REVIEW ARTICLE

RELEASE AND METABOLISM OF THE NEUROHYPOPHYSIAL HORMONES

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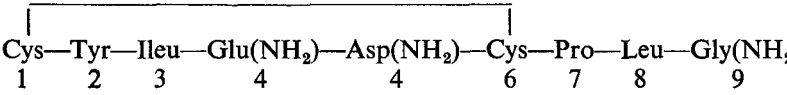
This article attempts to review a subject which is in a state of rapid expansion. The literature has not been covered completely, and the choice of works mentioned has been intentionally selective. Furthermore, points which are in process of elucidation have been omitted as their inclusion, at this stage, might have introduced controversial arguments.

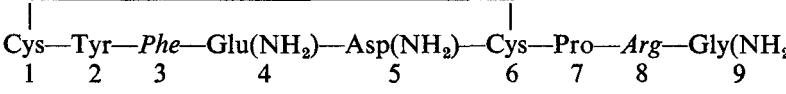
Definitions

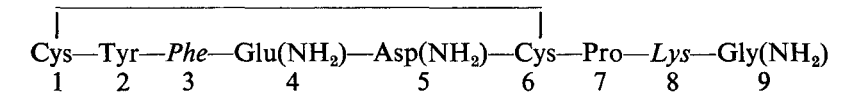
A substantial amount of confusion exists in the nomenclature of the neurophysical hormones, due mainly to the fact that commercial preparations have been used without any reference to their composition or even standardisation. For instance, preparations labelled as "vasopressin, B.P." suggest to the British research worker that they conform to the recommendations laid down by the British Pharmacopoeia, whereas in fact some of them are made according to the U.S. Pharmacopeia. The British Pharmacopoeia, in agreement with the Permanent Commission on Biological Standardisation of the League of Nations Health Organisation (Bangham and Musset, 1958), requires that the "Standard Preparation is a quantity of acetone-dried powder obtained from the posterior lobes of fresh pituitary bodies of *oxen*"; but the U.S. Pharmacopeia stipulates that the "Posterior Pituitary is a powder prepared from the clean, dried, posterior lobe of the pituitary body of *domestic animals* used for food by man," thus allowing the use of pigs' pituitaries as a source of vasopressin solution. With regard to the standardisation, it must be remembered that according to the British Pharmacopoeia the only suggested methods for the extract of the posterior pituitary lobe powder are assays of its oxytocic activity, with the result that it is not necessary to state its antidiuretic or pressor activity.

Oxytocin is the name given to the oxytoxic hormone extracted from the posterior lobe of the pituitary gland. Its formula is:



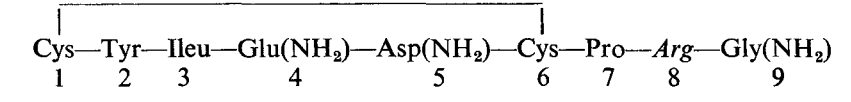
and, as far as it is known, its chemical structure is the same in all vertebrates. The name of the antidiuretic hormone of higher vertebrates is vasopressin. There are, however, at least two vasopressins, the arginine[8]-vasopressin and the lysine[8]-vasopressin, with the following formulae:





Arginine-vasopressin has been found in man, ox, horse, sheep, rat, dog, monkey, camel and marsupial (Acher and Chauvet, 1953; Acher, Chauvet and Lenci, 1959; Light and du Vigneaud, 1958; Sachs and Barrett, 1959; Sawyer, Munsick and van Dyke, 1960) whereas lysine vasopressin exists in hog and hippopotamus (Heller and Lederis, 1960; Popenoe, Lawler and du Vigneaud, 1952). Physiologically the two vasopressins differ markedly: the pressor activity of arginine-vasopressin is 600 u./mg. and that of lysine-vasopressin 300; when lysine-vasopressin and arginine vasopressin are injected intravenously in equipressor amounts in dog, rat or man, the latter has a much greater antidiuretic activity than the former (Dicker and Eggleton, 1961; Nielsen, 1958; Thorn, 1959; van Dyke, Engel and Adamson, 1956). It is therefore important that the antidiuretic hormone should be specifically defined as arginine-, or lysine-vasopressin. Works in which the secretion of antidiuretic hormone has been estimated using as standards commercial preparations of posterior pituitary extract or vasopressin solutions without reference to their composition or origin, must be considered with the utmost misgiving.

Finally, it must be remembered that the antidiuretic hormone of fish, amphibian and bird is arginine[8]-vasotocin and different from that of other vertebrates (Heller and Lederis, 1958; Pickering and Heller, 1959; Sawyer, Munsick and van Dyke, 1959). Its formula is:



As for the term "neurohypophysis" it will be used in this text with the meaning of a morphological unit comprising the hypothalamic neurones of certain nuclei, their axones which run down the infundibular stem to end in the posterior lobe and the posterior lobe of the pituitary gland, also known as "pars neuralis." The hypothalamic nuclei are the supra-optic and paraventricular nuclei, which in the dog contain together some 100,000 neurones.

Sites of Formation of the Neurohypophysial Hormones

A controversy has been going on for many years concerning the relative importance of the hypothalamus and the posterior lobe. According to Fisher, Ingram and Ranson (1938), both the oxytocic and the antidiuretic hormones are elaborated in and liberated from the posterior lobe, their secretion and liberation from the pars neuralis being controlled by the hypothalamic neurones. According to modern views, however, the sites of formation of the hormones are the neurones of the hypothalamic nuclei. According to this theory, the hormone-containing secretion of the neurones migrates in or along the axones and is finally

deposited in the posterior lobe. Both groups of workers, however, hold that liberation of the hormones from the posterior lobe is controlled by hypothalamic neurones. As happens so often in science a reinterpretation of the facts may lead to a conception which, though a compromise, may be more nearly correct.

The first question is to examine the distribution of the hormones in the posterior lobe and the hypothalamus and to see what light such studies can throw on the problem under consideration. In adult animals the amounts of antidiuretic and oxytocic activities found in the posterior lobe vary according to the species and do not bear any obvious relation either to their size or to their needs for water conservation. Expressed in terms of weight, the posterior pituitary gland of the dog contains an average of 4,500, that of man 760, that of rat 300 m.u./mg. of each hormone. But for a few exceptions such as the guinea-pig, the wallaby and the camel, in which the amount of oxytocin is smaller than that of vasopressin, in all adult animals and man the vasopressin : oxytocin ratio (= V/O) is unity. The posterior pituitary gland of the camel, an animal which is known to be able to withstand a much more severe degree of dehydration than any other mammal, contains 2,000 m.u. antidiuretic hormone per mg. of tissue, whereas that of the dog contains as much as 4,500 m.u./mg. gland; on the other hand, the concentration of the antidiuretic hormone in the posterior lobe of the kangaroo rat (*Dipodomys merriami*) is more than three-fold that of the ordinary rat.

