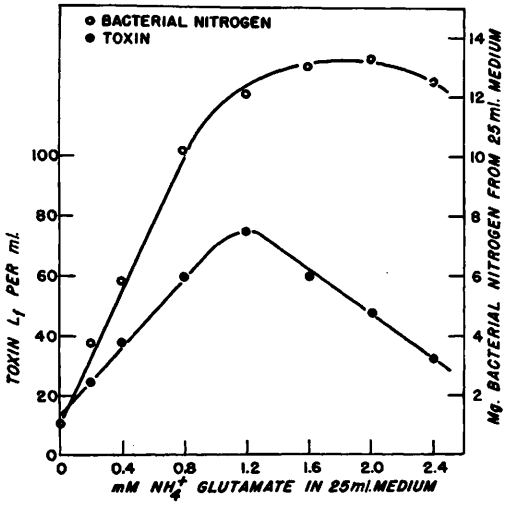

FIG. 2. Growth and toxin production on basal medium with added ammonium glutamate.

observed that the test organism grew exceptionally well on media containing 0.8 to 2.4 mM of ammonium glutamate, as evidenced by the rapidity of pellicle formation. Fig. 2 indicates that ammonium glutamate was readily utilized by the Toronto strain for growth and toxin production. Toxin production reached a maximum on medium containing 1.2 mM, but fell off sharply with increasing amounts of ammonium glutamate; the decrease in toxin production was probably due to inhibitory amounts of iron since the particular lot of glutamic acid used contained iron as impurity. The extreme sensitivity of the toxin-producing mechanism of the diphtheria bacillus to traces of iron is well recognized. In repeated experiments on iron-controlled medium, ammonium glutamate has been found to be equally as good as the combination of ammonium sulfate and sodium glutamate for growth and toxin production. Glutamine has consistently proved to be less efficient for toxin production on a low iron medium.

Discussion. The experimental results do not warrant the conclusion that glutamine is the metabolite required, and that in the absence of glutamine, ammonia and glutamic acid are essential for the synthesis of glutamine. The fact that high concentrations of glutamine are required for maximum growth makes it seem more probable that glutamine is broken down by the organism to ammonia and glutamic acid, which, of themselves, are readily utilized in a favorable pH environment. This conclusion is supported by the evidence that ammonium glutamate proved to be an efficient substitute for ammonium sulfate and sodium glutamate, or glutamine. The apparent synergism between ammonium salt and sodium glutamate may be simply a function of pH since the addition of ammonium sulfate prevents the accumulation of alkali resulting from sodium glutamate metabolism. Likewise, the acidity resulting from ammonium sulfate metabolism can be prevented by the addition of sodium glutamate.

Summary. The utilization of inorganic nitrogen in addition to organic nitrogen by the Toronto Strain of Park-Williams no. 8 *Corynebacterium diphtheriae* has been investigated. Ammonium nitrogen in the form of a simple ammonium salt (ammonium sulfate) is shown to be utilized by the organism. For optimum growth and toxin production on chemically defined medium, suitable concentrations of sodium glutamate and ammonium sulfate, or ammonium glutamate are required.

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Effect of Sanguinin, an Antibacterial Agent from Blood,* on Streptococcal Infection in Mice. (19144)

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In our earlier studies(1) the antibacterial activities of tissue extracts of several species of ticks were demonstrated. It was found that

* Patent pend.

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the intestinal tract of blood-engorged ticks exhibited a significantly higher antibacterial titer than of those which had not been fed. Further investigations to determine the role of the blood *per se*(2) revealed that enzymatic hydrolysates of the erythrocytes of various animals showed marked antibacterial effect *in vitro,* covering a relatively wide spectrum of most gram-positive and a few gram-negative organisms. The active principle of the hydrolysate was thought to be a peptide-amino acid complex. Since this product, which has been given the name Sanguinin, strongly inhibits the growth of various organisms *in vitro* including streptococci, it was obviously of interest to ascertain its effectiveness *in vivo.*

This inquiry was stimulated by the observations of other investigators on the antibacterial properties of extracts from normal animal tissues(3,4) and by the growing interest in antimicrobial substances of animal origin(5). Recently, Cline *et al.*(6) studying anticatalase-producing substances, found that Sanguinin injected into mice significantly lowers the liver catalase.

