of these substances. The mode of action is not clear but presumably may be dissociated from an activation of the plasma fibrinolytic system, inasmuch as chymotrypsin, which is active in reducing the granuloma reaction, is ineffective as an activator of the plasma fibrinolysin system(9,10). Since the effect upon granuloma size occurs in hypophysectomized animals (Table I) this action cannot be ascribed to a stimulation of the pituitary-adrenal system with consequent hypersecretion of cortical steroids. This indicates that the enzymes probably exert their action either through their own proteolytic or depolymerase properties, or by the activation of an anti-inflammatory mechanism distinct from the profibrinolysin-fibrinolysin system. Martin(11) has suggested that these enzymes may be adsorbed or concentrated at the site of fibrin deposits and may exert their anti-inflammatory properties by depolymerization or digestion of these fibrin deposits.

During the course of these experiments it was also observed that the incision area of the trypsin and chymotrypsin treated animals healed with less granulation tissue than did the similar areas in the untreated control group.

Summary. The parenteral administration of trypsin and chymotrypsin to animals carrying subcutaneous implants of cotton pellets, significantly reduces the size of the granulomas formed around these pellets. Heat inactivated enzyme does not manifest this property.

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Effect of Antimetabolites on Growth of *Endamoeba histolytica*. II. Pantetheine and Coenzyme A Antagonist.* (22593)

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The identity of a number of growth factors for *Endamoeba histolytica* has recently been established by Nakamura and Baker (1,2). Purine and pyrimidine analogues were found to be potent inhibitors of the amebas by Nakamura and Jonsson(3). Aside from this report and that of Kun *et al.*(4), who studied the effect of metabolic inhibitors on carbon dioxide and hydrogen sulfide production by *E. histolytica*, no other information is available with regard to the effect of antimetabolites on the amebas. This situation arises from the fact that until recently very little was known regarding the specific nutritional requirements of this protozoan. Since coenzyme A has been identified as a growth factor for *E. histolytica*(2), it became desirable to confirm this observation using a coenzyme A antagonist.

Boxer *et al.*(5) reported that β-*d*-pantoyl-

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aminoethanethiol (PAET) was an antagonist of the pantetheine-coenzyme A system *in vitro*. PAET inhibited the synthesis of coenzyme A from pantetheine and also the activity of coenzyme A itself. Structurally PAET contains all the functional groups of pantetheine but in a different molecular spacing.

The present studies have shown that the disulfide of PAET, bis (N-pantoyl-β-aminoethyl) disulfide (PAED),† is a potent inhibitor of *E. histolytica*.

Materials and methods. All experiments were performed with the NRS strain of *E. histolytica* under bacteria-free conditions employing technics described earlier(1-3). Briefly, the basal assay medium consisted of egg slants overlaid with serum-Ringer solution, dextrose, penicillin G, streptomycin, rice powder and sodium thioglycollate. The antagonist, PAED, was studied in the basal medium and also in the basal medium fortified with the growth factors adenosinetriphosphate (ATP) and ribose-5-phosphate (R-5-P). Calcium pantothenate and coenzyme A (coA) used for reversal studies were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, and pantetheine was obtained from the California Foundation for Biochemical Research, Los Angeles, Calif.

Results. PAED inhibited *E. histolytica* under bacteria-free conditions in dilutions of 1/1,000 to 1/50,000 (1 mg/ml to 0.02 mg/ml). This inhibition, as shown in Table I, was completely reversed by pantetheine and partially reversed by coA. Calcium pantothenate was inactive. PAED prevented growth of amebas even in media fortified with the growth factors ATP and R-5-P. In cultures of amebas containing associated bacteria (stock cultures), PAED was totally inactive due perhaps to an adequate supply of pantetheine or coA being continuously produced by the bacteria.

Discussion. Since both pantetheine and coA have been reported as growth-stimulatory substances for amebas(2), it is understandable that PAED was a potent inhibitor of *E. histolytica*. The reversal of inhibition by pantetheine and coA suggests strongly that PAED is an antimetabolite and not a nonspecific chemical toxic agent; however, these data do not allow us to determine whether this inhibition is competitive or non-competitive. Boxer *et al.*(5) have suggested possible mechanisms of action of the antagonist: (1) the antagonist prevents synthesis of coA from pantetheine, and (2) that an analogue of coA, containing the PAED moiety instead of pantetheine, is enzymatically synthesized. In amebic metabolism it is probable that the antagonist functions by blocking conversion of pantothenic acid to pantetheine, although it is also possible that it acts by preventing the conversion of pantetheine to coA. Al-

TABLE I. Inhibition of Bacteria-Free Cultures of *E. histolytica* by PAED and Reversal of Inhibition by Pantetheine and coA.

Material assayed	Total No. of determinations	Avg count of amebas per low power field at end of 3 days
Basal (control)	40	1
Basal + ATP + R-5-P	36	133
Basal + calcium pantothenate (1 mg/ml)	10	5
Basal + pantetheine (1 mg/ml)	9	64
Basal + coA (1 mg/ml)	7	41
Basal + PAED (.1 mg/ml) + ATP + R-5-P	4	0
Basal + PAED (.02 mg/ml) + ATP + R-5-P	4	0
Basal + PAED (.01 mg/ml) + ATP + R-5-P	4	11
Basal + PAED (.02 mg/ml) + ATP + R-5-P + calcium pantothenate (1 mg/ml)	4	0
Basal + PAED (.02 mg/ml) + ATP + R-5-P + pantetheine (.1 mg/ml)	6	101
Basal + PAED (.02 mg/ml) + ATP + R-5-P + coA (.5 mg/ml)	6	70
Basal + PAED (.02 mg/ml) + pantetheine (.1 mg/ml)	4	62
Basal + PAED (.02 mg/ml) + coA (.5 mg/ml)	4	37
Stock cultures (bacteria present)	70	51
Stock cultures + PAED (1 mg/ml)	4	53

† Obtained through the courtesy of Dr. Andrew N. Wilson, Chemical Division, Merck and Co., Rahway, N. J.

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)

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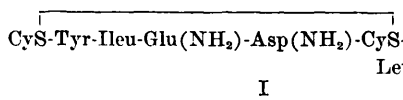
The structure of oxytocin, the principal oxytocic hormone of the posterior pituitary gland, has been established through its synthesis(1,2) as the cyclic disulfide of L-cysteinyl - L-tyrosyl - L-isoleucyl - L-glutaminyl-L-asparaginyl - L-cysteinyl - L-prolyl - L-leucylglycinamide, which is represented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



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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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