Preliminary work indicates that the compound present in eluate J is a *C*-glycoside of eriodictyol and this appears to be the first report of the occurrence of a flavanone *C*-glycoside. The compound has been named aspalathin (Koeppen, 1961).


## SUMMARY

1. Orientin (lutexin) was isolated from the leaves of rooibos tea (*Aspalathus acuminatus*) and crystalline tetra-*O*-methyl, octa-*O*-acetyl and tetra-*O*-acetyltetra-*O*-methyl derivatives were prepared. Analyses indicate that the structure proposed for this compound by Hörhammer *et al.* (1959*b*) is correct.

2. Homo-orientin [named lutonaretin by Seikel & Bushnell (1959)] was isolated in crystalline form for the first time. The compound failed to form a crystalline acetate but analyses of the compound itself together with crystalline tetra-*O*-methyl and hexa-*O*-acetyltetra-*O*-methyl derivatives supported the structure proposed by Hörhammer *et al.* (1959*b*).

3. Isoquercitrin and rutin were also isolated, in low yield, from the leaves.


The authors are indebted to Professor L. Hörhammer for a gift of orientin and also to Professor M. Seikel for a gift of lutonarin. Thanks are also due to Professor R. I. Nel, Director of the Western Province Fruit Research Institute, for facilities, and to the Department of Agricultural Technical Services for permission to publish the results. The authors gratefully acknowledge the technical help of Mr P. de K. du Preez.

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# The Catabolism of Some Physiologically Active Polypeptides by Homogenate of Dog Hypothalamus

BY K. C. HOOPER
*Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge*

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The biosynthesis of polypeptides has been less studied than that of proteins, and, except for glutathione, little is known of their synthesis. Physiologically active polypeptides may be classified into two groups: first, those such as angiotensin and bradykinin which are produced by enzyme action on inactive protein substrate, and, secondly, those such as oxytocin, substance P and vasopressin which appear to be produced in nerve tissue by mechanisms as yet unknown.

According to current concepts, oxytocin and vasopressin are formed by neurones of the hypothalamus and are transported to the posterior pituitary for storage and release (Bargmann & Scharrer, 1951). Attempts to show a synthesis of the hormones in cultures of posterior pituitary (Hild, 1954) and of neurosecretory material in hypothalmic tissues (Borghese, 1954) were unsuccessful. Sachs (1960) followed the incorporation *in vivo* of L-[$^{35}$S]cystine into vasopressin, and attempted to find the site of synthesis of the polypeptide by comparing the specific activities of the hormone in the hypothalamus and pituitary.

Substance P is widely distributed in the central nervous system (Amin, Crawford & Gaddum, 1954) but little is known of its production.

Meagre information is available about the catabolism *in vitro* of oxytocin and vasopressin by brain tissue. Heller & Urban (1935) found that minced guinea-pig brain inactivated vasopressin slightly, and Itoh & Kikuchi (1959) stated that vasopressin was inactivated by homogenate of rat brain only in the presence of pyridoxal. The inactivation of oxytocin does not appear to have been studied; however, Krivoy (1957) found that acetone-dried powders of guinea-pig brain did not contain enzymes destroying the hormone. The destruction of substance P has been studied in more detail (Gullbring, 1943; Pernow, 1953; Eber & Lembeck, 1956; Krivoy, 1957).

In the work presented here, the catabolism of four physiologically active polypeptides, namely bradykinin, oxytocin, substance P and vasopressin, by homogenate of dog hypothalamus has been studied.

## METHODS

*Preparation of tissue.* Dogs of either sex and weighing 9–25 kg. were anaesthetized with ether or with Pentothal Sodium and killed by bleeding from the neck vessels. The brain was removed from the skull and the hypothalamus dissected out after removal of the optic nerves and the corpora mamillaria. A few brains were obtained from dogs previously used for an abdominal operation under chloralose anaesthesia. Hypothalami were homogenized in 10 vol. of double-distilled water and dialysed, with agitation, against approx. $10^6$ vol. of water for 6 hr. at 4°.

*Conditions of incubation.* Homogenate was incubated at 37° for various times, and at different pH values, with bradykinin, oxytocin, substance P and vasopressin; the substrate concentrations were 2 $\mu$g. of bradykinin/ml., 1 International Oxytocic Unit (i.u.) of oxytocin/ml., 40 Euler (1942) units of substance P/ml. and 1 International Pressor Unit (i.u.) of vasopressin/ml. Enzyme action was stopped by immersion in boiling water for 15 min.