In infant animals and babies the situation is different. Their posterior lobe contains a much larger proportion of antidiuretic than oxytocic activity. The disproportion is even more marked in foetuses. For instance, the V/O ratio was found to be 15 in a human foetus of 160 days and 6 in a foetus of 195 days; though at term the V/O ratio was near unity (Dicker and Tyler, 1953a). Both activities increase in amounts with age, but the rate at which the oxytocic activity increases is greater than that of vasopressin. Whether in foetuses, newly born or adult animals, there is however no known instance of a V/O ratio smaller than unity; that is to say, there is never more oxytocic than antidiuretic activity in the posterior lobe of the pituitary gland.

The presence of both activities in the hypothalamus was first reported by Abel (1924) and has been confirmed by several workers (Chamorro and Minz, 1957; Dicker and Tyler, 1953b; Kovacs and Bachrach, 1951; Sato, 1928; Trendelenburg, 1928; Vogt, 1953), the oxytocic activity being confined in the posterior part of the hypothalamus, whereas the antidiuretic activity was located in the anterior part of it. A careful study of this region of the brain in the dog has shown that the paraventricular nuclei contain on the average 465 m.u. antidiuretic activity and 30 m.u. oxytocic activity, the supraoptic nuclei 1,700 m.u. antidiuretic activity and 60 m.u. oxytocic activity and the tuber cinereum 2,000 and 145 m.u. of both activities respectively. Thus, while the pars neuralis of the adult dog has equal amounts of both hormones (16,000 m.u./gland) the whole of the hypothalamus contains some 4,000 m.u. of antidiuretic activity but only 230 m.u. of oxytocic activity; that is, the oxytocic

activity of the hypothalamus represents about 5 per cent of its antidiuretic activity. The question then is whether the small amount of oxytocic activity in the hypothalamus represents the first appearance of the hormone and if not, what is implied? As a result of the observation that arginine-vasporessin exhibits some 5 per cent of intrinsic oxytocic activity, it has been suggested that the small amount of oxytocic activity found in the hypothalamus belongs intrinsically to the antidiuretic hormone. As the hog's antidiuretic hormone exhibits a much smaller intrinsic oxytocic activity than arginine-vasopressin, it would be interesting to see whether the amount of oxytocic activity in the hog's hypothalamus is similarly reduced in relation to its antidiuretic content. Such an investigation is long overdue. It might provide the answer to the question whether the oxytocic activity of the hypothalamus is that of a separate hormone or whether it belongs to the antidiuretic hormone. Indirect evidence supporting the latter hypothesis comes from the observation that the posterior pituitary gland of nursing animals contains less oxytocic than antidiuretic activity (Acher, Chauvet and Olivri, 1946; Acher and Fromageot, 1957; Dicker and Tyler, 1953a,b; van Dyke, Adamson and Engel, 1955) whereas no decrease of oxytocic or antidiuretic activity in the different parts of the hypothalamus has been observed. The implication of these findings is that most probably vasopressin is a precursor of oxytocin. The question of the origin of oxytocin remains, however, unanswered. Two possibilities have been suggested: it is either formed during the movement of the hypothalamic neurosecretion from the neurones to the posterior lobe or its site of formation is not in the hypothalamus, but possibly in the posterior lobe itself (Vogt, 1953).

The evidence so far reviewed based on physiological and pharmacological experiments is on the whole corroborated by results from anatomical and histochemical investigations. The nervous path along which it is assumed that the hormones from the hypothalamic nuclei travel consists of two parts, the supraopticohypophysial tract running in the ventral wall of the stalk and the tuberohypophysial tract which lies dorsally to it. Little is known about the latter. As to the former, it appears to have its origin in the supraoptic and paraventricular nuclei. According to histologists, the ganglionic cells of these nuclei appear to have some secretory activity and produce a substance of protein nature (Divry, 1934; Scharrer and Gaupp, 1933). The amount of the secretory material increases with age. It can be stained with chrome-alum-haematoxylin, a stain which according to Gomori (1941) is specific for neurohypophysial secretions. Granular secretions, Gomori-positive, have been described in the ganglion cells of the hypothalamic nuclei and are distributed in bead-like fashion along the entire length of their axones. Tracts laden with this material have been identified in all vertebrates as going from the paraventricular to the supraoptic nuclei and from there to the pituitary stalk, and Gomori-staining substances have been identified over most of the posterior lobe of the pituitary gland in adults, though more rarely in newborn animals. These findings have led the histochemists to believe

that the Gomori-staining granular formation represents the hormones, either during their migration from the hypothalamic nuclei, or in a stored form in the posterior lobe.

In favour of the neurosecretory theory are, *inter alia* the following data. Gomori-stainable material has been found in greater abundance in the neurohypophysis of dogs than in that of man (Hild and Zetler, 1952); similarly, the concentration of antidiuretic activity is greater in the former than in the latter. After pituitary stalk section in the dog there is a decrease of both hormonal content and neurosecretory Gomori-positive formation in the neural lobe, whereas on the hypothalamic side of the section, there is an increase above the control values of both. A similar correlation has been described in newborn animals, whose degree of maturity at birth varies according to their species. The degree of maturity is usually determined by the eruption of teeth, the development of locomotion, the opening of the eyes, the tolerance to anoxia, and the development of renal functions. By these criteria, the newborn hamster (Auer, 1951) and rat (Heller and Lederis, 1959) are less mature than the seal (Amoroso, Harrison, Harrison-Matthews, Rowlands, Bourne and Sloper, 1958) or the guinea-pig (Dicker and Heller, 1951). The small amount of antidiuretic activity in the pituitary gland of the newborn hamster is about the same as that found in the newborn rat; whereas that of the seal and of the guinea-pig is markedly higher. Similarly, there is much more Gomori-stainable material in the posterior pituitary gland of the newborn seal and guinea-pig than in the infant hamster or rat (Palay, 1957).

