verse rows of granules and the generally longitudinal connecting fibers are more regularly arranged. Smears of silver-stained (*1, 7*) salivary gland chromosomes were also examined in a microscope using visible light. It was found that the band regions were strongly stained by silver, whereas the interband regions were either slightly stained or not stained at all. The total lengths of the chromosome arms were not appreciably altered by the silver-staining procedure, but the widths of the arms were considerably reduced. The nucleolus was observed to be stained slightly by silver.

From the results obtained thus far, it is evident that the technique of transferring smears to electron microscope screens by embedding them in collodion films is applicable to smears treated chemically by a wide variety of methods so as to disclose details of the internal structure of chromosomes. The technique has the further advantage that in itself it involves only a few easily performed steps and a minimum number of reagents. Further electron microscope studies of chemically treated chromosome smears are in progress.

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Fatal *Trypanosoma cruzi* Infection in the White Rat[1]

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This is a preliminary report on the laboratory infection of white rats with *Trypanosoma cruzi,* running an acute course and terminating in the death of the animal.

Many animals, such as rats, mice, lemurs, guinea pigs, monkeys, dogs, and cats, may be experimentally infected with *T. cruzi.* Mice and guinea pigs are especially useful in diagnostic and research work on Chagas' disease (*1*). Inoculations of white rats with citrated blood from the patients have been successful in some cases. The guinea pig is less useful because it is less susceptible to infection than the white rat (*2*). Baby rats inoculated intraperitoneally with large doses of *T. cruzi* cultures show a few trypanosomes in the blood after an incubation period of 10 days to 2 weeks. This mild transient parasitemia, lasting about 2 weeks, was associated with leishmania forms in the cardiac muscle and in the reticuloendothelial cells, and ended in the complete recovery of the animal (*3*).

For the past 10 years, we have been attempting to find a method of inducing acute fatal infection in a laboratory animal with cultures of *T. cruzi.* Different animals and various methods were tried. A mild and transient invasion of the blood stream with trypanosomes was observed in most animals. One method was to inoculate *T. cruzi* cultures into animals following the blocking of the reticuloendothelial system with India ink or colloidal metals. Transient parasitemia with mild infection was induced, but most of the animals recovered (*4*).

Week-old, hybrid baby rats which were still being nursed by the mother were used routinely in our experiments. The skin was thin, tender, and completely devoid of fur. One to two million actively growing trypanosomes in 7–10-day-old cultures of *T. cruzi* from bi-phasic Seneca hemoflagellate medium were injected intraperitoneally in 0.2 ml saline with a 24-gauge needle. Since the abdominal wall was very thin, there was leakage of the injected culture through the hole made by the needle. To obviate this, the site of the inoculation was rubbed with cotton dipped in alcohol. Concomitantly, 6.25 mg cortisone acetate (Merck, 0.25 ml) was injected subcutaneously in the back. Twenty-four hr later, a second dose of 6.25 mg cortisone acetate was injected. There were two sets of controls for each litter. One set was injected with two doses of cortisone, and the second with *T. cruzi* intraperitoneally. All the baby rats were put together, and nursed by the mother rat. Both wet blood smears, and smears stained with Wright's stain were frequently examined.

About a week after the inoculation, an occasional trypanosome was seen in the cortisone-trypanosome group. Gradually the number of trypanosomes increased, so that in 3–4 weeks, there were 25–100 trypanosomes per high-power field of the microscope. Some of the animals died of severe parasitemia in 3 weeks after inoculation. Inhibition or retardation of growth, loss of appetite, loss of weight, tendency to sluggishness, rough texture of the fur, eye infections, and gradually increasing paralysis of the hind legs were observed. Within 5 weeks all the inoculated animals died of muscular paralysis, which gradually spread to the muscles of respiration. Rats inoculated with trypanosomes only (controls) showed a mild transient parasitemia about 2 weeks after injection; the parasitemia lasted 10 days to 2 weeks, and terminated in complete recovery. Cortisone controls showed inhibition and retardation of growth, poor texture of fur, and reduced resistance to bacterial infection, but continued to live.

Post-mortem examination of the cortisone-trypanosome group showed congestion of the meninges, brain, and liver. The spleen was enlarged, and the heart dilated. No gross pathological changes were observed in the other organs. Sections of the tissues showed

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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