side C. in rat urine is described. The renal excretion of Lanatoside C. in the rat is considerably greater than that of digitoxin.

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Complement-Fixation Studies with Poliomyelitis in Humans.* (19430)

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Specific complement-fixing (CF) antigens have been processed from cotton rat tissues infected with Lansing poliomyelitis virus (1-3). The CF antigens prepared by Casals and Olitsky(4,5) from MEF_1 infected tissues of new-born mice have appeared to cross somewhat with serum from Brunhilde-immune monkeys. More recently(6), specific CF antigens have been processed from nerve tissues of rhesus monkeys infected with Brunhilde and with Leon prototype poliomyelitis viruses. The latter antigens have been tested thus far only with serums from monkeys immunized with virus and the adjuvant recommended by Salk(7). The chronological antibody response of Lansing-immunized cotton rats appeared to follow a pattern in which the CF test was transitory, whereas the neutralizing property of their serum was of longer duration(1,2). The exact duration of the latter effect is uncertain but is thought to last for years. Some evidence has indicated that immunized monkeys may demonstrate a similar chronological antibody pattern.

When human cases of poliomyelitis recover from the infection, specific neutralizing antibodies rapidly appear in the blood(8,9). There has been suggestive evidence that such infection may elicit a non-specific rise of Lansing neutralizing antibody, even though the infection was of non-Lansing type(9). Lansing CF antibody has been demonstrated in human serums, but their specificity and clinical interpretation have not been studied extensively(1,5). The possibility of a non-specific rise in Lansing CF antibody during the course of infection with non-Lansing virus has not been studied. It is the purpose of this paper to describe the Lansing CF antibody response elicited in the human following infection with poliomyelitis virus.

Methods. Antigen. CF antigens were processed from Lansing poliomyelitis-infected nerve tissues of mature cotton rats (LCR) by two technics: One by precipitation of clarified nerve tissue suspensions with methanol and use of the final eluate as antigen(2); and secondly, by sedimentation of the virus in the ultracentrifuge and use of the resuspended sediment as antigen(3). The antigens were tested for specificity with antiserums prepared against Brunhilde and against Leon poliomyelitis, as well as against rabies and St. Louis encephalitis viruses. The LCR CF antigens processed by the ultracentrifugation technic appeared to be superior to those prepared by methanol precipitation because of the constancy of results and because such antigens were sufficiently sensitive to detect CF antibodies in immunized monkeys. *Serum.* Serum specimens were collected aseptically from 131

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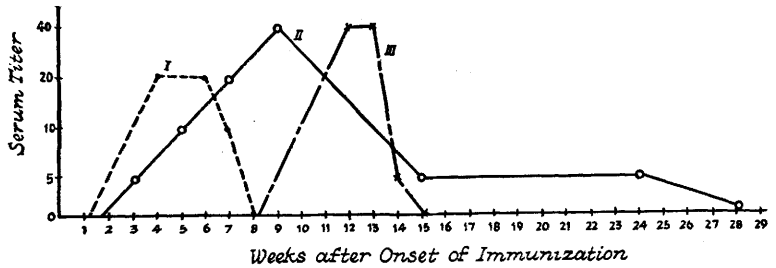

FIG. 1. Complement-fixing antibody response of Lansing + adjuvant immunized rhesus monkeys to Lansing antigen.

