amplifies the observations of Seastone,[2] who showed that while strains from various animal sources, exclusive of the rabbit, were serologically the same, they were different from a single rabbit strain which he studied. More recently, it was shown by Schultz, Terry, Brice, and Gebhardt[6] that 2 serological groups occurred among 11 different strains of *Listerella monocytogenes* derived from various animals, including man.

While the number of strains employed in this study is too small to indicate more than a possible eventuality, it is tempting to speculate whether it may not be that these types actually indicate the ultimate source of the organism—Type I, for example, being essentially derived from rodent animals (rabbit, gerbille[7]), and Type II from ruminant animals (sheep, cow, goat). If future work should confirm this hypothesis, then it may be predicated that human strains originate from some animal source, and the source in question may be surmised from the agglutinative type.

Preliminary experiments suggest that immunity to infection is broader than the type-differentiation demonstrable by agglutination. Thus, animals recovering from experimental conjunctivitis induced by *Listerella monocytogenes* become resistant to further infection following instillation of either the homologous or heterologous strain. In this connection, it is curious that while recovery from conjunctivitis insures resistance to conjunctival reinfection, intravenous immunization resulting in high agglutinin-titers (1:1260 to 1:5120), on the other hand, offers no similar protection. Conversely, the immunity localized in the conjunctiva is more frequently than not unaccompanied by circulating antibodies.

10417 P

Flocculation with Staphylococcal Toxin and Antitoxin.

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Bronfenbrenner and Reichert[1, 2] found that antitoxin obtained by immunizing animals against toxic filtrates of 24-day cultures of *B.*

[6] Schultz, E. W., Terry, M. C., Brice, A. T., Jr., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 605.

[7] Pirie, J. H. H., *Pub. S. African Inst. Med. Research*, 1927, **3**, 163.

botulinus precipitated the filtrate while antitoxin prepared against toxic filtrates of 4-day cultures did not precipitate. They concluded that the precipitation in the Ramon test[3] may be influenced by the antibacterial antibody in the antitoxic serum. A similar observation was reported by the author[4] using the tetanal toxin-antitoxin system. It seemed interesting to see whether these findings might also influence the practical application of the Ramon test in the standardization of staphylococcal toxin and antitoxin.

In a preliminary experiment it was found that an appreciable amount of toxin was present in the 48-hour filtrate of a toxin-producing strain of staphylococcus cultivated in the broth described by Parker, Hopkins, and Gunther.[5] The cultures were incubated at 37°C in an atmosphere of 10% CO_2. A potent toxin was produced by this strain after 20 days' incubation at 37°C. White mice were killed almost immediately by the intravenous injection of 0.3 cc of undiluted filtrate. Rabbits were immunized with the formolized filtrates of 48-hour and 20-day cultures, respectively, as well as with filtrates of 48-hour and 20-day cultures of a cream-colored atoxic variant of the same strain of staphylococcus. Isolation of the atoxic variant was accomplished by repeated sub-culturing in lithium-chloride broth. As expected, the antisera prepared against the non-toxic filtrates were found to be entirely devoid of antitoxin as determined by the protection-test on mice, since even 0.5 cc of undiluted serum failed to neutralize the lethal toxin contained in 0.3 cc of a 6-day culture-filtrate.

Rane and Wyman,[6] instead of using weak toxins and relatively large amounts of antitoxin, obtained true flocculation with a strong toxin (hemolytic streptococcus) and relatively few units of antitoxin. They suggested that when large quantities of antitoxin were employed in the test "it is conceivable that a bacterial protein rather than a neutralized toxin-antitoxin mixture was precipitated." In order that this point be considered in the present set of experiments, a constant amount of antigen was mixed in the *in vitro* tests with varying amounts of serum covering a very wide range so that the zone of precipitation, if narrow, might not be missed.

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1 Bronfenbrenner, J., and Reichert, P., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 391.

