duration. It would be interesting to determine the threshold of dosage for an active form as *Polistes*. The above animals were kept in a dark constant temperature cage with uniform conditions of moisture, so that only the basal rate was obtained. In all probability the loss in weight in other forms after prolonged feeding of thyroid found by previous workers[1] was due to an increase in oxidation and consequently a decreased fat deposition in the larval state.

It should also be mentioned here that feeding Lugol's solution (5% I + 10% KI) did not produce any marked change in the rate.

Five wasps and two roaches were used in the thyroid-feeding experiments. Twenty-five normal readings were taken with the wasps and seven experimental ones. Four normals and two experimentals were taken with the roaches. The same insects were used as checks before and after thyroid feeding. Checks were also made by feeding honey syrup alone with no thyroid or thyroxin present.

Three wasps and four roaches were used in the thyroxin experiments. Nine normal tests and three experimental ones were made with the wasps; nine normals and three experimentals also with the roaches.

5159

Toxin-Antitoxin Reactions on the Surface of Collodion Particles.

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Because of its theoretical and practical significance the mechanism of the neutralization of toxins by antitoxins is one of the most extensively investigated problems of immunology. According to Arrhenius and Madsen the toxin-antitoxin neutralization may be a chemical reaction governed by the law of mass action. On the other hand Bordet thinks that it does not follow the quantitative laws of simple chemical reactions but is an adsorption phenomenon controlled by the electrical charge of the toxins and antitoxins.[1]

[1] Kopec, S., *Biol. Bull.*, 1926, **50**, 339. Kunkel, B. W., *J. Exp. Z.*, 1918, **26**, 255. Terao, A., and Wakamori, N., *Japan Med. World*, 1924, **4**, 68.

[1] Wells, H. G., "The chemical aspect of immunity." Second edition. 1929. Bayne-Jones, S., chapter in "The Newer Knowledge of Bacteriology and Immunology" by Jordan, E. V., and Falk, I. S., 1928. Eisler, M., chapter in *Handb. path. Mikroorganismen* IV. 2 by Kolle, W., Kraus, R., and Uhlenhut, P., 1928.

It seems probable that the recent progress in the study of physico-chemical aspects of the antigen-antibody reactions[2] particularly those of agglutination, precipitation and phagocytosis is due to the circumstance that these reactions can be studied on the surface of particulate matter. Similarly it seems desirable to investigate the phenomenon of the neutralization of toxins by antitoxins on the surface of simple particles.

Loeb and his associates, Northrop, Hitchcock and Kunitz,[3] have shown that the adsorption of proteins by collodion particles makes possible the use of certain physico-chemical methods that can not be applied to proteins in solution. The possibility of studying immunological reactions on the surface of collodion particles occurred to me in connection with a study of physico-chemical aspects of the agglutination of tubercle bacillus.[4] In that work it was observed that collodion particles are acid-fast when stained by the Ziehl-Neelsen method and that they remain acid-fast even if they are coated with protein. The method of demonstrating the presence of protein film on the collodion particles by agglutinating the coated collodion particles with anti-egg-white precipitins, was not described in the paper. Jones[5] showed that collodion particles coated with various proteins are agglutinated by specific precipitins, and similar experiments were described by Hulshoff Pol,[6] who employed zinc stearat and colophonium instead of collodion particles. Bedson[7] successfully employed the method of adsorption to collodion particles in his studies on neutralization of vaccinia virus. Recently Mudd, Lucke, McCutcheon and Strumia[8] have reported that collodion particles treated with precipitogen are phagocytosed in a manner that is essentially identical with the phagocytosis of bacteria treated with bacteriotropins.

Thus far I have studied the reactions between diphtheria and tetanus toxins and antitoxins* by this method and the results can be summarized as follows:

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[2] Northrop, J. H., chapter in "The Newer Knowledge of Bacteriology and Immunology," by Jordan, E. V., and Falk, I. S., 1928.

[3] Loeb, J., "Proteins and the Theory of Collodial Behavior," 1922.

[4] Freund, J., *Am. Rev. Tuberc.*, 1925, **12**, 124.

[5] Jones, F. S., *J. Exp. Med.*, 1927, **46**, 303, 1928, **48**, 183.

[6] Hulshoff Pol, J. P. G., *Z. f. Immunitatsf.*, 1929, **61**, 99.

[7] Bedson, S. P., *Brit. J. Exp. Path.*, 1929, **10**, 364.

