PERSISTENCE OF ANTIBODY IN CHILDREN AFTER A THIRD DOSE OF POLIOMYELITIS VACCINE AND THEIR RESPONSE TO A FOURTH

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During the 1956 field trial of inactivated poliomyelitis vaccine in the United Kingdom, 196 children between 1 and 10 years of age who had no antibody to any of the three types of poliovirus were tested for their response to vaccination by titrating the neutralizing serum antibodies induced by two doses of vaccine. A year later 107 of these children were given a third dose, and the antibody response was again determined (Medical Research Council, 1957a, and 1957b).

Further tests have now been made on 27 of these children to find out whether they still had antibody two years after the third dose. They were all given a fourth dose of vaccine and the antibody response of 25 of them was tested. Tests were also made to detect the presence of inhibitors to poliovirus in the faeces of these children.

Methods

Serum samples were taken at the same time as the children received the fourth dose of 1 ml. of poliomyelitis vaccine (Burroughs Wellcome Batch 17) intramuscularly into the upper arm. A second serum sample was taken 14 days later.

Most sera were titrated both by the cytopathic tests used routinely in the Virus Reference Laboratory, Colindale, and by the galactose colour test used in the Biological Standards Control Laboratory, Hampstead, in order to compare the titres obtained by each method.

In both methods, each serum was titrated, using two culture tubes for each dilution, except those from eight subjects whose sample of the post-third dose serum was still available. These sera were tested by the colour test together with the previous samples, using four tubes at each fourfold serum dilution. This was done in order to obtain a more accurate result of the antibody decline between the third and fourth doses from the few pairs of sera available. Titres are expressed as the reciprocal of the initial dilution of serum protecting 50% of tubes from virus action.

Colindale Cytopathic Test.—0.1 ml. of each fourfold serum dilution was mixed with 0.1 ml. of virus solution containing 200 TCID₅₀ of poliovirus of the type being used in the test. After three hours' incubation in a 37° C. water-bath, the serum-virus mixtures were

inoculated in 0.1-ml. amounts into monkey-kidney-cell roller cultures containing 1 ml. of Parker 199, and incubated at 37° C. for six days. The end-points of neutralization of virus by the serum dilutions were determined microscopically.

Hampstead Galactose Colour Test.—0.25 ml. of each fourfold serum dilution was mixed with 0.25 ml. of virus suspension (100 TCID₅₀) and placed in a 37° C. air incubator for six hours and then held overnight at 4° C. 0.5 ml. of a suspension of secondary monkey-kidney cells in lactalbumin solution were then added to each tube and the cultures sealed with liquid paraffin and incubated at 37° C. As galactose replaces glucose in the technique no colour change occurs during incubation. After five days a glucose solution was added and end-points were determined by the colour change. Cultures containing enough antibody to neutralize the virus, and protect the cell sheet, produced acid and turned the phenol-red included in the medium yellow. Those in which the virus had not been neutralized and destroyed the cells remained red.

Examination of Faeces.—10% suspensions of faeces were made in Parker 199 solution. After centrifugation at 5,000 r.p.m. for one hour the supernatants were removed, inactivated at 56° C. for half an hour, and tested for inhibitors to each of the polioviruses by the Colindale cytopathic test.

Results and Conclusions

The figures of the serum titrations by both methods are given in Table I. The results were parallel within the limits of the experimental error of each test. The actual titres were usually higher by the colour test, owing to the larger amounts of serum used at each dilution to neutralize the same amount of poliomyelitis virus and the longer time of neutralization before adding cells. Thus the sera found negative by the Colindale cytopathic test had low antibody levels by the Hampstead colour test. There was no evidence to suggest that the colour test was detecting low-avidity antibody and the cytopathic test high-avidity antibody (Sabin, 1958). If there are two such antibodies, either test can be adjusted to measure them by varying the temperature and length of incubation of the virus-serum mixtures before they are brought into contact with the monkey-kidney cells. Higher titres of poliomyelitis antibody can more easily be obtained with all sera containing them if neutralization is allowed to run at 37° C. for a few hours than if only one hour at room temperature is selected (Perkins *et al.*, 1958).

