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IMMUNOLOGICAL RESPONSE TO TRIVALENT ORAL POLIOMYELITIS VACCINE

BY

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A survey of the literature to date on feeding living attenuated polioviruses for the immunization of man reveals that relatively little has been reported on simultaneous feeding of the three types of virus or the use of mixtures of them as a trivalent vaccine. This is apparently due to the fact that certain strains of poliovirus were found to interfere with the establishment and multiplication of other type strains in the human gut. Thus, Koprowski (1955) reported that type I, SM virus, interfered with the immunizing effect of type II, TN strain, when the two were fed simultaneously. In a subsequent study Koprowski *et al.* (1956) attempted to overcome this interference by feeding much larger doses of type II virus in a mixture with type I. Again interference was found to occur between the two types, though, under the conditions of the latter study, type I strain was not always dominant.

In 1955 Sabin stated that simultaneous feeding of approximately 10 million tissue-culture 50%-infective doses (TCD_{50}) of attenuated polioviruses of all three types to chimpanzees completely suppressed the multiplication and immunizing effect of only type III virus. In 1956 Sabin reported that when he fed approximately one million TCD_{50} of the naturally occurring attenuated strains (P 2149, P 712, and Glenn) simultaneously, he found no significant interference in four volunteers fed a mixture of types I and II, nor in three volunteers fed a mixture of types I and III, nor in three persons fed types II and III, nor in one person fed all three types together. He did note that, in some of the individuals fed, the appearance of antibodies was delayed and titres were in the lower range. In later studies carried out with his "optimum single plaque" strains, Sabin (1957) reported that the type II strain was the dominant one and that quite often, though not always, it interfered with the multiplication of the other two types when fed as a mixture. This finding prompted him to state that "immunization against all three types of poliovirus by a single administration of a mixture of them is not feasible" (Sabin, 1958). He therefore recommended that his three "optimal" strains be fed separately at intervals of three weeks in the order of types I, III, and II.

In contrast to some of the above findings Smorodintsev *et al.* (1959), working with some of Sabin's earlier strain variants (type I, LS of 22/12/55; type II, P 712-10ab of 5/9/56; and type III, Leon 14ab of 5/4/56), have reported a single experiment in which a trivalent vaccine containing one million TCD_{50} (a mixture of equal volumes of monovalent strains) was fed to eight staff members of the Institute's Virology Department* and to 29 children, 7 to 15 years old. No comments are made about the serological results obtained in the eight staff members, but the children with low or medium antibody levels before immunization showed "an increase in antibodies to all three types of virus."

Our interest in the possibility of using a trivalent vaccine for simultaneous immunization of humans against all three types of poliomyelitis by a single feeding was renewed about a year ago, when one of us (M. R.-G.) found that it was possible to maintain a mixed infection of types II and III polioviruses for at least 20 serial passages in monkey-kidney-tissue culture. Both types of virus were present in the stool specimen of a Minnesota child who had been fed the Lederle strains of attenuated polioviruses (Roca-Garcia, unpublished observations). This observation suggested that it might be possible to immunize against the three types of poliomyelitis by feeding the Lederle strains either in the form of capsules taken simultaneously or as a mixture in a stabilized fluid preparation, and a number of studies were initiated. Other studies in this connexion will be reported independently.

This paper presents results obtained to date in feeding different dosages of a liquid, trivalent, oral poliomyelitis vaccine to approximately 550 persons—principally employees of Lederle Laboratories, Pearl River, New York, who voluntarily requested the vaccination, and, in a few cases, members of their immediate families. All these individuals live in a semi-urban area located within approximately a 15-mile radius of Pearl River, New York, chiefly Bergen County (northern New Jersey) and Rockland County (southern New York). The study was well controlled, since all persons involved

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had ready access to the Medical Department of Lederle Laboratories, so that a good history and follow-up was obtainable in each case. The data presented here consist of the results of a serological study carried out on 241 persons out of the 550 fed; the serological studies of the remaining 309 vaccinated individuals are not yet completed, and will be reported at a later date.