*Materials.* Pitocin and Pitressin (Parke, Davis and Co., London,) were sources of oxytocin and vasopressin respectively. Bradykinin was a pure synthetic sample (lot 4447 × 116) supplied by courtesy of Parke, Davis and Co., Detroit, Mich., U.S.A. Substance P was kindly supplied by Dr J. H. Gaddum; it was a horse-gut preparation extracted according to Pernow (1953) and assayed at 40 Euler units/mg.

Phosphate buffers ($NaH_2PO_4$–$Na_2HPO_4$) were used in the pH range 5·5–8·0; below pH 5·5 and above pH 8·0 acetate and borate buffers respectively were used. The concentration of buffers in incubates was made approximately isotonic with blood to obviate osmotic effects on the bioassay preparations.

*Methods of biological assay.* Oxytocin was measured by the avian-depressor method of Coon (1939) or with the isolated rat uterus. The rat uterus was always used where hormone concentrations were less than about 20 milliunits/ml. Vasopressin was measured by the pressor method of Dekanski (1952) on rats (sometimes respired artificially) anaesthetized with urethane. Atropine-treated animals were used for the assay of samples containing di-isopropyl phosphorofluoridate. Bradykinin and substance P were estimated with isolated guinea-pig ileum in the presence of atropine sulphate (0·1 $\mu$g./ml.) and mepyramine (1 $\mu$g./ml.). A bracketing type of assay was used (Hooper & Jessup, 1959). The fiducial limits of assays were similar to those obtained in previous work (Hooper, 1959) and are given only where relevant.

## RESULTS

*Nature of the inactivation of oxytocin and vasopressin by homogenate of dog hypothalamus.* Preliminary work showed that no detectable hormones were formed when homogenate was incubated alone at pH 7·0 and 37°. Probably, therefore, the system was intrinsically incapable of producing oxytocin and vasopressin, or, if hormones were produced, they were inactivated soon after formation. Added oxytocin or vasopressin were inactivated on incubating with homogenate. However, hormones were not inactivated on incubation with homogenate previously heated at 100° for 15 min.; extensive dialysis of homogenate against water did not affect hormone inactivation. Although such properties suggested enzyme action, apparent loss of hormones owing to reaction with heat-labile non-dialysable material, or a pharmacological antagonism during bio-assay could not be excluded. The enzymic nature of the inactivation of the polypeptides was confirmed by measuring the simple kinetics of the system.

*Optimum* pH *for inactivation of bradykinin, oxytocin, substance P and vasopressin.* Four hypothalami were used to determine the optimum pH of destruction; of these, two were combined to compare, approximately, the rates of destruction of oxytocin and vasopressin. Different homogenates were used for bradykinin and substance P experiments and rates of destruction are therefore not directly comparable. The dialysed homogenate was incubated with polypeptides at selected pH values for 3 hr. at 37° with the substrate concentrations given in the Methods section. The pH of the incubates was then adjusted to approx. 7 (with narrow-range indicator paper) before heating at 100° for 15 min., and the residual biological activity was measured by the appropriate method.

The relation between destruction and pH is shown in Fig. 1. The logarithm of the ratio of the initial peptide concentration ($A_0$) to the residual polypeptide concentration ($A$) is plotted as ordinate. The curves obtained for bradykinin, oxytocin and vasopressin are similar, showing maxima between pH 7 and 8, the peak of optimum inactivation of substance P being somewhat lower. The known instability of substance P in alkali (Gaddum & Schild, 1934) is probably reflected in the increased inactivation observed at pH 9. In subsequent work

a compromise between optimum pH of inactivation and a pH suitable for bio-assay purposes was selected (pH 7·3). The biological activities of the four peptides were not altered, within experimental error, by heating at 37° for 3 hr. at pH 7·3 and at 100° for 15 min. Fig. 1 also shows that oxytocin is destroyed more rapidly than is vasopressin.

