though Boxer *et al.*(5) found some inhibition of coA itself by PAET, we have found that supplementation of the media with coA allowed the amebas to overcome inhibition by PAED. This establishes the importance of coA in the nutrition of *E. histolytica*.

Summary. 1. PAED, a pantetheine antagonist, inhibited bacteria-free cultures of *E. histolytica*. 2. Reversal of inhibition was possible with pantetheine and coenzyme A, but not with pantothenic acid. 3. The possible mechanism of action of PAED is discussed.

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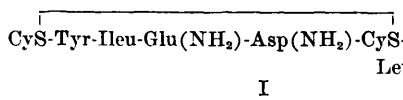
The Cyclic Disulfide Ring of Oxytocin.* (22594)

CHARLOTTE RESSLER. (Introduced by V. du Vigneaud.)
Department of Biochemistry, Cornell University Medical College, New York City.

The structure of oxytocin, the principal oxytocic hormone of the posterior pituitary gland, has been established through its synthesis(1,2) as the cyclic disulfide of L-cysteinyl - L-tyrosyl - L-isoleucyl - L-glutaminyl-L-asparaginyl - L-cysteinyl - L-prolyl - L-leucylglycinamide, which is represented by I. On the basis of structural studies, it has been



I

proposed that the antidiuretic hormone from oxen is structurally similar to oxytocin(3,4), with L-phenylalanine and L-arginine in vasopressin replacing the L-isoleucine and L-leucine, respectively, of oxytocin. An octapeptide amide synthesized according to this structure has been found qualitatively to have the expected biological activity(5). Moreover, vasopressin isolated from the hog has been found to differ in composition from beef vasopressin by the substitution of lysine for arginine(6). It has been suggested that both these vasopressins are otherwise structurally identical(3), and this has received strong support from the results of recent studies on the synthesis of lysine vasopressin(7).

Although some of the more well established physiological activities of oxytocin, namely, the oxytocic, milk-ejecting, and avian vasodepressor effects, are strikingly different from the pressor and antidiuretic activities of vasopressin, it may be noted that qualitatively these hormones possess several biological activities in common. Thus the vasopressins also have some oxytocic, avian vasodepressor(8,9) and milk-ejecting activity(9-11), and oxytocin also possesses low but definite degrees of pressor and antidiuretic activity(12), in addition to the other physiological activities of each of these hormones.

A striking structural feature of both of these hormones is a 20-membered cystine-containing peptide ring. It is of interest that in the structure of insulin as postulated by Sanger and his associates, an intrachain disulfide ring of the same size as that which occurs in oxytocin and vasopressin has also been proposed(13).

One interesting speculation resulting from these observations is that the ring structure itself of the posterior pituitary hormones might possess intrinsic biological activity. Ac-

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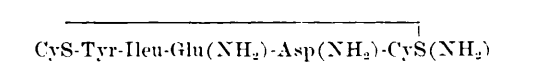
tivity inherent in the ring might then account for the presence of many of the same physiological activities in oxytocin and vasopressin. In this connection and as part of a general study in this laboratory of the relationship of structure to the biological activity of the posterior pituitary hormones, the synthesis of the compound, the cyclic disulfide of L-cysteinyl - L-tyrosyl - L-isoleucyl - L-glutaminyl-L-asparaginyl - L-cysteinamide, represented by II, was undertaken.



II

This compound represents the ring moiety of oxytocin, where the prolylleucylglycinamide side chain linked to the cyclic portion of oxytocin has been replaced simply by an amide grouping. The C-terminal residue of the resulting peptide is therefore a half-cystine amide residue which forms part of the 20-membered ring, being linked through its amino group to the carboxyl group of the asparagine residue and through its sulfur to the other half-cystine residue which bears the free amino group.

Of the several routes possible for preparing this compound the one described was chosen because of its similarity to the route used originally in the synthesis of oxytocin(1,2). By the use of a parallel approach, it has been possible to take advantage of the fact that several intermediates common to both these compounds could be used. Thus the tripeptide and dipeptide derivatives, tosyl-L-isoleucyl - L-glutaminyl - L-asparagine(14) and carbobenzoxy-S-benzyl - L-cysteinyl - L-tyrosine(15), respectively, which had been used previously in the synthetic oxytocin work also served as intermediates in the synthesis of the cyclic disulfide II. However, for the tetrapeptide amide S-benzyl - L-cysteinyl - L-prolyl - L-leucylglycinamide(16) used in the former synthesis, the amino acid derivative S-benzyl - L-cysteinamide has now been substituted. The yields in this synthesis, however, were somewhat lower.