[8] Mudd, S., Lucke, B., McCutcheon, M., and Strumia, M., *J. Exp. Med.*, 1929, **52**, 299, 313.

* The toxin and antitoxin preparations were obtained through the courtesy of Dr. F. M. Huntoon and Dr. T. S. Githens of the H. K. Mulford Co., Philadelphia.

I. Reaction Between Diphtheria Toxin and Antitoxin. Collodion particles treated with diphtheria toxin were flocculated by diphtheria antitoxic horse-serum. When the collodion particles were treated with toxin that had been heated for 2 hours at 55° C., the flocculation by antitoxin was reduced considerably. Collodion particles treated with formalin-toxoid were also flocculated by antitoxin and previous heating of the toxoid did not affect the flocculation phenomenon.

The following is a brief description of a typical flocculation experiment: To 10 cc. of diphtheria toxin No. 296 (400 M.L.D., 0.15 L + Dose) one cubic centimeter of a heavy suspension of collodion particles was added. The mixture was gently shaken for 5 minutes and kept over night in a refrigerator. Then the collodion particles were washed 3 times by centrifugalization and suspended in 3 cc. of saline. Three drops of the suspension of treated collodion particles were mixed with one cubic centimeter of dilution of antitoxic horse-serum (not concentrated, approximately 500 units), the mixture kept in water bath of 37° C. for 2 hours and then over night in a refrigerator. The reaction was read while the tubes were gently shaken.

TABLE I.

Mixture	Dilution of Serum					
	1:10	1:20	1:40	1:80	1:160	1:320
Collodion particles treated with toxin + antitoxin	Large flocculi; clear fluid	Large flocculi; clear fluid	Large flocculi; clear fluid	Large and medium flocculi; clear fluid	Medium flocculi; clear fluid	Negative
Collodion particles treated with heated toxin + antitoxin	Large flocculi; fairly clear fluid	Large flocculi; turbid fluid	Very few large flocculi; turbid fluid	Negative	Negative	"
Collodion particles treated with toxin + normal horse serum	Negative	Negative	Negative	"	"	"

Collodion particles treated with diphtheria toxin injected into the skin of guinea pigs produces redness, edema and superficial necrosis of the skin. The inflammation was prevented when antitoxin was injected simultaneously.

In interpreting the flocculation reactions the following possibilities may be taken into consideration. The reaction may be due to the interaction (1) between the toxin and antitoxin, and essentially similar to Ramon's flocculation reaction or Hoehn's and Tschertkow's[9] precipitation reaction, or (2) between bacterial agglutinogen and agglutinins, or (3) between other antigens and antibodies (lipoid antigen[10]). The following evidence seems to support the first possibility. (a) The collodion particles are coated with a film of toxin, for they have a toxic action that can be neutralized by antitoxin; (b) heating of the toxin reduced the flocculation materially; (c) in this experiment the toxin can be substituted by formalin-toxoid; (d) heating of the toxoid has little if any influence upon the flocculation of the particles treated with toxoid; (e) the antitoxic horse serums did not agglutinate a suspension of diphtheria bacilli; (f) serum obtained from rabbits immunized with washed and heated diphtheria bacilli did not cause flocculation; (g) the flocculating capacity of antitoxic serums was not changed by contact (absorption) with diphtheria bacilli. However it should be mentioned that serums of 2 rabbits treated with formalin-toxoid did not bring about flocculation.

II. Neutralization of Tetanus Toxin Adsorbed Upon Collodion Particles by Antitoxin. Collodion particles treated with tetanus toxin were not flocculated by antitoxin. Indeed it was observed repeatedly that such particles could be more readily suspended in various dilutions of antitoxin than in saline. However, the presence of tetanus toxin upon the treated collodion particles and the neutralizing action of antitoxin was very clearly demonstrated by animal experiment. To 10 cc. of tetanus toxin (M.L.D. 0.0001 cc.) 2 cc. of a heavy suspension of collodion particles were added and the suspension gently shaken for 5 minutes at room temperature. The particles were then separated by centrifugalization and after they had been washed in saline they were resuspended in 1 cc. of saline. One tenth of a cubic centimeter of the suspension was mixed with 1 cc. of antitoxin dilution, gently shaken for 5 minutes and centrifugalized. After the supernatant fluid had been discarded, the particles were carefully suspended in 4 cc. of saline, spun again and suspended in 0.4 cc. of salt solution. Two-tenths of a cubic centimeter of the suspension was injected into the right hind leg of mice.