*Progress curve of inactivation of bradykinin, oxytocin, substance P and vasopressin.* The curves shown in Fig. 2 were obtained by incubating the polypeptides at pH 7·3 and 37° with dialysed homogenates obtained from four hypothalami and measuring the residual biological activity at different times. The results are plotted for a first-order reaction. The rates of destruction of the four peptides do not follow precisely that predicted by first-order kinetics, since the rate of inactivation, indicated by the slope of the lines, decreases with time. The destruction of substance P shows the same pattern as the other peptides for the first 3 hr. After 3 hr., however, the residual biological activity remained constant at about 5% (Fig. 2). The specimen of substance P used contained small amounts of other physiologically active materials (J. Cleugh, J. H. Gaddum & K. C. Hooper, unpublished work), and the 5% activity remaining after 3 hr. is probably due to these contaminants. The absence of a definite peak in Fig. 1 is probably also due to contaminants. The rates of destruction of bradykinin, oxytocin and vasopressin did not follow a composite zero- and first-order reaction, and large deviations from linearity were obtained when using the expression derived by Elkins-Kaufman & Neurath (1948). It is possible that a first-order reaction would have been followed with different concentrations of substrate, but such studies would be more meaningful if made with pure preparations of enzyme and substrate.

*Relation between tissue concentration and inactivation of bradykinin, oxytocin, substance P and vasopressin.* Four hypothalami were used. Two of these were combined, and the dialysed homogenate was incubated with oxytocin and vasopressin to compare the rates of destruction of the two hormones. For the destruction of bradykinin and substance P, tissues from different animals, presumably with different concentrations of enzyme, were used. Fig. 3 shows an approximately linear relation between tissue nitrogen and the logarithm of the

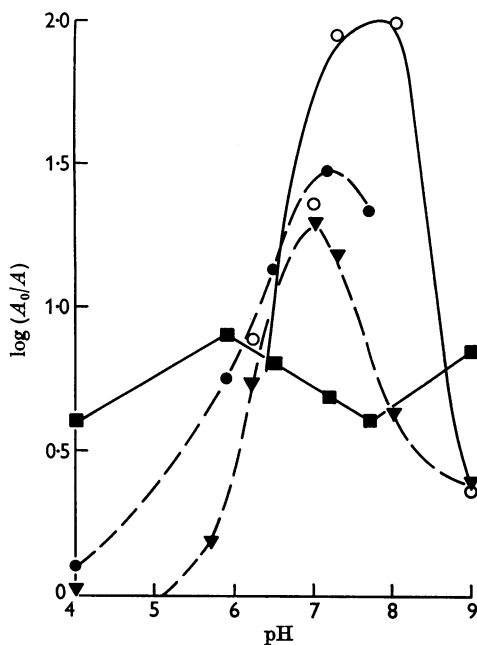


Fig. 1. Destruction of polypeptides as a function of pH. Polypeptides were incubated with homogenate of dog hypothalamus for 3 hr. at 37° at the pH values indicated. Reaction was stopped by heating at 100° for 15 min. and the residual biological activity measured as described in text. $A_0$ and $A$ are initial and residual polypeptide concentrations respectively. ●, Bradykinin; ○, oxytocin; ■, substance P; ▼, vasopressin.

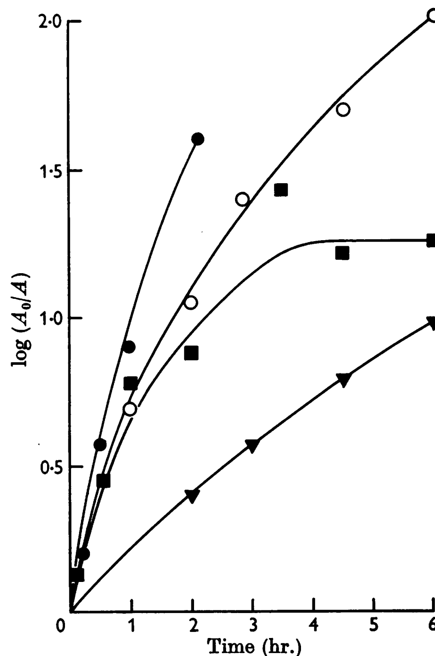


Fig. 2. Progress curves for destruction of polypeptides by homogenate of dog hypothalamus. Polypeptides were incubated with tissue at pH 7·3 and 37°. Samples were taken at different times and residual biological activity was measured and plotted as described for Fig. 1. Curves for oxytocin and vasopressin were obtained with the same homogenate.

residual concentrations for bradykinin, oxytocin and vasopressin. The curve for substance P deviates slightly from linearity owing probably to the presence of enzyme-resistant impurities. The rates of destruction of the peptides could not readily be defined in terms of kinetic constants (Fig. 2). It is convenient therefore to express enzyme activity in arbitrary units, with Fig. 3 as the reference curve, a unit of activity being defined as the amount of enzyme required to destroy 80% [$\log (A_0/A) = 0{\cdot}70$] of substrate when incubated under specified conditions, i.e. substrate (initial concentration as described in the Methods section) incubated at pH 7·3 for 3 hr. and 37°.

