

POSTERIOR PITUITARY-LIKE ACTIVITIES IN THE HYPOTHALAMUS

Species	Type and amount (in I.U.) of activity in hypothalamus (means)			Ratio pressor/ oxytocic	Ratio neurohypophysis /hypothalamus (pressor activity)	Remarks	Reference
	Pressor	Anti-diuretic	Oxytocic				
Sheep	Present	—	Present	—	—	Ant. hypothalamus	Abel[99,100]
Dog	—	Present	—	—	—	Ant. hypothalamus including supraoptico-neuroh. tract	Sato[101]
Dog	—	1·5–5·0	—	—	6·7–4·0*	Whole hypothalamus and supra-optic region separately	Trendelenburg[102] Melville and Hare[103]
Rat	—	Present	—	—	2·0	?	Melli[104]
Rat	—	Present	—	—	—	Whole hypothalamus	Kovacz and Bachrach[105]
Dog	3·11	2·40	0·67	4·6	5·8*	Ant. hypothalamus	Hild and Zetler[106]
Cattle	0·23	0·20	0·095	2·4	450·0	Ant. hypothalamus	Hild and Zetler[106]
Pig	0·25	0·14	0·18	1·4	160·0	Ant. hypothalamus	Hild and Zetler[106]
Man	0·23	0·14	0·26	0·9	50·0	Ant. hypothalamus	Hild and Zetler[106a]
Dog	3·8†	3·7†	0·20†	19·0	31·0	Ant. hypothalamus	Vogt[80]
Dog	—	0·76	0·01–0·05	—	10·0	Whole hypothalamus (?)	Dicker and Tyler[107]
Dog	7·62†	—	0·70†	9·5	—	Whole hypothalamus (?)	Schlichtegroll[108]
Cat	0·16†	—	0·19	1·0	—	Whole hypothalamus (?)	Schlichtegroll[108]

* Antidiuretic activity. † Units per g. fresh tissue, adult animals only.

TABLE II

EXCRETION OF ANTIDIURETIC ACTIVITY IN THE URINE OF "NORMAL" ADULT ANIMALS AND MEN AFTER THE INTRAVENOUS ADMINISTRATION OF POSTERIOR PITUITARY EXTRACTS OR PITRESSIN

Species	Mode of administration	Dose	Per cent. of administered activity excreted		Reference
			Range	Mean	
Man	Single injection	0·12 to 1·5 U.	0–7·4	5·0	Burn and Singh Grewal[148]
Man	I.v. infusion	3 to 5 U./10 min.	4·5–30	12·8	Noble and Taylor[149]
Dog	I.v. infusion	0·5 to 2·5 mU./min.	7–15	—	O'Connor[258]
Rat	Single injection	100 mU./100 g.	4·9–8·0	6·7	Ginsburg and Heller[142]
Rat	I.v. infusion	0·3 to 1·5 mU./100 g./min.	3·1–22·0	8·1	Dicker[170]
Rat	I.v. infusion	5 mU./100 g./min.	10·2–28·5	—	Ginsburg[171]

in the hypothalamus[109]. The presence of these "contaminations" may introduce errors into pressor and oxytocic assays. Introduction of significant inaccuracies into estimations of antidiuretic potency seems less likely since very high dilutions of the extracts can be used.

