amino acid composition. In all, six electrophoretically homogeneous globulin fractions with R_f 0.34 were obtained.

The investigation of the protein fractions in phosphate buffer (pH 8.05, $\mu = 0.5$) on a Spinco ultracentrifuge showed that only fraction I (μ of extraction 0.3) was homogeneous, having a sedimentation coefficient of 8.2S. In all

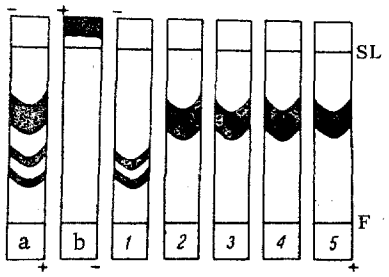

Fig. 1. Electrophoregrams of the total globulin (a, b) and of fractions 1–5. SL—starting line; F—electrophoresis front.

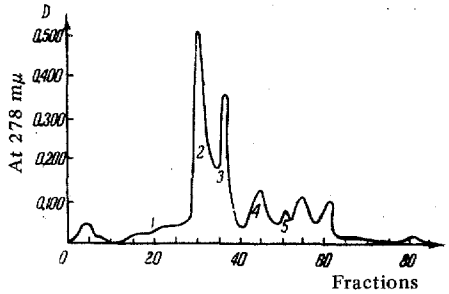

Fig. 2. Fractionating curves of the total globulin: fractions 1–5.

the other fractions, besides the 8.2S component there was a heavier component with a sedimentation coefficient of 11.8S, its content being higher in the later fractions. In fraction VI (μ of extraction 2), we found a third component, heavier than 11.8S. However, it was impossible to determine its sedimentation coefficient because of the diffuseness of the sedimentation peak.

The total nitrogen contents in the fractions were determined by the Kjeldahl and Conway method [2] (% on the absolutely dry weight): fraction I—16.05, II—13.27, III—16.04, IV—14.49, V—16.83, and fraction VI—14.76.

All six fractions differed considerably in amino acid composition, which was determined on an amino acid analyzer (% on the absolutely dry weight of the homogeneous 8.2S globulin component): alanine 3.6; arginine 10.7; aspartic acid 7.2; glutaric acid 12.5; glycine 3.6; histidine 3.3; isoleucine 3.2; leucine 6.1; lysine 3; phenylalanine 8.1; proline 4.7; serine 5.2; threonine 3.3; tyrosine 3.3; and valine 5.2.

These figures show the differing properties of the proteins making up electrophoretically homogeneous globulin fractions.

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SPECIFIC INHIBITION OF HUMAN PEPSIN AND GASTRICSIN

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It has been shown previously that N-diazoacetyl-N'-dinitrophenylethylenediamine (DDE) inhibits porcine and hen pepsins in the presence of Cu^{++} at pH 5.0 [1, 2], one inhibitor residue being added to the molecule of the enzyme which inhibits the carboxy group of aspartic acid [3]. We have established that the proteases of human gastric juice—pepsin and gastricsin [4]—are also inhibited by DDE.

The inhibition was performed by the following method: to 10 mg of the enzyme in 5 ml of 0.04 N acetate buffer with pH 5.0 was added 0.05 ml of a 0.1 M solution of copper acetate; the mixture was kept at room temperature for 10 min, and, with stirring, 1 mg of DDE in 0.1 ml of moist acetone was added. After 15 min, the proteolytic activities of human pepsin and gastricsin (with respect to the degradation of hemoglobin) had fallen to 0.25–0.5% of their initial values. The inhibited enzymes were separated from the low-molecular-weight components of the mixture by filtration through Sephadex G-25. The UV spectra of the inhibited enzymes showed the two absorption maxima at 280 and 360 mμ

that are characteristic for the protein and the dinitroaniline group of the inhibitor. At a concentration of the inhibited enzymes of 1 mg/ml, the optical densities at 280 and 360 mμ were, respectively: for human pepsin, 1.3 and 0.465; for gastricsin, 1.3 and 0.480; and for porcine pepsin, 1.3 and 0.410. This showed that in human pepsin and gastricsin, and also in procine pepsin, there is one inhibitor residue per molecule of the enzyme. A comparison of the content of basic amino acids in the two enzymes and the absorption at 360 mμ gives a similar result. Thus, DDE inhibits human pepsin and gastricsin, porcine pepsin [1], hen pepsin [2], and calf rennin [5]. The data given in the papers mentioned permits the conclusion that DDE is apparently a general inhibitor for proteases of the pepsin group.

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N-ACETYL-3-HYDROXY-4-METHYLANTHRANILIC ACID FROM AN ACTINOMYCETE—THE PRODUCING AGENT OF THE ACTINOMYCIN ANTIBIOTIC AURANTIN

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In a study of the mechanism of the actinomycin antibiotics it was shown that the precursor of the phenoxazine part of the molecule is 3-hydroxy-4-methylanthranilic acid, detected in the culture liquid of the producing agent in trace amounts [1].

We have established that the producing agent of the Russian actinomycin antibiotic aurantin liberates into the medium an N-acyl derivative of 3-hydroxy-4-methylanthranilic acid (HMAA) which accumulates in considerable amounts [2]. The substance has been isolated and characterized, and its structure has been shown, apart from the organic acid acylating the amine group. The N-acyl-HMAA has the following elementary composition (%): C 58.23, 57.61; H 5.65, 5.68; N 6.51, 6.56. This agrees with the elementary composition of N-acetyl-HMAA (C 57.41; H 5.30; N 6.70) and differs little from the composition of N-propionyl-HMAA (C 59.19; H 5.82; N 6.27).

The choice between these two compounds was made on the basis of the chromatography of hydrolysates of the substance under investigation. The volatile organic acid formed as a result of hydrolysis was converted into the hydroxamate by a published method [3], as modified by us, and was chromatographed with reference materials. The N-acyl-HMAA was hydrolyzed with 2 N sulfuric acid at 105° C for 4 hr, the volatile acid was distilled off with steam, the distillate was neutralized and evaporated to dryness, and the residue was dissolved in 2 ml of ethanol and treated with 0.1 ml of thionyl chloride. After 1 hr, the mixture was neutralized with 12.5% alkali in methanol, and 1 ml of hydroxylamine solution was added (5% hydroxylamine hydrochloride in methanol was mixed with an equal volume of 12.5% caustic soda in methanol). After 18 hr, the precipitate was filtered off. The filtrate was evaporated in vacuum, and the residue was chromatographed in the solvent system amyl alcohol—acetic acid—water (4:1:5). The hydroxamates were revealed on the chromatogram with a 15% aqueous solution of ferric chloride. In the solvent used, the chromatograms showed the presence of acetohydroxamic acid. This means that the hydrolysate of the N-acyl-HMAA contained acetic acid. Consequently, the producing agent of the actinomycin antibiotic aurantin synthesizes and liberates into the medium N-acetyl-3-hydroxy-4-methylanthranilic acid.