### *Inactivation of mixed substrates*

*Destruction of a mixed substrate of oxytocin and vasopressin by homogenate of dog hypothalamus.* Oxytocin and vasopressin are present in hypothalami of animals killed by methods which do not cause a depletion of the hormones, and, in dogs, the ratio of vasopressin to oxytocin is about 14:1 (Vogt, 1953). As is shown in Figs. 1 and 3, homogenate of dog hypothalamus destroys oxytocin more rapidly than it does vasopressin, and it was of interest therefore to measure the rates of destruction of the two hormones by homogenate when incubated separately and as a mixture. Hypothalami from two dogs were combined and incubated with hormones with substrate concentrations of 1 unit of each hormone/ml. Fig. 4 shows that when incubated singly oxytocin was inactivated approx. 2·8 times as rapidly as vasopressin. The rates of destruction of the two peptides obtained with mixed substrates, however, shows that the rate of inactivation of oxytocin is reduced and is now about the same as that of vasopressin. The rate of inactivation of vasopressin is only slightly reduced by the presence of oxytocin. No detectable interference from the mixed substrates was encountered during the bio-assay of hormones.

*Inactivation of a mixed substrate of substance P and vasopressin by homogenate of dog hypothalamus.*

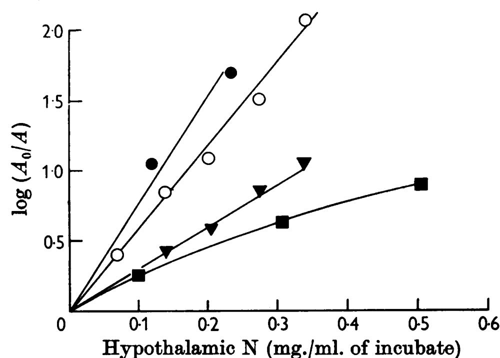


Fig. 3. Effect of homogenate concentration on destruction of polypeptides. Homogenate of dog hypothalamus was incubated at the concentrations indicated with the polypeptides for 3 hr. at 37°. Residual biological activity was measured and plotted as in Fig. 1.

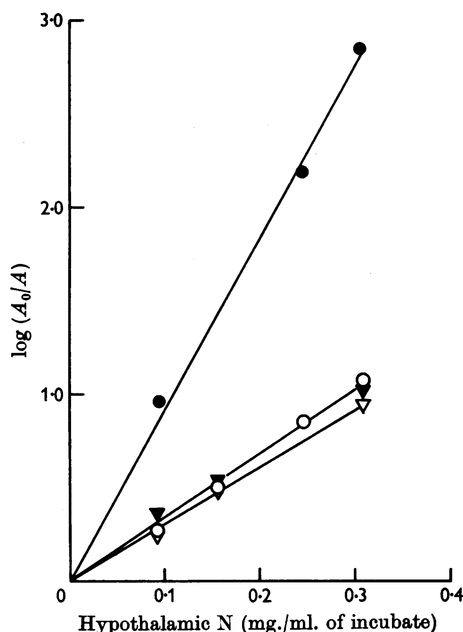


Fig. 4. Destruction of a mixed substrate of oxytocin (1 i.u./ml.) and vasopressin (1 i.u./ml.) by homogenate of dog hypothalamus. Hypothalami from two dogs were combined and the homogenate was used at the concentrations indicated. Incubation conditions and method of hormone estimation were as described for Fig. 3. ●, Oxytocin (sole substrate); ○, oxytocin (mixed substrate); ▼, vasopressin (sole substrate); ▽, vasopressin (mixed substrate).