The main arguments against the neurosecretory theory are: first, the chrome-alum-haematoxylin stain lacks specificity: it stains equally well the subcommissural organ and microglial cells which have no connections with the neurohypophysis (Wingstrand, 1953; Wislocki and Leduc, 1952). Second, a mixture of absolute ethanol and chloroform will extract the stainable material from the neurohypophysis without affecting the amount of hormonal activity present in the tissue. Third, neither the antidiuretic nor the oxytocic hormones can be stained by the Gomori technique (Acher and Fromageot, 1955). Fourth, the relation between the presence of neurohypophysial activities and the Gomori-stainable material does not always hold; for instance, no trace of Gomori-stainable material was found in the gland of human foetuses younger than 23 weeks (Benirschke and McKay, 1953), but antidiuretic activity has been estimated in pituitary glands of human foetuses from the 14th week of intra-uterine life onwards (Dicker and Tyler, 1953a). Similarly, whereas antidiuretic activity is present in chick embryos after 10 days of incubation, the Gomori-granules appear only 4 days later (Wingstrand, 1953). The posterior lobe of the newborn rat contains appreciable amounts of both antidiuretic and oxytocic activities (Acher, Chauvet and Olivry, 1956; Dicker and Tyler, 1953a; Heller and Lederis, 1959), but there is no evidence of Gomori-granules before the 6th day of postnatal life. Finally, according to Ortmann (1951) heavy hydration is accompanied by a marked increase of the Gomori-stainable material; however, no increase of the antidiuretic

activity in the neurohypophysis has ever been observed in those circumstances.

What then is the so-called secretory Gomori-positive material? Van Dyke, Chow, Greep and Rothen (1942) isolated from the posterior pituitary gland of ox a protein which had both antidiuretic and oxytocic activities; its molecular weight was estimated to be about 20,000 (Block and van Dyke, 1952) though the synthesis of both hormones has fixed their molecular weight at only 1,084 and 1,007, respectively. Van Dyke and others (1942) expressed the view that in the posterior lobe of ox, both hormones are stored in association with a homogenous protein and similar conclusions have been reached by Acher, Manoussos and Olivry (1955) who called the protein neurophysine. The biological activity of van Dyke's protein complex is destroyed by the same enzymes and chemical reagents that affect the active peptides and it could be further shown that the complex is formed by the association of one molecule of vasopressin and one molecule of oxytocin per molecule of protein, both the antidiuretic and the oxytocic hormones being attached to the inert protein either by simple adsorption or as a result of electrostatic force (Acher and Fromageot, 1955).

Recently, Chauvet, Lenci and Acher (1960) have shown that the complex could be dissociated in its three components oxytocin, vasopressin and neurophysine, from which they were able to reconstitute the original molecule; and they could even bring about associations between the hormones and neurophysine of different species. Acher and Fromageot (1955) had previously found that the neurophysine in the presence of the hormones take the Gomori-staining readily, whereas the hormones alone do not. Finally, according to Barrnett and Seligman (1954) the granules in the hypophysis and the hypothalamus which take Gomori's chrome-alum-haematoxylin staining can also be stained by reagents which are specific for the presence of disulphide bonds. As van Dyke's protein is known to be rich in cystine it is reasonable to conclude that van Dyke's protein, Acher's neurophysine and Gomori's granules are the same substance, and cannot therefore be identical with the hormones. It may be of interest to mention here that according to Pardoe and Weatherall (1955) the oxytocic and vasopressor material in the rat's pituitary gland is present in cytoplasmatic particles which behave like mitochondria, and that the two substances do not appear to be contained in the same particles. These observations, however, are at variance with those of Lederis and Heller (1960).

Little is known about the nature of the bonds by which the hormones are attached to the protein-carrier. There are, however, some observations which suggest that the bond that fixes oxytocin is much more labile than that which fixes vasopressin. Acher and Fromageot (1955) confirming an old observation by Dudley (1923) found that oxytocin is preferentially extracted by acetone from the gland of adult animals, and is more soluble in organic solvent than the antidiuretic hormone, a fact which led to the separation of the two peptides by the counter-current distribution method. Heller and Lederis (1959, 1960) also found that the"solubility"

of oxytocin in acetone was greater in newborn than in adult rats, in lactating females than in males. Since the variations in the amount of oxytocin extracted by acetone cannot be attributed to changes in actual solubility of the molecule of oxytocin, this can be explained only either by changes in the nature of the bond between the carrier and the hormone or by physicochemical changes of the protein carrier. The observation by Dawson (1953) that the neurosecretory material in the pituitary gland of infant rats stains differently from that of adult animals may be worth remembering. Though the reason for the greater lability of oxytocin is still not clear, the fact that it can be separated from the protein-carrier more easily than vasopressin is of interest and will have to be borne in mind when considering the mechanism which regulates the release of the hormones from the posterior pituitary gland.

It may then be concluded that in mammals the antidiuretic activity (hormone?) is manufactured somewhere in the hypothalamus and travels with an inert protein (neurophysine?) along some nervous path, until it is stored in the posterior pituitary gland. As for oxytocin, it is not known where it is formed; however, when in the neural lobe, it appears to be bound on the same protein as vasopressin. In the pars neuralis of adult animals, both hormones are stored in equal amounts, one molecule of oxytocin and one molecule of vasopressin being associated with one molecule of the inert neurophysine.

Estimation of the Hormones

Though it may not be necessary to use highly sensitive methods to study the fate of large amounts of intravenously injected vasopressin or oxytocin, they are the limiting factor when it comes to measuring the minute amounts of the hormones normally present in the peripheral blood or in the urine. Since there are no suitable chemical methods for estimating the concentrations of these substances in the body fluids, they must be assayed biologically. An ideal method of assay would be completely specific, extremely sensitive and precise. Simplicity, though not absolutely necessary, is also an important consideration. It is safe to say that no known method fulfils all these requirements.

How specific are any of the methods? One mg. of pure oxytocin assayed in terms of the International Standard has 500 units of oxytocic (rat uterus), depressor (fowl's blood pressure), and milk ejecting (rabbit) activity, and exhibits at the same time 7 units pressor (rat) and 3 units of antidiuretic (dog) activity. One mg. of pure arginine-vasopressin has 600 units of antidiuretic (dog), and pressor (rat) activity, 120 units of milk ejecting (sow) activity and 30 units of oxytocin (rat uterus) activity. Thus there is no complete specificity of an assay method for either hormone; vasopressin is quantitatively the more versatile; however, as 1 mg. of pure oxytocin has 3 units of antidiuretic activity, whereas vasopressin has as much as 600 units, it can be said that the antidiuretic action of vasopressin approaches true specificity. The same thing can be said about the oxytocic activity of oxytocin, but not of its milk ejecting activity. The specificity is limited further by the choice of techniques

used. For example, if oxytocin is injected in a non-pregnant woman it will be less potent than vasopressin in stimulating the uterus muscle. Likewise, when small amounts of antidiuretic hormone such as those which exist in plasma are assayed by intraperitoneal or subcutaneous injections into hydrated rats, non-specific antidiuresis is likely to occur (Dicker and Ginsburg, 1950); for instance, the titre of endogenous antidiuretic hormone of plasma can be up to 25 times higher if assayed by intraperitoneal instead of by intravenous injections (Ames and van Dyke, 1952).

