COMPLEMENT-FIXING ANTIBODIES TO TYPE 2 (LANSING) POLIOMYELITIS VIRUS IN A NORMAL POPULATION OF A SUBTROPICAL AREA*

By NATAN GOLDBLUM,‡ Ph.D., and JOSEPH L. MELNICK, Ph.D.

(From the Section of Preventive Medicine, Yale University, School of Medicine, New Haven)

(Received for publication, April 26, 1952)

Following the adaptation of the MEF1 strain of poliomyelitis virus to infant mice, Casals, Olitsky, and Anslow (1) were able to prepare from the central nervous system of these mice an antigen for the detection of complement-fixing (c-f) antibodies to Type 2 poliomyelitis virus (2). Thus, another parameter different from the neutralizing antibody test, has been added for measuring poliomyelitis infection in man. It was felt that in addition to being another measure of "immunity," c-f antibodies might perhaps prove to differ from neutralizing antibodies in their persistence in man. And, if this were true, determinations of both antibodies might indicate more precisely the time of exposure to poliomyelitis virus.

This paper deals with: (*a*) The age distribution of c-f antibodies to Type 2 poliomyelitis virus in a normal population of a subtropical area. (*b*) The correlation, if any, between c-f and neutralizing antibodies to the same immunological type of virus. (*c*) The fate of c-f antibodies in the same individuals in the course of an 18 month period.

In order to investigate these problems, sera previously collected in 1950 in the vicinity of Cairo, Egypt, have been studied.[1] Although poliomyelitis epedemics have not been reported in the native population in Cairo, the disease is endemic and Paul *et al.* (3, 4) have commented in two previous reports on the large number of infantile cases seen at one local children's hospital. In the population of this area, neutralizing antibodies to all three immunological types of poliomyelitis virus were acquired early in life indicating that virtually all children above the age of 3 had been exposed (4). The almost universal presence of antibodies in those older than 3 years partially explains the infantile nature of the disease in Cairo. Because virtually all the population

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

‡ Fellow of the Israeli Government.

[1] We are indebted to Dr. John R. Paul for making these valuable sera available to us. The circumstances under which they were collected have been reported previously (4), together with acknowledgments to the various people and organizations who assisted in making possible their collection.

was exposed here early in life, it was felt that a study of c-f antibodies in Cairo could be interpreted more easily than in an area in this country where the acquisition of antibodies is delayed over a longer period in life (5, 6).

In addition to the sera collected by Dr. Paul, a second bleeding was obtained from 16 children, 18 months after they were first bled in 1950. The latter sera were kindly furnished us by Dr. Richard M. Taylor of The Rockefeller Foundation.[2]

Materials and Methods

The MEF1 "infant mouse–adapted" strain of poliomyelitis virus was used as a source of antigen. This strain was kindly supplied by Dr. Casals and Dr. Olitsky in the 48th and 79th mouse passage. Brains of infected mice in the 80 to 85th passages were mainly used as these materials gave higher infectivity titers and yielded more potent antigens than the earlier ones. Such brains yielded ID_{50} titers in infant mice, following intracerebral inoculation, between $10^{-3.6}$ and $10^{-4.7}$, and 10^{-5} in adult mice. Antigens were prepared by intracerebral inoculation of mice less than 2 days old. When inoculated with a 20 per cent suspension of infected infant mouse brains, the mice came down with limb paralysis, on the average as follows: 23 per cent on the 2nd day after inoculation, 57 per cent on the 3rd day, and the remaining 20 per cent on the 4th day. After testing antigens prepared from infant mouse brains harvested on the 2nd and 3rd day respectively, we found no significant difference in the titers of the antigens whether paralyzed or non-paralyzed mice were used for harvest. Consequently we made it a practice to harvest all mice approximately 60 hours after inoculation. For the preparation of antigens we used brains exclusively. Casals, Olitsky, and Anslow (2) found that spinal cords of infected mice yielded slightly more potent antigens than brains but the difference was not great. As it is so much easier and more convenient to use only brains, we adopted it as a method of choice. Specific reactions were obtained using acetone-ether extraction of the brains as described by Casals (7). As about 1 to 2 per cent of human sera reacted with the antigen from normal infant mouse brains prepared by the latter method, normal infant mouse brain antigens had to be included in each test, or, sera which gave fixation of complement in the presence of the MEF1 antigen had to be retested with the normal antigen. Sera were stored frozen from the time of collection; they were thawed occasionally for antibody tests. Before use, all sera were inactivated at 60° C. for 20 minutes.