Clinical Observations

Even though a very careful follow-up was made, only 3 of the 550 persons who were fed reported any clinical signs of illness during the observation period of more than two months. At the time of the second bleeding one individual reported that on the seventh or eighth day after ingestion of the vaccine she had a rather severe headache, associated with stiffness of the neck, lasting for approximately 24 hours. During this time she felt flushed, but remained afebrile and had no sore throat. This condition cleared up spontaneously, rather abruptly, and no further sequelae have been reported. Serological testing revealed this person to have been a triple negative before vaccination and to have responded to types I and III polioviruses.

The second and third patients experienced diarrhoea which lasted for a day or two during the third and fourth week, respectively, following ingestion of the oral vaccine. Since a diarrhoeal condition was being reported frequently in unvaccinated subjects in the community, it is believed that these latter cases should be completely dismissed from association with the vaccine.

Laboratory Data

Materials and Methods

Strains of Virus.—The viruses used in this study were the Lederle strains SM (type I), MEF_1 (type II), and Fox (type III) isolate #P 1149. These are the same strains which were used previously in studies carried out in Minnesota, Colombia, Uruguay, Cuba, and Finland. The properties and behaviour of these strains are now being reported separately by Cabasso *et al.* (1959).

Neutralization Tests.—Neutralization tests were carried out on the pre- and post-vaccination sera simultaneously, using the *p*H or colour method according to the procedure of Salk, *et al.* (1954). All sera were first inactivated for 30 minutes at 56° C. in a constant-temperature water-bath. The serum samples were then prepared in fourfold dilutions in duplicate, using 0.25 ml. per dilution. Approximately 100 to 300 TCD_{50} of the representative strains of virus per 0.25 ml. were added to the respective serum dilutions, and the mixtures were held at room temperature for three hours. Trypsinized monkey-kidney-tissue cell suspensions containing approximately 600,000 cells per 0.25 ml. were then added to each of the serum–virus mixtures, along with appropriate controls, and the tubes then were held at 37° C. until the sixth or seventh day, when they were read. Antibody titres were calculated by the method of Reed and Muench (1938).

Results

Results of the first experiment, using a liquid trivalent vaccine, carried out on two individuals in June, 1958, are shown in Table I (subjects 1 and 2). Subject 1 was a triple negative (no antibodies against any of the three types of poliovirus). Subject 2 showed no antibodies against two types of poliovirus (I and II). Titration of the post-vaccination sera, obtained approximately three months later, indicated that both individuals responded well to the vaccine, as shown by the antibody levels attained against all three types of poliovirus. Further, it is seen from the results obtained on the blood sample taken in March, 1959, from Subject 2 that these antibodies persisted for at least nine months. Each of these individuals was fed liquid vaccine consisting of equal volumes of each of the three types of poliovirus, and the dosage was approximately $10^{5.5}$ (300,000) TCD_{50} of each type of virus.

TABLE I.—*Antibody Response of 10 Persons Fed 0.5 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{5.5}$ TCD_{50} Each Type Virus Per Dose)*

Subject	Age in Years	Vaccine Fed	Bleeding-Date	Antibody Titres		
				Type I	Type II	Type III
1	24	*	12/6/58	<1:4	<1:4	<1:4
			13/9/58	1:512	1:256	1:64
2	36	*	12/6/58	<1:4	1:512	<1:4
			12/9/58	1:256	>1:1024	1:32
			4/3/59	1:512	>1:1024	>1:1024
3	12	7-1238-801†	28/11/58	<1:4	<1:4	1:32
			3/2/59	1:128	1:8	1:512
4	18	,,	28/11/58	1:32	1:128	1:8
			3/2/59	1:512	1:512	1:128
5	29	,,	28/11/58	<1:4	<1:4	1:512
			29/1/59	1:32	1:128	>1:1024
6	29	,,	28/11/58	1:512	<1:4	<1:4
			21/1/59	>1:1024	<1:4	1:128
7	29	,,	28/11/58	<1:4	1:8	<1:4
			21/1/59	1:128	1:32	1:8
8	5	,,	13/1/59	<1:4	1:128	1:8
			15/2/59	1:32	>1:1024	>1:1024
9	10	,,	13/1/59	<1:4	1:512	1:8
			15/2/59	1:128	>1:1024	1:512
10	13	,,	13/1/59	1:512	1:512	1:32
			15/2/59	1:512	1:512	1:512

* A mixture of equal volumes of type I (7-1231-121), type II (7-1232-216), and type III (7-1233-318).
† A mixture of equal volumes of type I (7-1231-115), type II (7-1232-217), and type III (7-1233-319).

