polioviruses; and (*b*) to avoid the potential neutralizing effect of monkey antisera against simian viruses. Undiluted culture fluid was mixed with an equal quantity of the type-specific rabbit antiserum, and after an incubation period of one hour at room temperature 0.2 ml. was distributed in each of 10 monkey-kidney-culture tubes. At the end of seven days the medium is changed to permit observation of the tubes for another seven days for the detection of minimal amounts of non-poliovirus cytopathogenic agents. It is essential that the fresh medium contain 0.1 ml. of the specific rabbit antiserum per tube to prevent the emergence of poliovirus. Complete neutralization was obtained, and no other cytopathogenic agent was found. However, if a cytopathogenic effect had appeared, it would be necessary to check whether it was poliovirus that escaped neutralization or some other agent.

Tests for Residual Neurotropism in Monkeys.—The fluid was tested in 35 cynomolgus monkeys by intracerebral (1 ml.) and intraspinal (0.1 ml.) inoculation of the undiluted culture fluid and of the dilutions indicated below. The diluted, as well as undiluted, culture fluids are tested as a check against a zone phenomenon, which has been observed with certain strains or with cultures containing mixtures of virus of differing degree of neurotropism. The intracerebrally inoculated monkeys must be observed for at least four weeks, and the intraspinally inoculated monkeys for at least three weeks. The results of these tests, published in *J. Amer. med. Ass.*, 1957, **164**, 1216, were as follows:

Paralytic Effect in Monkeys Inoculated Intracerebrally with 1 ml. of Indicated Dilution		Paralytic Effect in Monkeys Inoculated Intraspinally with 0·1 ml. of Indicated Dilution		
Undiluted	10^{-1}	Undiluted	10^{-1}	10^{-2}
0/10	0/5	1/10	0/5	0/5

Test for Absence of Neurotropism in Spinally Inoculated Chimpanzees.—Although this need not be a standard test, fluid was tested in three chimpanzees devoid of spontaneously acquired antibody for any of the three types of poliovirus. Chimpanzees with spontaneously acquired antibody for any of the three types of poliovirus might also have some lesions in the spinal cord as a result of the natural infection, and therefore are not suitable for this type of test. Each chimpanzee was inoculated intraspinally in the region of the lumbar enlargement with 0.2 ml. of undiluted culture fluid (approximately 8×10^6 P.F.U.). They were observed for at least three weeks prior to sacrifice for histological examination. Neither paralysis nor lesions were observed in any of the nine chimpanzees used in these tests, and sections through the lumbar spinal cord indicated that the inoculum had been properly placed in the grey matter.

Tests in Human Beings.—Aliquots were fed to human beings between January and May, 1957, as described in *J. Amer. med. Ass.*, 1957, **164**, 1216. The dose consisted of 0.01 ml. (0.1 ml. of a 1:10 dilution) of culture fluid added to a teaspoonful of cherry syrup just before administration. No illness resulted and no cytopathogenic agents, other than the administered polioviruses, were recovered from the stools. The type 2 virus multiplied well in the alimentary tract and antibody formation was demonstrated. A summary of the results obtained in five children, without prior antibody for any of the three types of poliovirus, is shown in the Table. The results of extensive tests on the neurotropism of the excreted virus were published in *J. Amer. med. Ass.*, 1957, **164**, 1216. Further human tests with aliquots of these lots are now in progress in the U.S.A. by three different groups of investigators.

Excretion of Virus and Development of Antibody by 5 Children Without Low-avidity or High-avidity Neutralizing Antibody for Any of the 3 Types of Poliovirus Following Feeding of 0.01 ml. of Culture Fluid (100,000 TCID) of the Type 2 Attenuated Strain at 3-Week Intervals

Child	Age in Years	Excretion of Indicated Type of Virus		Antibody Development† for Indicated Type	
		2		Low-avidity+ High-avidity *p*H Test	High-avidity Cytopathogenic Test
		Peak Titre	Duration Days	2	2
A. S.	5	5·2	53*	256	200
D. S.	7	4·7	32	512	320
S. F.	5	5·7	32	512	320
P. F.	9	5·7	52†	512	320
K. F.	11	4·2	21	512	320

* Titre = Log_{10} TCD_{50} per gramme of faeces.
† Serum obtained 77 days after feeding of first type and 36 days after last.