Every batch of antigen was tested for antigenic potency as well as for anticomplementary activity. Usually the latter activity was low, while the antigenic potency was high; when a "box titration" against homologous monkey antiserum was carried out, the antigen diluted to 1:8 still reacted with the antiserum at a dilution of 1:256. 4 antigenic units were used for screening human sera, 1 antigenic unit being defined as the highest dilution of antigen (contained in 0.1 ml.) that reacted to a high titer (at least 1:32) with homologous monkey antiserum.

The type of complement fixation used was a modified Kolmer test based on 100 per cent hemolysis. Serum, antigen, and complement (2 units) were added in 0.1 ml. increments each and after overnight incubation at 4° C., 0.2 ml. of a sensitized suspension of sheep red blood cells was added, the mixture kept for 30 minutes at 37° C. and read.

In the neutralization tests, the Lansing strain of poliomyelitis virus was used; the details of the qualitative tests have been described in a previous paper from this laboratory (4).

---

[2] We are also indebted to Capt. James J. Sapero (MC), U.S.N., Commanding Officer of Naval Medical Research Unit (NAMRU) No. 3, for providing certain laboratory facilities and for transporting the sera to this country.

For the quantitative tests carried out on a selected number of sera, a fixed dose of 100 ID_{50} (final dilution) of virus was used against fivefold serial dilutions of serum, ranging from 1:2 to 1:250. The titer of neutralizing antibody was determined using the 50 per cent end-point calculated by the Reed-Muench method.

RESULTS

In order to find the range of reactivity of human sera with the MEF1 antigen, preliminary titrations were run, and soon it became clear that the reactivity ranged from 1:4 to 1:16 serum dilutions; sera positive at 1:2 and negative at 1:4 were an unusual finding and so were sera with titers of 1:32 or higher. We tested, therefore, human sera by screening at 1:4 and titrating

TABLE I

Neutralization and Complement Fixation Tests in Sera Collected in Cairo, 1950

Age	Lansing neutralization		MEF1 complement fixation		
	No. tested	Positive	No. tested	Positive at 1:4	Positive at 1:16
mos.		*per cent*		*per cent*	*per cent*
0–3	10	80	3	0	0
4–6	11	55	8	0	0
7–12	15	7	9	0	0
yrs.					
1	30	57	22	45	27
2	34	79	27	41	30
3–4	35	88	31	51	26
5–9	37	89	21	43	5
10–14	27	93	21	10	5
15–29	22	95	19	15	5
30+	27	96	10	0	0
Total.....	248		171		

all the positive reacting sera at 1:16 (as well as testing them with normal infant mouse brain antigen).

The number of sera tested in each age group is shown in Table I. A total of 171 Cairo sera were included in the complement fixation tests and the results obtained are represented in Fig. 1 (together with the neutralization tests results obtained in an earlier study from this laboratory (4)). It will be noted that c-f antibodies are completely absent in children below the age of 1 year. The absence of c-f antibodies in this age group parallels their scarcity in the higher ages, from 10 years on, and may be explained by the fact that the mothers are devoid of these antibodies and therefore cannot transfer them to their offspring. The rise of c-f antibodies starts steeply at the age of 1 year, after which a sort of plateau, at the level of 50 per cent, is formed until the