From Table I it can be seen that two years after a third, booster, dose of poliomyelitis vaccine all except perhaps one (V.C.) out of 27 children still had detectable serum antibody to all three types, as determined by the colour test. With type 1 the levels ranged from 4 to 1,024, with type 2 from 64 to 1,024, and with type 3 from 8 to 4,096. In the one exception, the amount of serum available was so small that a full type 1 titration could not be done; however, type 1 antibody was not detectable at a dilution of 1/16 and in all probability was entirely absent, since an indifferent response was obtained to the fourth dose in both tests. With exception of this subject, the response to all the components of the vaccine was good after the fourth dose and similar to that after the third (M.R.C., 1957b). Only one of the subjects had lost antibody before the third dose, and

TABLE I.—Comparison of Antibody Titres Obtained by Two Methods of Titration Before and After a Fourth Dose of Poliomyelitis Vaccine

Subject	Type 1				Type 2				Type 3			
	A1	B1	A2	B2	A1	B1	A2	B2	A1	B1	A2	B2
P.C.A.	64	16	128	256	128	64	512	512	128	64	128	256
M.A.A.	32	32	512	2,048	128	256	256	4,096	32	64	512	1,024
A.J.A.	<4	4	64	1,024	64	64	1,024	4,096	32	64	512	1,024
A.C.B.	128	256	1,024	4,096	512	512	1,024	1,024	64	512	512	2,048
B.K.B.	32	256	128	256	128	256	256	1,024	32	64	128	1,024
G.R.B.	16	32	128	256	32	64	256	1,024	<4	64	64	512
B.A.B.	32	256	512	—	256	1,024	1,024	—	128	128	512	—
A.E.B.	128	512	512	4,096	128	256	512	4,096	32	16	512	2,048
B.F.B.	32	512	128	4,096	512	256	4,096	4,096	1,024	4,096	8,192	8,192
J.F.B.	<4	16	128	4,096	128	256	512	2,048	64	256	1,024	4,096
M.J.B.	<4	8	16	256	32	64	2,048	1,024	128	256	128	128
C.J.B.	64	64	512	4,096	32	64	2,048	4,096	32	16	256	512
P.S.B.	128	256	128	1,024	128	256	256	2,048	8	128	128	1,024
S.C.	<4	4	256	512	256	256	512	1,024	128	64	128	128
V.C.	—	<16	32	128	—	128	64	1,024	—	16	128	1,024
D.A.D.	512	1,024	512	2,048	128	64	256	1,024	128	64	512	2,048
G.J.E.	<4	4	32	—	128	256	256	—	<4	16	256	—
S.N.G.	<4	16	128	1,024	64	128	512	2,048	<4	16	128	1,024
A.B.H.	64	256	256	2,048	128	256	1,024	4,096	256	1,024	512	1,024
B.R.H.	8	64	128	2,048	128	512	256	1,024	128	64	256	1,024
H.N.	64	256	64	—	64	64	128	—	—	256	—	—
C.E.S.	8	32	512	1,024	8	64	1,024	4,096	256	32	256	2,048
D.S.S.	128	1,024	256	1,024	512	256	1,024	4,096	512	512	2,048	4,096
D.B.V.	16	128	128	1,024	256	256	256	1,024	64	256	128	1,024
F.A.	4	64	—	—	128	256	—	—	128	512	—	—
P.A.	<4	4	—	—	128	1,024	—	—	32	128	—	—
F.B.	128	1,024	256	—	128	512	1,024	—	128	256	256	—
Geometric mean titre*	16	69	137	1,060	112	195	535	1,860	53	103	309	1,060

* Only those sera titrated by both methods used for the mean titre calculation.

A1 and A2: Titres obtained before and after vaccine by Coliadale cytopathic test. B1 and B2: Titres obtained before and after vaccine by Hampstead galactose colour test.

to type 1 only. It can therefore be concluded that the three vaccines used in the British field trial produced a good level of primary immunization and were as potent as any that have been used since in this country.