TABLE II.—*Pre- and Post-vaccination Status of 8 Antibody Negatives Among 10 Persons Fed 0.5 ml. Trivalent Oral Poliomyelitis Vaccine ($10^{5.5}$ TCD_{50} Each Type Virus Per Dose)*

Status of Subjects	Negative for Types	Age in Years	Post-vaccination Results Negative for Types
Triple negative 1 ..	I II III	24	0
Double negative 5			
2	I II	12, 29	0
1	II III	29	II
2	I III	29, 36	0
Single negative 2 ..	I	5, 10	0
Total : 8 ..	Sum total all negatives: pre- 15; post- 1		

Eight additional persons were fed a second preparation of liquid trivalent vaccine bearing the Lot No. 7-1238-801 and containing approximately $10^{5.5}$ (300,000) TCD_{50} of each type virus per 0.5 ml. This vaccine has been used for all the additional trials reported in this paper. From data presented in Tables I and II it is seen that a good antibody response was induced by the 0.5-ml. dose of vaccine in all but one of the individuals. These include one person who was a triple negative, two who were double negatives for types I and II, one who was a double negative for types II and III, two who were double negatives for types I and III, and two additional persons who were negative for type I.

After feeding the trivalent vaccine, only one person was left without demonstrable antibodies to any one of the three types of poliovirus (see Table II). Prior to vaccination this individual had been a double negative for types II and III polioviruses. He apparently failed

to respond to the type II component of the vaccine. No persons were left as double or triple negatives after being fed the trivalent preparation.

Table III shows the results obtained in a single individual, 38 years old, who first was fed capsules containing approximately 10^5 (100,000) TCD_{50} of each of the three types of poliovirus at three-week intervals. This man, who had no antibodies against types II and III polioviruses, failed to get an immune response following this feeding procedure. Subsequently, in November, 1958, he was fed a 0.5-ml. dose of liquid trivalent vaccine, and again he failed to show a demonstrable serological response. Finally, in January, 1959, he was fed a 2-ml. dose of the same liquid vaccine which contained approximately $10^{6.1}$ (1,200,000) TCD_{50} of each of the three poliovirus strains. Titration of the serum sample taken on February 11, 1959, showed a good serological response for all three types of polioviruses; antibody titres were 1:1024 for type I; 1:512 for type II; and 1:32 for type III.

TABLE III.—*Antibody Pattern of an Adult After the Feeding of Different Dosages of Oral Poliomyelitis Vaccine (Dr. A. T., 38 years)*

Vaccine	Material Fed	Virus Titre of Vaccine	Pre- or Post-feeding	Bleeding-date	Antibody Titre†		
					Type I	Type II	Type III
Each of the 3 polio types at 3-week intervals	Capsules	10^5 TCD_{50} per capsule	Pre-	9/9/58	32	<4	<4
			Post-	20/10/58	64	<4	<4
0·5 ml. trivalent ǂ7–1238–801*	Liquid	$10^{5.5}$ TCD_{50} each virus type	Pre-	19/11/58	128	<4	<4
			Post-	19/12/58	128	<4	<4
2 ml. trivalent ǂ7–1238–801	,,	$10^{6.1}$ TCD_{50} each virus type	Pre-	5/1/59	128	<4	<4
			Post-	11/2/59	1024	512	32

* ǂ7–1238–801 = equal volumes of type I (ǂ7–1231–115), type II (ǂ7–1232–217), and type III (ǂ7–1233–319).
† Reciprocal of the serum dilution.

Following these encouraging results, a comparative study was set up in which one group of patients received a 1-ml. dose of trivalent vaccine representing $10^{5.8}$ (600,000) TCD_{50} of each strain of poliovirus while a second group received a 2-ml. dose representing $10^{6.1}$ (1,200,000) TCD_{50} of each type. Table IV, Charts 1, 2, and 3, and Fig. 1 summarize the data which were obtained on the 42 subjects who received the 1-ml. dose of trivalent vaccine, Lot No. 7-1238-801. The volunteers ranged in age from 8 to 65 years, with the majority between the ages of 11 and 50. Among this group were one triple negative, two double negatives for types I and II, one double negative for types II and III, three double negatives for types I and III, a single negative for type I, five single negatives for type II, and three single negatives for type III. Thus there were 16 persons showing a total of 24 "negatives" for all three types of poliovirus.