Table 1. *Effect of pyridoxal phosphate and cyanide on the inactivation of vasopressin by homogenate of dog hypothalamus*

Dog hypothalamus was homogenized and dialysed as described in the Methods section. Homogenate from 60 mg. of tissue was incubated with vasopressin (2 i.u.) and 0·4M-phosphate buffer, pH 7·3 (0·4 ml.), for 3 hr. at 37°, in a total volume of 2 ml. Pyridoxal (1 $\mu$g./ml.) and cyanide (mM) were added as indicated. Reaction was stopped by heating at 100° for 15 min., and residual hormone was assayed by the rat-pressor method. Fiducial limits are given for a probability of 0·95.

| Preparation | Residual vasopressin (%) | Fiducial limits |
|---|---|---|
| Enzyme | 9·7 | 8·7–10·7 |
| Enzyme + coenzyme | 9·7 | 9·2–10·2 |
| Enzyme + cyanide | 7·6 | 5·8–9·4 |
| Enzyme + coenzyme + cyanide | 8·1 | 6·8–9·4 |

Table 2. *Effect of enzyme inhibitors on the inactivation of vasopressin by homogenate of dog hypothalamus*

Homogenate was prepared as described in the Methods section. In group (*a*) the homogenate from 68 mg. of tissue was diluted with 0·4 M-phosphate buffer, pH 7·3 (0·8 ml.), and aqueous solution of inhibitor to give the inhibitor concentrations shown below. After 15 min. at room temperature, vasopressin (4 i.u.) was added to make the total volume up to 4 ml. The mixture was incubated at 37° for 3 hr., heated at 100° for 15 min. and the residual vasopressin measured. The same procedure was used for group (*b*) except that the homogenate was prepared from 52 mg. of tissue obtained from a different animal. Fiducial limits are given for a probability of 0·95.

| Preparation | Residual vasopressin (%) | Fiducial limits | Inhibition (%) |
|---|---|---|---|
| (*a*) Enzyme | 13·9 | 10·4–17·4 | — |
| Enzyme + $Fe^{3+}$ (mM) | 13·6 | 12·5–14·7 | 0 |
| Enzyme + $Zn^{2+}$ (mM) | 12·9 | 7·1–18·7 | 0 |
| Enzyme + $CN^{-}$ (mM) | 12·4 | 9·8–15·0 | 0 |
| Enzyme + $F^{-}$ (mM) | 14·7 | 14·0–15·4 | 0 |
| Enzyme + EDTA (disodium salt) (mM) | 12·8 | 11·3–15·3 | 0 |
| (*b*) Enzyme | 21·8 | 19·7–23·9 | — |
| Enzyme + $Cu^{2+}$ (mM) | 43·9 | 43·1–44·7 | 46 |
| Enzyme + iodoacetamide (10 mM) | 23·3 | 22·2–24·4 | 0 |
| Enzyme + di-isopropyl phosphorofluoridate (10 mM) | 37·9 | 36·0–39·8 | 33 |

Substance P and vasopressin were incubated separately and mixed with homogenate at peptide concentrations of 40 Euler units/ml. and 1 i.u./ml. respectively. The presence of vasopressin caused a slight decrease (14 %) in the rate of inactivation of substance P, in contrast with the much larger effect it had on that of oxytocin (Fig. 4). The effect of substance P on the rate of destruction of vasopressin could not be assessed owing to limitations of the bio-assay method: substance P causes a vasodepressor effect on rat blood pressure, and, in the mixed substrate experiments, the peptide was present in sufficient amounts to make a pressor method of assay invalid.

*Effect of pyridoxal on the inactivation of vasopressin by homogenate of dog hypothalamus.* Itoh & Kikuchi (1959) stated that the antidiuretic activity of vasopressin was not lost on incubating the hormone with homogenate of rat brain at pH 7·2; on addition of pyridoxal to the incubation medium, however, a loss of antidiuretic activity was noted. From this, it seemed possible that pyridoxal might be a cofactor of the enzyme present in dog hypothalamus, even though the enzymes was not inactivated by extensive dialysis. The concentration of pyridoxal in dog brain does not appear to be known, but Rabinowitz & Snell (1948) examined rat brain and found $9{\cdot}2 \pm 0{\cdot}9\,\mu g.$ of pyridoxal hydrochloride/g. of dry tissue. Experiments in which pyridoxal phosphate (33 $\mu$g./g. of wet tissue) was added were therefore carried out. Table 1 shows the effect of incubating homogenate with vasopressin with and without pyridoxal, and the effect of cyanide which is an inhibitor of pyridoxal-dependent enzymes: the enzyme is neither pyridoxal-dependent nor cyanide-sensitive.