As to sensitivity, the rat under ethanol anaesthesia (Jeffers, Livesey and Austin, 1942) kept with a constant water load (Dicker, 1953; J. Heller, 1959; Thorn, 1957) appears to be the most sensitive animal preparation for antidiuretic assays; its sensitivity varies from 0·001 to 0·005 m.u. Other preparations using dogs, rabbits or mice have a range of sensitivity varying from 0·5 to 2·5, from 0·1 to 0·4 and from 0·01 to 0·05 m.u., respectively. For oxytocin, the isolated rat's uterus (Holton, 1948) is by far the most suitable preparation, though it has obvious limitations: not less than 1 to 2·0 m.u. of oxytocin can reliably be estimated; furthermore, body fluids with low concentrations of the hormone will modify the uterine response, producing non specific contractions.

In view of the lack of sensitivity of any of these methods, attempts at concentrating the hormones have been made either by extraction (Bisset and Lee, 1957; Ginsburg and Smith, 1958) or chromatographic methods (Arimura and Dingman, 1960), combined with one of the methods of assay already mentioned; for example, the rat under ethanol anaesthesia with constant water load for the antidiuretic hormone, or the isolated superfused rat uterus (Gaddum, 1953) for the oxytocic hormone.

The use of unsuitable methods of assay (Birnie, Eversole, Boss, Osborn and Gaunt, 1950) or the lack of a suitable standard of comparison (Mirsky, Stein and Paulisch, 1954) has led to unrealistic claims for the concentration of the neurohypophysial hormones in the peripheral blood. For instance, reports of 140 to 530 m.u. of antidiuretic activity for 100 ml. plasma have repeatedly been made (Hawker, 1953). To assess such results critically it must be borne in mind that an intravenous injection of as little as 1·0 m.u. of arginine-vasopressin ($= 1{\cdot}7 \times 10^{-6}$ mg.) causes a clear cut antidiuresis in a hydrated human subject. Assuming that none of the antidiuretic hormone injected is either inactivated, destroyed or excreted, its concentration in the plasma would be about 0·03 m.u. per 100 ml. plasma (see also p. 458). As a matter of fact, very few methods, if any, have a sufficient sensitivity to estimate such small amounts.

It is because the methods of assaying the hormones in biological fluids are so unsatisfactory that research workers have tried to equate the reactions which follow a stimulation of the neurohypophysis with that produced by a suitable intravenous infusion of the hormone under consideration. This method of matching the response to a stimulation with that produced by the administration of the hormone has proved very useful in animals (Harris, 1947) and in man (Burn and Singh Grewal, 1951; Lauson, 1951). It has, however, obvious limitations and lacks the

precision of a biological assay. Furthermore, some experimental procedures may produce the secretion of hormones in much greater concentration than normally required, and it is a well known observation that very large doses of posterior pituitary extracts may have effects other than those ascribed to vasopressin and oxytocin.

Lastly, when some oxytocic or antidiuretic activity has been found in body fluids or their extracts, its identification with one or the other neurohypophysical hormone must be achieved. The extract will need to possess all the pharmacological characteristics of oxytocin or vasopressin; thus, in the instance of oxytocin it will not only have to contract an isolated rat's uterus but produce milk ejection and exhibit some antidiuretic activity in the required proportion; likewise for vasopressin, it will have to have 5–7 per cent of oxytocic activity besides its antidiuretic activity. When assaying either substance, the dose-response curve will have to be parallel with that of the hormones.

Release of the Hormones

It is common knowledge that the release of the antidiuretic hormone is controlled, *inter alia*, by suitably adjusted osmotic stimuli. In theory, the stimulus could be produced either by a lowering or by an increase of the osmotic pressure. In the former hypothesis, an increased water load would alter the degree of stimulation of osmoreceptors and so produce a reflex inhibition of the secretion of the antidiuretic hormone. According to the other theory, the osmoreceptors are stimulated by an increased osmotic pressure, after water restriction, and so produce an enhanced secretion of the hormone. Whichever point of view is adopted, dehydration is accompanied by a release of the antidiuretic hormone.

The release of neurohypophysial hormones can also be achieved by other means such as electrical stimulations of the supraopticohypophysial tract and of the supraoptic and paraventricular nuclei, by haemorrhage and administration of anaesthetics, by emotional stimuli of various kinds, and more specifically in the female by stimulation of the mammilla, dilatation of the cervix and of the body of the uterus as well as by coitus. At first sight it is difficult to see how such different stimuli can produce the same effect. All these stimuli can, however, be divided into two main groups, one concerned with the regulation of body water (for example, dehydration, intravascular injections of hypertonic saline solutions, haemorrhages); the other related to physical or emotional stress. As it would appear that the neurohypophysis is fully dependent on its nerve supply represented by the supraopticohypophysial tract, the broad outline of the mechanism regulating its activity can be summarised in the following way. First, its activity depends on its connections with the hypothalamus. Second, it is influenced, directly or indirectly, by changes in the internal environment. Third, superimposed on the latter, comes the influence of the central nervous system in response to changes in the external environment, which are closely associated with states of stress and act by nervous reflex paths involving the hypothalamus. The supraoptic nuclei are assumed to function as minute osmometers (Jewell, 1953) more specifically