2 Bronfenbrenner, J., and Reichert, P., *J. Exp. Med.*, 1926, **44**, 553.

3 Ramon, G., *Compt. Rend. de la Soc. de Biol.*, 1922, **86**, 711; *Ann. de l'Institut Pasteur*, 1923, **37**, 1001.

4 Sulkin, S. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 555.

5 Parker, J. T., Hopkins, J. G., and Gunther, A., PROC. SOC. EXP. BIOL. AND MED., 1925-1926, **23**, 344.

6 Rane, L., and Wyman, L., *J. Immun.*, 1937, **32**, 321.

The sera containing antitoxin and those obtained against the non-toxic filtrates were used in the precipitative test with the following antigens: (1) Filtrates of 48-hour cultures of the toxic and atoxic variants; (2) filtrates of 20-day cultures of the toxic and atoxic variants; and (3) 20-day filtrates of tetanus toxin (control). *In vivo* neutralization tests were also carried out at the same time, and in Table I, the letter "N" indicates the neutral point as determined by this method. Although the antiserum obtained against the 48-hour filtrate of the toxic variant neutralized the toxin in the *in vivo* tests, no precipitation occurred in the presence of the various filtrates tested. Similarly, no precipitation occurred when the antiserum prepared against the 48-hour filtrate of the atoxic variant was combined with the respective antigens. On the other hand, copious precipitation occurred when the antitoxic serum prepared against the

TABLE I.

		Antigen (2.0 cc in each tube)				
	cc	Filtrate of 48-hr culture of toxic strain*	Filtrate of 48-hr culture of atoxic variant	Filtrate of 20-day culture of toxic strain*	Filtrate of 20-day culture of atoxic variant	*Control* 20-day filtrate tetanal toxin (20,000 MLD per cc)
Antiserum	.10	—	—	+	—	—
against	.09	—	—	++	+	—
20-day	.08	—	—	+++	+	—
filtrate	.07	—	—	+++	++	—
of toxic	.066	—	—	++(N)	++	—
variant	.06	—	—	++	+++	—
	.05	—	—	+	++	—
	.04	—(N)	—	+	+	—
	.03	—	—	++	+	—
	.02	—	—	—	—	—
Antiserum	.10	—	—	+	—	—
against	.09	—	—	++	+	—
20-day	.08	—	—	++	+	—
filtrate	.07	—	—	++	+	—
of atoxic	.06	—	—	++	++	—
variant	.05	—	—	+	++	—
	.04	—	—	+	++	—
	.03	—	—	—	++	—
	.02	—	—	—	+	—
	.01	—	—	—	+	—

*White mice were killed almost instantaneously by intravenous injection of 0.3 cc of undiluted 20-day filtrate, while 0.5 cc of the 48-hour filtrate was necessary to produce acute and fatal toxemia in mice.

(N) = Neutral point in protection test; +++ = copious precipitation; ++ = moderate precipitation; + = slight precipitation; — = no precipitation.

NOTE: Antisera against 48-hour toxic as well as those against 48-hour atoxic filtrates failed to precipitate any of the antigens and, therefore, are omitted from this table.

20-day formolized filtrate was mixed with either its homologous toxic filtrate or with the 20-day filtrate of the atoxic variant. Likewise, the antiserum prepared against the 20-day filtrate of the atoxic variant produced a wide zone of precipitation in the presence of both the homologous filtrate and the 20-day filtrate of the toxic strain.

The results of these experiments indicate that (1) the filtrates of the young cultures of the toxic variant are relatively free from bacterial protein and hence their antisera contain antitoxin and no detectable antibacterial antibody; (2) that the filtrates of the old cultures of the toxic variant contain bacterial protein in addition to the toxin, thereby stimulating the production of both antibacterial antibodies and antitoxins; (3) that the usefulness of the Ramon test in the *in vitro* standardization of staphylococcal toxin and antitoxin is limited by the fact that the flocculating power is not strictly parallel to toxicity but depends upon the presence of bacterial proteins in the antigen and antibacterial antibodies in the antitoxic serum.

10418 P

Auto-Injection of the Biliary Passages from the Gall Bladder in Rabbit.

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Contraction of the rabbit gall bladder may drive its contents back into the biliary passages if the bile papilla is tonically contracted or if the choledochus is clamped, and dyes previously injected into the gall bladder may then be demonstrated in the liver and arterial blood.

Experimental Procedure. 300 mg sodium barbital subcutaneously per kg animal; clamp choledochus; expose gall bladder and withdraw 1 cc of bile; through the same hypo needle kept *in situ* inject 0.5 cc of 5% sodium fluoresceinate solution; clamp the puncture in the gall bladder by means of a small narrow-jawed bulldog clamp; to contract the gall bladder, 0.5 cc per kg of a crude secretin preparation containing histamin was injected intravenously.

Within one or 2 minutes after the secretin injection the gall bladder usually becomes tense, decreases in volume and the choledochus and hepatic ducts appear as bulging, rounded, green cords; the choledochus may be so distended that it is palpable. In 15 minutes ap-