hemoglobin determinations were made by the method of Sahli. There were 14 anemic rats and 15 littermate controls. The results are shown in Table I.

TABLE I.
Data Comparison of Anemic Rats with Litter Mate Controls. Each Value Is Followed by Its Standard Deviation.

	Litter mate controls	Anemic rats
Blood hemoglobin, %	16.5 ± 1.1	4.1 ± 0.8
Ventricular wt., mg.	429.3 ± 76.3	820.1 ± 136.4
Body wt., gm.	160. ± 28.	92. ± 19.
Ventricular wt./body wt. ratio	2.7 ± 0.38	9.07 ± 1.45
Muscle hemoglobin in ventricles, mg. %	389. ± 60.	363. ± 58.
Total muscle hemoglobin in ventricles, mg.	1.63 ± 0.14	2.90 ± 0.25
Total muscle hemoglobin in ventricles, mg./100 gm. body wt.	1.04 ± 0.18	3.27 ± 0.67

The concentration of muscle hemoglobin in the myocardium is not significantly different in the 2 series. The total amount of muscle hemoglobin is nearly twice as great for the anemic rats, owing to the great hypertrophy present in these cases. It is evident that in spite of the severe anemia, growing rats have the ability to store manufactured muscle hemoglobin in their hearts in amounts not only sufficient to meet the demands of normal heart growth, but indeed to maintain normal muscle hemoglobin concentration during the development of abnormal hypertrophy.

8731 C

Staphylococcal Antihemolysin-Titers Following Staphylococcal Toxoid in Chronic Osteomyelitis.

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The progress of immunization following the therapeutic administration of staphylococcal toxoid to patients with staphylococcal infections may be followed in the laboratory by the titration of staphylococcal antitoxin in the sera of these individuals. It is the purpose of this communication to record the serologic response, as measured by repeated titrations of antihemolysin (antitoxin?), in a series of 38 patients with chronic osteomyelitis who received therapeutic injections of staphylococcal toxoid.

For the purpose of comparison, it is essential to know the amount of circulating antitoxin in the sera of normal individuals, and of persons with chronic staphylococcal osteomyelitis before treatment with toxoid. A common basis for reference has been made available by the establishment of a provisional International Standard Staphylococcal Antitoxin.[1] The range of staphylococcal antihemolysin in normal, healthy individuals has been reported as about ⅓ to 1 standard International Unit (I.U.) by Dolman,[2] as 0.4-0.7 to 2 I.U., by Murray,[3] as 0.15-1.5 by Nélis and Poncelet,[4] and as 1 or less by Blair and Hallman.[5]

The general opinion is recorded in the literature that sera from cases of osteomyelitis possess antihemolytic titers definitely above the normal range. Thus, in 59 cases of subacute and chronic osteomyelitis, Dolman[2] reported that titers of 5-6, and occasionally of 10, I.U. were commonly obtained. Murray[3] found an average initial titer of 11.7 I.U. in 7 cases of osteomyelitis. In 5 cases reported by Nélis and Poncelet,[4] titers of 0.8-1.5 I.U. were found in 3 and titers of 3 or more units in 2. Blair and Hallman[5] have demonstrated, in a series of 80 patients with chronic osteomyelitis, that the titers of sera of these persons generally fell within the range established as normal, that is, 1 I.U. or less. Only one-fifth of the sera from these individuals had an initial titer definitely above normal, namely 3 or more units.

In the present work, staphylococcal toxoid was administered to a group of 38 patients with chronic staphylococcal osteomyelitis. The majority of the patients presented frank bone-lesions which were subjected to radical saucerization, followed by maggot-therapy, during the administration of the toxoid. The staphylococcal toxoid employed was supplied through the courtesy of Drs. A. F. Coca and E. F. Roberts, of the Lederle Laboratories. Following an initial injection of 20 "units" of toxoid, the dosage was gradually increased in 10 successive subcutaneous injections to a maximum of 1000 "units". The maximal dose was repeated in most of the cases for 10 or 15 injections. A "unit" of staphylococcal toxoid is described by the manufacturer as "the toxoid obtained from a dermonecrotizing unit of toxin (the least amount of toxin which

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[2] Dolman, C. E., *Lancet*, 1935, **1**, 306.