Experimental.† S-benzyl - L-cysteinamide was prepared by the amination of ethyl S-benzyl-L-cysteinate‡ in methanolic ammonia. A suspension of 7.9 g of ethyl S-benzyl-L-cysteinate hydrochloride in 50 cc of ethyl acetate was treated with 7.9 cc of triethylamine for 30 minutes. It was then filtered and concentrated and the residue was dissolved in 135 cc of anhydrous methanol saturated with ammonia. After 3 days the solvent and NH_3 were removed and the residue was crystallized from ethyl acetate, 5.5 g (92%); m.p. 78.5-79.5°. A sample of the free base was recrystallized from toluene; m.p. 80°, $[\alpha]_D^{23}$ + 5.65° (*c* 3, acetic acid).

Anal. Calcd. for $C_{10}H_{14}ON_2S$: S, 15.3; N, 13.3. Found: S, 15.2; N, 13.3.

The S-benzyl - L-cysteinamide (0.56 g) was coupled with tosyl-L-isoleucyl - L-glutaminyl-L-asparagine(1.0 g) at 95° for 1 hour in 10 cc of diethyl phosphite by the use of 1.4 cc of tetraethyl pyrophosphite(17). The protected tetrapeptide tosyl-L-isoleucyl-L-glutaminyl - L-asparaginyl-S-benzyl - L-cysteinamide was precipitated with water and washed with warm methanol; wt. 1.0 g, m.p. 249-250°. After several crystallizations from acetic acid or diethyl phosphite a sample melted at 273-274°.

Anal. Calcd. for $C_{32}H_{45}O_8N_7S_2$: C, 53.4; H, 6.3; S, 8.9. Found: C, 53.7; H, 6.3; S, 8.8. Removal of the S-benzyl and N-tosyl protecting groups and rebenzylation was effected in 70% yield by treatment of 100 mg of the protected tetrapeptide with sodium in liquid ammonia followed by 0.05 cc of benzyl chloride in 0.5 cc of toluene(18,19). The isoleucyl-glutaminyl-asparaginyl - S - benzyl-cysteinamide‡‡ obtained was purified by countercurrent distribution(20) in the system 2-butanol-0.01 *M* NH_3 in which the partition coefficient was approximately 1.0. A suspen-

† The reported melting points are corrected, capillary melting points.

‡ This compound was prepared according to Harington, C. R., and Pitt Rivers, R. V.(15). It melted at 161-162°; reported, 156-157°.

‡‡ When the tetrapeptide was used without purification, several byproducts were isolated along with the final product. Studies on these products will be reported separately.

sion of 100 mg of this tetrapeptide in 0.5 cc of diethyl phosphite containing 0.1 cc of tetraethyl pyrophosphite was allowed to react with 135 mg of N-carbobenzoxy S-benzyl-L-cysteinyl - L-tyrosine at 85° for one-half hour. The resulting protected hexapeptide N-carbobenzoxy-S-benzyl - L-cysteinyl - L-tyrosyl - L-isoleucyl - L-glutaminyl - L-asparaginyl-S-benzyl-L-cysteinamide, which was also prepared by the use of the coupling agent N, N'-dicyclohexylcarbodiimide(21), was washed with warm methanol; wt. 93 mg. When it was reprecipitated from formamide it melted at 249-250°. This was treated with sodium in liquid ammonia to remove the protecting groups(18) and after evaporation of the ammonia the sulfhydryl product was aerated in aqueous solution at pH 6.5(1,2), after which the solution was adjusted to pH 3.5, concentrated under reduced pressure and finally dried by lyophilization. The material was isolated by the use of countercurrent distribution in the system 0.05% acetic acid-2-butanol. The distribution of material was determined by following the absorption in the ultraviolet at 275 mμ and the distribution of weight. The contents of a peak having the partition coefficient $K = 0.19$ were concentrated and lyophilized and yielded a white powder, $[\alpha]_D^{23} - 20.5°$ (*c* 0.51, water).

For analysis the compound was dried at 100°C *in vacuo* and showed a loss in weight of 3.9%. The analytical values are calculated on the basis of the dried sample and are corrected for 1.0% ash.

Anal. Calcd. for $C_{30}H_{45}O_9N_9S_2 \cdot C_2H_4O_2$: C, 48.1; H, 6.2; N, 15.8. Found: C, 47.8; H, 6.5; N, 16.0. When the material was hydrolyzed in acid and analyzed on the starch column according to the quantitative procedure of Moore and Stein(22), it showed the expected amino acid composition in the following ratio: isoleucine 1.0, tyrosine 0.8, glutamic acid 1.0, aspartic acid 1.1, cystine 1.0, ammonia 3.6. Like oxytocin, this polypeptide underwent cleavage into two peptides on treatment with bromine water(2).

