maintenance of the myelin sheaths.

Summary. Nervous tissues of thiamine deficient and control pigeons were examined histochemically in respect to both alkaline and acid phosphatases. In this deficiency the nerve cells shrink and acquire increased amounts of alkaline phosphatase, whereas the neuropil shows only a slightly increased reaction. Acid phosphatase of the axis cylinders and cytoplasm of the nerve cells is diminished in amount. After administration of thiamine to the deficient pigeons, a considerable return to normal is observed. Accompanying restoration of the acid phosphatase a network, similar to the pattern of "neurokeratin", becomes evident in the myelin sheaths.

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Failure to Demonstrate Antibody in Feces of Monkeys Vaccinated with Poliomyelitis Virus, Lansing Strain.*† (18310)

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There is evidence that antibodies for various infectious agents occur in certain of the secretions and excretions of humans and animals. For example, antibodies have been found for influenza virus(1-3) and the Lansing strain of poliomyelitis virus(4-6) in nasal secretions. In feces, antibodies have been reported for cholera(7,8), several enteric bacilli(9-11) and possibly for one strain of the Lansing type of poliomyelitis virus(12).

The present study was made in order to determine whether antibodies occurred in the feces of monkeys during and after vaccination with the Lansing strain of poliomyelitis virus. Monkeys were chosen for this study since it has been shown that high antibody titers may be obtained in their sera by vaccination(13,14).

Materials and Methods. Eight young Macaca mulatta monkeys were used. The monkeys were examined daily, and daily rectal temperatures were recorded. For titration of the virus-neutralizing capacity of stool suspensions and serum specimens, white Swiss mice 3-4 weeks of age and 12-16 g in weight were used; these were examined daily for a month. The day of onset of paralysis, the character of the paralysis and the day of death were recorded. At the end of one

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month all survivors were discarded.

The Lansing strain of poliomyelitis virus was used(15). The line§ used for vaccination had been in continued monkey passage and the pool had a titer of $10^{-2.5}$ PD_{50}. For neutralization tests 2 pools of the line¶ maintained in mice in this laboratory were used, with average LD_{50} titers of $10^{-4.0}$. Preparation and storage of the virus suspension has been described(16).

Four monkeys (18-61, -63, -65, -84) in individual cages were vaccinated with Lansing virus IIIa, while 4 were maintained as controls. Before each inoculation the animals were anesthetized with ether and blood samples of 5 cc were drawn from a femoral vein. After intracerebral challenge 20 to 40 cc of blood were drawn from the heart at each bleeding. The sera were stored at 4°C. Three (18-61, -63, -65) received 5 biweekly intravenous injections‖ of 4 ml of 5% virus suspension, representing a total intravenous inoculation of 1.0 g of infected nervous tissue. Each of these, and monkey 18-84, then received a total of 1.4 g of infected cord tissue intramuscularly, in doses of 2 ml of 10% suspension inoculated into the calf muscles once a week for 7 weeks. Intracerebral challenge with 1.0 ml of 10% virus suspension (140 PD_{50}) was given to 18-84 two weeks after the end of the course of vaccination, and to the others 3 weeks after the end of the course.

Monkey stools were collected daily and stored individually at −2 to −10°C. Each pool of feces represented 5 to 7 days' collection, except for the last 2 collections of 2 days each, from individual monkeys. A 10% suspension in physiological salt solution was prepared from each pool and kept at 4°C while centrifuged first at low speeds and finally for 1 hour at 15,000 r.p.m. (approx. 18,000 g) in an anglehead centrifuge(17). The final clarified supernate was kept at −2 to −10°C during precipitation with cold ethanol, according to methods used by Felton(18) and others(19). Cold 95% ethanol was added dropwise with constant agitation to yield a final ethanol concentration of 30%; frequently ethanol was added to another aliquot to yield a final concentration of 20% or 40%. The precipitate was removed by centrifugation (approx. 1450 g), and resuspended in 1-2 cc of supernate, dried in vacuum, and stored at −2 to −10°C. The efficiency of the alcohol procedure is indicated by the following considerations: 1), in one experiment, an average of 74% of the Lansing virus neutralizing antibody was recovered in the precipitate resulting from alcohol treatment of 7 human sera with original titers of 5 to 133 ND_{50} against 40 LD_{50} of the virus; 2) in another experiment, when 2 cc of human serum with a titer of 200 ND_{50} against 100 LD_{50} of virus was added to 50 cc of normal monkey stool suspension, over half of the virus neutralizing activity of the serum was recovered in the precipitates obtained with alcohol treatment, while simultaneous treatment of the same serum mixed with physiological salt solution resulted in a 60% recovery of the neutralizing activity of the unaltered serum. In addition, the pH values of the crude stool suspensions, as well as of the resuspended precipitates, were found to range from 6.1 to 7.5, in which range there is essentially no deleterious effect on immune globulins. In addition to tests of feces from immunized monkeys, stools were tested from