Table II shows the decline in the antibody titre of eight children during the two years between their third and fourth doses. The titres declined at different rates in different subjects, but the rate for all types was approximately similar in any individual. The average fall was 4.8-, 4.6-, and 4.7-fold for types 1, 2, and 3 antibody respectively, a result similar to that found in adolescents and adults by Kendall *et al.* (1960). Two of the children showed a rise in antibody to one type between the third and fourth doses, a rise which was greater than could be accounted for by the error of the colour test (Mussett and Tobin, to be published) and could have been due to natural infection; they were therefore excluded from the calculation of the mean titre.

The question of natural infection augmenting the response to vaccine must be considered when studying persistence of antibody after vaccination. Only when

samples of serum taken at the beginning and end of the observation period are tested, and the subjects themselves belong to a community free from infection during the study period, can results be conclusive. These criteria have been realized in the studies conducted at public schools (Medical Research Council, 1959; Kendall *et al.*, 1960), but are difficult to obtain in those of the general public. However, the levels of titres recorded here before revaccination are such that they are unlikely to be due to natural infection but are rather what one would expect after the good response obtained to the third dose in this group of children (M.R.C., 1957b).

No inhibitors to any of the poliovirus types were found in the faeces of any of these subjects, either before or after vaccination, in spite of the high antibody titres appearing in the blood of most subjects. Thus vaccination does not appear to produce the faecal inhibitors described by Steigman and Lipton (1959) after natural infection. Similar experiments made on the saliva of vaccinated schoolboys also gave negative results except in rare cases, when the serum antibody titre was exceptionally high (Kendall *et al.*, 1960). These findings support the conclusions of Gelfand *et al.* (1957) and of Davis *et al.* (1958), that vaccination with killed vaccine is unlikely to reduce the spread of the poliomyelitis virus in the community and that protection against paralysis must come directly from the adequate immunization of each individual.

Summary

The poliomyelitis antibody level in 27 children was measured two years after they had received a third dose of inactivated poliomyelitis vaccine. The children still had demonstrable antibody in their serum against the three polioviruses except for one who appeared to lack type 1 antibody. The antibody fall during the two years was estimated to be 78–79% for all three virus types.

TABLE II.—Decline in Poliomyelitis Antibody During a Two-year Period Between the Third and Fourth Doses of Vaccine

Subject	Type 1		Type 2		Type 3	
	Post 3rd Dose Titre	Pre 4th Dose Titre	Post 3rd Dose Titre	Pre 4th Dose Titre	Post 3rd Dose Titre	Pre 4th Dose Titre
P.C.A.	512	32	1,536	512	1,024	128
A.J.A.	256	8	1,024	64	512	64
A.C.B.	1,024	256	256*	768*	768	512
B.K.B.	48*	256*	512	256	512	96
G.R.B.	256	32	512	64	512	96
B.A.B.	512	256	2,048	512	512	128
B.F.B.	512	256	512	512	2,048	1,536
C.J.B.	256	32	1,024	64	1,024	64
Geometric mean titre	420	87	890	192	763	163
Ratio of means	4.8		4.6		4.7	

* Rise greater than could be accounted for by error in colour test. Excluded from calculation of mean titre.

A good response to a fourth dose of the inactivated vaccine was found in most of the children.

No poliovirus inhibitors could be found in the faeces of these children either before or after the fourth dose.

A comparison was made of two different methods of titrating poliomyelitis antibodies.

Our thanks are due to the children who participated in this investigation and to their parents for permission to take serum samples.

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NUCLEAR CHANGES IN SQUAMOUS CELLS FROM BUCCAL MUCOSA IN PERNICIOUS ANAEMIA

BY

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In a previous communication evidence was briefly presented which confirmed the existence of abnormally large nuclei in oral squamous mucosa in pernicious anaemia (Farrant, 1958a). The present paper gives the results obtained by examining a series of patients with pernicious anaemia before and after treatment. In addition, further comparison of patients with pernicious anaemia and normal subjects has disclosed differences in nuclear shape and in variability of size.