TABLE IV.—*Pre- and Post-vaccination Status of 16 Antibody Negatives Among 42 Persons Fed 1 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{5.5}$ TCD_{50} Each Type Virus Per Dose)*

Pre-vaccination Antibody Negatives		Post-vaccination Status			
Negative to Type	No. of Subjects	Negative to Type			Converted
		I	II	III	
I, II, III	1			1	
I, II	2				2
I, III	3	1			2
II, III	1				1
I	1	1			
II	5		3		2
III	3			1	2
Total	16	2	3	2	9

Sum totals all negatives, pre- 24; post- 7.

Table IV also gives the post-vaccination antibody pattern of these 42 persons approximately 30 days after ingestion of the 1-ml. dose of trivalent vaccine. Note that no triple or double negatives remained after vaccination and only seven persons were left as single negatives for any of the three types of poliovirus. One of these subjects remained without antibody to type I; he previously had been a double negative for types I and III. A second individual, originally a single negative to type I, was left without antibodies to type I. Three persons, previously negative to type II virus only, were still without antibodies to this type virus. One individual who had been a triple negative was left without antibodies to type III virus only, and one individual who previously was a single negative for type III virus did not respond to the type III component of the triple vaccine. For detailed information concerning individuals who were fed the trivalent vaccine see Charts 1, 2, and 3, which depict the antibody response of these 42 persons to types I, II, and III polioviruses, respectively.

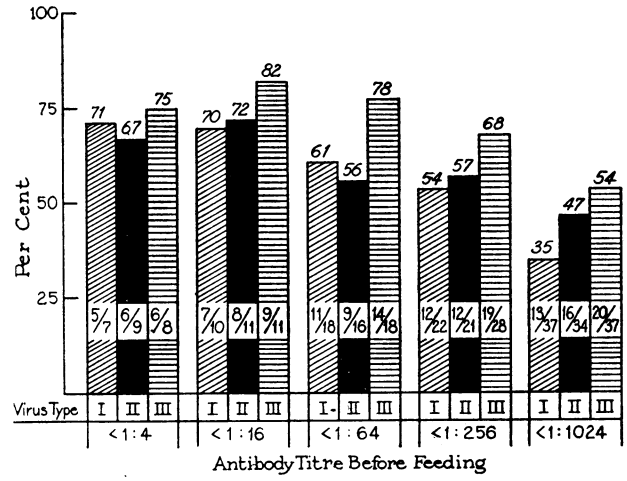

FIG. 1.—Percentage of 42 persons with fourfold or greater antibody rise following feeding of 1 ml. of trivalent oral poliomyelitis vaccine ($10^{5.8}$ TCD_{50} of each type of virus per dose).

Fig. 1 shows the number and percentage of persons who manifested a fourfold or greater antibody rise after the feeding of the 1-ml. dose of trivalent vaccine. While the numbers are small, it is seen from this figure that, of those individuals with pre-feeding antibody titres of <1:4, 71% responded to type I, 67% to type II, and 75% to type III. Of those with antibody titres of <1:16 either a primary or a booster response was shown by 70% to type I, 72% to type II, and 82% to type III. As would be expected, a decreasing proportion of those persons with greater initial antibody titres manifested a response, but even of those persons showing initial antibody titres <1:1024, 35% showed either a primary or a booster effect to type I, 47% to type II, and 54% to type III.