Stability of the Vaccine.—The activity of the viruses is maintained for long periods of time by storage at approximately −20° C. Tests carried out by Dr. P. A. D. Winter, of the Poliomyelitis Research Institute, Johannesburg, South Africa, while a visiting investigator in this laboratory, indicated that the 1:10 dilution of the vaccine used for feeding can be maintained in the fluid state in ordinary wet ice without loss of titre for at least one week.

NOTE.—This experimental vaccine fulfils the requirements of the Expert Committee on Poliomyelitis of the World Health Organization (Report of July, 1957) for an attenuated live poliovirus vaccine for use in large-scale tests on human beings (see W.H.O. Technical Report Series No. 145).

BRITISH STANDARD POLIOMYELITIS ANTISERA TYPES 1, 2, AND 3

BY

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There is clearly a need for standard antisera to each of the three poliomyelitis virus types. Such sera would enable the neutralizing potency of other sera to be expressed in terms of the standard and allow a valid comparison to be made between the results obtained by different workers. In 1955 reference poliomyelitis antisera were prepared to serve as controls in titrating poliomyelitis antibodies in both human and animal sera (Medical Research Council, 1957; Biological Standards Control Laboratory, 1957; Perkins, 1957; Perkins, Sousa, and Tobin, 1958). These antisera were used essentially in ensuring the consistency of the titration system and not in assaying sera in terms of units. For a number of reasons they were unsuitable for use as standards to which a unitage could be assigned. New freeze-dried preparations of antisera to each of the three poliomyelitis virus types were therefore made, but before establishing them as standards a collaborative study* was arranged. The study was designed to determine whether different workers were able to obtain similar potency values when sera were assayed in terms of the freeze-dried preparations. As a result of the study, which is described in this report, it

*Participants in the study.—Cytopathic test: Miss J. O. R. Day, Glaxo Laboratories, Sefton Park, Stoke Poges, Bucks; Dr. R. Heath, Messrs. Pfizer Ltd., Richborough, Sandwich, Kent; Mrs. J. McCapra, Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London; and Dr. F. T. Perkins and Miss R. Yetts, with the assistance of Miss K. South and Miss P. Tumber, Biological Standards Control Laboratory, Hampstead, London. Colour test: Dr. J. O'H. Tobin and Miss O. Stanbridge, Biological Standards Control Laboratory, Hampstead, London.

was agreed by those who participated that the freeze-dried preparations should be established as British Standard Poliomyelitis Antisera types 1, 2, and 3.

Preparation of the Standard Antisera

Monospecific antiserum to each type of poliomyelitis virus was prepared in rhesus monkeys by immunization with live virus suspensions prepared in monkey-kidney-cell cultures. The virus strains used were Mahoney (type 1), MEF-1 (type 2), and Saukett (type 3). For each type the high titre serum was diluted in 6% aqueous dextran solution to give a titre with respect to its neutralizing antibody of the order of 500 to 1,000. Approximately one litre of each diluted antiserum was obtained, and was distributed in 1-ml. volumes in neutral hard glass ampoules and freeze-dried, all the serum to one type being dried in one run. The serum was finally dried over P_2O_5 for 14 days, sealed in dry nitrogen, and stored at $-10°$ C. in the dark.

In the collaborative study the sera were designated S-1, S-2, and S-3, and were reconstituted for use as follows: 10 ml. of Hanks solution containing 0.01% sodium bicarbonate was measured into a test-tube and an aliquot transferred to an ampoule to completely immerse the dried serum, which was then incubated at 37° C. until the serum completely dissolved (about 10 minutes). The serum was transferred to a 10-ml. cylinder using a Pasteur pipette, and with the same pipette the remainder of the 10 ml. of Hanks solution was used to wash out the ampoule, transferring the

TABLE I.—*Poliomyelitis Antibody Titres of 21 Sera Obtained in Five Laboratories*