[3] Murray, D. S., *Lancet*, 1935, **1**, 303.

[4] Nélis, P., and Poncelet, F., *Compt. rend. Soc. biol.*, 1935, **118**, 312.

[5] Blair, J. E., and Hallman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 382.

on intradermal injection in a susceptible rabbit will produce an erythema with a central necrosis at least 5x5 mm. in diameter)".* An average of 10515 "units" of toxoid was administered, in an average of 17.9 injections, over periods varying from about 1 to 4 months. The administration of toxoid, and the clinical management of the cases was done under the supervision of Dr. Joseph Buchman, of the Department of Orthopedic Surgery, service of Dr. Samuel Kleinberg.

The course of immunization of each patient was followed in the laboratory by the determination at varying intervals of the antihemolytic titer of the patient's serum. An initial titration was done in each case prior to the beginning of toxoid-therapy, and from 2 to 9 titrations were done during and after the course of treatment. The titrations were originally done by determining that dilution of serum which just inhibited hemolysis of 1% rabbit erythrocytes by one M.H.D. of staphylococcal toxin, after incubation for one hour at 37°C. Subsequently, a sample of the International Standard Antitoxin was obtained through the courtesy of Dr. Hartley, and after suitable preliminary tests, our sera were titrated in terms of that standard. The titers recorded in this paper are in International Units.

Preliminary to titrating sera in terms of the International Standard, the toxin employed was first standardized. This was done by setting up a series of tubes, each containing decreasing quantities of toxin, made up to 0.5 cc. with physiologic salt-solution, and one unit of Standard Antitoxin (so diluted that one unit was contained in 1 cc.). After incubation at 37°C. for 15 minutes, 0.5 cc. of a twice-washed suspension of 4% rabbit-erythrocytes was added to each tube, giving a final concentration of 1%, and incubation was continued for one hour at 37°C. The amount of toxin which just caused hemolysis represented the "test-dose" of toxin.

In titrating serum, one cc. each of varying dilutions of the serum was placed in a series of tubes, and to each was added the test-dose of previously standardized toxin, made up to 0.5 cc. After incubation for 15 minutes at 37°C., 0.5 cc. of 4% erythrocytes was added to each tube, the tubes were shaken, and incubated for one hour at 37°C. The dilution at which hemolysis was just discernible represented the titer of the serum. As controls, from 4 to 6 previously titrated sera were always run with each lot of new sera. Agreement of the controls with previous results was required before accepting the titrations of the new sera.

* This description is given in a folder accompanying each vial of toxoid.

In both tests we have found it convenient to centrifugate the tubes when they were removed from the waterbath for final reading, as the clear supernatants allowed more precise reading of small degrees of hemolysis. It is essential to use rabbit-erythrocytes in all hemolytic titrations of staphylococcal toxin and antitoxin.

The toxin used was prepared from the "Ha" strain of *Staphylococcus aureus,* isolated by Dr. Burky. Our culture was obtained through the courtesy of Dr. Roberts, of the Lederle Laboratories. Cultures were grown in semisolid (0.3%) agar, in an atmosphere of 30% CO_2 for 48 hours at 37°C. The culture was then drained through gauze, and filtered through a Seitz filter. The potency of the filtrate (toxin) was demonstrated by dermonecrotic and lethal tests on rabbits, and by hemolytic tests.

We found a definite rise in antihemolytic titer in the sera of all of the 38 cases following administration of staphylococcal toxoid. The increase from initial to maximal titers is recorded in Table I.

TABLE I
Increase of Antihemolytic Titer Following Staphylococcal Toxoid-Therapy in Chronic Osteomyelitis.

No. Sera	Initial Titer	Maximal Titers 1.1	2.2	3.3	4.4	5.5	6.6	7.7	8.8	10	12.2	13.3	14.4	15.5	17.7	28.8
8	0.25		2		2	1	3									
9	0.5	2		2	1	1	1		1						1	
9	1.1				3	2			1	2		1				
5	2.2				1			1		2			1			
3	3.3						2							1		
2	4.4													1		1
1	5.5										1					
1	6.6															1
38		2	2	2	7	4	6	1	2	4	1	1	1	2	1	2

Titers are recorded in terms of International Units.