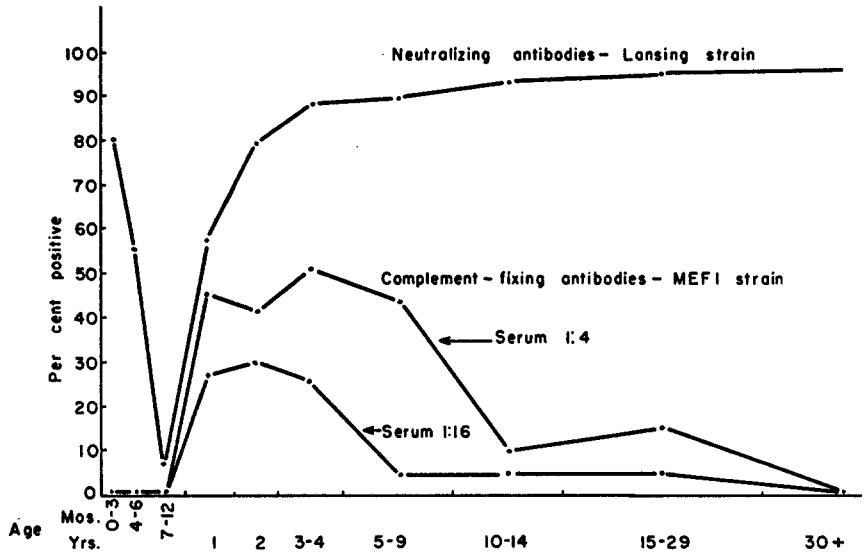

FIG. 1. Neutralizing and complement-fixing antibodies to Type 2 poliomyelitis virus in the native population of Cairo.

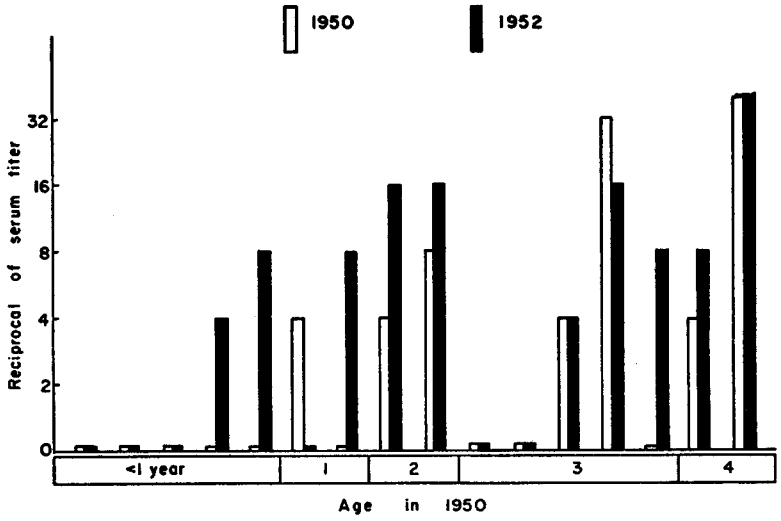

FIG. 2. MEF1 complement fixation titers of sera from 16 Cairo children bled in 1950 and again in 1952.

age of 5 to 9 years. After that age there is a steep fall in the antibody level, nearly to 0, the older age groups being practically devoid of c-f antibodies. The curve at serum dilution of 1:16 gives additional information by showing

that the percentages of positives are lower than that obtained with a serum dilution of 1:4 and that at the age of 5 to 9 years the percentage of reactors dropped very significantly, almost to 0.

TABLE II

MEF1 Complement Fixation Results with Sera from 16 Children Bled in 1950 and Again in 1952

	1950		1952	
	+	−	+	−
No. of children	7		6	1
No. of children		9	4	5

TABLE III

Neutralization and Complement Fixation Tests with Sera from 16 Cairo Children Bled in 1950 and Again in 1952

No. of serum	Age in 1950	MEF1 complement fixation serum titer		Lansing neutralization serum titer*	
		1950	1952	1950	1952
	mos.				
288	7	0	0	0	25
291	7	0	0	0	50
270	7	0	8	0	>250
285	8	0	4	0	>250
286	11	0	0	0	150
253	13	4	0	0	5
219	18	0	8	0	>250
268	25	8	16	150	>250
277	33	4	16	>250	100
234	36	0	0	30	10
272	36	0	0	0	150
218	36	4	4	250	150
228	36	32	16	250	>250
238	36	0	8	30	>250
201	48	4	8	<5	100
222	48	>32	>32	>250	>250

* 100 ID_{50} of virus used in each test. Sera were used at final dilutions of 1:2, 1:10, 1:50, 1:250.

0 indicates no neutralization at 1:2. The other titers are given as 50 per cent protection end-point.

Previous results show that Type 2 neutralizing antibodies, also represented in Fig. 1 and Table I, follow quite a different course (4). Following the loss of the parental antibody, there was a very sharp rise, paralleling at this stage the c-f antibodies, and reaching an 80 per cent level at the age of 2 years, and this high level was retained at 88 to 96 per cent throughout life.