paralytic poliomyelitis cases in humans:† Eighty-five were collected 2 weeks after onset of illness; and 46, at onset and at weekly intervals thereafter, when possible. Serum specimens were collected at weekly intervals for 6 months from rhesus monkeys which were immunized by 3 weekly I.M. injections of Lansing virus plus adjuvant‡ emulsion. All serums were stored in screw-top vials at −20°C. When prepared for CF test they were diluted 1 in 5 with 0.15 M NaCl solution and heated at 61°C for 20 minutes. *Complement-fixation test.* Antigen, antiserum (1:5) and complement (2 units) were mixed in 0.2 cc quantities each and incubated in the refrigerator overnight. Hemolysin (2 units) and 2% washed sheep red blood cells (0.2 cc each) were added to each tube and incubation continued for 30 minutes at 37°C, after which the reactions were recorded. The reactions were generally clear cut and 1+ or 2+ reactions were seldom encountered. Reactions read as 3+ and 4+ were recorded as positive. Serum dilutions were mixed with constant dilutions of antigen and 2 units of complement in order to determine their end-point titers. Each CF test was repeated at least twice. *Neutralization test.* Undiluted serum was added to an equal amount of Lansing virus which was so diluted that each inoculum contained approximately 100 LD_{50} of virus. After incubation of the serum-virus mixtures overnight in the refrigerator, eight mature Swiss mice were inoculated intracerebrally with 0.03 cc of the mixture. After 21 days of observation, if 6 or more mice survived, the test was positive; if 3 to 6 survived, the test was equivocal; if less than 3 survived, the test was negative.

Results. When monkeys were inoculated with Lansing virus in adjuvant their CF antibody response was transitory but followed a similar curve pattern (Fig. 1). One showed a rapid rise and decline, one a delayed rise and quick decline, and a third one a rapid rise and slow decline. The latter monkey was positive for 6 months and was negative thereafter. Each of 3 monkeys similarly immunized against Brunhilde and 3 against Leon viruses were negative for Lansing CF antibodies during the 6 months examination period.

Eighty-five of the specimens of human serum were collected at approximately two weeks after onset of poliomyelitis symptoms. They were tested both for MEF_1 neutralizing and for CF antibodies, the latter with methanol precipitated MEF_1 antigen. Fig. 2 indicates that 38 of these were positive and 47 negative for neutralizing effect on MEF_1 poliomyelitis virus. None of these negative serums contained CF antibodies for the same virus, while 11 of the former were positive in the 1:5 dilution of serum examined.

Forty-six specimens of serum were collected from paralytic poliomyelitis cases immediately after admission to the hospital and at weekly intervals thereafter, when possible. They were examined for CF antibodies with LCR CF antigen processed by ultracentrifuga-

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‡ "Pendil" manufactured by Endo Products, Richmond Hill, N. Y.

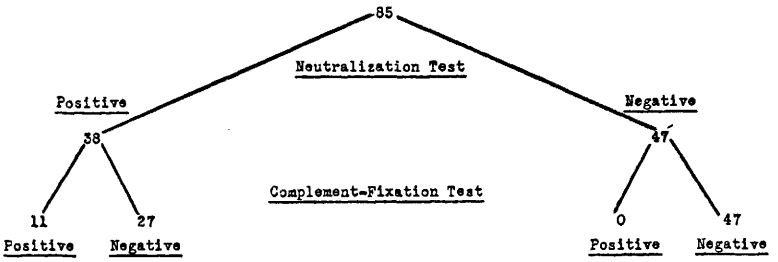

FIG. 2. Serologic studies on 85 poliomyelitis cases with MEF_1 virus. Serum collected two weeks after onset of illness.

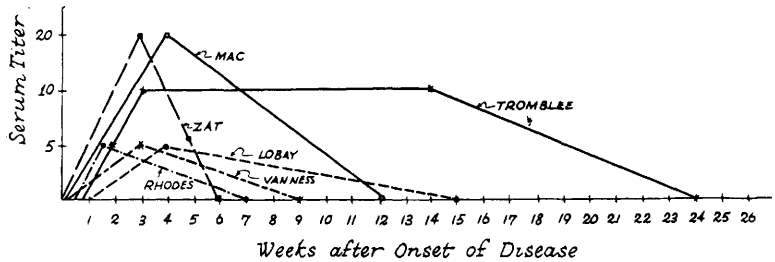

FIG. 3. Complement-fixing antibody response of paralytic poliomyelitis cases to Lansing antigen.

tion. Ten were positive and 6 of these are outlined in Fig. 3. The CF antibody titer appeared to reach its peak within the first 4 weeks of illness and declined thereafter. One case was positive during the first week, 3 during the second and third week, and one during the fourth week. The latter case could not be tested earlier. The CF reactions remained positive from 6 weeks to 3½ months after onset. The latter case was not tested between the 3½-month period and 6 months, but became negative for Lansing CF antibody some time between these two periods. The remaining 36 cases in this group of paralytic poliomyelitis were negative for LCR CF antibodies during the acute and convalescent stages of infection.