adapted to regulate the secretion of the antidiuretic hormone, when stimulated by changes in the osmotic pressure. For instance, an increase of 1 per cent in the osmotic pressure of the aortic blood lasting over a period of 10 to 40 min., will reduce a water diuresis to about 10 per cent of its initial value, corresponding to a release of about 50 μu. of vasopressin ($= 2 \times 10^{-9}$ mg. of arginine-vasopressin) per min. Such a change in the osmotic pressure of arterial blood is within physiological ranges. As for all other stimuli (physical, traumatic, emotional stress or excitement) it is likely that they activate nervous pathways to the supraoptic nuclei. Though the anatomy of these afferent nerves is still unknown, there is evidence that certain fibres end on and modify the activity of the supraoptic nuclei and that at least some of them are cholinergic in nature. Intravenous injections of acetylcholine produce antidiuresis. As this can be prevented by removal of the neural lobe, but not by atropine, it can be concluded that the mechanism of antidiuresis is similar to that produced by the nicotinic action of acetylcholine (Burn, Truelove and Burn, 1945; Pickford, 1939). Pickford (1947) localised the site of action of acetylcholine by showing that the injection of 2–40 μg. of acetylcholine directly into the supraoptic nuclei of dogs produces a release of the antidiuretic hormone. Further evidence for a central action of acetylcholine comes from the observation that much smaller amounts are effective when injected into the carotid artery rather than intravenously. All these results suggest that it is the cells of the supraoptic nuclei which are sensitive to acetylcholine and not the cells upon which the supraopticohypophysial tract terminates, an interpretation which is entirely consistent with the findings that very low values for choline acetylase (an enzyme concerned with the formation of acetylcholine) are found in the neurohypophysis, but substantially higher amounts of it in the supraoptic nuclei (Feldberg and Vogt, 1948). It is interesting to note that small injections of adrenaline, in amounts corresponding to those likely to be liberated from the adrenals during physical or emotional stress, can prevent the liberation of the antidiuretic hormone. Though the inhibitory action of adrenaline has not yet been explained satisfactorily, it is likely that it acts through some specific interference in the chain of chemical reactions initiated in the nervous system by emotional stimuli, and there are grounds to believe that the adrenaline may act on the supraoptic nuclei.

While the sites from which a stimulus produces a release of the antidiuretic hormone from the pars neuralis appear to be located in the supraoptic nuclei, those responsible for the liberation of oxytocin appear to be situated in the paraventricular nuclei (Olivecrona, 1957). Bilateral destruction of these nuclei produces a loss of extractable oxytocic activity from the neurohypophysis, though the antidiuretic activity remains unaffected; conversely, electrical stimulation of the paraventricular nuclei produces a release of oxytocic activity, as estimated by milk ejection, without any decrease of the antidiuretic activity. Whether acetylcholine is the chemo-transmitter for the release of oxytocin (Walker, 1957) is still under discussion (Chaudhury, 1960).

As in the neurohypophysis both oxytocin and vasopressin are bound in the proportion of 1 : 1 to one molecule of inert protein, the possibility of a separate release of one hormone without the other is difficult to conceive, unless it can be shown that the so-called neurosecretory material which contains the hormones breaks up into its constituents before the entry of the hormones into the blood stream. According to Hanström (1952), Rothballer (1953) and Scharrer and Scharrer (1954) stainable granules of the same kind as those in the nerve endings of the supra-opticohypophysial tract have been seen within the blood vessels of the posterior lobe, suggesting that the neurosecretory substance is discharged as such into the circulation. In the blood stream, however, vasopressin is adsorbed on a protein (Heller, 1957; Hipsley and McKellar, 1960) which has been identified as a beta globulin (Thorn and Silver, 1957) and cannot therefore be identical with the protein carrier in the neurohypophysis, which has a molecular weight of about 20,000 only (Block and van Dyke, 1952). If this is so, one has to accept the possibility of the hormones being released from the gland with an inert protein (Scharrer and Scharrer, 1954) eluted from the latter (where and when is not known) and readsorbed in the blood on another protein.

Whatever the final explanation, one thing is certain: any stimulus that releases one hormone releases the other simultaneously. For instance, intracarotid or intravenous injections of hypertonic NaCl or sucrose solutions into a bitch inhibits a water diuresis by liberating the antidiuretic hormone and produces increased uterine activity as a result of the release of oxytocin (Abrahams and Pickford, 1954). Similar results have been observed after electrical stimulation of the hypothalamus in the conscious rabbit (Harris, 1947). Likewise the release of oxytocin which follows suckling or coitus is accompanied by that of vasopressin, in animals and man (Cross, 1951; Friberg, 1953; Kalliala and Karvonen, 1951; Kalliala, Karvonen and Leppänen, 1952; Peeters and Coussens, 1950; Peeters, Coussens, Bouckaert and Oyaert, 1949).

In an attempt to estimate the amount of hormones released after the stimulation of the neurohypophysis, its effects (antidiuresis, uterine activity) have been matched with those obtained after adequate intravenous injections of the hormones. All the results agree and show that whatever the stimulus, both hormones are released together, but that the amount of oxytocin released always exceeds that of vasopressin. Electrical stimulation of the neurohypophysis of conscious rabbits causes antidiuresis and uterine contraction, which could be reproduced by the intravenous injections of a mixture of the hormones in which the oxytocin concentration was more than twenty times that of vasopressin (Harris, 1947). In other experiments specifically designed for estimating the amount of oxytocin released in cows and rabbits, it was found that suitable stimuli produced a simultaneous secretion of both hormones, in a ratio of vasopressin : oxytocin = 1/100 (Cross, 1956; Peeters and Coussens, 1950). In bitches an intravenous injection of hypertonic saline solution causes the release of 15 to 20 times as much oxytocin as vasopressin (Abrahams and Pickford, 1954).

These results raise a series of problems. From the evidence reviewed above, according to which both hormones are bound together on one molecule of inert protein in the pituitary gland, it is difficult to see why any stimulus releases more oxytocin than vasopressin. It may be that the "bonds" which fix oxytocin to the protein carrier are looser than those of vasopressin: evidence to that effect has already been mentioned. Alternatively, it may be that, in agreement with the results of experiments during which the glands of nursing animals have been depleted of their hormones, vasopressin is a precursor of oxytocin, the latter being released as soon as manufactured. As for the discrepancy between the ratio of oxytocic and antidiuretic activities released, it must be remembered that all the above experiments were made before pure hormones were available, and thus the authors used as standards of comparison commercial preparations of unspecified composition. For instance, it is possible that if arginine-vasopressin had been used instead of Pitressin solutions (which in all likelihood contained lysine-vasopressin) the ratio of oxytocin to vasopressin observed in dogs (Abrahams and Pickford, 1954) would be different from that stated. Finally it must be borne in mind that no satisfactory quantitative estimation of plasma hormonal content has yet been achieved. Also, the comparisons of the actions of two different hormones on different effector organs assumes, among other things, that the two hormones under investigation are metabolised, inactivated or excreted at a similar rate. That this is unlikely to be so will be shown later on. It is clear, however, that whatever the interpretation both oxytocin and vasopressin are released simultaneously and that whenever they are released the amount of oxytocin secreted exceeds by far that of vasopressin.