Vogt[80] in a careful investigation has taken most of these difficulties into account. She extracted hypothalamic tissue of dogs with ethanol acidified with hydrochloric acid, and separated the sympathomimetic amines by chromatography from the other active constituents. The pressor, antidiuretic and oxytocic effects found were completely abolished after incubation with sodium thioglycollate[110] which suggests strongly that she was dealing with posterior pituitary-like activities only. However, the hypothalamic extracts differed from those of the neural lobe in having a much lower oxytocic potency; the ratio pressor to oxytocic activity varied from 12 to 40 in adult dogs, and from 10 to 20 in dogs aged 10 to 11 weeks. The possibility that the oxytocic values were too low because the eluates contained inhibitory substances could be excluded by testing extracts to which oxytocin had been added after treatment with thioglycollate. Hydroxytryptamine as the oxytocic agent could be ruled out, since the activity persisted after dihydroergotamine (which blocks the action of 5-hydroxytryptamine) had been added to the uterus bath. Likewise, since substance P is not inactivated by thioglycollate[111], the oxytocic activity in dog's hypothalamus is unlikely to have been due to contamination with that substance. However, it will be remembered (p. 232) that there is strong evidence that vasopressin has some intrinsic oxytocic activity and hence Vogt made the interesting suggestion that the substance extracted from the hypothalamus was pure vasopressin. Where then could the oxytocin in the posterior lobe of the dog originate? Theoretically there would seem to be the following possibilities: Firstly that oxytocin—as in contrast to vasopressin—is elaborated by the gland. Secondly that the active principle extracted from dog's hypothalamus is a precursor of both vasopressin and oxytocin which is modified on its way to or in the neurohypophysis and thirdly that "hypothalamic" vasopressin is converted into oxytocin in the neurohypophysis. The latter process meets the difficulty that two of the amino-acids in vasopressin would have to be substituted by two others, in other words a radical reshaping of the molecule would be necessary. However, before exploring these possibilities experimentally it may be as well to investigate whether oxytocin or an oxytocin-like substance occurs in the hypothalamus of other species. The results of Hild and Zetler[106] and Schlichtegroll[108] suggest such species differences. Thus, while the similarity of distribution in the HNS between the "neurosecretory material" (= Gomori-positive substance) and biological activity has been demonstrated, it is not certain whether the same "hormone"* or "hormones" occur in the hypothalamic nuclei and in the posterior lobe.

* The question may be raised whether the term "hormone" should be applied to the pituitary-like principles extracted from the hypothalamus. The original meaning of hormone[112] was that of a substance entering the blood and acting as "messenger" in the effector organ. Since it cannot be excluded that—at least in some species[80,108]—the hypothalamic substance(s) are chemically altered before they reach the circulation, some such term as pro-hormone may have to be used.

Age dependance of the distribution of neurosecretory material and biological activity. One of the ways by which the parallelism between the occurrence of Gomori-positive granules and posterior pituitary-like activities has been explored consisted in the study of the HNS before and shortly after birth. Bargmann[78] failed to find Gomori-positive material in the supraoptic nucleus or the neural lobe of the newborn dog though there were a few poorly staining granules in the pituitary stalk. In puppies aged one week secretory granules were seen in isolated ganglion cells; the supraoptic nucleus of dogs aged 25 days showed signs of vigorous neurosecretion and the neurohypophysis contained "storage material" resembling that of adult animals. Scharrer[113] found material stainable with Gomori's method in the neurohypophysis of dog fœtuses about a week before birth. Later, but still a few days before birth granules in cells of the supraoptic and paraventricular nucli were just discernable and occurred quite frequently in the axons. In puppies 5 days after birth, storage of neurosecretory material was demonstrable but did not compare in intensity with that of dogs aged $5\frac{1}{2}$ months. These findings agree with Vogt's[80] results of pressor and oxytocic assays on extract from the ventral hypothalamus of puppies, in so far as much less activity per unit weight was found than in hypothalamic extracts from adult dogs. They would also agree with the observation of Dicker and Tyler[107] that in the dog's neurohypophysis, pressor and antidiuretic activity can be found some time before birth.

The human fœtus at 16 weeks has already well definable supraoptic and paraventrivular nuclei but though there was some diffuse staining with Gomori's chrome-alum hæmatoxylin, no definite granules could be identified[96]. Well defined granules were first seen at 19 to 20 weeks of gestation. All later stages showed numerous ganglion cells filled with blue granules which also extended into the dendrites. In the pituitary, Gomori-positive material first appeared in fœtuses older than 23 weeks; the amounts in the very young fœtus were small but a steady increase was seen up to the 34th week of gestation. Whether the quantitative distribution of Gomori-stainable substance at birth compares with that in the adult neurohypophysis has not been stated. Both pressor and oxytocic activities have been reported to be demonstrable in the pituitary of human fœtuses as young as 10 to 16 weeks[114] but quantitative assays were only possible in fœtuses older than 16 weeks. At that stage and up to 28 weeks of gestation pressor activity exceeded oxytocic activity considerably but at birth the ratio approaches parity[115]. However, even in the pituitary of the newborn infant antidiuretic and oxytocic activity per mg. neurohypophysial tissue is about 5 times lower than in the adult gland[115].