Materials and methods. The following technic was used for the preparation of Sanguinin. Blood from cattle or from human blood bank was mechanically defibrinated, centrifuged, and the serum discarded. The packed cells were then submitted to dialysis according to the Anson and Mirsky(7) procedure for the preparation of hemoglobin. After freeze-drying, 30 g of the dark-red powder were suspended in 2,000 ml sodium phosphate buffer (pH 8.0) and 3 g trypsin (Pfanstiehl, 1-110) added. The mixture was then incubated for 10 hours at 37°C and heated in a 65°C water bath for 30 minutes. The material was then filtered through filter paper and the filtrate dialysed in cellulose tubing against running tap water for 48 hours and against cold distilled water for the next 24-48 hours to a pH 7.2. Sufficient N/6 HCl was added to the pooled tubing content to bring the pH to 5.0 with resulting voluminous precipitate. This is then centrifuged and the pale yellow supernate discarded. The sediment is redissolved in an adequate amount of phosphate buffer to pH 7.2 and lyophilized. This dark-brown amorphous substance is Sanguinin in its crude form. For the treatment of mice Sanguinin solution of 20 mg/ml in saline was prepared fresh daily and heated at 56°C for 20 minutes prior to administration. Subcutaneous injections were routinely given. Treatment with Sanguinin usually began one to four days before the infection and was followed after infection by daily inoculations (0.25 to 1.0 mg per mouse) for 2 or 4 days. The beta-hemolytic strain of *Streptococcus zooepidemicus* used in this study was furnished by Dr. C. E. Lankford, Department of Bacteriology, University of Texas, Austin. The cultures were maintained in Difco brain heart broth with weekly passages through mice. Broth dilutions of 1:100 to 1:1000 were prepared with the incorporation of 4% gastric mucin (Nutritional Biochemical Corp.) in early experiments until an LD50 at 3 to 4 days could be established with streptococci alone. Mice were infected intra-abdominally, usually with an inoculum of 0.5 ml of a 1:1000 dilution, which dose represented an average of 300,000 bacteria. Depending upon the pretest virulence, the dosage per mouse was sometimes lowered to 0.2 ml. A total of 489 albino mice, weighing 17 to 25 g (averaging 20 g) obtained from an inbred stock were used. Serial experiments varying as to time-dose relationship of Sanguinin administration were performed on groups of 10-15 mice and accompanied by adequate controls. The latter were simultaneously and uniformly infected with the test animals.

Results. The rates of survival time of all groups of treated and untreated mice are expressed in percentages in Table I. The observations on the rapid progress of the acute

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TABLE I. Percentage Survival of Mice Infected with Beta-Hemolytic *Streptococcus zooepidemicus* and Treated with Sanguinin Subcutaneously.

Series	No. of mice		Sanguinin, mg/mouse			% survival on days after infection					
			Prior to infection		After infection, daily	Treated mice			Untreated controls		
	Test	Controls	Days	Daily		2nd	4th	6th	2nd	4th	6th
1	43	40	0	0	.25	88.3	23.2	6.9	89.8	22.4	2
2	30	30	1	1	.25	93.3	66.6	36.6	85.3	44.1	5.8
3	60	50	2	1	1	100	70	23	85	25.7	5.6
4	88	71	3	.75	.25	70.4	48.8	43	47.7	18.1	0
5	47	30	4	.75	.25	95.5	63.7	53.2	75	33.3	16.6

streptococcal septicemia were recorded daily and terminated on the 6th day. No treatment prior to infection was given to mice of series I, but shortly after the infection each mouse was inoculated with 0.25 mg of Sanguinin; this was continued daily over 4 days. The test mice of this series began dying approximately at the rate of the controls until the 6th day when the survival rates of the test groups were only slightly higher than in the untreated. In the next series (2, 3, 4, and 5, Table I) Sanguinin was given prior to infection. A single dose of 1.0 mg was inoculated into each mouse of the second series 24 hours before the infection. This was followed by daily inoculations of 0.25 mg beginning with the first day of infection and continuing for the next 3 days. As seen from the table, series 2, 3 and 4 were all pretreated with 0.75 or 1.0 mg/mouse daily for 2 to 4 days preceding the infection. This was followed by doses of 0.25 to 1.0 mg daily until half of the infected controls were dead. Further analysis of series 2 shows that whereas only 5.8% of the untreated controls survived the infection, 36.6% of the treated mice were alive on the 6th day. When pretreatment was begun 2 days prior to infection, the survival rates on the 6th day were 23% as compared with 5.6% of the controls. Still more significant were the results when Sanguinin was administered over 3 days before infection. The survival rate of this series rose to 43% against no survivals in the controls (series 4, Table I). Furthermore, in a 4-day pretreatment schedule of series 5, more than half of the experimental animals survived as contrasted to 16.6% in the untreated mice. The appraisal of the results expressed in survival percentages on the 6th day indicates 38.9 for the combined series of the pretreated mice against 6.0 of the total of untreated controls.