Role of Age on the Mechanism of Release of Hormones

It is well known that the kidneys of newborn animals and babies concentrate their urine less well than those of adults and that in old age there is a decrease in the concentrating power of the kidneys. The lack of urinary concentration immediately after birth may be due to an inadequate amount of antidiuretic hormone available, a deficiency in the mechanism of its release, an inability of the kidneys to respond to the hormone, or a combination of these factors. There is some difficulty in assessing the adequacy of available antidiuretic activity present in the neural lobe of newborn infants and animals, due mainly to the difficulty of choosing the right parameter in which to express it: hormonal contents have been expressed in terms of glandular weight, wet or dry, in terms of kidney weight or body surface. However, irrespective of the parameter adopted, the amounts of antidiuretic and oxytocic activities in the neurohypophysis of newborn babies or animals is smaller than that of adults (Dicker and Tyler, 1953a,b; Heller and Lederis, 1959; Heller and Zaimis, 1949): it is about 1/8th of that in adults. This is more than twice the minimum amount needed by adults to prevent the occurrence of diabetes insipidus. It is therefore unlikely that the lack of urinary concentration can be attributed to an inadequate amount of available hormone. Is the

mechanism of release deficient? The most powerful physiological stimulus producing the liberation of the antidiuretic hormone in adults is dehydration, after prolonged water deprivation. According to J. Heller and Stulc (1960) whereas there is a tenfold rise in the plasma antidiuretic activity after 24 hours of dehydration in adult rats, in newborn animals no similar rise has been noted and no plasma antidiuretic activity could be estimated. However, as antidiuretic activity, presumably of neurohypophysial origin, has been repeatedly found in the urine of newborn animals and babies (Ames, 1953; Dicker and Eggleton, 1960) its absence in the blood can be explained only by a lack of sensitivity of the method used. It is possible, however, that the antidiuretic hormone is synthetised at a slower rate in newborn animals than in adults, a hypothesis which would agree with the results obtained by comparing the neurohypophysial content during dehydration in newborn and adult animals: water deprivation for 24 hours leads to a decrease of the antidiuretic activity of the neurohypophysis of new born rats (Heller and Lederis, 1959) whereas in adults it produces a marked increase of it (Ames and van Dyke, 1950; Dicker and Nunn, 1957). Thus, there is no clear evidence of a faulty mechanism in the release of the antidiuretic hormone in newborn animals or babies, and thus the inability of infants to concentrate their urine may be attributed more plausibly to the immaturity of their kidneys (Dicker and Eggleton, 1960; Heller, 1944; McCance, Naylor and Widdowson, 1954; McCance and Widdowson, 1954).

In ageing animals, the pattern of salt and water excretion resembles that of animals with diabetes insipidus. This has been attributed to a decreased ability of the neurohypophysis to respond to osmotic stimuli (Friedman, Hincke and Friedman, 1956). Dicker and Nunn (1958), however, were unable to find any change in the responsiveness of the neurohypophysis to osmotic stimuli and concluded that the decreased ability to concentrate the urine arose from changes in the kidney functions.

Fate of the Neurohypophysial Hormones

Gilman and Goodman (1937) found that after dehydration some antidiuretic material appeared in the urine. This was confirmed by Boylston and Ivy (1938) and then refuted by Arnold (1938), Walker (1939) and Krieger and Kilvington (1951). The apparent conflict between these opinions was due to unsatisfactory methods of assay. It is now accepted that antidiuretic activity is present in the urine of dehydrated animals, or in that of animals which have been injected with the antidiuretic hormone (O'Connor, 1951; Dicker, 1954; Dicker and Eggleton, 1960; Dicker and Nunn, 1958; Ginsburg and Heller, 1953; Thorn, 1959). When a commercial preparation of the antidiuretic hormone is injected intravenously into a conscious rat, about 10 per cent of its activity can be recovered from the urine (Dicker, 1954; Ginsburg, 1954; Heller, 1952). The antidiuretic material excreted in the urine of dog or rat after stimulation of the osmoreceptors or after an intravenous injection of vasopressin would appear to be a large molecule (Ames, Moore and van Dyke, 1950; Thorn, 1959). Whether this large molecule is the same as that described

by Thorn and Silver (1957) in the blood or whether it represents the pituitary octapeptide adsorbed on to a urinary protein is still unknown. Since the antidiuretic activity in the urine can be inactivated by trypsin, indicating the presence of a basic peptide, and can be destroyed after treatment with sodium thioglycollate in the same way as purified vasopressin (Thorn, 1959) it is likely that the kidneys do excrete the hormone.

Since about 10 per cent only of the injected antidiuretic hormone or of the secreted endogenous polypeptide can be recovered from the urine, the question is what happens to the remainder. The likelihood is that it is used up, destroyed or inactivated by tissues. This problem has been studied in *in vitro* and *in vivo* experiments. From *in vitro* experiments it is known that vasopressin is inactivated after incubation with slices or homogenates of kidney (Dicker and Greenbaum, 1954), of liver (Birnie, 1953; Eser and Tuzunkam, 1950; Eversole, Birnie and Gaunt, 1949), of spleen and of duodenum (Christlieb, 1940) but not by that of muscle (Dicker and Greenbaum, 1956). Most of the renal enzyme responsible for the inactivation of vasopressin can be extracted from the particle free supernatant fluid of the tissue homogenate from which it can be precipitated at pH 8 with 40–50 per cent $(NH_4)_2SO_4$ saturation. The inactivation of vasopressin by this fraction can be partly reversed by oxygenation. It could be further shown that the inactivation of the antidiuretic activity of vasopressin is partly due to the reduction of the –S–S–bond of the polypeptide to –SH and that the renal enzyme is in all likelihood an –SH enzyme with a maximum activity in the –SH form (Dicker and Greenbaum, 1958; Fong, Silver, Christman and Schwartz, 1960). However, the inactivation of vasopressin by the renal enzymic preparation cannot be due solely to the reduction of the disulphide link because by treating the reduced vasopressin with agents such as oxidised glutathione or cysteine, only a partial restoration of the hormone's initial antidiuretic activity can be obtained. This suggests that the enzymic preparation has therefore other possible inactivation mechanisms. As it is known that the kidney is rich in amide splitting enzymes, such as glutaminase or asparaginase, it is reasonable to assume that a point for enzymes to attack the vasopressin molecule might be at the amide linkage of the three amides present: glycineamide, asparagine and glutamine. When vasopressin and the crude enzymic preparation are incubated in the presence of an excess of one, two or three amides, for example, under conditions of competitive inhibition, the addition of each of the amides —glutamine, asparagine or glycineamide—causes a reduction in the inactivation process and when all three amides are present there is an almost complete inhibition of the enzymic destruction. It follows that the amide groups of the vasopressin are necessary for its antidiuretic activity and that one of the mechanisms by which the kidney destroys it, besides the reduction of the –S–S–link, may be an attack on the amide groups of the molecule (Dicker, 1960).