The neurohypophysis of newborn rats contains much less (and histochemically apparently differing[115a]) neuro-secretory material than that of adult animals[88] which is in accordance with the small amounts of antidiuretic activity found in rats killed 18 to 42 hours after birth[116]. It may be that the postnatal production or storage of active principles in this species increases rapidly since the figures for hormone content given by Dicker and Tyler[114] for rats aged 5 days are considerably higher than those for

"newborn" animals. However, it is difficult to say from the results of these authors whether the amounts they found in animals aged five days approach those in the glands of adult rats considering that their figures for hormone content in the glands of adult animals are much lower than those usually found[116–123]

Experimental changes of the quantitative distribution of neurosecretory material and biological activity. Section of the pituitary stalk. It has already been mentioned (p. 233) that section of the pituitary stalk results in the accumulation of Gomori-positive material in the rostral stump. A corresponding increase in the yield of posterior pituitary-like activities from this area has recently been reported[124]. The increase in neurosecretory substance at the ends of the cut nerve fibres is only a temporary phenomenon: In dogs which had been killed 1 to 48 days after stalk section, maximal accumulation of stainable material was observed about a week after the operation, later the swollen ends of the cut fibres and the neurosecretory material disappeared[85]. If identity or close relationship of neurosecretory material and neurohypophysial activities is accepted this finding would argue strongly against a release of antidiuretic hormone from anywhere but the neurohypophysis since diabetes insipidus may follow neurohypophysectomy in dog within the period during which the neurosecretory material is accumulating[125,126]. Stalk section causes polyuria lasting 1–11 days after the operation but this "temporary phase" is followed by a "normal interphase" during which the fluid exchange is within normal limits for 2–11 days before the "permanent phase" of diabetes insipidus sets in. The "normal interphase" is regarded by Heinbecker and White[127] as a period of normal function of the neurohypophysis between its temporary inactivation by the operation and degeneration. O'Connor[126], however, suggests that the release of antidiuretic hormone during this phase is one stage of the process of degeneration of the denervated gland. It could perhaps also be argued that antidiuretic material is freed from the degenerating rostral end of the stalk but the question requires study of the vascular connections of the stump after stalk section and removal of the neurohypophysis. In the rat, Stutinsky[128,129] found increased amounts of Gomori-positive material in the remains of the pituitary stalk after hypophysectomy and this phenomenon persisted up to two months after the operation. Antidiuretic activity in extracts from the cut stalk and the region of the supraoptic nucleus likewise persisted but was lower than in non-operated controls. It is difficult to interpret these results since the author stresses himself that the hypophysectomies were not complete. His report that the supraoptic nucleus did not degenerate completely and that the water balance of his animals was maintained[87] would be in accordance with this fact.

Dehydration. The amount of antidiuretic hormone in the pituitary of thirsting rats appears to depend on the length of the period of dehydration. Ames and van Dyke[130], who deprived their animals of fluid for 3 days found a significant increase of antidiuretic activity in the neurohypophysis. A similar increase was found in thirsting kangaroo rats. Simon[117] reported a "slight decrease" in the glands of rats kept on a dry diet for

four days. After 6 or 7 days of fluid withdrawal the hormone content decreased markedly to about 25 per cent. of the control values, both the pressor and the oxytocic activity being affected. Much the same result was obtained in cats, guinea-pigs and rabbits[131]. Gersh[69] and Hickey, Hare and Hare[71] confirmed Simon's results in rats, but in contrast to Ames and van Dyke[130] found a decrease in potency from the second day of dehydration. These results may be compared with those of Ortmann[132] who, by photoelectric measurements of the colour intensity of stained serial sections, attempted to obtain a quantative estimate of the effect of