[9] Hoehn, E., and Tschertkow, L., *Z. f. Immunitatsf.*, 1929, **62**, 201.

[10] Bouquet, A., and Negre, L., *Ann. Inst. Pasteur*, 1923, **37**, 787. Freund, J., *J. Immun.*, 1927, **13**, 161. Witebsky, E., and Krah, E., *Z. f. Immunitatsf.*, 1930, **66**, 78. Krah, E., and Witebsky, E., *Ibid.*, 1930, **66**, 59.

TABLE II.

Days after injection	Dilution of Antitoxin							
	1:10	1:100	1:1000	1:10,000	1:50,000	1:100,000	1:1,000,000	Control
2	normal	normal	normal	normal	normal	local tetanus	local tetanus	local tetanus
4	"	"	"	"	"	dead	dead	dead

Mice injected with the *supernatant* fluid of the control suspension, *i. e.,* collodion particles treated with toxin, remained free from tetanic symptoms; therefore the tetanus observed in these experiments was due to the toxin adsorbed by the collodion particles before and released from them after injection into the mouse.

This experiment shows clearly that tetanus toxin adsorbed upon collodion particles is neutralized in 10 minutes even by a very high dilution of antitoxin (1 in 50,000).

The question arises as to whether collodion particles treated first with antitoxic serum and then with toxin produce tetanus in mice. Collodion particles were treated (a) with antitoxic serum and then with toxin; (b) with normal horse serum and then with toxin; (c) with antitoxic serum, then with toxin and then with antitoxin again; (d) with toxin alone.

Repeated experiment showed that the order of the toxicity of particles was as follows: a>d>b>c. In other words the antitoxin probably adsorbed on collodion particles (there is no direct evidence for the adsorption of the antitoxin on the particles) failed to neutralize the toxin subsequently adsorbed, moreover collodion particles treated first with antitoxin and then with toxin were slightly but definitely more toxic than particles treated with toxin alone. In contrast to this collodion particles treated first with normal horse serum and then with toxin were less toxic than those treated with toxin alone. Collodion particles first treated with antitoxin, then with toxin and then again with antitoxin were found to be neutralized by the second contact with antitoxin.

It is interesting to compare this observation with Jones' experiment on particles treated with precipitin-serum and then suspended in precipitinogen. Jones found that "Particles exposed to immune serum and subsequently washed fail to agglutinate in the presence of antigen although some of the protein constituents of the immune serum are fixed upon them and its antibody content diminishes."

Conclusions. (1) Collodion particles treated with diphtheria toxin or formalin-toxoid are flocculated by antitoxic horse serum. This phenomenon is shown to be very probably due to a reaction

between toxin and antitoxin and not to a combination of other antigens and antibodies. (2) Tetanus toxin adsorbed to collodion particles can be neutralized by high dilutions of antitoxin.

5160

The Experimental Production of Chronic Empyema in Dogs.

W. M. FIROR. (Introduced by Ferdinand C. Lee.)

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In 1929 we became interested in a new treatment for chronic empyema. The procedure, however, was such a radical departure from the accepted ones that it seemed desirable to test its value on experimental animals, rather than on human beings. Chronic empyema virtually never occurs spontaneously in dogs. We found a paucity of literature on this subject; namely, that chronic empyema in dogs is difficult to produce.

We first opened the pleural cavity and injected into it 2 or 3 cc. of fluid abundantly infected with a variety of organisms. To secure the persistence of this infection a bit of gauze was placed in the cavity before expanding the lungs and closing the wound. These animals all died within 48 hours. At necropsy both pleural cavities were found to be heavily infected. In some there was an accumulation of hemorrhagic, purulent fluid which partially compressed both lungs; in others there was a small amount of thick pus covering all the pleural surfaces. A similar result was obtained when cultures of a single pathogenic organism were used in place of those with several types of organisms. It seemed apparent, then, that a possible explanation of the non-occurrence of chronic empyema in dogs is found in the inability of this species to localize acute pyogenic infections within the pleural cavity. The mediastinum in these animals is a thin, filmy structure, and organisms pass with ease and rapidity from one pleural cavity to the other. The pericardial sac is totally different in this respect; in all of our animals this tissue resisted the penetration of the infecting organisms. Another possible explanation for the failure of empyema to occur spontaneously in dogs lies in the fact that these animals seldom have lobar pneumonia.

The next experiments were devised with the idea of securing adhesions between the visceral and parietal pleurae previous to the