Results. The purified peptide was assayed for the principal physiological activities normally associated with oxytocin, namely, the oxytocic, milk-ejecting, and avian vasodepressor activities, the latter being the effect on the blood pressure of the fowl. The USP Standard posterior lobe powder served as the reference in each of these determinations. The cyclic peptide was found to have a low but definite degree of oxytocic activity. When measured on the isolated rat uterus according to the method of Burn(23[a,b]), it assayed approximately 3.3 U/mg. It was therefore roughly 1/150 as active as oxytocin and approximately 1/10 as active as beef vasopressin in this respect. The cyclic peptide was also found to be active in effecting the let-down of milk,§ the potency being approximately 1.1 U/mg. These activities, while low, are considered real because of the great sensitivity of the assay methods. The sensitivity of the uterus method is approximately 2 mU, and in the assay for milk-ejecting activity, 0.5 mU can be detected(12). The material was also assayed for strepogenin activity by the method used previously for related peptides(24) and had a potency of 55 U/mg.‖

The most striking aspect of the pharmacology of the cyclic peptide was concerned with the avian vasodepressor effect. The depressor effect of oxytocin on the blood pressure of the chicken has been associated with its oxytocic activity(25) and in pure oxytocin the former activity is present to the same degree as the oxytocic activity. The official assay for the hormone adopted by the United States Pharmacopeia(26) is in fact based on the avian blood pressure depressor method. Assay of the cyclic peptide, however, for this effect on blood pressure by the method of Coon(27) using the anesthetized chicken showed that, although the compound pos-

§ The assays for milk-ejecting activity were kindly performed by Dr. H. B. van Dyke, Mr. S. L. Engel, and Dr. K. Adamsons, Jr. of the College of Physicians and Surgeons, Columbia University, to whom appreciation is expressed. The assay procedure involved measurements of the pressure within the lactating mammary gland of the rabbit.

‖ Strepogenin activity was determined through the kindness of Dr. D. W. Woolley and Dr. R. B. Merrifield of the Rockefeller Institute for Medical Research.

sessed significant oxytocic and milk-ejecting activities, the avian depressor effect was absent within the limits of the assay method. Any avian depressor activity, if present, would therefore be less than approximately 0.03 U mg. The small pressor effect which oxytocin has been found to possess(12) was also not detectable in the cyclic peptide.

Considerable interest arises from a comparison of the ratio of the potencies of the biological effects of the peptide with that ratio in oxytocin. Thus, while the oxytocic, milk-ejecting, and avian depressor activities of the hormone are equipotent in terms of the standard, the corresponding ratio of these activities in the cyclic peptide is approximately $3:1:<0.03$. There is, therefore, 3 times as much oxytocic as milk-ejecting activity per unit of weight of the peptide. Simplification of the structure of oxytocin has thus resulted in a markedly disproportionate change in the potencies relative to each other of the several physiological activities of the hormone. It is conceivable that this type of change may be a general consequence of structural alteration when dealing with compounds that exhibit multiple physiological activities.

The virtual absence of avian depressor activity in the cyclic peptide and the difference in the relative oxytocic and milk-ejecting activities afford a pharmacological differentiation of it from the hormone. The compound has been differentiated from oxytocin in another way, *i.e.*, by means of a comparison of the degree of potentiation of the oxytocic effect brought about by the presence of magnesium ion. This *in vitro* effect of magnesium has been described by Fraser(28) in experiments in which the oxytocic activity of various preparations of oxytocin and vasopressin of low potency as well as of mixtures of these was determined using the guinea pig uterus in a medium containing graded amounts of magnesium. The cyclic peptide was assayed directly against a solution of pure synthetic oxytocin diluted to approximately the same potency in modified Locke solution(23[b]) using the rat uterus. A similar assay was performed in Tyrode solution (29), which contained 10 mg% $MgCl_2$. The oxytocic activity of the cyclic peptide was increased approximately 10 times in this concentration of magnesium ion, while the synthetic oxytocin underwent approximately 63 fold potentiation.

It could be suspected that the relatively low degree of biological activity in this peptide may not be a property of the compound itself, but may be due possibly to the presence of a small amount of oxytocin picked up as a contaminant in the laboratory. However, the absence of avian depressor activity in the cyclic pentapeptide, the differences between oxytocin and the cyclic peptide with respect to the ratio of the oxytocic/milk-ejecting potencies and the degree of potentiation of the oxytocic effect by magnesium ion, as well as the fact that the final product was extensively purified, make this possibility extremely unlikely.

