marked parasitic invasion of the cardiac muscle cells, and the brain cells (nerve and glia). The bone marrow and the liver were moderately heavily parasitized, and the spleen and the lungs had mild invasion. The kidney and the striated muscle were not invaded.

The second and third passages of the trypanosomes from the infected baby rats to healthy baby rats, which were also given cortisone, resulted in an increase in the virulence of the trypanosomes, so that the height of parasitemia was reached in about 10–15 days, and the animal died in 2–3 weeks. Cortisone and trypanosome controls were also included in these experiments. The former showed retardation or inhibition of growth. Trypanosome controls showed a mild transient parasitemia with complete recovery.

Further studies are in progress with other laboratory animals, and different strains of trypanosomes and leishmania. A complete report will be published at a later date.

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The Action of Some Water-Soluble Poly-α-Amino Acids on Fibrinolysis

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During our study of the action of water-soluble poly-α-amino acids on blood clotting (*1*), it was observed that the basic poly-amino acids: poly-lysine (*2*), poly-ornithine (*3*) and poly-arginine (*3*), retard fibrinolysis of human clotted blood. A more detailed analysis of this phenomenon was therefore undertaken.

Fibrinolytic activity of oxalated human plasma was induced by mixing the plasma with a suspension of β-hemolytic streptococci (*4, 5*), by treatment with a cell-free broth containing streptokinase (*6*), or by shaking the plasma with chloroform (*6, 7*). The activated plasma was then treated with the poly-amino acids (prepared in this laboratory), and a fibrin clot obtained by the addition of thrombin. The final mixtures were incubated at 37° C for 15–24 hr to determine if lysis occurred. When the preparations used did not interfere with fibrinolysis, dissolution of the clot occurred. Inhibition of fibrinolysis was indicated by the maintenance of the fibrin clot, obtained as described above, for 24 hr.

A typical experiment with poly-L-lysine and a streptococci-activated plasma is described below.

Oxalated human plasma (0.5 ml) was mixed with a suspension of β-hemolytic streptococci (0.4 ml), and the mixture added to 1 ml saline solution containing 40γ poly-L-lysine hydrochloride. Clotting was induced by adding 4 units of bovine thrombin (Upjohn Company) in 0.1 ml saline with vigorous shaking. The clot was incubated at 37° C for 24 hr. No visual change in the clot was observed. In the control experiment where the 1 ml poly-lysine solution was substituted by saline, a complete lysis of the clot was evident within 30 min.

The fibrinolytic activity of plasma activated by β-hemolytic streptococci was not inhibited either by the neutral poly-DL-alanine (*8*) or by the acidic poly-L-aspartic (*9*) and poly-D-glutamic (*10*) acids up to concentrations of 500γ/ml final test mixture. The basic poly-α-amino acids, poly-DL-lysine hydrochloride (average chain length $n = 35$) (*2*), poly-DL-ornithine hydrochloride ($n = 30$) (*3*), and poly-DL-arginine sulfate ($n = 30$) (*3*), on the other hand, prevented fibrinolysis at concentrations greater than 30γ–40γ/ml test mixture.

In the presence of the basic poly-amino acids, fibrinolysis was inhibited equally well when the streptococcal culture suspension was replaced (in the test mixture) by a cell-free supernatant containing streptokinase. Furthermore, it has been demonstrated that the fibrinolytic activity of chloroform-treated plasma and of menstrual blood was also inhibited by relatively low concentrations of poly-DL-lysine and poly-DL-arginine. It thus seems justified to assume that the basic poly-amino acids are capable of inhibiting hydrolysis of fibrin by plasma fibrinolysin (plasmin) under the experimental conditions used.

Preliminary experiments indicated that the average molecular weight of the basic poly-amino acids plays a profound role in the determination of their antifibrinolytic properties. L-lysine monomer, as well as L-lysyl-L-lysine (*11*), did not show any antifibrinolytic activity up to a concentration of 750γ/ml. A tetra-L-lysine showed slight antifibrinolytic activity at 750γ/ml, whereas poly-L-lysine of average chain length $n = 7$, 35, and 100 showed distinct antifibrinolytic activity at concentrations of 500γ, 40γ, and 35γ/ml test mixture, respectively.

No great difference was observed in the antifibrinolytic activity of poly-L-, poly-D-, and poly-DL-lysine of similar average molecular weights.