Pre-feeding	Antibody Titres: Post-feeding											Total No. Fed	No. with Fourfold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	2				4		1					7	5
1:4			1									1	0
1:8							1	1				2	2
1:16				1	1						1	3	1
1:32					1	1		1	1	1		5	3
1:64						1						1	0
1:128							2			1		3	1
1:256							1	5	2	1		9	1
1:512								1	4	1		6	0
1:1024									1			1	
>1:1024											4	4	
Totals	2	0	1	1	6	2	5	8	8	4	5	42	13

CHART 1.—Poliovirus Type I Antibody Response of 42 Persons Fed 1 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{5.8}$ TCD_{50} of Each Type per Dose)

Pre-feeding	Antibody Titres: Post-feeding											Total No. Fed	No. with Fourfold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	3	3	1		1				1			9	6
1:4									1			1	1
1:8									1			1	1
1:16				1	1							2	0
1:32				1	1					1		3	1
1:64											1	1	1
1:128							1	1	1		1	4	2
1:256							1	2	1			4	0
1:512								1	3	1	4	9	4
1:1024										1	1	2	
>1:1024									1		5	6	
Totals	3	3	1	2	3	0	2	4	9	3	12	42	16

CHART 2.—Poliovirus Type II Antibody Response of 42 Persons Fed 1 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{5.8}$ TCD_{50} of Each Type per Dose)

Pre-feeding	Antibody Titres: Post-feeding											Total No. Fed	No. with Fourfold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	2	1	2		2				1			8	6
1:4				1								1	1
1:8							1	1				2	2
1:16					2			1			1	4	2
1:32							1		1	1		3	3
1:64								1			1	2	2
1:128							3	2	2		1	8	3
1:256							3	1				4	0
1:512									3	1	1	5	1
1:1024										2		2	
>1:1024										1	2	3	
Totals	2	1	2	1	4	0	8	6	7	5	6	42	20

CHART 3.—Poliovirus Type III Antibody Response of 42 Persons Fed 1 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{5.8}$ TCD_{50} of Each Type per Dose)

The response among these volunteers should be compared with that obtained in 188 volunteers fed the 2-ml. dose of the same vaccine, which represents $10^{6.1}$ (1,200,000) TCD_{50} of each type virus per dose (see Table V and Charts 4, 5, and 6). The age range of the 188 persons was from 4 to 60 years, with the greatest numbers ranging from 21 to 50. In this group there were 11 triple negatives (aged 18 to 44); 18 double negatives to types I and II (17 to 39); 7 double negatives to types II and III (19 to 38); and 10 double negatives to types I and III (23 to 54). There were 14 single negatives to type I poliovirus (20 to 50); 6 single negatives to type II (22 to 39); and 30 single negatives to type III (10 to 58). Thus there were 96 persons showing a total of 153 "negatives" for all three types of poliovirus. Results when the sera of these same individuals were tested about 30 days after taking 2 ml. of the trivalent vaccine are shown in Table V. No triple or double negatives and only 11 single negatives remained. Two individuals were still negative to type I; nine were left negative to type II; and none remained negative to type III.

The pre-vaccination status of those who failed to show an antibody response to the triple vaccine is presented in the left-hand column of Table V. One of the persons left without antibody to type I had been a single negative to type I. The second individual left without antibody to type I had been a double negative to types I and III. One of the subjects who remained negative to type II had been a single negative to type II. Another person left without type II antibody had been a type II and III double negative.

In addition, three persons who were originally type I and II double negatives and four persons who were triple negatives were left without type II antibody. Charts 4, 5, and 6 show the antibody response of the 188 persons fed the 2-ml. dose of the trivalent vaccine to types I, II, and III polioviruses.

A summary of antibody conversion effect as well as booster response of the 188 persons fed 2 ml. of vaccine is shown in Fig. 2. Of those individuals with antibody titres originally <1:4, 96% responded with antibodies to type I, 78% to type II, and 100% to type III. Likewise, of all individuals who showed pre-vaccination antibody titres of <1:16, 90% showed either a primary or booster effect to type I, 83% to type II, and 95% to type III. Corresponding primary or booster effects are shown for those groups of individuals whose initial antibody titres were <1:64, <1:256, or <:1024. It is remarkable that of all persons who showed initial antibody titres <1:1024, 62% showed either a primary or a booster response to type I, 67% to type II, and 71% to type III.