These views on the mechanism of inactivation of vasopressin are in good agreement with results of *in vivo* experiments. Ginsburg (1953)

showed that the decreased concentration of intravenously injected vasopressin followed an exponential course, but that the tying up of the coeliac and mesenteric arteries or the removal of kidneys, or both, retarded the rate at which the hormone disappeared. It could be calculated that the kidneys account for 50 per cent of the vasopressin cleared, and the splanchnic area for about 40 per cent, the remaining 10 per cent being excreted in the urine. If tissues such as intestine, spleen, liver and kidneys all take part in the inactivation of vasopressin, how is it that 10 per cent of either exogenous or endogenous vasopressin escapes destruction and can be recovered from the urine, especially as it would appear that the kidneys extract in a single circulation all the vasopressin from the blood which passes through them? (Crawford and Pinkham, 1954). In animals killed 3 min. after an intravenous injection of vasopressin, no antidiuretic activity can be found in the renal tissues, though some of it can be found in the bladder, ureters and kidney dead space (Heller and Zaidi, 1957). As the process of inactivation appears to be confined to the tubular cells, it may be either that some vasopressin is filtered unaltered through the glomeruli, or alternatively, since vasopressin circulates bound to some proteins (Thorn and Silver, 1957), that some of it is secreted by the renal tubules (Ginsburg, 1957).

When oxytocin is injected intravenously into rabbits it disappears quickly from the blood stream but its half life is twice as long as that of vasopressin (Heller, 1960). This suggests that the mechanisms of inactivation of the two hormones are different in spite of the similarity of their molecules. The ligation of the splanchnic vascular area does not affect the rate of disappearance of injected oxytocin, though it retards the elimination of vasopressin considerably. When both kidneys and splanchnic vascular area are excluded, the plasma concentration of oxytocin falls exponentially during the first 7 min. after administration, but thereafter remains constant. Heller's (1960) interpretation of these experiments is that the initial fall in concentration is due to equilibration of the hormone in the extravascular space, but that once this process is completed no inactivation takes place. The volume of distribution of oxytocin at equilibrium is 43·5 ml./100 g. weight as compared with 11·8 ml./100 g. for inulin, suggesting that the hormone distributes itself in a volume markedly greater than the extracellular fluid space (Heller, 1960).

The differences between the rates of inactivation of vasopressin and oxytocin *in vivo* are corroborated by *in vitro* experiments. Whereas both cysteine and glutathione reduce the antidiuretic activity of commercial preparations of vasopressin (Dicker and Greenbaum, 1958) they do not appear to inactivate oxytocin significantly (Dicker, 1960). This agrees with Sealock and du Vigneaud's (1935) observation that a reduction of the disulphide bond of the oxytocin molecule does not result in loss of activity, since the disulphide bridge of oxytocin is constantly reformed under the action of oxygen (du Vigneaud, Ressler, Swan, Roberts and Katsoyannis, 1954). If, however, no oxygen is present, cysteine will in fact reduce oxytocin (Audrain and Clauser, 1958). Other differences are that whereas trypsin destroys vasopressin, it does not attack oxytocin

(Lawler and du Vigneaud, 1953) and that, in contrast with vasopressin, the addition of asparagine, glutamine and glycineamide singly or together does not appear to influence the process of inactivation of oxytocin (Dicker, 1960).

Influence of Sex, Pregnancy and Lactation

Ginsburg and Smith (1959) showed that the half life of oxytocin was about the same in male rats, in female rats in oestrous, and in female rats during the last week of pregnancy but that in lactating animals the rate of disappearance of oxytocin from the blood was significantly faster than in any other group. Furthermore, while in female rats in oestrous or during pregnancy, as in male rats, the exclusion of both the renal and splanchnic vascular beds resulted in a steady plasma concentration of injected oxytocin, no constant level was achieved in lactating rats under similar conditions. Thus though there is no evidence of preferential uptake of oxytocin by the uterus of the rats, whether pregnant or not, the hormone is readily inactivated in the lactating animal, presumably by the contractile cells responsible for the let-down of milk.

Though plasma from men or non-pregnant women does not inactivate either vasopressin or oxytocin, both hormones are readily inactivated by plasma of pregnant women. This inactivation is of enzymic nature. As the inactivation of oxytocin by plasma of pregnant women takes place in the presence of oxygen, it is clear from the work of Sealock and du Vigneaud (1935), Audrain and Clauser (1958) and Dicker (1960) that this inactivation cannot depend on the reduction of –S–S–bond, and Tuppy and Nesvadba (1957) were able to show that it was due to an amino-peptidase which split the molecule between its cystine and tyrosine components. Whether this aminopeptidase can inactivate the anti-diuretic hormone in a similar way is still under discussion. Hooper and Jessup (1959) using homogenates of placenta found that the enzyme responsible for the inactivation of oxytocin is present in the soluble fraction of the homogenate, whilst the enzyme destroying vasopressin is located in the mitochondria and microsomes only. Copper inactivates both enzymes, but silver and zinc inactivate oxytoxinase only. TEPP and DFP inhibit the enzymic activity of oxytocinase but not that of vasopressinase. These results suggest that oxytocinase, the enzyme responsible for the destruction of oxytocin, is an esterase with peptidase activity and is different from vasopressinase, the enzyme responsible for the inactivation of vasopressin, which is a peptidase and not an esterase (Hooper, 1960).

It is interesting to note that oxytocinase exists in the plasma of pregnant women and some anthrapoid monkeys only (Caldeyro-Barcia and Poseiro, 1958), but does not exist in the plasma of pregnant rats (Sawyer, 1954), rabbits, dogs and rhesus monkeys (Dicker and Whyley, 1960; 1961). Plasma oxytocinase appears to originate from the placenta.

