combined with one mg. of eserine, peristalsis resulted, almost constantly in all 3 viscera. Intramuscular injection of larger amounts, 2-3 mg., of eserine regularly induced generalized intestinal motility but caused undesirable concomitant effects as well.

7819 P

Virus Adsorbed to Alumina-gel for Production of Antipoliomyelitis Serum in Sheep.

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Early in the history of experimental poliomyelitis it was demonstrated that the serum of both monkeys and humans, convalescent from the disease, had the property of neutralizing the action of the virus when the 2 were mixed *in vitro*. As therapeutic value has been ascribed to such serums a number of attempts have been made to produce antiserum with similar properties in large, poliomyelitis-refractory animals. In most instances horses have been employed and the results, although sometimes successful, have been irregular and seem to depend upon individual differences in responsiveness of the animals. The immunization of sheep has met with success in some hands (Howitt[1]) and failure or irregular results in others (Stewart and Haselbauer[2]). The production of poliomyelitis antiserum in goats has also been reported.

In these various types of animals, virus inoculations have usually been continued over a long period of time, in some instances years, before potent neutralizing serums were demonstrated. A method of immunization in which the time as well as the number of injections could be decreased would be a distinct improvement.

We were subjecting a sheep to a series of virus injections for the purpose of producing an antiserum when it occurred to us that virus adsorbed to a colloidal carrier might prove to be a better immunizing agent than the crude virus. There is evidence that the antigenicity of substances may be enhanced by injecting them in combination with a colloid. Hektoen and Welker[3] have reported the continued maintenance of a high precipitin level in rabbits following one injection of protein adsorbed to alumina-gel. Since the virus of

[1] Howitt, B. F., *J. Inf. Dis.*, 1932, **50**, 26.

[2] Stewart, F. D., and Haselbauer, P., *J. Exp. Med.*, 1928, **48**, 449.

[3] Hektoen, L., and Welker, W. H., *J. Inf. Dis.*, 1933, **53**, 309.

poliomyelitis has been adsorbed to this substance by Sabin[4] and others, we decided to investigate the suitability of such a combination as an immunizing agent in sheep. Virus-alumina-gel complex has been used as an immunizing agent for susceptible animals in poliomyelitis by Rhoads[5] and by us.[6] It is perhaps noteworthy that 2 horses which produced good neutralizing serum within 5 months were injected with a preparation in which 0.1% alum had been incorporated in the saline (Weyer, Park and Banzhaf[7]).

Our sheep (L) had been given 16 weekly injections of crude virus over a period of 4 months. After a rest period of 7 weeks, 2 injections of virus-alumina-gel complex were given with an interval of 3 weeks. The crude virus injections consisted of pooled glycerolated 10% virus given both subcutaneously and intramuscularly, usually 50 cc. by each route. The virus-alumina-gel was prepared by shaking together equal parts of ether-treated virus, at a pH of 6.5, and alumina-gel (Willstätter, Type C). The first injection consisted of 50 cc. and the second of 65 cc., both given subcutaneously.

TABLE I.

Production of poliomyelitis antiserum in sheep.

Treatment	Blood collected	Serum Neutralization Tests		
		Dilution of serum	Sheep L Virus injection	Sheep R normal tissue injection
——	Before injections	undiluted	—	—, —, —
16 weekly injections of crude material	3 weeks after last injection	"	+	—
2 injections of gel preparation	1 month after last injection	"	+	+, —
		1:5	+	—, —
		1:25	+	—
		1:50	+	
		1:100	+	
	5 months after last injection	undiluted		+, —
		1:5		—, —
		1:25	+	+, —
		1:50	+	—
		1:100	+	
		1:250	+	
		1:500	+	

+ indicates neutralization; —, lack of neutralization. Each sign represents one monkey.

[4] Sabin, A. B., *J. Exp. Med.*, 1932, **56**, 307.

[5] Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 399.

[6] Gordon, F. B., Harrison, J. A., and Hudson, N. P., paper read before Soc. Am. Bact., Dec. 29, 1934.

[7] Weyer, E. R., Park, W. H., and Banzhaf, E. J., *J. Exp. Med.*, 1931, **53**, 553.

As a control, another sheep (R) had been given parallel injections of a crude emulsion of the central nervous system of normal monkeys. It also was given 2 similar injections of alumina-gel which had been treated with nervous tissue from normal monkeys in the same manner that the virus-alumina-gel was prepared. Table I gives the results of neutralization tests made with serum from different bleedings of both sheep.

Attention should be called to the fact that we have not found an end-point in the serum titre of the sheep (L) injected with virus. In every instance neutralization was effected by the highest dilution of serum used, which in one instance was 1:500. We were gratified to find that the serum possessed good neutralizing power 5 months after the last immunizing injection. Irregular results were obtained with the serum of the sheep (R) that received normal nervous tissue. This is consistent with the reports of several workers that normal sheep serum may in some cases neutralize the virus, and that this property may fluctuate in individual sheep.[2]

7820 P

Mechanism of Death in Bile Peritonitis.

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The question of the mechanism of death in so-called "bile peritonitis" due to the leakage of moderate or large amounts of bile into the peritoneal cavity has puzzled investigators for some time. The resultant peritonitis has been attributed to a toxic action or the action of anaerobic organisms. Mason[1] and Andrews[2] noted that a large amount of exudate was formed in the peritoneal cavity of animals dying from liver autolysis and experimentally produced bile peritonitis. Blalock,[3] Underhill,[4] Harkins,[5] and others have shown that

[1] Mason, E. C., and Davidson, E. C., *J. Lab. and Clin. Med.*, 1925, **10**, 622.

[2] Andrews, E., Rewbridge, A. G., and Hrdina, Leo, *Surg. Gynec. and Obst.*, 1931, **53**, 176.

[3] Beard, J. W., and Blalock, A., *Arch. Surg.*, 1931, **22**, 617.

[4] Underhill, F. P., Kapsinow, R., and Fisk, M. E., *Am. J. Physiol.*, 1930, **95**, 302.

[5] Harkins, H. N., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 994; and articles on freezing, in press.