Discussion

From the above results it is apparent that a much better neutralizing antibody response was obtained in individuals fed the 2-ml. dose of trivalent poliovirus vaccine than in those receiving the 1-ml. dose. While the results obtained with the 0.5-ml. dose are good, it must be admitted that the number of people involved is small, It is important that these highly favourable results were obtained primarily in adults. We believe that this should be noted, because the study carried out in Minnesota in 1958 clearly showed that children could be more

Pre-feeding	Antibody Titres: Post-feeding											Total No. Fed	No. with Fourfold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	2	2	9	5	10	5	12	1	7			53	51
1:4							1		1			2	2
1:8			2	3	1		2	2	2			12	7
1:16					1			2		1		4	3
1:32					2	5	6	3	4	1		21	14
1:64						1	5	2	1	1	3	13	7
1:128					1	2	10	9	10	2	2	36	14
1:256								5	5	1	2	13	3
1:512								2	8	3	5	18	5
1:1024									1	7	4	12	
>1:1024											4	4	
Totals	2	2	11	8	15	13	36	26	39	16	20	188	106

CHART 4.—Poliovirus Type I Antibody Response of 188 Persons Fed 2 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{6.1}$ TCD_{50} of Each Type per Dose)

Pre-feeding	Antibody Titres: Post-feeding											Total No. Fed	No. with Fourfold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	9	5	6	4	2	2	7	3	2	1	1	42	33
1:4							1	1	1			3	3
1:8			1		4	1		3	3	1	2	15	14
1:16			1		1		1	1	3		1	8	6
1:32					3	1	3	3	2	2		14	10
1:64						1	4		1	1	3	10	5
1:128						1	11	4	6		7	29	13
1:256								3	3	1	5	12	6
1:512									6	3	14	23	14
1:1024									3	2	10	15	
>1 1024										3	14	17	
Totals	9	5	8	4	10	6	27	18	30	14	57	188	104

CHART 5.—Poliovirus Type II Antibody Response of 188 Persons Fed 2 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{6.1}$ TCD_{50} of Each Type per Dose)

Pre-feeding	Antibody Titres: Post-feeding											Total No. Fed	No. with Fourfold or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4		2	8	13	17	5	6	3	2	1	1	58	58
1:4			1									1	0
1:8			1	2	5		2	1	3			14	11
1:16				1	1	2	2	3	4			13	11
1:32					3	5	5		4	4	3	24	16
1:64					1	2	5	1	3		3	15	7
1:128							6	2	10	1	3	22	14
1:256								3	4		4	11	4
1:512									8	4	5	17	5
1:1024									1	3	2	6	
>1:1024										1	6	7	
Totals	0	2	10	16	27	14	26	13	39	14	27	188	126

CHART 6.—Poliovirus Type III Antibody Response of 188 Persons Fed 2 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{6.1}$ TCD_{50} of Each Type per Dose)

readily immunized with the oral vaccine than could adults (Barr *et al.*, 1959). That is one of the reasons why the decision was made to use adults primarily in carrying out the study with the trivalent vaccine reported here.

It is obvious, of course, that from the standpoint of ease and cost of administration a trivalent oral vaccine has many advantages to offer over the procedure of feeding the three virus strains separately. There is no doubt that a trivalent oral vaccine would be of great advantage to public-health workers, who ordinarily have limited time and funds with which to carry out mass-immunization programmes.

TABLE V.—*Pre- and Post-vaccination Status of 96 Antibody Negatives Among 188 Persons Fed 2 ml. of Trivalent Oral Poliomyelitis Vaccine ($10^{6.1}$ TCD_{50} Each Type Virus Per Dose)*

Pre-vaccination Antibody Negatives		Post-vaccination Status			
Negative to Type	No. of Subjects	Negative to Type			Converted
		I	II	III	
I, II, III	11		4		7
I, II	18		3		15
I, III	10	1			9
II, III	7		1		6
I	14	1			13
II	6		1		5
III	30				30
Total	96	2	9	0	85

Sum total all negatives, pre- 153; post- 11.