15. Armstrong, C., *Pub. Health Rep.*, 1939, v54, 1719.

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¶ Received from Dr. S. D. Kramer in 1941.

16. Brown, G. C., and Francis, T., Jr., *J. Exp. Med.*, 1945, v81, 161.

‖ Immediately after the second or third and succeeding intravenous injections the monkeys developed respiratory arrest, accompanied by a rapid, thready, or imperceptible femoral pulse and cardiac thrust. This was treated by artificial respiration for 2 or 3 minutes, by which time the animals once more began to breathe, at first irregularly with gasping, and later rapidly and deeply with rapid recovery of consciousness. These incidents had no permanent ill-effects, and none was observed after intramuscular inoculation.

17. Pickels, F. G., *Rev. Scient. Instr.*, 1942, v13, 93.

18. Felton L. D., *J. Immunol.*, 1931, v21, 357.

19. Cohn, E. J., *et al., J. Am. Chem. Soc.*, 1946, v68, 459.

four paralyzed poliomyelitic patients and two normal persons, all of whose sera had antibody titers of 600 to 2000 against 10 LD_{50} of Lansing virus.

The contents of each vial of dried material recovered by precipitation from the stool specimens were resuspended in 1.0 cc of physiological salt solution. The suspensions were tested in final dilutions of ½, ¼, 1/8, and 1/16 against final concentrations of virus suspensions of 5 or 1% at first (400 or 80 LD_{50}) and later against 0.5 and 0.15% (40 and 10 LD_{50}). Equal volumes of diluted serum, serum precipitate, or stool precipitate, were mixed with virus, incubated for 30 minutes at 37°C, and stored at 4°C for 30-90 minutes before inoculation. A volume of 0.03 cc of the mixture was inoculated intracerebrally into each of 8 mice lightly anesthetized with ether. A complete virus titration accompanied every test except for a few of the initial experiments with stool preparations in which a control was employed only for the infectivity of the virus concentration used. For all titrations and neutralizations, 50% endpoints were calculated according to the method of Reed and Muench(20). Neutralization results were expressed as reciprocals of the final dilution of serum giving that endpoint. Neutralization indices represent the product of the reciprocal of the final dilution of serum giving a 50% endpoint and the number of LD_{50}'s neutralized.

Results. Immunization of Monkeys. Results obtained with 10 LD_{50} of virus will be considered here, although most sera were also tested using 400 and 100 LD_{50} (Fig. 1). As seen in Fig. 1, virus neutralizing antibodies appeared in the serum of 4 monkeys after the twenty-first day. In three monkeys (18-61, -63, -65) antibody levels ranging from 1000 to 4000 were found in the blood towards the end of the 71-day period of immunization (Fig. 1). The antibody titer in the serum of monkey 18-84 rose to 813 two weeks after the end of the 56-day intramuscular course. A challenging intracerebral inoculation of 1.0 ml (140 PD_{50}) of the same 10% virus

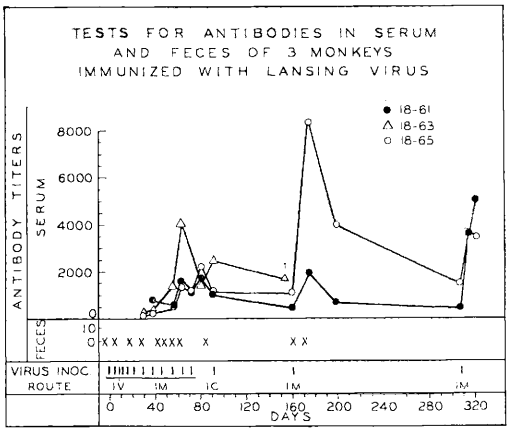

FIG. 1.