Laboratory	Test Used	Titres Obtained in Individual Tests and Geometric Mean Titres of Sera							Calculated Ratio	
		A-1	B-1	C-1	D-1	E-1	G-1	S-1	A-1/B-1 (Theoretical =5)	A-1/C-1 (Theoretical =25)
Biological standards control	Cytopathic	1,536	512	48	192	256	<8	1,120	3	32
		2,048	256	64	128	256	<8	960	8	32
		1,024	256	96	192	192	<8	800	4	12
		1,024	256	48	128	256	<8	800	4	24
	G.M.T.	1,348	304	61	157	238	<8	910	4·5	22
Glaxo	Cytopathic	512	192	32	128	128	<8	960	3	16
		768	128	48	64	64	<8	960	6	16
		1,536	192	24	96	128	<8	960	8	64
		768	192	32	128	192	<4	640	4	24
	G.M.T.	825	174	33	100	119	<8	868	4·8	25
Pfizer	Cytopathic	512	192	32	64	128	<8	640	3	16
		1,024	64	48	64	128	<8	450	16	24
		768	128	32	48	32	<4	450	6	24
		768	192	32	96	96	<4	420	4	24
	G.M.T.	746	132	35	66	84	<8	483	5·7	21
Virus reference	Cytopathic	1,024	384	32	96	128	<8	768	3	32
		1,024	384	32	128	128	<8	640	3	32
		1,536	256	24	96	192	<8	640	6	64
		1,024	256	24	96	128	<8	540	4	48
	G.M.T.	1,132	314	28	103	142	<8	642	3·6	40
Biological standards control	Colour	512	256	16	48	32	<8	280	2	32
		1,024	192	64	64	128	<8	320	6	16
		1,024	192	64	64	128	<8	320	6	16
		1,024	256	32	96	64	<8	320	4	32
	G.M.T.	860	222	38	66	76	<8	310	3·9	23

Type 2

Laboratory	Test Used	A-2	B-2	C-2	D-2	E-2	G-2	S-2	A-2/B-2 (Theoretical =5)	A-2/C-2 (Theoretical =25)
Biological standards control	Cytopathic	1,536	192	32	32	384	<8	640	8	48
		1,024	128	24	12	128	<8	320	8	48
		768	128	32	24	96	<8	320	6	24
		768	128	24	32	96	<8	320	6	32
	G.M.T.	980	141	28	23	146	<8	380	6·9	35
Glaxo	Cytopathic	512	96	48	48	512	<8	480	6	12
		512	128	32	96	256	<8	640	4	16
		1,024	128	32	64	512	<8	960	8	32
		1,024	256	32	96	384	<4	768	4	32
	G.M.T.	724	142	35	73	400	<8	690	5·1	21
Pfizer	Cytopathic	512	128	24	256	256	<8	256	4	24
		1,024	192	48	192	128	<4	256	6	24
		768	96	16	96	256	<4	192	8	48
		768	128	16	128	128	<4	256	6	48
	G.M.T.	745	132	23	157	181	<8	238	5·7	32
Virus reference	Cytopathic	512	64	16	12	64	<8	384	8	32
		512	64	16	8	96	<8	320	8	32
		768	48	16	12	96	<8	384	16	48
		512	64	16	16	64	<8	384	8	32
	G.M.T.	567	60	16	12	78	<8	367	9·4	35
Biological standards control	Colour	256	96	12	64	96	<8	320	3	24
		512	128	24	48	128	<8	240	4	24
		512	128	24	48	128	<8	240	4	24
		1,024	64	32	64	256	<8	240	16	32
	G.M.T.	512	101	22	55	142	<8	258	5·1	23

(*Continued on next page*)

washings to the cylinder. The volume in the cylinder was finally made up, if necessary, to 10 ml., giving a 1/10 dilution of the serum, which was stored at 4° C.

The Collaborative Study

Five different laboratories took part in the study; two of them were in the Biological Standards Control Laboratory, but on this occasion they worked independently and can be regarded as two separate laboratories. Each laboratory received the three standard sera and 18 other sera, six to each type, the potencies of which were unknown to the participants. All the sera were from rhesus monkeys. The six sera to type 1 were coded A-1, B-1, C-1, D-1, E-1, and G-1; those to type 2, A-2, B-2, C-2, D-2, E-2, and G-2; and those to type 3, A-3, B-3, C-3, D-3, E-3, and G-3. For each type, sera B and C were made from serum A by diluting it 1/5 and 1/25 respectively with normal monkey serum. Sera G-1, G-2, and G-3 were normal monkey sera containing no neutralizing antibody. There were thus 15 positive sera. All sera were inactivated at 56° C. for 30 minutes, and each laboratory stored the sera at 4° C. during the investigation.

