logically. Preliminary work suggests that androgens may also be excreted in the saliva.

Summary. Estrogenic substance has been biologically demonstrated in the saliva of a male and female patient receiving 1000 mg of stilbestrol potassium sulfate intravenously.

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Specificity of Cathepsin III. (21193)

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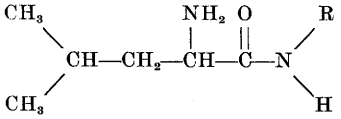
The cathepsins(10,11) have been extensively studied by Bergmann, Fruton, and their co-workers(3-5). Several distinct enzymes have been identified by their specific substrate requirements, pH optima, and their different requirements for activation by cysteine or ascorbic acid. This investigation deals with the substrate specificity of cathepsin III (leucine aminopeptidase) of beef kidneys. Several substrates which differ by the size of the group attached to the nitrogen atom of leucine have been utilized to determine the effect of chain length and of polarity upon the activity of the enzyme. The structures of the substrates used are shown by the formula below in which R represents, alternatively, –H, $-CH_2COOH$, $-CH_2CH_2COOH$, and $-CH_2CH_2CH_2COOH$.



Methods. Three different enzyme preparations were made by the procedure described by Fruton and Bergmann(3) and were stored at –10°C. Preparation B was activated prior to storage; preparations A and C were activated immediately before use. The enzyme was activated by incubating it for 2 hours at 40°C with 0.01 M cysteine at pH 5. During activation a heavy flocculent precipitate formed which was removed by centrifugation. The substrate solution was added to the buffered and activated enzyme solution and maintained at 40°C and pH 5 throughout the reaction period. Citrate buffer at a final concentration of 0.04 M was used to keep the pH constant. The initial concentration of substrate in the reaction mixture was 0.05 M. The rate of enzymic hydrolysis was followed by measurement of the liberated carboxyl groups by the micro-titration method of Grassman and Heyde(6). The enzyme concentration is expressed as milligrams of protein nitrogen per milliliter of test solution. The protein nitrogen was determined by a micro-Kjeldahl method described in the Official Methods of Analysis of the A.O.A.C.(8). Non-protein nitrogen was determined on filtrates prepared after precipitation of the protein with 5% trichloroacetic acid. The proteolytic coefficients C_0 and C_1 were calculated from the equation $C = K/E$ where K represents, respectively, the zero order and first order specific reaction rate constants and E is the enzyme concentration. The first order specific rate constant was calculated in decimal logarithms from the formula $K_1 = \frac{1}{t} \log a_0/a$, where t is the reaction time expressed in minutes. The zero order specific rate constant was calculated from the equation $k_0 = \frac{1}{t} (a_0 - a)$.

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TABLE I. Kinetic Data for Hydrolysis of L-leucinamide by Enzyme Preparations A, B and C.

Preparation A *C = .861				B .214				C .552			
t, min.	Hydrolysis, %	$k_1 \times 10^4$	$k_0 \times 10^4$	t, min.	Hydrolysis, %	$k_1 \times 10^4$	$k_0 \times 10^4$	t, min.	Hydrolysis, %	$k_1 \times 10^4$	$k_0 \times 10^4$
60	17.1	13	1.4	60	23.9	19	1.9	30	27.8	47	4.6
120	32.3	14	1.3	120	36.9	16	1.5	60	52.4	53	4.4
180	45.3	14	1.3	180	42.6	13	1.2	90	62.4	47	3.5
240	60.2	19	1.3	240	50.2	12	1.0	120	68.2	41	2.8
300	71.3	18	1.2	300	57.2	12	.9	180	77.6	36	2.2
360	83.1	20	1.1	360	59.9	11	.8	240	78.4	28	1.6

* C = Enzyme concentration (mg protein nitrogen/ml of test solution).

The initial substrate concentration and the substrate concentration at time, t, is represented by a_0 and a, respectively. *Preparation of substrates*—L-leucinamide HCl(9), L-leucylglycine(1), and L-leucyl-β-alanine(7) were prepared by methods described in the literature. L-leucyl-γ-aminobutyric acid was prepared by the same method described by Smith(7) for the preparation of L-leucyl-β-alanine. The crystals of L-leucyl-γ-aminobutyric acid melted at 164°-165°C.