*Effect of some enzyme inhibitors on the inactivation of vasopressin by homogenate of dog hypothalamus.* Homogenate was left for 15 min. with inhibitor at room temperature and at pH 7·3 before the addition of substrate. The effect of the inhibitors is listed in Table 2. The enzyme appears to have no requirement for metal ions since cyanide, fluoride and EDTA (disodium salt), at millimolar concentration, do not inhibit. The ineffectiveness of iodoacetamide indicates that the enzyme does not possess thiol groups which are essential for activity. Di-isopropyl phosphorofluoridate (mM) appeared to have some inhibitory action, but results were not significantly different from those obtained with untreated enzyme; 10 mM-di-isopropyl phosphorofluoridate did possess inhibitory action. However, the enzyme is unlikely to belong to the 'serine' group of enzymes in view of the high concentration of inhibitor required to produce the effect. Of the metal ions tested, $Cu^{2+}$ ion was the only one which produced any definite inhibition. The effect of these inhibitors on the stability of vasopressin, and on the biological assay of the hormone, has been shown to be small in the presence of homogenate of placenta (Hooper, 1959), and control experiments were therefore not done with brain preparations.

## DISCUSSION

In addition to producing oxytocin and vasopressin (for references see Ortmann, 1960) and possibly substance P, dog hypothalamus also contains enzymes destroying the peptides. When looking for such enzymes, it was necessary to minimize the effects of endogenous materials contributing to substrate concentrations or interfering with bio-assay procedures. Hypothalamus contains several physiologically active compounds

eliciting responses in bio-assay preparations used to measure bradykinin, oxytocin, substance P and vasopressin. Dialysable materials which would interfere have been reduced to approximately one-millionth of their initial concentration. The amount of endogenous posterior-pituitary hormones is reduced in dogs killed by bleeding under ether anaesthesia, for both haemorrhage and ether are known to deplete storage depots of oxytocin and vasopressin. The amount of vasopressin in hypothalami of dogs killed with the minimum of disturbance varies greatly; Van Dyke, Adamsons & Engel (1955) found an average of approx. 4·07 units/hypothalamus in a group of eleven animals. It was not possible to measure the concentration of vasopressin in the homogenates used in the present work, since toxic effects were noted before the threshold concentration of hormone was reached. However, each millilitre of incubate corresponded, in most cases, to less than a twenty-fifth of a hypothalamus. The amount of hormone in a twenty-fifth of an undepleted hypothalamus would be approx. 80 milliunits or 16 % of added hormone. The amount of hormone in the hypothalamus of an animal killed as described would be expected to be less. The ratio of vasopressin to oxytocin in dog hypothalamus is approx. 14:1 (Vogt, 1953; Van Dyke *et al.* 1955); the effect of endogenous oxytocin on substrate concentration would therefore be negligible.

The hydrolysis of synthetic peptides by brain and pituitary has been examined by several workers (Pope & Anfinsen, 1948; Adams & Smith, 1951; Ansell & Richter, 1954*a*; Hanson & Tendis, 1954), although in many instances the brain peptidases themselves have not been well characterized. Adams & Smith (1951) examined pig pituitary and found, amongst other peptidases, leucine aminopeptidase and a prolidase. Leucine aminopeptidase and prolidase are inhibited by EDTA (Smith & Hill, 1960) and thiol reagents (Smith, 1960) respectively, whereas the enzyme destroying vasopressin is unaffected by these reagents. By definition, di- and tri-peptidases would not hydrolyse the physiologically active peptides tested here.

The hydrolysis of haemoglobin has frequently been used as a measure of brain proteolytic activity. Most proteolytic activity appears to be associated with cathepsins having an optimum pH near 3·5 (Kies & Schwimmer, 1942; Adams & Smith, 1951; Ansell & Richter, 1954*a*; Palladin, 1961). A second group of proteolytic enzymes has an optimum pH near 7. Ansell & Richter (1954*b*) described an unstable neutral proteinase which could not be detected 1½ hr. after death, and which was inhibited by iodoacetate. Adams & Smith (1951) found an enzyme hydrolysing haemoglobin, edestin, and human serum albumin having an optimum pH of 8·3 and which was inhibited by 0·1 M-phosphate. It is probable therefore that vasopressin is destroyed by an enzyme different from those previously shown to be present in brain and pituitary.