Repeat Studies on Certain Children First Bled in 1950.—In order to follow the fate of c-f antibodies in the very same individuals, 16 of the children, from 7 months to 4 years old in July, 1950, were bled again by Dr. R. M. Taylor in 1952, 18 months later. Sera of the 1950 and 1952 bleedings were titrated

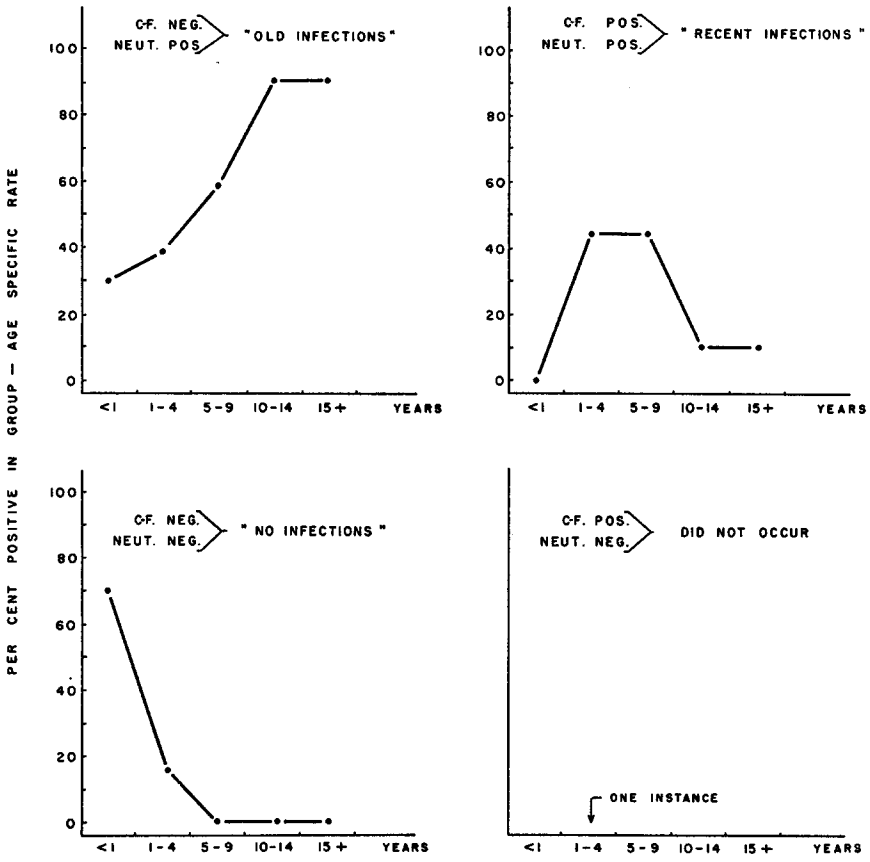

FIG. 3. Combined neutralization and complement fixation results using in the c-f tests serum diluted 1:4.

simultaneously in twofold dilutions, from 1:2 to 1:32. The findings are shown in Fig. 2; each pair of columns represents the titer of both sera of 1 child in 1950 and 1952, respectively. Though the number of sera is small, the results show that c-f antibodies are not at all in a static state. Some children who had no c-f antibodies in 1950 developed them by 1952; in others rises and falls in titer occurred. The over-all changes have been summarized in Table II: of 7 children who were positive in 1950, 6 remained positive in 1952 and 1 became

negative; of 9 negative in 1950, 5 remained negative and 4 developed c-f antibodies.

Titrations of neutralizing antibodies using a fixed amount of Lansing virus were also carried out on the same pairs of sera, and the results are presented

