The antithrombin of the serum and plasma of various species was then determined (Table I). Human serum and plasma contain between 74 and 115 units per cubic centimeter. In human subjects with the plasma prothrombin within the hemorrhagic zone the prothrombic unitage usually approximated or was lower than the antithrombic unitage.

Summary. A method is devised for the quantitative determination of antithrombic activity of serum and plasma. One unit of antithrombin is defined as that amount which will inactivate or neutralize 1 unit of thrombin in 4 minutes' incubation at 28°C. There is little or no quantitative difference in the antithrombin of serum and plasma. The antithrombic activity of normal serum and plasma is considerably greater than has been previously described.

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Diphtheria-Antitoxin Production After Intravenous or Subcutaneous Injection of Alum-Toxoid.

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The purpose of the present study is to compare the effectiveness of intravenous and subcutaneous injections of alum-precipitated diphtheric toxoid in stimulating antitoxin-formation in the rabbit. The general opinion that the subcutaneous is superior to the intravenous route in antitoxin-production is based on experiments with horses[1] and guinea pigs.[2] Further important difference between the previous and the present experiments is that in the previous studies the antigen, toxin or toxoid, was used in solution and in the present study the toxoid was employed as particulate material.

When bacterial suspensions are employed as antigen the vascular route is the more effective one. This is true not only in regard to pneumococci that rapidly become gram-negative when introduced into the skin,[3] but also in regard to heat-killed tubercle bacilli that

1 Kolle, W., and Wassermann, A., *Handbuch der path. Mikroörganismen*, 1912, Verlag Gustav Fischer, Jena.

2 Neill, J. M., Sugg, J. Y., and Richardson, L. V., *J. Immunol.*, 1935, **28**, 363.

3 Julianelle, L. A., *J. Exp. Med.*, 1930, **51**, 441, 449; Dubos, R. J., and MacLeod, C. M., *J. Exp. Med.*, 1938, **67**, 269.

are quite resistant.[4] The question can be raised whether toxins or toxoids would behave like bacteria, if they are injected as particulate material. By the subcutaneous route toxins and toxoids in combination with particulate material or as precipitates were employed by Ramon[5] and Glenny and his associates.[6]

Four rabbits received intravenous and 5 rabbits subcutaneous injections of 1 cc of alum-precipitated diphtheric toxoid containing 25 L_f on 4 successive days and after a rest of 3 days a similar course of 4 injections. The antigen was diluted 1:10 with salt solution for the intravenous injections. The animals were tested 12, 18, and 25 days after the first course of 8 injections. Thirty-two days after the first course of injections the rabbits were given a new series of injections, but this time 5 days apart. The rabbits were bled approximately 5 days after each injection. The titrations were carried out by the intracutaneous method.

The results are presented in Table I. The first 3 samples of blood taken at different intervals after the first series of 8 injections show that the rate of antitoxin-formation was strikingly higher in the group with intravenous injections, since at the time of the first bleeding the average level in this group was 1.8 and in the group with subcutaneous injections only 0.1 antitoxin unit per cc. At the

TABLE I.

Antitoxin Units per cc of Serum in Rabbits Immunized with Alum-precipitated Diphtheric Toxoid.

Route	Rabbit No.	Days after the first course of injections			During the second course of injections. After						
		12	18	25	1st	2d	3d	4th	5th	6th	7th
Intravenous	1	5.0	4.0	2.0	15.0	15.0	20.0	15.0	12.5	12.5	10.0
	2	1.0	1.0	2.0	11.0	15.0	20.0	—	18.0	18.0	15.0
	3	1.0	2.0	1.0	11.0	12.0	15.0	15.0	10.0	10.0	7.5
	4	1.0	1.0	0.5	3.0	7.0	7.5	—	7.5	7.5	5.0
Avg		*1.8*	*2.0*	*1.3*	*10.0*	*12.2*	*15.6*	**16.0*	*12.0*	*12.0*	*9.3*
Subcutaneous	5	.1	2.0	.5	3.0	7.0	5.0	5.0	3.0	2.5	2.5
	6	.1	1.0	.5	3.0	6.0	5.0	2.5	2.0	3.0	2.5
	7	.1	2.0	.5	3.0	5.0	4.0	2.5	3.0	2.5	2.5
	8	.1	1.0	.5	2.0	5.0	4.0	2.5	3.0	2.5	2.5
	9	.1	0.1	.1	1.0	3.0	3.0	2.5	2.0	3.0	2.5
Avg		*.1*	*1.2*	*.4*	*2.4*	*5.2*	*4.2*	*2.5*	*2.7*	*2.8*	*2.5*

*Pooled sera.

[4] Freund, J., and Opie, E. L., *J. Exp. Med.*, 1938, **68**, 273.

[5] Ramon, G., *Revue d'Immunol.*, 1939, No. 5, 385.

[6] Glenny, A. T., Pope, C. G., Waddington, H., and Wallace, U., *J. Path. and Bact.*, 1926, **29**, 38.

time of the second and third bleedings the level was only slightly higher in the animals with intravenous injections. The 2 injections of the antigen that followed the rest-period stimulated a sharp rise in the titers in both groups while the following injections produced a rise only in the group with intravenous injections. At the time of all of the 7 bleedings during the second course of injections the antitoxin levels were conspicuously higher in the group that received intravenous injections.

Conclusion. Antitoxin-formation in the rabbit is more rapid and abundant after intravenous than after subcutaneous injections of 25 L_f alum-precipitated diphtheric toxoid.

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Experiments on Cultivation of Virus of Infectious Avian Encephalomyelitis.*

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In connection with investigations on the virus of infectious avian encephalomyelitis (A.E.),[1] we sought to enhance antigen quantitatively for the preparation of immunizing vaccines, by cultivating the virus in developing chick embryos and in their tissues *in vitro*. The results relating to epidemiology and to additional properties of this newly discovered virus were considered of sufficient importance to be offered in the present paper.

Materials and Methods. The strain of virus employed was that kindly sent to us by Dr. Van Roekel and which was described by him[1] as well as by Olitsky.[1] The procedures followed closely those which had been employed by the latter. Ten percent infected-chick-brain suspension in broth was used to initiate the various cultures. Tests for virus, except as noted otherwise, were made by intracerebral inoculation of 0.05 to 0.1 cc of tenfold dilutions of the material to be examined into 2- or 3-weeks-old Rhode Island, or New Hampshire, Red chicks.

* We express our debt to Mr. P. Haselbauer for his invaluable aid.

1 Olitsky, P. K., *J. Exp. Med.*, 1939, **70**, 565; Olitsky, P. K., and Bauer, J. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 634. For earlier descriptions, see: Jones, E. E., *J. Exp. Med.*, 1934, **59**, 781, and Van Roekel, H., Bullis, K. L., and Clarke, M. K., *J. Am. Vet. Med. Assn.*, 1938, N.S. **46**, 372.