Itoh & Kikuchi (1959) found that homogenates of rat whole brain, and of rat hypothalamus, were unable to inactivate vasopressin unless pyridoxal was added to the incubation medium. It was suggested that inactivation was due to absorption of hormone by protein, facilitated by the presence of pyridoxal. The incubation conditions used by Itoh & Kikuchi (1959) were such that the ratio of the weights of pyridoxal to vasopressin was 250:1. The stability of vasopressin in the presence of such large amounts of coenzyme was therefore measured. After incubating 2 $\mu$g. (1 i.u.) of hormone with 200 $\mu$g. of pyridoxal phosphate for 3 hr. at 37° at pH 7·3 no vasopressin could be detected. It seemed likely that the inactivation of vasopressin under these conditions was due to the formation of a Schiff base between the hormone and the cofactor.

Although homogenate of hypothalamus inactivated the four biologically active peptides tested, the number of enzymes involved is not known. Results obtained from mixed substrates suggest that oxytocin and vasopressin are destroyed by the same enzyme. Vasopressin markedly decreased the rate of inactivation of oxytocin, and there are several possible reasons for this. First, the hormones are destroyed by an enzyme having a higher affinity and a lower maximum velocity of destruction for vasopressin. Secondly, the hormones are inactivated by different enzymes, the observed decrease in the rate of inactivation of oxytocin being due to the presence of competitive substrates present in the impure sample of vasopressin. Thirdly, if two enzymes are involved, each may be inhibited by the substrate for the other, or by substances present in the impure samples of the hormones. The first of these possibilities appears to be the more likely, owing to the similarity of the pH–activity curves and the common source of both polypeptides. The fact that vasopressin did not interfere with the destruction of substance P, and also the difference between the optima pH of inactivation of the polypeptides, suggests that different enzymes are responsible for the destruction of these two substances. More convincing evidence for the existence of separate enzymes is that dorsal roots from the lumbar region of the dog's spinal cord did not contain detectable amounts of enzyme destroying vasopressin whereas substance P was inactivated. Krivoy (1957) stated that acetone-dried powder of guinea-pig brain inactivated substance P but not oxytocin.

The presence of enzymes destroying oxytocin, substance P and vasopressin in the hypothalamus may have some physiological significance. The

enzymes may prevent diffusion of the peptides into surrounding tissue, and serve to limit them to the particles in which they occur. The enzyme destroying substance P may possibly serve to terminate its action if substance P does act as a transmitting agent (Lembeck, 1953). Finally, the enzymes may assist in the maintenance of the amino acid pool in the hypothalamus, and the destruction of the four peptides may be incidental.


## SUMMARY

1. Incubation of bradykinin, oxytocin, substance P and vasopressin with homogenate of dog hypothalamus at pH 7·3 and 37° caused a reduction in the biological activity of the polypeptides.

2. The inactivation of the polypeptides is due to enzymes; bradykinin, oxytocin and vasopressin are destroyed most rapidly at pH 7–8, and substance P at pH 6.

3. The general properties of the enzyme destroying vasopressin, taken in conjunction with the effect of some enzyme inhibitors, suggest that it is different from enzymes previously found in brain.

4. Results obtained with mixed substrates suggest that oxytocin and vasopressin are substrates for the same enzyme; substance P appears to be inactivated by a different enzyme.


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# Carbohydrases of the Rumen Ciliate *Epidinium ecaudatum* (Crawley)

## ACTION ON PLANT HEMICELLULOSE

BY R. W. BAILEY, R. T. J. CLARKE AND D. E. WRIGHT
*Plant Chemistry Division, D.S.I.R., Palmerston North, New Zealand*

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The fermentation in the rumen of the hemicellulose fraction of pasture plants is well established. Several species of rumen bacteria, notably a *Butyrivibrio* species (probably *Butyrivibrio fibrisolvens*) (Howard, Jones & Purdom, 1960) and *Bacteroides amylogenes* (Howard *et al.* 1960), can utilize the xylan which is a major constituent of hemicelluloses. So far as we are aware, however, no species of rumen ciliate protozoa has been reported to hydrolyse or ferment this type of polysaccharide. Four species of rumen ciliates, *Dasytricha ruminantium*, *Isotricha prostoma*, *Isotricha*