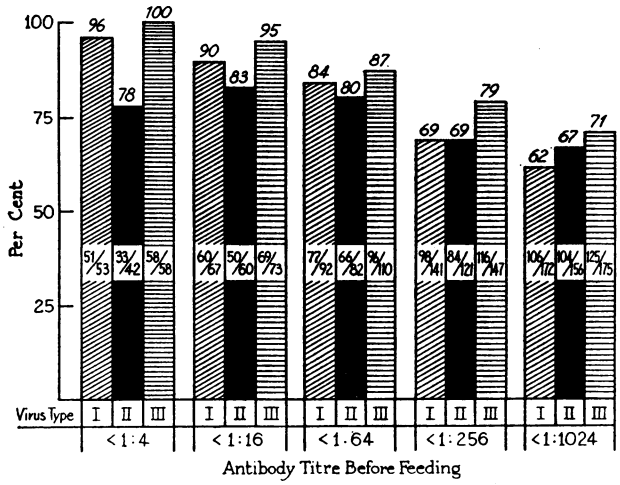

FIG. 2.—Percentage of 188 persons with fourfold or greater antibody rise following feeding of 2 ml. of trivalent oral poliomyelitis vaccine ($10^{6.1}$ TCD_{50} of each type of virus per dose).

It is of interest to note that the percentage of persons responding to the trivalent vaccine appeared to be highest for types III and I and somewhat less for type II. From the frequency of the serotypes of virus seen in the disease as it is encountered under natural conditions, a lesser response to type II would represent the least degree of risk. All knowledge to date, of course, indicates that the most important type to protect against is type I, followed probably by types III and II in that order. Certainly use of the trivalent vaccine as reported here gave excellent results in immunizing against types I and III, and, though the rate of response to type II was not as good, it is nevertheless encouraging, inasmuch as these results were obtained by feeding adults a single 2-ml. dose.

It must be pointed out also that in this study the vaccine was fed at various times of the day and no special effort was made to determine if a better rate of response could be obtained by feeding the vaccine under more nearly optimal conditions, such as before or after meals. Perhaps feeding the vaccine after a meal, at which time the gastric acidity could be partially reduced by food, might give better results. Perhaps an even higher rate of response could be obtained by giving a second dose of trivalent vaccine at a later interval, such as six to eight weeks after the administration of the first dose. Such a procedure might fill in the "negative gaps" among those individuals who failed to respond to the first feeding.

These problems are being explored at the present time.

Finally, similar studies are now under way with trivalent vaccines prepared from sub-line strains derived from the strains of poliovirus discussed here. These particular sub-line strains show less neurotropic activity in monkeys, and their immunogenicity for man is under investigation. In any event, there seems to be little doubt that by using the strains of poliovirus reported here it is entirely possible to induce in adults, and presumably in children also, a high state of immunity to all three types of poliovirus by a single feeding of trivalent oral poliomyelitis vaccine.

Summary and Conclusions

To date a serological study has been completed on 241 paired sera obtained from 550 volunteers fed various dosages of a liquid trivalent poliovirus vaccine. The majority of subjects in the study were employees of Lederle Laboratories, American Cyanamid Company, or their immediate families, and all resided in a semi-urban area within a 15-mile radius of Pearl River, N.Y.

Good neutralizing antibody responses, as judged by primary conversions or a titre increase of at least four-fold, were elicited when each of the three types of virus was present in the mixture at concentrations of $10^{5.5}$ (300,000), $10^{5.8}$ (600,000), and $10^{6.1}$ (1,200,000) TCD_{50}. The most favourable results, however, were obtained in the group of 188 persons fed the largest dose—namely, $10^{6.1}$ (1,200,000) TCD_{50} of each of the three types of poliovirus in 2 ml. of vaccine. Prior to vaccination with the single oral dose of vaccine this group of 188 persons included 11 triple negatives, 35 double negatives, and 50 single negatives. After vaccination, no double or triple negatives and only 11 single negatives remained. The conversion rate, therefore, was 93%. Two persons were left without antibodies to type I, and nine without antibodies to type II, while none was left without antibodies to type III.

These results indicate that it is both feasible and practicable to immunize against all three types of poliovirus by a single feeding of a trivalent oral poliovirus vaccine. The advantages of this type of vaccine are discussed.

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ACRYLIC INVESTMENT OF INTRACRANIAL ANEURYSMS

BY

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My original intimation of this subject was presented to give preliminary notification of a new method of treating bleeding intracranial aneurysms (Dutton, 1956), unsuitable for clipping or ligation, and previously considered manageable only by the uncertain method of muscle wrapping. Logue (1956) comments thus: "... surrounding the sac wall with a coat of hammered muscle to support it from the outside, a procedure which surely must have more effect on the surgeon's peace of mind than on the aneurysm." He also notes in 9% of his cases "contraindications to surgery" due to "anomalies of the anterior part of the circle of Willis, so that both anterior cerebral arteries arise from one internal carotid trunk, with the result that occlusion of the anterior cerebral is not feasible." Hook and Norlén (1958) wrapped 8 out of an operative series of 57 middle cerebral aneurysms.