Our previous observations on the relatively high tolerance of mice to Sanguinin(2) were corroborated in the course of the present experiments. Except for an induration at the site of the inoculum and a brown discoloration of the subcutaneous tissue, no visible untoward clinical effects were observed. This refers to the dosage of 1.0 mg daily over a period of 5 days. No acute toxicity was recorded when Sanguinin was inoculated at a single dose of 5.0 mg to a mouse of 20 g body weight (J. K. Cline, unpublished data)(8).

Discussion. Its relatively low toxicity distinguishes Sanguinin from the highly toxic hematin with which it was recently identified by van Heyningen(9) and who previously found that the growth of certain aerobic sporing bacilli is inhibited by hematin *in vitro* (10). Prior to this, Kammerer(11) who was probably the first to investigate the effect of the blood derivatives on bacteria, found hematin incorporated in nutrient media stimulating rather than inhibiting the growth of various organisms. Although the presence of hematin among other components of blood hydrolysis from which Sanguinin derives is feasible, chemical evidence is lacking to identify Sanguinin with hematin.

The prolongation of survival time in all series of mice when treatment with Sanguinin began one or more days before infection, points to bacteriostatic action leading to sup-

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11. Kämmerer, H., *Verh. Deut. Kongr. innere Med.*, 1914, v31, 704.

pression of the disease process. Although curative treatment with Sanguinin gave only insignificant results, the survival percentages increased approximately 6-fold over the controls when the infection was preceded even by one single dose of Sanguinin (series 2, Table I). It seems, therefore, that the pretreatment is the decisive factor influencing the course of the streptococcal infection. Whereas, it is improbable that the suppression of the infection is due to a direct action of Sanguinin on the agent, it appears that the mode of action is indirectly eliciting the body defense mechanism by a non-specific stimulation. The newly established property of Sanguinin as a powerful enzymatic inhibitor(6) deserves attention in the further analysis and interpretation of its antibacterial activities.

Summary and conclusions. The blood hydrolysate Sanguinin was tested *in vivo* in 268 albino mice accompanied by 221 untreated controls for its effectiveness against the beta-hemolytic *Streptococcus zooepidemicus*. Sanguinin solution (20 mg/ml) was inoculated subcutaneously (0.25 to 1.0 mg/mouse daily, usually for 4-6 days) either prior or after infection. Suppressive effect on the course of the infection was noted in all series of treated mice. A significant increase in survival time was noted in the pretreated series with an average survival percentage of 38.9 compared with 6.0 of the total of untreated controls. It does not appear that a direct correlation can be established between the survival rates and the intake of Sanguinin or the length of its administration, however, pretreatment seems to be of decisive value.

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Pharmacological Properties of a New Parasympathetic Blocking Agent, N,N Dimethyl 4-Piperidylidene 1,1 Diphenylmethane Methyl Sulfate (Prantal) (19145)

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The investigations of Acheson and Moe(1), Longino, Grimson *et al.*(2,3) and Hambourger, *et al.*(4) have stimulated the search for new substances capable of blocking synaptic transmission. Interest has been furthered by encouraging clinical results reported for parasympathetic blocking agents in the management of peptic ulcer(5,6). During the study in this laboratory of a series of new quaternary salts, several compounds were found to have autonomic ganglion blocking properties. One of these, N,N dimethyl 4-piperidylidene 1,1 diphenylmethane methyl sulfate (Prantal) (7), exhibits some unusual pharmacodynamic properties which distinguish it from previously known orally active atropine-like drugs. Pharmacological data and preliminary clini-

* The authors are indebted to S. W. Lee for valuable suggestions, and to S. Neuwirth for the biometric evaluation of data.

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