Mechanism of Action of Vasopressin and Oxytocin

Until recently the action of the antidiuretic hormone has been described as restoring "water balance by promoting the reabsorption of the

osmotically free water left by the distal reabsorption of Na. Under the action of the hormone this water is reabsorbed; in its absence this water is excreted" (Smith, 1956). According to Wirz (1956) and to Gottschalk and Mylle (1959) the fluid in the first half of the distal tubule is hypotonic to plasma under all conditions of hydration, but in dehydrated animals it becomes isotonic in the second half of the distal tubule and hypertonic in the collecting ducts. In the kidney producing a concentrated urine, the tubular fluid comes into osmotic equilibrium first with the cortical tissue and later, in the collecting tubules, with the medullary tissue which is known to be increasingly hypertonic towards the papilla. Thus, according to this theory, the essential action of vasopressin is to increase the permeability to water of the distal parts of the nephron and collecting ducts. In 1958, Ginetzinsky found that the urine of several mammals contained hyaluronidase: this disappeared during water diuresis, but was present during osmotic diuresis, in the dog. In a histological study in rats, Ginetzinsky observed that the cement substance between the cells of the collecting tubules reacted as hyaluronic acid when the animals were water-loaded, but as its depolymerisation products when they were dehydrated. He concluded that when stimulated by the antidiuretic hormone, the cells of the collecting tubules secrete hyaluronidase, which in turn depolymerises the mucopolysaccharide complex of the basement membrane of the tubules, hence making "the structures separating the tubule lumen from the interstitial tissue permeable to water. The hypotonic fluid in the tubules then follows the osmotic gradient and undergoes facultative reabsorption" (Ginetzinsky, 1958). This is a new approach to a process which had hitherto been difficult to envisage. In a series of recent papers Dicker and Eggleton (1960a,b; 1961a,b) have shown that (*a*) normal human subjects excrete hyaluronidase when their urine is concentrated, but fail to do so during a water or alcohol diuresis, (*b*) the intravenous administration of either lysine- or arginine-vasopressin at the peak of a water diuresis leads to the excretion of hyaluronidase, (*c*) the concentration of hyaluronidase in the urine is quantitatively related to the degree of antidiuresis produced, (*d*) in cases of nephrogenic diabetes insipidus in which the administration of vasopressin does not produce an antidiuretic response, there is no urinary excretion of hyaluronidase. This suggests that in this rare renal syndrome the lack of response to either exogenous or endogenous vasopressin may be attributed to a failure of the antidiuretic hormone to release hyaluronidase, the absence of which would prevent the urine in the collecting tubules to equilibrate with its hypertonic surroundings. While this is a step forward, it still does not explain how the antidiuretic hormone works. Confirming previous work (Dicker and Greenbaum, 1958; Dicker, 1960) Fong and others (1960) have shown that the antidiuretic hormone is attracted to a receptor in the renal tubules and have postulated that this would involve electrostatic interactions of opposite charges. The hydroxyl group of tyrosine and the amide group of glutamine, asparagine and glycineamide appear in this respect to have a prominent attractive force. When the interactions between attractive and repulsive forces

have produced a suitable alignment of the hormone with its receptor, the thiol-disulphide exchange reaction takes place. This results in a hormone-receptor disulphide, a bond which is ultimately cleft by a reductase in conjunction with one or more enzymes (Dicker and Greenbaum, 1958). The reaction is then reversed and the receptor-sulphydryl group regenerated. From this it would appear that it is the series of sulphydryl-disulphide reactions which underlies the mechanism of increased passive transport of water through the tubules. Whether this is mediated by the secretion of hyaluronidase is not yet known, but is under investigation.

As for oxytocin, it would appear that its primary action on uterine muscle is the lowering of the membrane potential (Jung, 1957) which is followed by a series of tetanic action potentials accompanied by mechanical contractions. The latent period before these discharges occur depends on the hormone concentration. With increasing doses of oxytocin, there is a decrease of both the amplitude of the action potentials and of the time that elapses before the peak potential is reached. As the muscle remains contracted a long time after the action potentials have disappeared, it has been suggested that what has been called uterine tetanus is a contracture. Recent studies by Evans, Schild and Thesleff (1958) however, indicate that the action of oxytocin does not necessarily depend on changes of membrane potentials: an isolated rat uterus, immersed in a Ringer solution in which sodium has been replaced by potassium has no measurable membrane potential. It will contract, however, in response to a number of smooth muscle stimulants including oxytocin.

Two questions which have not yet been answered satisfactorily are, what is the function of oxytocin in the male, and whether in the female it is really of importance in the process of labour? The action on renal functions has been discussed and investigated recently in both sexes by Cross, Dicker, Kitchin, Lloyd and Pickford (1960), with on the whole, negative results. The view that oxytocin plays a part in labour finds its support in the analogy between normal, spontaneous uterine contractions and uterine motility elicited by oxytocin administered in correct dosages (Caldeyro-Barcia and Poseiro, 1958) and in the observation of an increased sensitivity to the hormone as term approaches (Fitzpatrick, 1957; Nixon and Smyth, 1958). Against it are the facts that no increased concentration of oxytocin has been found in plasma of women in labour and that the fate of the hormone in animals does not appear to be affected by the presence of absence of the uterus since it is the same in pregnant females and males (Ginsburg and Smith, 1959). The sole action of oxytocin which has been established with certainty is that on milk ejection. In 1948, Gunther observed that in lactating women in labour each uterine contraction was accompanied by a spontaneous ejection of milk. The milk ejection reaction, which is accompanied by a measurable rise of pressure in the mammary gland is the result of the direct action of oxytocin on the myoepithelial cells (Cross and Harris, 1951; Folley, 1956; Labouche, 1957). The rise in pressure in the ducts converging on the nipple in response to oxytocin is so characteristic that it has been used for the estimation of small amounts of the hormone (Berde and Cerletti,

1957; van Dyke, Adamson and Engel, 1956). It will be noted that these facts are in good agreement with the finding of a decreased oxytocin content of the neurohypophysis of lactating animals (Dicker and Tyler, 1953a) and with the observation that the rate of disappearance of oxytocin is significantly accelerated in lactating rats (Ginsburg and Smith, 1959).

This short review makes no claim to completeness. It is hoped, however, that it will have shown the progress achieved in this field of research and that it will allow an unbiased reader to form an independent judgment devoid of theoretical and dogmatic beliefs on some of the problems under discussion. A few of these are in the process of elucidation, many more await further investigation, the most challenging of them all being the mechanism of action of vasopressin and the role of oxytocin in the male.

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