Four of the laboratories were asked to follow the cytopathic titration method described by the Biological Standards Control Laboratory (1957), details of which are given below, and to titrate on four occasions the standard to one type in parallel with the six corresponding sera, each time using cell cultures prepared from different monkeys. It was also required that a separate pipette should be used in preparing each serum dilution. The fifth laboratory (one of the two in the Biological Standards Control Laboratory) was asked to follow the general instructions given to the others, but used, in place of the cytopathic test, a metabolic inhibition test referred to as the galactose colour test. Each of the five laboratories used their own virus suspensions prepared from the Brunenders, MEF-1, and Saukett strains.

Cytopathic Test

Six- or seven-day monkey-kidney-cell cultures in test-tubes are emptied of medium and rinsed with Hanks solution (1 ml. per tube) to remove traces of serum used in initiating cell growth. The Hanks solution is then replaced by Parker 199 medium (1 ml. per tube) containing 0.2% sodium bicarbonate to ensure that the pH of the medium remains above 7 for the duration of the test.

Twofold dilutions of both test and standard sera are made in Hanks solution containing 0.01% sodium bicarbonate using 1-ml. volumes for the test sera and 2-ml. volumes for the standards, and changing the pipette between each dilution step. An equal volume of virus suspension, containing approximately 100 $TCID_{50}$ per 0.25 ml., is added to each serum dilution, and, after shaking, the mixtures are incubated at 37° C. for three and a half hours in an air incubator. The mixtures are inoculated into monkey-kidney-cell culture tubes either immediately or after being held at 4° C. overnight. Each serum–virus mixture is inoculated in 0.5-ml. volumes into each of two cell cultures for the test sera and each of four cell cultures for the standards. The inoculated cultures are "rolled" at 37° C, and finally examined microscopically for cytopathic degeneration at seven days. Neutralizing antibody levels are calculated as the dilution of serum required to neutralize virus in 50% of tubes (Reed–Muench or other method). The dilution of serum is taken as that in the serum–virus mixtures.

Galactose Colour Test

This test is essentially the metabolic inhibition test (Salk, Youngner, and Ward, 1954) modified by using galactose instead of glucose in the maintenance medium (Tobin, to be published). Serum–virus mixtures are added to monkey-kidney-cell suspensions, and the end-point of neutralization is determined by the change in colour of the phenol red contained in the nutrient medium. Monkey-kidney-cell cultures which have been

TABLE I (*Continued*)

Type 3

Laboratory	Test Used	Titres Obtained in Individual Tests and Geometric Mean Titres of Sera							Calculated Ratio	
		A–3	B–3	C–3	D–3	E–3	G–3	S–3	A–3/B–3 (Theoretical =5)	A–3/C–3 (Theoretical =25)
Biological standards control	Cytopathic	1,024	256	64	64	256	<8	1,024	4	16
		2,048	128	32	48	256	<8	640	16	64
		1,024	256	64	96	256	<8	800	4	16
		1,024	192	32	48	192	<8	640	6	32
	G.M.T.	1,218	200	45	61	238	<8	761	6·1	27
Glaxo	Cytopathic	1,536	1,536	192	128	192	<8	512	1	8
		2,048	768	96	64	256	<8	960	3	24
		1,536	384	128	128	256	<8	960	4	12
		2,048	384	96	64	96	<4	640	6	24
	G.M.T.	1,774	646	122	91	186	<8	741	2·7	15
Pfizer	Cytopathic	1,024	192	48	32	128	<8	450	6	24
		512	256	64	64	128	<4	450	2	8
		1,536	96	24	32	128	<4	420	16	64
		512	256	48	96	256	<4	420	2	12
	G.M.T.	802	186	43	50	152	<8	435	4·3	19
Virus reference	Cytopathic	1,536	192	48	32	192	<8	540	8	32
		1,536	256	32	32	256	<8	540	6	48
		1,536	256	32	48	256	<8	450	6	48
		1,024	256	32	48	192	<8	450	4	32
	G.M.T.	1,388	238	35	39	222	<8	493	5·8	40
Biological standards control	Colour	512	128	48	32	256	<8	280	4	12
		768	192	64	32	384	<8	240	4	12
		768	192	64	32	384	<8	240	4	12
		768	128	64	48	192	<8	280	6	12
	G.M.T.	694	157	60	35	292	<8	259	4·4	12