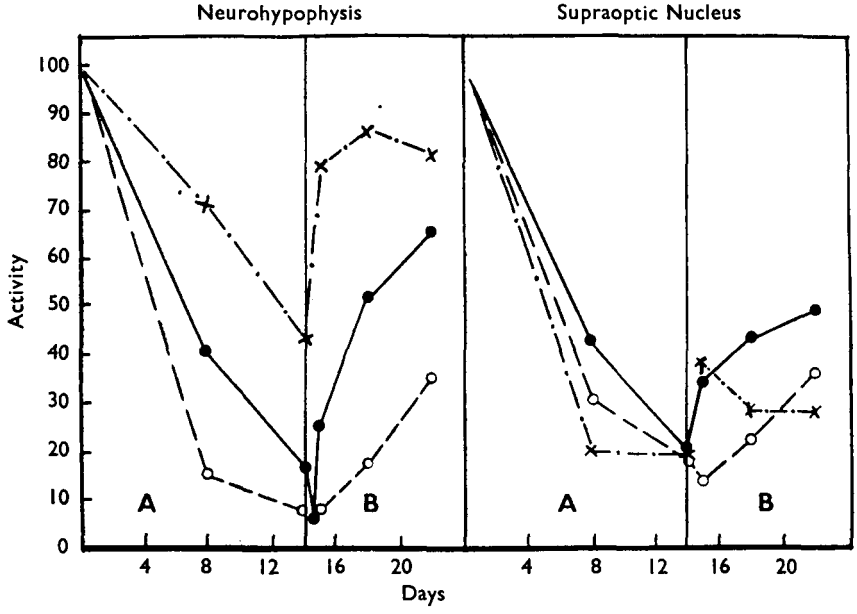

FIG. 4. Effect of A, dehydration and B, rehydration on the content of Gomori-stainable material (———), antidiuretic (— — —) and oxytocic (— · — · —) activity in the neurohypophysis and the supraoptic nucleus of the dog (mean results in 10 animals). Drawn from data of Hild and Zetler[86].

dehydration on the Gomori-positive material in the rat pituitary. He found a doubtful decrease in neurosecretory material during the first days of dehydration. After 8 days however, the Gomori-positive substance in the glands was much diminished and had disappeared completely after 13 to 16 days. The amount of stainable material increased again when access to water was permitted. Sawyer and Roth[135] found that the oxytocic potency in the pituitary of rats after 10 days of dehydration decreased from 219 to 75 mU/100 g. bodyweight and that both the hypothalamus and the neurohypophysis showed depletion of Gomori-stainable material. Hild and Zetler[86], using a similar method as Ortmann[132], measured the effects of dehydration on the quantity of Gomori-stainable substance in the neurohypophysis and the hypothalamic nuclei of dogs. Pressor, antidiuretic and oxytocic activities were measured in the gland and nuclei of the same animals. Figure 4 summarises some of their

results. There is clearly considerable resemblance between the rate of decrease in the biological activities and that of stainable material during dehydration and similarly between the rate of repletion after access to fluid has been permitted. But the combined errors of the methods used are too large to assess the degree of parallelism with sufficient confidence. In an important extension of this work[86] the authors furnished probably the best evidence for the hypothalamic origin of posterior pituitary activities. When the pituitary stalk was cut in dogs whose neurohypophysis and nuclei had been depleted by dehydration, there was no repletion of the neurohypophysis when the operated animals were again allowed to drink, but both the biological activity and the stainable material increased in the structures rostral to the cut.

Response to the administration of hypertonic salt solutions. Loss of antidiuretic activity in the pituitaries of animals receiving sodium chloride solutions has been reported by Kuschinsky and Liebert[118], Chambers[133], Kovacz and Bachrach[105] and Cavallero, Dova and Rossi[120]. The morphological counterpart to these investigations has been supplied by Ortmann[132], Stutinsky[94] and Leveque and Scharrer[134].