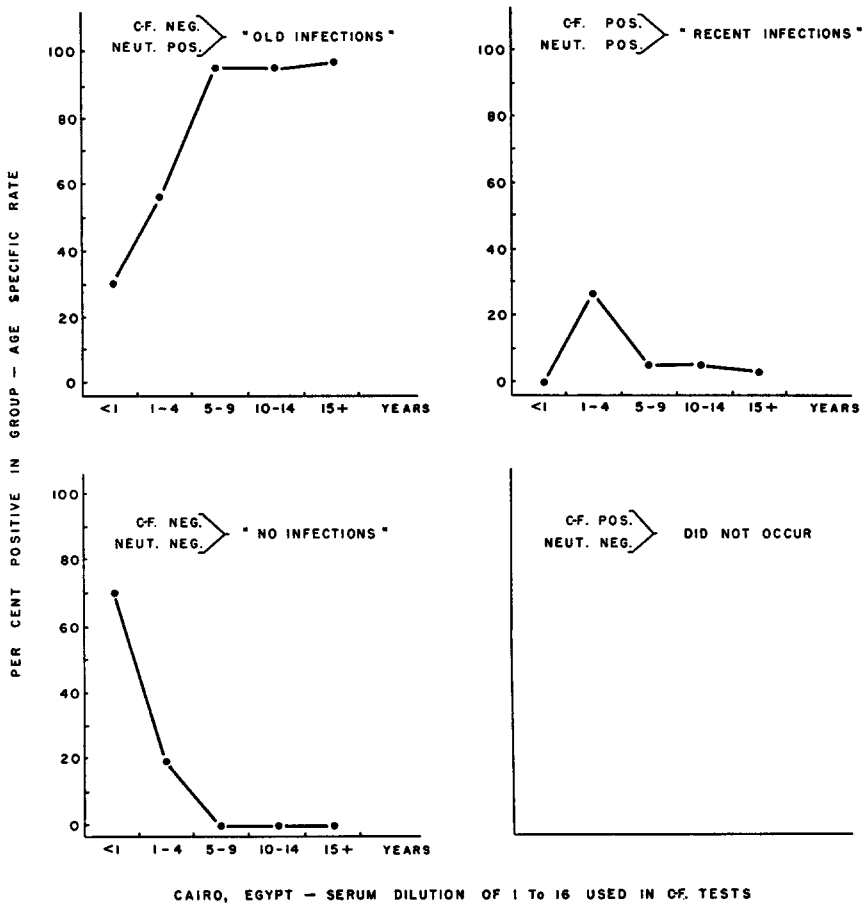

FIG. 4. Combined neutralization and complement fixation results, using in the c-f tests serum diluted 1.16.

in Table III, together with the results of the c-f tests. In general there is a correlation between the two antibodies if one considers only those sera with a high titer of neutralizing antibodies. All children who were negative in 1950 and who developed c-f antibodies by 1952, also developed neutralizing antibodies to a titer of more than 1:250 during the same period (sera 219, 238, 270, 285).

It may also be seen that some children (sera 288, 291, 286, 272) who failed

to develop c-f antibodies, did develop neutralizing antibodies although of lower titer than found in children in whom c-f antibodies made their appearance. There seem to be two possible explanations for the latter finding: (*a*) c-f antibodies may have formed during the 18 month period and then disappeared by the time of the second bleeding, or (*b*) the infection with poliomyelitis virus had been so minimal that c-f antibodies had not yet reached a detectable level at the time of the second bleeding.

TABLE IV

Calculation of Age-Specific Rates for Each Antibody Pattern

Antibody pattern	Serum dilution 1 to 4 in C-f tests											Serum dilution 1 to 16 in C-f test										
	Observed Nos. in each antibody pattern						Age-specific rate					Observed Nos. in each antibody pattern						Age-specific rate				
	Age in years					Total	Age in years					Age in years					Total	Age in years				
	<1	1–4	5–9	10–14	15+		<1	1–4	5–9	10–14	15+	<1	1–4	5–9	10–14	15+		<1	1–4	5–9	10–14	15+
C-f neg., neut. pos.	6	31	12	19	26	94	30	39	57	90	90	6	45	20	20	28	119	30	56	95	95	97
C-f pos., neut. pos.	0	35	9	2	3	49	0	44	43	10	10	0	21	1	1	1	24	0	26	5	5	3
C-f neg., neut. neg.	14	13	0	0	0	27	70	16	0	0	0	14	14	0	0	0	28	70	18	0	0	0
C-f pos. neut. neg.	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total.....	20	80	21	21	29	171	100	100	100	100	100	20	80	21	21	29	171	100	100	100	100	100