In our previous study on the action of water-soluble poly-amino acids on blood clotting (*1*), it was demonstrated that the acidic poly-amino acids, poly-D-glutamic acid and poly-L-aspartic acid, as well as heparin, are capable of neutralizing the anticoagulant activity of the basic poly-amino acids. A similar relationship was found to hold for the antifibrinolytic effect of the basic poly-amino acids. Heparin, as well as poly-L-aspartic acid ($n = 50$), was found to obviate the antifibrinolytic activity of poly-lysine. The neutralization of the antifibrinolytic activity of the basic poly-amino acids occurred when approximately equivalent concentrations of the basic and acidic poly-amino acids were applied.

The ability of the basic synthetic peptides to inhibit

reversibly the proteolytic activity of fibrinolysin resembles the antiproteolytic properties of some natural peptides, such as the pepsin inhibitor and the trypsin inhibitors (*12*). The interaction of the natural as well as the synthetic peptides with the different proteolytic enzymes is probably determined in both cases by some specific groups present in the enzyme and the inhibitor, as well as by the electrostatic forces prevailing between the enzyme and the relatively high molecular weight inhibitor. Further studies with the synthetic amino acid polymers may contribute to our basic knowledge of the mode of action of naturally occurring polypeptides on enzyme behavior.

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Action of Penicillin on Streptococci: Enhancement of Sensitivity *in vivo*?

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The bacteriostatic and bactericidal action of penicillin on growing bacterial cells may under certain conditions continue after the complete removal of the penicillin (*1*). On the other hand, it has been stated by Grunberg, Unger, and Eldridge that streptococci exposed to penicillin *in vivo* are temporarily more susceptible to the action of penicillin than are cells of the same culture not so exposed (*2, 3*). Because of the evident significance of this phenomenon, and because of the increasing use of the method of sensitivity testing upon which the conclusion was based, an attempt has been made to confirm the observation.

In brief, the experimental procedure used by Grunberg, Unger, and Eldridge was as follows. Mice were injected subcutaneously with a broth culture of a hemolytic streptococcus and were immediately treated by injection into the same site of sodium penicillin G. At intervals thereafter the mice were sacrificed, cultures were made of the tissues at the site of inoculation, and the sensitivity of the surviving organisms tested by the use of "gutter" plates. It was found that with advancing time the susceptibility to penicillin of the surviving streptococci progressively increased. The increase in sensitivity was deduced from the fact that with a constant concentration of penicillin in the gutter, the zone of inhibition, or distance from the edge of the gutter to the nearest streptococcal colony, became greater. It was stated, however, and examination of the protocols confirms it, that with time there was a marked decline in the number of streptococci in the lesion, and therefore in the number of streptococcal colonies on the test plate.

TABLE 1

EFFECT OF DENSITY OF INOCULATION ON DIAMETER OF "ZONE OF INHIBITION"

Expt. 1		Expt. 2	
Dilution of culture	Diameter of zone of inhibition (mm)	Dilution of culture	Diameter of zone of inhibition (mm)
Undiluted	32	Undiluted	42
10^{-1}	34	10^{-1}	40
10^{-2}	43	10^{-2}	44
10^{-3}	48	10^{-3}	62
10^{-4}	50	10^{-4}	76
10^{-5}	55		

In the present experiments the same strain of hemolytic streptococcus was used as was employed by Grunberg *et al.* It was used, however, without exposure to penicillin or injection into mice. Experiments were designed to test the effect of dilution of the culture on the apparent sensitivity of the organism to penicillin. Constant 0.1 ml portions of serial decimal solutions of an 18-hr broth culture were spread over the surface of a blood agar plate. A ceramic porcelain cup ("Penicylinder"®) was then fixed in place on the surface of the agar and filled with a solution containing 100 units penicillin/ml. After overnight incubation the diameter of the zone of inhibition which surrounded the penicillin cup was measured. The results are set forth in Table 1. It is evident that a very direct relation exists between the diameter of the zone of inhibition and the density of the inoculum.

These data indicate, therefore, that in order to explain the results which were seen following *in vivo* exposure of streptococcus to penicillin it is not necessary to invoke damaging effects associated with the *in vivo* situation. A simple decrease in number of streptococci present would serve equally well to explain them. The data also indicate the possible hazards associated with the use of the various disk and tablet methods for assaying the sensitivity of bacteria to various antibiotics in the clinical laboratory. Difficulties in interpretation of results would most easily arise when the disk method is applied to an initial mixed culture in which the absolute numbers and the proportions of various organisms are uncontrollable.