Adrenalectomy. Kovacz and Bachrach[105] and Cavallero *et al.*[120] found that bilateral adrenalectomy in rats decreased the antidiuretic activity in the pituitary. Sawyer *et al.*[135] on the other hand, found an increase in oxytocic potency. A decrease of neurosecretory material in the pituitary of adrenalectomised rats has been observed by Eichner[136] and by Malandra and Corbetta[137], but not by Sawyer and Roth[135].

Other secretory stimuli. High environmental temperatures have been reported to lead to the discharge of antidiuretic hormone[122] and the disappearance of Gomori-positive substance[132] from the neurohypophysis. It would also appear that pain produces a noticeable depletion of the neurosecretory substance in the rat hypophysis within a few minutes of the application of the stimulus[98,88]. This would be compatible with a very rapid release of the Gomori-positive substance into the circulation —though the possibility of some transformation which renders it less visible cannot be excluded.

A relationship between the distribution of "neurosecretory" material and the biologically active substances in the HNS at different stages of its development and under a variety of experimental conditions is evident from these investigations. But there are several reasons why no more than a rough parallelism can be expected: when discussing the first appearance of Gomori-stainable substance in the neurons of the hypothalamic nuclei, Scharrer[89] points out that "synthesis of the neurosecretory material must have preceeded the appearance of microscopically visible granules." Similarly, it may be suspected that some of the neurosecretory substance may be present in a sub-microscopical form at later stages of development. Even more important in this connection are the reports of Hild[138] and Hild and Zetler[139] that the Gomori-positive substance in the neurohypophysis can be separated from the biological activities by extraction with absolute ethanol or ethanol-chloroform mixtures. The stainable material is therefore regarded as a carrier[139,89] with the properties of a lipid[139] or a

lipoprotein[140,141] which prevents the release of the water soluble active polypeptides from the cell. The possibility can obviously not be discounted that, depending on the metabolic state, some of the activities in the cell are linked to carriers other than the Gomori-stainable material or not "bound" at all, or again that hormone is released into the circulation while some of the carrier substance remains in the pituitary.

Secretory Stimuli

Acceptance of the theory of the hypothalamic origin of neurohypophysial activity or activities requires re-definition of the nature of stimuli to the HNS. Present concepts may be summarised as follows: a variety of stimuli (for review see Heller[50]) which may be of peripheral or central origin, and which may be nervous or humoral, are conducted to the hypothalamic nuclei. From there nervous impulses pass through the supraoptico-hypophysial tract and cause the release of hormone(s) from the neurohypophysis. This view leads to the endowment of the neurosecretory cell with a double function namely that of receiving and conducting impulses, and that of elaborating and transporting the substance which it releases. Division into conducting and secretory neurones is another possibility. However, it is difficult to assume that all stimuli received by the supraoptic (and paraventricular) nuclei are only concerned with the release (or the inhibition of the release) of the neurohypophysial active principles. One may assume—on the analogy with other glands—that some stimuli are also concerned with the rate at which the neurosecretory material is elaborated. In other words *trophic* stimuli may have to be distinguished from *tropic* ones, i.e., from stimuli for the release of hormone from the pituitary. The functional linkage and inter-dependance of these stimuli is unknown but it will have to be considered in investigations concerned with the amounts and the ratio of active principles in the hypothalamus and the neurohypophysis. For example, Ginsburg and Brown[123] in recently completed experiments, have found in confirmation of previous findings[142] that withdrawal of blood from anæsthetised rats leads to the increased release of antidiuretic hormone into the circulation. However, when the amount of activity in the posterior pituitary of such animals was estimated, there was no depletion of the gland beyond that observed in animals which had been anæsthetised only. Indeed in rats anæsthetised with pentobarbitone there was a significant increase of pressor and oxytocic activity in the neurohypophysis. It may therefore be inferred that hæmorrhage besides being a tropic stimulus also increases production in the hypothalamus and/or the rate of transport of hormone or hormone precursors to the neurohypophysis.