grown in bottles for six days are removed from the glass surfaces with versene. The cell suspensions are centrifuged at 500 r.p.m. for 20 minutes and suspended in a medium of lactalbumin, galactose, inorganic salts, and bovine albumin or serum, to give a count of 150,000 cells per ml. Each serum–virus mixture is dispensed in 0.5-ml. quantities into each of four tubes, and 0.5 ml. of cell suspension is added to give 75,000 cells per tube. After shaking, 0.75 ml. of liquid paraffin is added and the tubes are incubated at 37° C. for six days. During this time the colour of all tubes remains pink, and on the sixth day 0.04 ml. of a yeast-extract solution containing 10% glucose is added to each tube. After a further period of 48 hours' incubation at 37° C, metabolism of the glucose by the viable cells changes the colour of the medium to yellow. Thus, only in those serum–virus mixtures with excess antibody is a red-to-yellow colour change observed. Neutralizing antibody levels are calculated as in the cytopathic test.

TABLE II.—*Poliomyelitis Antibody Potencies of 15 Test Sera in Relation to Standard Antisera Obtained by Five Different Laboratories*

Laboratory	Test Used	Potency of Sera in Units per ml. in Terms of the Appropriate Standard				
		A–1	B–1	C–1	D–1	E–1
B.S.C. ..	Cytopathic	14·8	3·3	0·67	1·7	2·6
Glaxo ..	,,	9·5	2·0	0·38	1·2	1·4
Pfizer ..	,,	15·4	2·7	0·73	1·4	1·7
Virus reference	,,	17·6	4·9	0·44	1·6	2·2
B.S.C. ..	Colour	27·7	7·2	1·23	2·1	2·5
		A–2	B–2	C–2	D–2	E–2
B.S.C. ..	Cytopathic	25·8	3·7	0·73	0·61	3·8
Glaxo ..	,,	10·5	2·1	0·51	1·06	5·8
Pfizer ..	,,	31·3	5·6	0·97	6·60	7·6
Virus reference	,,	15·5	1·6	0·44	0·33	2·1
B.S.C. ..	Colour	19·8	3·9	0·85	2·13	5·5
		A–3	B–3	C–3	D–3	E–3
B.S.C. ..	Cytopathic	16·0	2·6	0·59	0·80	3·1
Glaxo ..	,,	23·9	8·7	1·62	1·23	2·5
Pfizer ..	,,	18·4	4·3	0·99	1·15	3·5
Virus reference	,,	28·2	4·8	0·71	0·79	4·5
B.S.C. ..	Colour	26·8	6·1	2·32	1·35	11·3

B.S.C. = Biological Standards Control.

TABLE III.—*Poliomyelitis Antibody Titres of Standard Antisera Obtained by the Cytopathic Test*