The passage of time has allowed of the addition of further cases for study, and this paper contains an appraisal of this method consequent on a three-year follow-up. Certain additional advantages are also discussed, especially in relation to accidental rupture during surgical operation. Details of experimental work and aseptic techniques are also presented.

Experimental Work

The method was originally developed to provide an immediate unyielding investment for the sac and parent vessels. "Simplex rapid" cold curing acrylic made by Dental Fillings Limited was finally selected, because its physical properties were most suitable, and though certain drawbacks were known (Lefkowitz *et al.*, 1949; Zander, 1951; Kramer and McLean, 1952; Coy *et al.*, 1952; van Huysen and Boyd, 1953; Müller and Maeglin, 1953; Massler and Silberkweit, 1954; Nygaard-Östby, 1955; Kramer, 1955, 1956), relating to the use of "self-curing" acrylics in experimental dental caries, its use was thought to be practicable. This material was known to withstand immersion in salinous fluids for long periods, to set with a smooth surface, to show minimal shrinkage only during polymerization of the order of 6% by volume, corresponding to approximately 2.7% linear, and investigations on heat production revealed that small quantities—that is, 1–2 ml.—when allowed to polymerize, reached 115° F. (46.1° C.). With intracranial use such temperature production was thought to be of small moment, especially in the presence of cerebrospinal fluid and saline wash. The problem of incomplete polymerization, amounting to between 1% and 5%, was also considered to be amenable to the same method of dissipation.

In vivo experiments were then conducted, goats being found entirely suitable. Initially, collars of acrylic were made around peripheral limb vessels, and the immediate, early, and late results noted. There were no immediate effects on the vessel or limb circulation, no infection or foreign-body reaction, the collar was well accepted by the host; and, most important, there was no encystment, the collar being firmly felted. Segments of the vessel and collar were studied, and histologically the intima was found to be healthy, the media was normal, and fibrosis of the adventitia was found to be not great. There was no sign whatsoever of thrombus deposition in the vessel.

Acrylic and bone-wax (as control) implants and vessel investments were then made into the cerebral sulci of goats, to study the immediate and delayed vascular and cellular effects. Specimens were examined at varying post-implant periods ranging from seven days to seven weeks. Two blocks of cortex removed after seven days were frozen and stained for nerve-cell bodies (carbol azure) and for lipoid (scharlach R and haematoxylin). In a few sections from one block a narrow wedge-shaped zone of increased cellularity was seen around one of the small cortical vessels, which passed through the nerve-cell layer into the subcortical white matter. No other sign of damage to the brain underlying the implanted material (acrylic) was found. In a control block from the same animal in which bone wax had been inserted a similar zone of perivascular reaction was seen. Here the course of the vessel was similarly marked out by macrophage cells containing granules of neutral fat.

Blocks of cortex removed seven weeks later were also examined. In all, the molecular layer of the cortex showed increased cellularity (Figs. 1 and 2) below the site of insertion. In this situation a mild proliferation of astrocytes had occurred accompanied by an increase in the number of microglial cells, the processes of which were often slightly swollen and could be visualized in ordinary nerve-cell preparations. At one point the superficial part of the grey matter showed a slight increase in microglial cells, but nowhere had there been any detectable destruction of neurones. The microglial cells did not contain lipoid.

Summarizing, one can say that the insertion of acrylic had produced a mild localized microglial and astrocytic reaction in the molecular layer of the cortex, but its presence had not proved detrimental in any way to the underlying nerve cells. The narrow wedge-shaped areas of perivascular damage are to be regarded as the consequence of the surgical procedure and cannot be directly related to the effect of the acrylic, since similar appearances were seen in the control sections.

Bacteriology, with Special Reference to Cobalt Irradiation

The powder (polymer) as received from the manufacturers is probably sterile; all tested specimens have failed to produce any organisms, but contamination is easily effected, and the addition of monomer to effect