suspension used for vaccination was given the latter monkey two weeks after, and to the 3 others, (Fig. 1) whose sera maintained antibody levels of 1000 to 2500, three weeks after the end of the course of vaccination. Monkey 18-84, which had been vaccinated with a total of only 1.4 g of tissue (2000 PD_{50}) given intramuscularly and had the lowest titer of serum antibody, became paralyzed after the intracerebral challenge inoculation, as did 4 control animals which included 18-64 and 18-70. Histological study of the spinal cord confirmed the diagnosis of poliomyelitis in each case. The other 3 animals, each of which had been vaccinated with a total of 2.4 g of tissue (3400 PD_{50}) given by both the intramuscular and intravenous routes, developed no fever or other signs of poliomyelitis (Fig. 1). Approximately two-fold drops of serum antibody titer were noted in 2 of the 3 surviving immunized animals 10 weeks after the challenge inoculation. One of them (18-63) died at this time from a hemopericardium and showed no histological evidence of poliomyelitis. Immediately after this bleeding an additional inoculation of 0.2 g of the same material was given intramuscularly to the 2 survivors, and 2 weeks later the antibody titers were 1900 and 8300, respectively, thus surpassing their previous peak levels. Progressively lower values were obtained with blood taken 5 and 21 weeks after the booster dose in each animal, demonstrating that the antibody levels 21 weeks after the booster were as low as those obtained just

20. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

before the booster. A second booster dose given 21 weeks after the first resulted in a 2 and 10-fold rise in titer to values of 3400 and 5100, respectively, within 2 weeks.

Antibody in the feces. The precipitates obtained with 20, 30 and 40% alcohol treatment of the stools collected from 4 vaccinated and 2 normal monkeys throughout the first 25 weeks of the test were consistently negative for the capacity to neutralize 10 or 40 LD_{50} of the Lansing strain of poliomyelitis virus. Using the technic of precipitation of stool extracts with alcohol in the cold, negative results were also obtained in tests with stools from two normal persons and four paralyzed poliomyelitic patients each of whose serum had antibody titers of 600 to 2000 against 10 LD_{50} of Lansing virus.

Discussion. Some observations were made concerning the development of antibody in the serum and of resistance to intracerebral challenge in vaccinated monkeys. The animal which received a total of 1.4 g of tissue (2000 PD_{50}) in 8 weeks was not immune to intracerebral challenge (140 PD_{50}) on the tenth week, when the neutralization index of the serum had reached 800. The other 3 monkeys received 2.4 g (3400 PD_{50}) in 10 weeks, and were immune when challenged intracerebrally in the thirteenth week at which time the neutralization indices of their sera were 10,000, 11,000 and 25,000 respectively. Additional inoculations on 2 subsequent occasions resulted in rises of antibody titer to levels considerably higher than those previously attained. The results of vaccination are similar to those reported by Morgan in extensive studies with the same strain of virus in monkeys(13,14).

There are several possible explanations for the failure to demonstrate Lansing antibody in the feces of immunized monkeys. One is that the procedures employed for its detection were not sufficiently sensitive. In this regard the evidence previously presented demonstrates the effectiveness of the method used: there was an average recovery of 74% of the neutralizing antibody for Lansing virus in precipitates resulting from alcoholic treatment of 7 human sera; there was approximately the same recovery of neutralizing substances by alcoholic precipitation of a mixture of serum and stool as by precipitation of a mixture of the same serum with saline. These results indicate that when specific antibody is present, in moderate amounts, at least, in serum or feces it can readily be detected by the methods employed. These methods might, of course, not be adequate for the demonstration of antibody if only a very small amount entered the intestine, especially since there is no known mechanism which would serve to concentrate antibody in the intestine. It is, therefore, conceivable that coproantibody might be detected if concentrates of the total feces excreted over a longer period of time were tested, rather than testing only the concentrates of smaller amounts as was done in the present experiments.