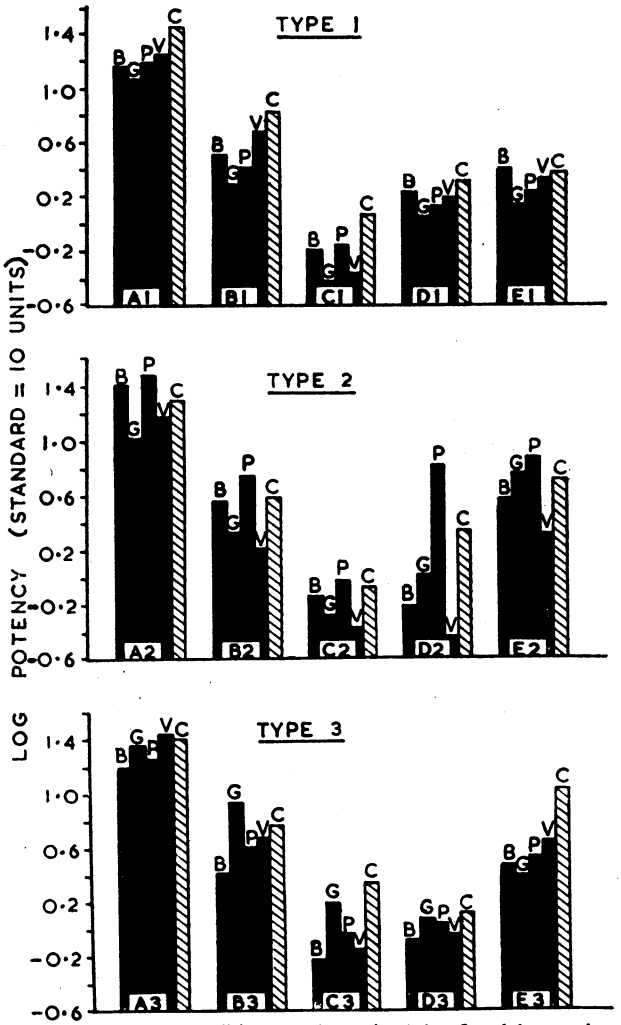
Test	Neutralizing Antibody Titres of Standard Sera		
	Type 1	Type 2	Type 3
Sera from different ampoules titrated in different tests immediately after reconstitution	960 1,280 1,280 1,920 1,280 1,280	960 1,280 1,280 960 640 480	960 960 1,280 1,280 1,280 1,280
Reconstituted sera titrated:			
Immediately after reconstitution ..	1,280	640	1,280
After storage at 4° C. for 4 months ..	1,280	960	960
,, ,, ,, ,, 5 ,, ..	1,280	960	960
,, incubation at 37° C. for 21 days ..	800	640	800
Sera titrated:			
From refrigerated ampoules ..	960	960	960
From 2 ampoules heated at 37° C. for 40 days	1,120 960	320 480	800 640
From 2 ampoules heated at 56° C. for 20 days	480 480	240 320	400 320
From ampoules heated at 100° C. for 5 minutes	640	640	480
From ampoules heated at 100° C. for 15 minutes	480	480	480

Results

Neutralizing End-points of Titrations

The results of the titrations from each laboratory are given in Table I. The five laboratories, in titrating the 15 positive sera and the three standards, produced 360 results consisting of 90 groups each containing four results. Considering each laboratory separately, it is evident that the variation in titre for any one serum was small; of the 90 groups of results, 70 gave a variation of twofold or less, 18 gave twofold to fourfold, and the remaining two groups fourfold. The variation occurring within each of the five laboratories with the standard preparations was never more than twofold.

The variation occurring between the laboratories was, however, slightly greater. Of the 18 sera tested, the mean titres for four varied within twofold, for 11 from twofold to fourfold, and for three greater than fourfold.



Potencies of poliomyelitis sera determined by five laboratories. B=Biological Standards Control Laboratory. G=Glaxo Laboratories. V=Virus Reference Laboratory. P=Pfizer Ltd. C=Biological Standards Control Laboratory. Black columns=Results by cytopathic test. Cross-hatched columns=Results by colour test.

TABLE IV.—*Weight of Total Solids Per Ampoule of the Standard Poliomyelitis Antisera*

Virus Type	Weight of Total Solids in Each of Nine Ampoules of Each of the Three Standard Antisera (g.)									Mean Weight (g.)	Standard Deviation (σ)	Coefficient of Variation
1	0·1084	0·1075	0·1076	0·1081	0·1079	0·1076	0·1087	0·1070	0·1074	0·1078	0·00053	0·491%
2	0·1062	0·1057	0·1043	0·1040	0·1047	0·1040	0·1042	0·1040	0·1039	0·1046	0·00084	0·798%
3	0·1054	0·1048	0·1045	0·1047	0·1058	0·1036	0·1062	0·1052	0·1026	0·1048	0·00111	1·058%

The calculated ratios of titres of serum A to B and A to C for each laboratory are also given in Table I, and it is interesting to note that they closely approximated to the theoretical values of 1:5 and 1:25.

The test dose of virus varied between the different laboratories from 30 to 350 $TCID_{50}$, but there was no evidence that the variation was in any way related to the variation in serum titres.