Materials and Methods

Films of oral mucosa were obtained by scraping the inside of the right cheek with an ordinary microscope slide. The resulting material was spread on to a further slide, dried in air, fixed, and then stained by a standard technique (May-Grünwald-Giemsa). An image of the buccal squamous cells and their nuclei was projected by means of a microscope and a powerful source of light on to a ground-glass screen arranged so as to give a magnification of 1,000. Measurements were made in the centre only of this optical system in order to minimize the effects of spherical aberration. It was found difficult to measure the size of the squamous cells themselves because of their irregular outline. The squamous-cell nuclei, on the other hand, have a relatively uniform oval shape, and accordingly 100 nuclei in each preparation were examined and measured. The measurements made were those of the long and short axes. For each axis the means of these 100 measurements were used for comparison. Though variation in mean nuclear size among normal controls is considerable, it was found that the mean nuclear measurements in different parts of the same slide, and

different slides taken consecutively from the same person, varied by only a small amount.

Twenty-five patients with pernicious anaemia were examined both before and after treatment. Repeat films of oral mucosa were taken at intervals varying from 2 to 32 months after successful treatment. Criteria of diagnosis were the presence of a typical macrocytic blood film and of a megaloblastic marrow, the demonstration of a histamine-fast achlorhydria, and a satisfactory response to treatment with vitamin B₁₂ or liver. In eight patients these diagnostic criteria were incompletely fulfilled. Four patients had no bone-marrow examinations made, one died as the result of a fractured femur before treatment of her anaemia became effective, while the rest had either no test meals or test meals which showed achlorhydria but without histamine stimulation. The haemoglobin concentrations in 24 patients after treatment varied from 12.5 to 17.2 g./100 ml., with a mean of 14.4 g. Blood films were either normal or showed minor degrees of anisocytosis. One further patient had a haemoglobin value of 11.5 g./100 ml., but developed some changes of iron deficiency in the blood film.

Results

The mean values of the nuclear measurements have been compared for each axis, both before and after treatment, and the differences calculated (see Table).

Effect of Treatment Upon Mean Nuclear Size

Case No.	Long Axis in Microns			Short Axis in Microns		
	Before Treatment	After Treatment	Difference	Before Treatment	After Treatment	Difference
1	11.93	12.34	+0.41	9.32	9.47	+0.15
2	13.15	12.48	-0.67	10.50	9.01	-1.49
3	12.88	11.57	-1.31	10.30	8.78	-1.52
4	9.74	10.92	+1.18	6.87	7.12	+0.25
5	11.53	11.71	+0.18	9.13	8.48	-0.65
6	12.09	11.64	-0.45	9.83	9.35	-0.48
7	12.64	10.27	-2.37	10.16	7.48	-2.68
8	10.48	10.95	+0.47	7.95	7.98	+0.03
9	12.25	11.51	-0.74	9.81	8.40	-1.41
10	12.57	11.83	-0.74	9.74	8.90	-0.84
11	13.08	12.03	-1.05	10.58	9.19	-1.39
12	13.06	11.65	-1.41	9.78	8.32	-1.46
13	12.12	11.98	-0.14	9.48	8.99	-0.49
14	11.90	11.59	-0.31	9.52	8.60	-0.92
15	12.18	11.91	-0.27	9.41	8.83	-0.58
16	12.45	11.42	-1.03	9.55	8.82	-0.73
17	15.02	11.44	-3.58	11.50	8.85	-2.65
18	12.32	11.27	-1.05	9.76	8.62	-1.14
19	12.39	11.87	-0.52	9.13	9.07	-0.06
20	12.89	11.68	-1.21	10.43	8.89	-1.54
21	12.21	12.61	+0.40	9.73	9.61	-0.12
22	12.26	11.49	-0.77	9.64	8.50	-1.14
23	14.04	12.05	-1.99	11.52	9.19	-2.33
24	11.99	10.30	-1.69	9.60	7.72	-1.88
25	11.15	11.55	+0.40	8.33	8.51	+0.18

In six patients the mean values in one or other axis are greater after treatment than before, but only in four of them is this effect shown in the measurements of both axes, the other two showing a diminution in size of the short axis. In three of the four cases (Nos. 4, 8, and 25) normal values were obtained for both axes before treatment and one would therefore hardly expect any diminution in nuclear size after treatment. The fourth case (No. 1) showed only a minor degree of anaemia (12.1 g./100 ml.), and has been treated and maintained on an oral preparation of vitamin B₁₂. In all these four patients the increase in nuclear size after treatment is comparatively small. The remaining 19 patients all show varying degrees of diminution in nuclear size in both axes.