The State of the Neurohypophysial Principles in the Body

Chemical and physicochemical evidence. It has already been pointed out that it is not certain in what way or with what constancy the active principles in the HNS are associated with Gomori-positive material. Nor do we know—in spite of du Vigneaud's brilliant work—in which form or forms these activities occur in the gland and in the circulation. It

has been held on one side that the antidiuretic-vasopressor and oxytocic principles occur as separate entities in the gland and that they can be differentially released, and on the other[143,144] that these activities form part of a large protein-like molecule which may also be encountered in body fluids[110,130]. The latter concept—Abel's "unitary hypothesis"—received support from the work of Rosenfeld[145,146] who compared the behavior of purified pressor and oxytocic preparations with that of untreated press juice from posterior lobes in the ultracentrifuge. The purified preparations showed very little tendency to sediment even after almost 6 hours at 61,000 r.p.m. On the other hand, both activities sedimented rapidly and at approximately the same rate when press juice was used instead. When press juice was mixed with 0·5 per cent. acetic acid and boiled, ultra-centrifugation of the filtrate showed the presence of both slowly and rapidly sedimenting particles. Van Dyke and his colleagues[144] went considerably further by isolating from bovine posterior pituitaries a protein which contained the active principles in constant amounts and in a ratio identical with that in fresh or acetone-dried glands. The molecular weight of the protein, as calculated from its sedimentation-constant in the ultracentrifuge, was about 30,000. Van Dyke and his associates adduced the following findings as proof for the purity of their material: (1) the protein gave a solubility curve typical of a pure substance; (2) its behaviour in the ultracentrifuge as judged from "Schlieren" patterns, was that of a pure protein; (3) assay of various fractions from solubility, electrophoresis and ultracentrifugation tests gave no indication of the presence of components of greater or lesser potency than the protein itself.

Extending their investigations to the antidiuretic material found in the urine of dogs after hormone release from the posterior pituitary had been stimulated by dehydration or by the intracarotid injection of hypertonic sodium chloride solution, van Dyke and his co-workers[110] found that the antidiuretic activity underwent sedimentation in the ultracentrifuge. So did the endogenous antidiuretic material in the urine of kangaroo rats[130]. These findings would be in harmony with the view that the neurohypophysial activities may occur as a large molecule not only in the posterior lobe but also in urine and therefore perhaps in the blood. Although van Dyke and his associates believed that their results were strongly in favour of the homogeneity of their "active protein" and that the activities form an integral part of its molecule, they did not deny the possibility of adsorption of the hormones to an unknown substance of high molecular weight. Du Vigneaud and Irving[147] after a critical discussion of the physicochemical criteria for purity used, concurred with this view and proposed further tests to examine homogeneity.

When characterising their "large molecular compound" van Dyke *et al.*[144] pointed out that it differed from the highly purified preparations of vasopressin and oxytocin in its behaviour towards the reducing action of cysteine and thioglycollic acid. It has been mentioned before (p. 226) that treatment of oxytocin and vasopressin, i.e., of the pure polypeptides with cysteine caused no loss of biological activity[20], whereas van Dyke

et al. were able to show that at *p*H 7·5 (i.e., at the same hydrogen ion concentration as that used in the experiments with the purified principles) cysteine, after reduction for 5 minutes, caused the loss of 75 to 85 per cent. of the oxytocic potency of their active protein. A chemical difference between the native neurohypophysial principles (or more correctly between neurohypophysial activities which have not been subject to a rigorous process of purification) and du Vigneaud's peptides is thus suggested but not proved since inactivation may have been due to the blockage of the reduced thiol groups (see p. 226) by some substance present in impure extracts. However, the different reaction to reducing agents can, in any case, not serve as support for the "unitary" hypothesis until we know how the separated but not purified active principles react to cysteine. Separation by a "mild" procedure like electrophoresis by which fractionation of the oxytocic and pressor activities can be obtained in untreated press juice from fresh posterior lobes[11,12] may be suggested. Since the commercial preparations Pitressin and Pitocin are easily inactivated by thioglycollic acid, inactivation of the less purified principles by cysteine would not be unexpected.