Potency of Sera in Relation to the Standard

Each of the three standards was assigned a potency of 10 units per ampoule of dry preparation. For each laboratory the potencies of the 15 positive test sera were calculated in terms of the appropriate standard (by dividing the mean titre of each serum by the mean titre of the standard and multiplying by 10) and expressed in units per millilitre. These potencies are given in the Chart and in Table II. It is evident that in general the agreement between the five laboratories was extremely close; with four of the 15 positive sera the variation in potency was less than twofold, and with all but two sera (D-2 and E-3) was within fourfold. It is also evident that the agreement was greater than that obtained when the potencies were expressed in terms of titre-dilutions.

Additional Tests With the Standard Preparations

A number of additional tests were made in the Biological Standards Control Laboratory, the results of which are given in Table III. These results indicate that there was little variation in antibody titre from ampoule to ampoule with each of the three standard preparations. The results also give an indication of the degree of stability of both the dry and reconstituted standards.

The weight of total solids per ampoule was determined for each of the three standard preparations (Table IV). Since each ampoule contains 10 units of antiserum, it is calculated that: 1 unit is contained in 0.01078 g. of the type 1 preparation; 1 unit is contained in 0.01046 g. of the type 2 preparation; and 1 unit is contained in 0.01048 g. of the type 3 preparation.

Conclusions

A collaborative study in which five laboratories took part showed that close agreement was obtained between the different laboratories when the type 1, 2, or 3 neutralizing potencies of poliomyelitis antisera were determined with reference to freeze-dried preparations of antisera. It was therefore decided, with the agreement of those who participated in the study, to establish the three freeze-dried preparations of antisera as British Standard Poliomyelitis Antisera types 1, 2, and 3, and to assign to each a value of 10 units per ampoule. It will now be possible to express the neutralizing potency of sera in terms of units and to abandon the unsatisfactory method of using titre-dilutions.

The standard antisera will be held by the Department of Biological Standards, National Institute for Medical Research, Mill Hill, London, and may be obtained on request by manufacturing and research laboratories.

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ACTION OF CHLOROTHIAZIDE AND "ORADON," ALONE AND IN COMBINATION

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It has been claimed by many that chlorothiazide is superior to other diuretics, that it is effective in all forms of oedema, and that it has no significant side-effects (Ford *et al.*, 1957, 1958; Laragh *et al.*, 1958; Watson *et al.*, 1958; Goodkind *et al.*, 1958; Wener *et al.*, 1958). Later communications, however, have not always been able to substantiate these claims, and caution in the indiscriminate use of the drug has been suggested (*Brit. med. J.*, 1957). Hypokalaemia (Laragh *et al.*, 1958; *Brit. med. J.*, 1958; Slater and Nabarro, 1958), failure to respond after prolonged administration (Slater and Nabarro, 1958; Read *et al.*, 1958), and the production of hepatic coma in cirrhotic patients (Read *et al.*, 1958) have all been reported, and as a result of these studies it would seem wise to carry out routine plasma electrolyte determinations during treatment. In this way oral supplements of potassium can be given if necessary or therapy curtailed if it proves impossible to maintain normal plasma levels.

Laragh *et al.* (1958), Bayliss (1958, personal communication), and Bayliss *et al.* (1958) noticed that the addition of mercurial injections to oral chlorothiazide significantly augmented the sodium and water diuresis. This observation prompted us to reassess the value of combined mercurial and chlorothiazide therapy, not only with injected mercurials but also with an oral form. Such a combination might give a therapeutic response with lower dosages and a lower incidence of side-effects, particularly hypokalaemia, which would be of advantage under the conditions of general practice. During the trial we were also able to evaluate the oral mercurial and to make some interesting observations on the potassium excretion pattern of subjects taking chlorothiazide with and without potassium supplements.

Material and Method

The five patients studied in detail had all been suffering from persistent oedema with only fair or poor response to injected mercurials; four of these had congestive heart failure from different causes and one had the nephrotic syndrome.

The usual dose of chlorothiazide, given alone or in combination, was 0.5 g. twice daily, although one patient (Case 5) required 2 g. daily.

The oral mercurial chosen for this study was 3-hydroxy-mercuri-2-methoxy-l-succinimidopropane, theophylline hydrate (known as "oradon"), which has been reported (Hvidberg and Nielsen, 1957) as having an absorption of up to 54% of ingested mercury as judged by urinary recovery and therefore comparing favourably with chlormerodrin, which is reported as

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