"Old" and "Recent" Infections.—An attempt has been made to classify the population sampled into three groups:—those who had "old" or "recent" infections, and those who had not been infected with the virus. This was based on the assumption that both complement-fixing and neutralizing antibodies are formed as a result of infection with Type 2 poliomyelitis virus, but that they run a different course with time, the neutralizing antibodies being maintained while the c-f antibodies gradually disappear. Thus, a person with both antibodies is regarded as having had a relatively recent infection, a person with only neutralizing antibodies as having had an old infection, and one with no antibodies of either kind, as not having been infected at all. Figs. 3 and 4 are graphic representations of the findings. The numbers plotted were obtained by calculating the age-specific rates in each antibody pattern,

as shown in Table IV. It will be noticed that: (*a*) the percentage of old infections tends to become greater with advancing age; (*b*) recent infections are predominantly contracted at the ages of 1 to 9; and "very recent" ones (serum dilution 1:16) at 1 to 4 years of age; (*c*) lack of infection is confined mainly to infants under 1 year; (*d*) persons with complement-fixing antibodies and no neutralizing antibodies are extremely rare (only one being found in this series).

DISCUSSION

It is too early to draw far reaching conclusions of epidemiological nature from the data reported above. But it is our belief that these findings throw some light on problems of infection and immunity in poliomyelitis. Whatever bearing c-f antibodies may have on immunity to poliomyelitis, there seems little doubt that they represent a reaction to an infection with poliomyelitis virus. Recent observations of Casals (8) and Svedmyr, Enders, and Holloway (9, 10) indicate that following infection with one type of poliomyelitis virus, transient c-f antibodies to at least two types may appear, while the longer lasting neutralizing response appears to be restricted to the virus type causing the infection. On the other hand, immune animal sera exhibit type-specificity in both neutralization and complement fixation reactions.[3] Similar findings have recently been made in human infections with the Coxsackie viruses (12). Whether or not the c-f response in the Cairo population is type-specific seems to be of little importance as far as the temporal pattern of Type 2 c-f antibodies is concerned. The reason for this is that infection with all three known types of poliomyelitis virus occurs early in life (4). In any case the c-f reaction is certainly different from the one measured by the neutralization test; for the c-f antibodies are confined to the years of 1 to 9 if a serum dilution of 1:4 is used, or to the years 1 to 4 if a serum dilution of 1:16 is used.

We know from morbidity and immunity data (4) that in Cairo the exposure to all three immunological types of poliomyelitis virus is confined to very early childhood, virtually all children having been exposed by the age of 3 years. This brings us close to the problem of whether single or multiple exposures are necessary for the maintenance of immunity to poliomyelitis, as ex-

[3] Lahelle (11), using an antigen prepared from brains of infant cotton rats infected with the Lansing strain, compared the titers of neutralizing and c-f antibodies in the sera of monkeys which received a course of intramuscular vaccinations with active Lansing virus. He found a close correlation in the rise of the two antibodies, but the c-f antibodies decreased earlier than the neutralizing ones. The c-f titers after increasing to a high level shortly after vaccination (1 to 4 weeks) decreased almost to 0 in a period of 3 months, the neutralizing antibodies remained at a relatively high level during this time. Lahelle also observed a correlation between the two kinds of antibodies in the few adult human sera that he tested; on the other hand, five sera from children at the age of 1 had no detectable c-f antibodies though all exhibited a slight neutralizing effect.

pressed by the presence of neutralizing antibodies. If we assume that reinfection after some years would be evidenced by a reappearance (or maintenance at detectable levels) of c-f antibodies, then the data presented are in favor of single rather than multiple stimuli. The "single" stimulus may not be confined to one contact with virus, but may represent several contacts over a relatively short period of time.

In this regard the children who were bled in 1950 and again in 1952 are of interest. Children who developed high titers of neutralizing antibodies in this period, also developed c-f antibodies, and may represent those individuals exposed to repeated and perhaps large doses of virus. On the other hand, children who developed low titers of neutralizing antibodies and no detectable c-f antibodies may represent those exposed to a single stimulus. Possibly such children on repeated exposure may show a rise in neutralizing antibody titers together with the appearance of c-f antibodies.

The results of the present study are in accord with the observations of Paul and Riordan (13) that a single stimulus (or multiple stimuli during a short period of time) is sufficient for the production of neutralizing antibodies and their maintenance over long periods of time. Experimental data on the oral infection of chimpanzees with poliomyelitis virus also show that neutralizing antibodies are maintained for long periods—at least 6 years (14).