Results. The data for the hydrolysis of L-leucinamide by each of the enzyme preparations are shown in Table I. During the first 45% of its course the hydrolysis of this substrate by enzyme preparation A appears to follow either zero order or first order kinetics; during the remainder of the reaction period it conforms more closely to zero order kinetics. The hydrolysis of L-leucinamide by either preparation B or preparation C follows neither first order nor zero order kinetics; nor does it adhere to the mixed first and zero order equation obtained by Elkins-Kaufman and Neurath(2). Calculation of the proteolytic coefficient from either the average zero order or the average first order reaction rate constants shows preparations B and C to be about 4 times as active as preparation A. The rapid decrease in the rate of hydrolysis of these 2 preparations may be caused by destruction of the enzyme in the more highly purified preparation.

With each of the enzyme preparations the kinetics of the hydrolysis of L-leucylglycine, L-leucyl-β-alanine, and L-leucyl-γ-aminobutyric acid were similar to the kinetic order found for L-leucinamide. The reaction is apparently more complex than a simple first or zero order kinetic. More exact studies require a more highly purified and more stable enzyme preparation.

Table II summarizes the results for the hydrolysis of the substrates by each of the enzyme preparations. In each instance L-leucinamide is hydrolyzed 2 to 3 times as fast as either L-leucylglycine or L-leucyl-γ-aminobutyric acid and about 4 times as fast as L-leucyl-β-alanine. The slower rate of hydrolysis of L-leucyl-β-alanine cannot be explained by a possible inhibitory effect of liberated β-alanine, since the addition of β-alanine to a test solution in which L-leucinamide was the substrate had no effect on the rate of hydrolysis of L-leucinamide.

Discussion. Since the rates of hydrolysis of L-leucylglycine, of L-leucyl-β-alanine and of L-leucyl-γ-aminobutyric acid were approximately the same, especially with enzyme preparations B and C, the decrease of the polarity of the substrate produced by the greater distance between the carboxyl group and the peptide bond has little effect on the rate of hydrolysis. The faster hydrolysis of L-leucinamide may be the result of a faster rate of enzyme-substrate complex formation permitted by the absence of relatively large groups adjacent to the amide group. In other words, L-leucinamide would be expected to have much less steric hindrance than any of the other substrates.

Summary. 1. A purified cathepsin III (leucine aminopeptidase) preparation hydrolyzed L-leucinamide from 2 to 3 times as fast as either L-leucylglycine or L-leucyl-γ-aminobutyric acid and about 4 times as fast as L-leucyl-β-alanine. The activity of the enzyme was not affected by the proximity to the pep-

TABLE II. Comparative Activity of Cathepsin III toward L-leucinamide and L-leucylpeptides.

Substrate	Preparation A			B			C		
	Enzyme conc.	$C_1 \times 10^4$	$C_0 \times 10^4$	Enzyme conc.	$C_1 \times 10^4$	$C_0 \times 10^4$	Enzyme conc.	$C_1 \times 10^4$	$C_0 \times 10^4$
L-leucinamide	.861	20	1.4	.202	76	7.3	.552	76	5.8
L-leucylglycine	.805	6.4	.60	.205	18	1.9	.565	18	1.8
L-leucyl-β-alanine	.852	3.9	.43	.205	13	1.4	.562	13	1.4
L-leucyl-γ-amino-butyric acid	.861	6.4	.63	.200	21	2.2	.567	18	1.8

Enzyme conc. = mg protein nitrogen/ml of test solution.
C_1 = First order specific reaction rate constant/enzyme conc.
C_0 = Zero " "

tide bond of the free carboxyl group of leucyl peptides. 2. The fact that L-leucinamide is hydrolyzed faster than are the peptides of leucine can possibly be explained by the hypothesis that the large groups attached to the peptide nitrogen of the substrates interfere with the formation of an enzyme-substrate complex. 3. The hydrolysis of the substrates by enzyme preparation A closely approximated zero order kinetics. The failure to observe a regular kinetic order for the reactions catalyzed by preparations B and C may be the result of destruction of the enzyme in the more highly purified preparations.

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Reversal of Cortisone Inhibition of Wound Healing by Tissue Culture Media.* (21194)

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The inhibition of normal wound healing by cortisone was demonstrated by Howes(1) and Spain(2). As early as 1911, Carrel demonstrated growth promoting and sustaining factors in embryo extracts as applied to tissue cultures of mammalian cells(3). The purpose of this paper is to present data on the influence of media containing Carrel's growth factors on cortisone inhibition of wound healing.

Methods. Under light ether anesthesia wounds 3 cm in length were incised through the skin to the fascia of the back of the neck of young adult male and female rats of the Sprague-Dawley strain. In one group single wounds were made. In a second group 2 parallel incisions 2 cm apart were made. The wounds were studied under 3 different conditions, as follows: 1) Wounds which were

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