Another explanation for the absence of demonstrable coproantibody is that it was present but was combined with fecal components so as to lose its identity. The control studies mentioned in the preceding paragraphs demonstrate that when the specific antibody in the form of whole serum is added to feces it is neither destroyed nor removed by adsorption onto particulate fecal material discarded in the procedure. Or possibly antibody could be specifically absorbed by virus in the intestinal tract; this seems unlikely since virus was not demonstrable at any time in the alcoholic precipitates of fecal extracts. Failure to detect virus does not seem attributable to the procedures employed, since the Lansing strain of poliomyelitis virus is not inactivated, but on the contrary is concentrated, by precipitation with 20 to 30% ethanol(21). Similar concentration of the MEF strain of Lansing-type poliomyelitis virus has been reported by the use of methanol (22).

A third explanation for the failure to demonstrate coproantibody is that it was absent. This is the most likely reason for the present results although it may be that the antibody

21. Unpublished data.

22. Pollard, M., Connolly, J., and Fromm, S., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 290.

titer of the serum has to be above the levels attained in the present experiments before a detectable quantity will be found in the feces. A trend toward such a threshold effect has been reported when the antibody to Lansing virus is found in nasopharyngeal secretions(4). But in the present experiments there was no evidence of this even when marked secondary rises in the antibody level of the serum were obtained.

It will be recalled that no neutralization of Lansing virus was demonstrable with extracts of feces from humans with moderate levels of antibody to Lansing virus in their sera. Morgan has also stated, without experimental details(23), that no neutralization of Lansing virus was obtained with the feces of 3 humans who had high titers of antibody in their sera. Although the response of human subjects to active infection might differ from that of monkeys to active immunization, the absence of coproantibody in both species suggests that the results obtained with the monkey may apply to man. Moreover, the antibody to Lansing in the serum of man is most probably acquired through earlier infection. Further studies with human subjects are, however, necessary to establish the absence of homologous coproantibody during subclinical or frank infection with poliomyelitis virus. One approach is indicated by a recent study of paralytic cases and their familial associates which suggests that the disappearance of poliomyelitis virus from the feces is related to the appearance or increase of homologous antibodies in the serum(24). Hammon has recently stated that feces from monkeys paralyzed with the MEF-1 strain of poliomyelitis virus neutralize the Lansing strain of virus(12). Since no experimental details are given a comparison of methods cannot be made. However, because of the present results one would assume that the effect reported by Hammon was probably related to something other than antibody unless the response of monkeys to active infection with the MEF-1 strain differs sufficiently from their response to immunization with the Lansing strain to account for the presence of coproantibody during paralysis.

No capacity to neutralize Lansing virus was demonstrated with extracts of pooled stools from each of 4 normal rhesus monkeys, nor with 11 pools from each of 2 other normal rhesus monkeys. These are similar to results mentioned by Hammon(12), but contrast with a report by Levaditi *et al.*(25) in which it was concluded that some neutralization of a monkey-adapted poliomyelitis virus was obtained with the stools of normal monkeys. However, Levaditi used cynomolgus rather than rhesus monkeys, and tested only crude stool suspensions.

Summary and Conclusion. Four monkeys were vaccinated with active preparations of the Lansing strain of poliomyelitis virus by parenteral routes. One animal received a total inoculum of 1.4 g of infected tissue, at the time of intracerebral challenge had an antibody titer in the serum of 800 against 10 LD_{50} of virus, but succumbed to an acute poliomyelitic infection. The remaining 3 received 2.4 g of inoculum, had antibody titers in their sera of approximately 2000 against 10 LD_{50} of virus and did not develop the disease. Each of 2 additional inoculations resulted in an increase in antibody of the serum to levels of 5100 and 8300 against 10 LD_{50} of virus. Despite the fact that Lansing virus antibody in the serum of three monkeys reached high titers, no evidence was obtained for the presence of antibody in the feces. Antibody was not detected in single specimens in the feces obtained from 6 humans whose sera had moderate titers of antibody to the Lansing strain of poliomyelitis virus. It is concluded on the basis of these data that antibody in feces is not an important factor in the resistance of the rhesus monkey, and probably of man, to infection with poliomyelitis virus.

23. Morgan, I. M., *Poliomyelitis*, J. B. Lippincott Co., Philadelphia, 1948, p264.

24. Brown, G. C., and Ainslie, John D., in press. (Abstracted in *Fed. Proc.*, 1950, v9, 378.

25. Levaditi, C., Kling, C., and Lepine, P., *Bull. Acad. Med.*, Paris, 1931, v105, 190.

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