However, it is clear that one cannot generalize from the Cairo results, for in a survey of c-f antibodies to Type 2 poliomyelitis virus in this country (Charleston, West Virginia, 1951) it was found (15) that the c-f curve displays a different course—pointing to different mechanisms of exposure from those in Cairo. The c-f antibody curve of Charleston rises at the age of 1 to 4 years to about 30 per cent (serum dilution of 1:4) and it remains close to this level through all subsequent ages; thus, unlike the population in Cairo, the period of exposure and infection with Type 2 (Lansing) poliomyelitis virus does not seem to be confined to early childhood alone, but appears to occur frequently in adults as well. This divergence in findings might be explained by the fact that children in Cairo, being exposed early in life, to repeated and heavy doses of virus, build up their antibodies rather quickly, while in Charleston the process of antibody production to poliomyelitis virus is a slow one lasting for many years. This last assumption has of course yet to be proven. Evidence for widespread prevalence of poliomyelitis virus in Cairo has recently been obtained by Ward who found that poliomyelitis virus could be readily detected in flies trapped in this area during a normal endemic period (16).

SUMMARY

Sera collected in 1950 from the native population in the vicinity of Cairo, Egypt, have been tested for complement-fixing antibodies to Type 2 (Lansing) poliomyelitis virus.

Complement-fixing antibodies are confined to the age of 1 to 9 years if a serum dilution of 1:4 is used in the test, or to the age of 1 to 4 years with a serum dilution of 1:16.

A comparison has been made of the findings obtained in this study with the results for neutralizing antibodies previously reported. Complement-fixing antibodies were found to be temporary in nature while neutralizing antibodies were maintained for long periods of time. On this basis, criteria for "recent," "old," or "no infection" in poliomyelitis have been established.

Sixteen of the children were bled again in 1952, 18 months after the first bleeding and both series of sera were compared in complement fixation and neutralization tests. All children who were negative in 1950 and who developed c-f antibodies by 1952, also developed neutralizing antibodies to a titer of more than 1:250 during the same period. Some children who failed to develop c-f antibodies, did develop neutralizing antibodies although of lower titer than found in children in whom c-f antibodies made their appearance.

These findings are discussed in the light of theories regarding the mode of acquisition of antibodies to poliomyelitis virus.

BIBLIOGRAPHY

1. Casals, J., Olitsky, P. K., and Anslow, R. O., *J. Exp. Med.*, 1951, **94,** 111.
2. Casals, J., Olitsky, P. K., and Anslow, R. O., *J. Exp. Med.*, 1951, **94,** 123.
3. Paul, J. R., Havens, W. P., and van Rooyen, C. E., *Brit. Med. J.*, 1944, **1,** 841.
4. Paul, J. R., Melnick, J. L., Barnett, V. H., and Goldblum, N., *Am. J. Hyg.*, 1952, **55,** 402.
5. Turner, T. B., Hollander, D. H., Buckley, S., Kokko, U. P., and Winsor, C. P., *Am. J. Hyg.*, 1950, **52,** 323.
6. Melnick, J. L., and Ledinko, N., *Am. J. Hyg.*, 1951, **54,** 354.
7. Casals, J., *Proc. Soc. Exp. Biol. and Med.*, 1949, **70,** 339.
8. Casals, J., Second International Poliomyelitis Conference, Copenhagen, 1951, data to be published.
9. Svedmyr, A., Enders, J. F., and Holloway, A., *Proc. Soc. Exp. Biol. and Med.*, 1952, **79,** 296.
10. Svedmyr, A., Enders, J. F., and Holloway, A., *Fed. Proc.*, 1952, **11,** 483.
11. Lahelle, O., *Am. J. Hyg.*, 1951, **54,** 391.
12. Kraft, L. M., and Melnick, J. L., *J. Immunol.*, 1952, **68,** 297.
13. Paul, J. R., and Riordan, J. T., *Am. J. Hyg.*, 1950, **52,** 202.
14. Melnick, J. L., Second International Poliomyelitis Conference, Copenhagen, 1951.
15. Yale Poliomyelitis Laboratory and Communicable Disease Center, U.S.P.H.S., unpublished data.
16. Ward, R., *Fed. Proc.*, 1952, **11,** 486.