Ames, Moore and van Dyke[110] have shown that the antidiuretic activity of Pitressin of the purity of 80 pressor units per mg. may show sedimentation in the ultracentrifuge which indicates that such extracts may contain the separated activity as a larger molecule than that of the pure octapeptide. Adsorption to impurities can again not be excluded but another possibility may perhaps be adduced: the molecular weight of insulin was originally reported as about 35,000[148,149] but it was suggested by Crowfoot[149] that this molecular weight was due to the presence of insulin in a polymeric form containing three sub-units. This conclusion has been substantiated by Gutfreund[150,151] and others[152,153] who described several examples of reversible dissociation into sub-units under the influence of *p*H, temperature, electrolyte concentration and the type of electrolyte present in the solution. It may not be too fanciful to consider the possibility of a similar agreggation of the neurohypophysial active peptides.

Biological evidence. If all neurohypophysial activities are attributable to one hormone, artificial and natural stimuli to the posterior lobe should produce a multiple simultaneous effect resembling that of an injection of mixtures of vasopressin and oxytocin. Such multiple effects have been found in many investigations: electrical stimulation of the pituitary stalk caused increased uterine activity and antidiuresis[154–158]; antidiuresis inducing measures (intracarotid or intravenous injections of hypertonic sodium chloride solution, intravenous injection of hypertonic sucrose, intracarotid injection of acetylcholine) produce milk-ejection[159] and stimulate uterine movements[160] and suckling is accompanied by a decrease in urine flow[161,162,163,164]. These results appear to favour the "unitary" hypothesis, but difficulties arise when the activities released are assessed quantitatively. In the investigations mentioned above, Peeters[165] and Cross[162] found an apparent release of pressor and oxytocic activity in the ratio of 1 to 100, Abrahams and Pickford[160], in experiments designed to

release antidiuretic activity, found antidiuretic/oxytocic ratios of 1 to 15 to 1 to 20 and Harris[157,158] using electrical stimulation found ratios of 1 to 4 to 1 to 10. There is thus general agreement that a variety of stimuli to the neurohypophysis causes a secretion which seems much richer in oxytocic than in pressor or antidiuretic potency. As pointed out by Abrahams and Pickford[160] it is difficult to understand how glands containing antidiuretic-pressor and oxytocic activity in equal proportions (or in the rabbit in the ratio pressor/oxytocic 2 : 1[166,108]), release these activities simultaneously in anything from a pressor to oxytocic ratio of 1 to 4 to 1 to 100.

Another indication that the main activities of the neural lobe may be released separately is furnished by recent reports[107,114] that in lactating rats, guinea-pigs, cats and dogs the ratio of pressor to oxytocic activity in the neurohypophysis increases in favour of the pressor potency. Similar experiments in lactating goats[167] failed to show alterations of the ratio.

Antidiuretic activity in urine. The renal excretion of antidiuretic activity after the injection of posterior pituitary extract or Pitressin has been studied by several investigators. Table II (see p. 235) summarises some recent results. It will be seen that while the mean values tend to approach 10 per cent., individual figures for percentage excretion vary widely. More systematic investigations, using a wider range of doses, seem indicated.

The chemical form in which the antidiuretic material, whether exogenous or endogenous, is excreted by the kidneys has not been ascertained. The possibility of a transformation of the neurohypophysial antidiuretic "hormone" in the kidney into a substance with less antidiuretic action than vasopressin has been considered[172,142] but it is also possible that most of the antidiuretic activity which reaches the renal circulation is rapidly destroyed and that small amounts escape unchanged into the urine. The former possibility, namely transformation of injected vasopressin into a substance with antidiuretic activity equivalent to about 10 per cent. of that of Pitressin, has recently been postulated by Dicker and Greenbaum[173,174] who base their conclusions mainly on inactivation experiments with rat kidneys *in vitro*. It has been shown[142,175] that in the rat a substantial fraction of intravenously injected Pitressin is cleared by the kidneys and that most of the remaining portion is removed in the splanchnic vascular bed though not necessarily by the liver. The assumption that about half of the dose injected is inactivated in (not merely cleared by) the liver is an important part of Dicker and Greenbaum's theory but the recent results of Mathé and Altman[176] which have been confirmed by Lauson[177], suggest that this premise is not securely based.

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