The results obtained confirm the findings of Dolman in cases of osteomyelitis, and of Murray and others in superficial staphylococcal infections. These workers have found that the titer usually increases 5- to 10-fold subsequent to the administration of 4 to 8 injections of toxoid.[2, 3] In our series of 38 cases, the titers of 19 were increased 5- to 10-fold; the titers of 31 were increased 2- to 10-fold, and the titers of 7 were increased 12 to 34 times. In every instance, however, the number of injections of toxoid necessary to raise the titer of sera to their maximum was greater than has been reported by others, the average number being about 13. The maximal titer obtained in the series was 28.8 I.U. This titer was obtained in the sera of 2 patients, and represented an increase of 4.5 and 7 times the initial titers.

It appears that certain individuals are better able than others to respond to the antigenic stimulus of infection or of artificially introduced toxoid. This is shown by the fact that those sera which reached the highest titers (10 units or more) attained their maximal levels in a definitely shorter time than those whose maxima were lower (4.4 units or less); the former group required an average of 44 days, while sera in the latter group reached their maximal level in about 71 days. In addition, most of the sera in the former group had initial titers above the normal range.

It has been previously observed that the titers obtained following toxoid-therapy do not remain maximal, but drop to a level which is usually higher than the initial titers.[2, 3] We found a similar drop in titers. This occurred about 1½-2 months after the cessation of toxoid-therapy in 29 individuals, and during the course of injections in 9. In the latter group the titer dropped and did not again rise in spite of continued injections of toxoid. In the entire group, the titers of 4 patients dropped to a level lower than the initial titers; the titers of 2 dropped to a level equal to the initial titers; and the titers of the remaining 32 dropped to a level higher than the original titers. The level finally reached in the majority of instances was from 2 to 8 times higher than the initial titer. We found no relationship between the maximal titers obtained and the final levels to which they fell. Neither was there any relationship between the extent of the rise of titers and the final levels. We have found that in general the higher titers fell somewhat more precipitously than the lower titers.

The authors submitted themselves to a course of administration of toxoid entirely comparable to an average course received by the patients. Their initial titers, which were 0.18 and 0.55 I.U., increased 4 and 6 times, respectively. Three months after the administration of toxoid had been stopped, the titer of one was still at the maximal level of 4 times the initial titer, while the titer of the other had dropped to a level 4 times the initial titer.

A detailed description of the clinical results following staphylococcal toxoid-therapy is to be made by Dr. Buchman. Briefly, it may be stated that the serologic response of the patients was not paralleled by a corresponding improvement in their clinical condition.

Summary. Following the administration of staphylococcal toxoid to a group of 38 patients with chronic staphylococcal osteomyelitis there was a definite increase in the staphylococcal antihemolytic titer, ranging from 2 to 34 times the initial titer. The titers fell, after a varying interval, from the maximum to a level which was generally higher than the initial titer. The definite increase in

antihemolysin titer was not paralleled by a corresponding improvement in the patients' clinical condition.

8732 P

Ascorbic Acid Metabolism in Tuberculosis.

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The literature relating to experimental animals indicates decreased resistance to tuberculous infection in animals on vitamin C deficient diets.[1-5] Conversely, animals with chronic disease are more easily precipitated into acute scurvy.[4] Clinical reports[1, 6, 7, 8] have appeared to the effect that scurvy renders a patient increasingly susceptible to tuberculosis, while the addition of vitamin C to the diet improves the prognosis in these cases. With the development of knowledge concerning subclinical vitamin deficiencies[9, 10] and of a method of judging the state of ascorbic acid nutrition in the human,[10] the study of this substance in its relation to tuberculosis was indicated.

Schroeder[11] has reported an increased requirement of vitamin C in tuberculosis. Three cases were studied: 2 for a period of 5 days during which they were fed 300 mg. of ascorbic acid daily. The report of Bullowa, *et al.*,[12] shows in one tuberculous patient a reduced excretion of vitamin C but with prolonged dosage of ascorbic acid a high peak was reached which fell rapidly on discontinuing the treatment.

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