The results of the bioassays of the peptide allow certain deductions with respect to some of the structural requirements for biological activity in oxytocin and closely related compounds. The significant degree of activity possessed by the peptide that represents the cyclic moiety of oxytocin makes it clear that the entire molecule of oxytocin is not necessary for the existence of milk-ejecting and oxytocic activity. The fact that the activity of the cyclic peptide is of a relatively low order of magnitude could suggest that the minimum requirements for milk-ejecting and oxytocic activities in oxytocin might possibly be defined by the cyclic part of the oxytocin molecule. The tripeptide side chain of oxytocin or portions of the side chain would then serve to enhance these biological activities. For significant avian depressor activity to be present in these peptides, however, there would appear to be different, perhaps more extensive structural requirements. It may be added in this connection that several other peptides closely related in structure to oxytocin have been tested and have been found not to have significant activity. Thus, the linear peptide the disulfide of L-isoleucyl-L-glutaminyl - L-asparaginyl - L-cysteinyl - L-prolyl - L-leucylglycinamide(24) which rep-

resents an even larger, although partly different, portion of the sequence of the amino acids of oxytocin than does the cyclic peptide has been found to possess negligible oxytocic, milk-ejecting,§ and avian depressor activity. Preliminary testing of an aqueous solution of the disulfide of L-isoleucyl - L-glutaminyl-L-asparaginyl - L-cysteinamide obtained by the treatment of the corresponding tosyl S-benzyl peptide with sodium in liquid ammonia followed by aeration without further purification showed that this compound also seems to have negligible oxytocic and avian depressor activity.

Comparison of the highly active oxytocin, the weakly active cyclic pentapeptide, and the inactive heptapeptide and tetrapeptide disulfides with respect to their potencies of oxytocic, milk-ejecting, and avian depressor activity show clearly that a considerable degree of biological specificity is involved here. However, inasmuch as oxytocin and the two vasopressins do share these activities to varying degrees and inasmuch as the cyclic peptide possesses two of them, it is apparent that certain structural changes are allowable. It would seem that perhaps within a necessary framework of the molecule, a minimum requirement of which may be the cyclic part, some change, such as the substitution of certain of the amino acids by others, may be tolerated without complete destruction of these three biological activities of oxytocin.

It is of interest that there appears to be a completely different type of structural specificity involved in the biological effect associated with strepogenin activity. Thus, the otherwise inactive heptapeptide disulfide was found to be almost as active as oxytocin itself in stimulating the growth of *L. casei*(24). On the other hand the cyclic pentapeptide which does have significant oxytocic and milk-ejecting activities also has considerable strepogenin activity, and this activity is less than the strepogenin activity of the heptapeptide disulfide. It has been indicated that chain length of the peptide may be of some importance in determining the strepogenin activity for *L. casei*(30), and the strepogenin activity of the cyclic peptide would not be inconsistent with such a viewpoint.

Summary. A cyclic pentapeptide amide, the disulfide of L-cysteinyl - L-tyrosyl - L-isoleucyl - L-glutaminyl - L-asparaginyl - L-cysteinamide, which represents the cyclic moiety of oxytocin has been prepared synthetically and some of its physical and chemical properties are given. This compound has been found to have low but significant oxytocic (3.3 U/mg) and milk-ejecting (1.1 U/mg) activity but no detectable avian depressor activity. Moreover, this simplification of the oxytocin molecule appears to have resulted in a change in the ratio of the potencies of oxytocic, milk-ejecting, and avian depressor effects, the ratio being $3:1:<0.03$ for the cyclic pentapeptide amide compared to 1:1:1 for oxytocin. Some of the possible structural requirements for biological activity in oxytocin have been discussed in the light of these results.

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Eight Culture Strains (Detroit) of Human Epithelial-Like Cells.*† (22595)

LAWRENCE BERMAN AND CYRIL S. STULBERG. (Introduced by Harry Eagle.)

Department of Pathology, Wayne University College of Medicine, and The Child Research Center of Michigan, Detroit.

Various investigators have reported the development of stable strains of human cells capable of growing on glass surfaces in relatively simple fluid media(1-8). Some strains have been derived from tumor tissue and others from presumably normal tissue. Most have been described as "epithelial" although there are also human fibroblast-like strains in existence. Virologists have given a great deal of attention to one of these human cell strains, the HeLa strain(1), and wide use of this strain has stimulated the development of other human cell strains which might have additional or different useful properties.

A total of 8 epithelial-like strains have now been isolated in these laboratories(9,10). The present report is concerned with histories of the cell strains, procedures used for their isolation and maintenance, and preliminary observations on their morphology and growth behavior.

Histories of the cell strains. Five strains (Detroit-6, -32, -34, -52 and -98) were derived from cultures of sternal bone marrow. Two (Detroit-30A and -56A) were cultured from carcinomatous ascitic fluids and one (Detroit-116P) was obtained from lymphomatous pleural fluid. Table I lists the origin of each cell strain, pertinent data on the

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