Discussion. It is apparent from the results described above that the LCR CF responses of Lansing-immunized monkeys and of naturally infected humans are of transitory nature. One might attribute the protracted curve of Monkey II (Fig. 1) to the effect of adjuvant mixture with the virus inoculum, but the CF curves of human case "Tromblee" (Fig. 3) appears to be protracted without such adjuvant being involved. Then, too, monkeys I and III exhibited shorter curves and their inocula and bleeding schedules were the same as for monkey II. We may thus be justified in saying that with the antigens used, the CF responses ranged from brief rises of 3-4 weeks duration to prolonged rises of 3-4 months. This strengthens the opinion previously suggested(2,3) that a positive LCR CF test might indicate current or recent Lansing infection, and that a negative CF test indicates either a remote exposure to the virus or none. The latter problem may then be unraveled by a virus neutralization test with the serum, a procedure which has become more feasible through application of the tissue culture technics of Enders *et al.*(10). It should be emphasized that the CF titer curves of Fig. 3 represent test points, joined at intervals of several weeks because the intervening specimens were unavailable. While the positive CF reactions would not be longer than demonstrated certainly they could be shorter, particularly in those cases designated as "Mac" and "Tromblee." If Fig. 3 accurately depicts the rise of LCR CF antibody in the human, then a specimen of serum collected 4 weeks after onset of illness should be the optimal period for the CF test. If a non-Lansing infection engenders a non-specific Lansing anti-

body response, this was not apparent in the 36 cases which were LCR CF negative during the entire period of examination. We must not insist that the LCR CF reactions here demonstrated in human serums mean that Lansing virus caused the paralytic syndrome. Since the CF antigens were prototype specific, the antibody which it demonstrated was undoubtedly Lansing induced. Therefore, these human cases were either Lansing infections or dual infections with a non-Lansing virus.

In the 85 human cases tested (Fig. 1) a CF antibody pattern was not determined, but one may note a correlation between CF and neutralizing antibody. The former was positive only among those serums having the latter antibody. Serums, negative for MEF_1 virus neutralizing effects, did not contain CF antibodies. The neutralizing antibody response of infected humans was rapid(9). In immunized cotton rats, both neutralizing and CF antibodies appeared almost simultaneously (2). If we indulge in an extrapolation of the latter data, a positive CF and negative neutralization test might be an unexpected finding in human serum.

It is not felt that the CF technic described above would be of great value in determining the past history of infection in a population survey; however, one might speculate as to its use in evaluating either prophylactic or therapeutic trials of vaccines or drugs, in identifying Lansing strains of virus, and possibly in establishing a clinical diagnosis of human Lansing infection. Further studies should delineate more accurately the CF pattern of response to infection. The CF antigens used above were prototype specific, and for such a test to be of added value to the investigator the Brunhilde and Leon prototype CF antigens must be developed further. Thus far, they are difficult to prepare routinely, probably due to the low titer of virus in the infected tissue from which such CF antigens have been processed.

Summary. The Lansing complement-fixing (CF) antibody response to immunization in monkeys and to infection in humans appears to be transitory. One hundred thirty-one paralytic poliomyelitis cases in humans were examined for CF antibody to Lansing virus. In the serums of 85 cases, collected 2 weeks after onset of clinical disease, 11 had both neutralizing and CF antibodies. CF antibody was not found in any of 47 cases which were negative for neutralizing antibody. In a series of serum specimens collected from 46 other cases, CF antibody appeared in 10. The CF antibody rose quickly and remained elevated for from 6 weeks to 3 months. A positive CF test may indicate recent infection; a negative, either no exposure or infection at a period remote in the past.

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