A POTENT ANTIPOLIOMYELITIC HORSE SERUM CONCENTRATE AND ITS EXPERIMENTAL USE IN INFECTED MONKEYS*

By ELLIOTT R. WEYER, PH.D., WILLIAM H. PARK, M.D., AND E. J. BANZHAF, PH.D.

(From the Laboratories of New York University and the Department of Health of the City of New York, New York)

(Received for publication, January 28, 1931)

Since the discovery by Netter (1) and Levaditi (2) of the presence of virus-neutralizing antibodies in the blood, both from human convalescents and from monkeys recovered from experimental poliomyelitis, several attempts have been made to immunize large animals with a view to quantity production of therapeutic serum.

The results of Aycock (3) and others who have used convalescent serum in the treatment of poliomyelitis tend to indicate that serum therapy is effective when used in the preparalytic stage of this disease. The difficulties encountered in obtaining ample quantities of human immune serum have hampered its widespread use.

Flexner (4), in 1910, reported unsuccessful attempts to bring forth an immunological response in a horse by injection over a period of many months of filtrates carrying the active virus of poliomyelitis.

In 1917, Banzhaf and Neustaedter (5) published the results of similar efforts, having injected subcutaneously and intramuscularly the supernatant fluid from emulsions of brains and spinal cords of monkeys dying of poliomyelitis. This antiserum gave some evidence of ability to neutralize virus in the few experiments then conducted.

Pettit (6), in 1918 described the preparation and subsequent use in human cases of a horse serum drawn from an animal which had received injections over a period of 2 years. He did not test this serum directly for virus-neutralizing antibodies but depended upon clinical results for an index of its usefulness. Later, Stewart and Haselbauer (7) investigated this preparation and found it to neutralize virus

*The expenses of this experimental study were largely defrayed by a gift of money to New York University by Mr. Jeremiah Milbank, this University being one of the group working under the International Committee for the Study of Infantile Paralysis.

only occasionally and then only in a ratio of 1 part of virus emulsion to 3 parts of serum. These workers also attempted without success to produce an antiserum from sheep. At the same time they also tested Rosenow's antistreptococcus serum of alleged therapeutic value in poliomyelitis and found it to be devoid of any virucidal principles.

Herein is reported the successful production of potent virus-neutralizing preparations drawn from immunized horses with evidence relative to its potency from the standpoint of prophylaxis and therapeutics.

Method

In general it has been found satisfactory to inject horses with virulent cord emulsion (10 per cent) in doses increasing from 1 cc. to 50 cc. The inoculations are given subcutaneously or intradermally and are administered on 4 successive days in each week. That certain horses fail to respond has been demonstrated. It has also been found that horses having no measurable native antibody may readily become producers of a high titre serum. Indeed, in no horse has there yet been found evidence of a specific antibody prior to the immunization treatments.

After 2 months of injections, horses which will satisfactorily respond to the immunization will have produced sufficient antibody to neutralize the active virus of poliomyelitis. Evidence of worth is determined by means of an *in vitro* neutralization test, the technique of which is as follows:

5 per cent virus emulsions are prepared by grinding virulent monkey spinal cord with saline. The serums to be tested are added in the desired proportion and after an hour's incubation at room temperature the mixtures are introduced into the cerebral hemispheres of susceptible monkeys. The amount of inoculum is so calculated that it represents at least two infective doses. The potency of the virus has apparently no effect upon the amount of a specified serum preparation needed to neutralize it. When using particularly potent strains of virus the inoculum often contains as many as several hundred killing doses of virus.

Using this procedure it has been possible to determine several relative values quite accurately. For example, pooled convalescent serum from human poliomyelitis cases will consistently neutralize 5 per cent virus emulsion in the ratio of 1 part of serum to 20 parts of virus but fails to do so when the ratio is 1:30. This has been demonstrated by the titration of two pools of convalescent serum each containing serum from sixteen individuals.

Individual serums from so-called normal human adults will often

TABLE I

Virucidal Properties of Various Human Sera

Monkey No.	Mixture injected	Virus used	Serum tested	Ratio virus to serum	Paralysis appeared	Death	Result
	cc.				*days*	*days*	
			Human convalescent serum pooled				
228	0.3	226	A	10:1	—	—	Not infected
220	0.3	226	"	20:1	—	—	" "
227	0.3	226	"	40:1	7	9	Poliomyelitis
214	0.3	89	B	20:1	—	—	Not infected
			"Normal" adult serum				
294	0.4	267	Donor 1	10:1	—	—	Not infected
293	0.4	267	" 1	20:1	8	9	Poliomyelitis
310	0.4	298	" 2	14:1	—	—	Not infected
309	0.4	298	" 3	14:1	10	15	Poliomyelitis
305	0.4	298	" 4	14:1	6	14	"
462	0.4	457	" 5	10:1	8	10	"
466	0.8	10	" 5	1:1	—	—	Not infected
384	0.4	457	" 6	10:1	9	12	Poliomyelitis
467	0.8	10	" 6	1:1	—	—	Not infected
385	0.4	457	" 7	10:1	—	—	" "
458	0.4	457	" 8	10:1	19	—	" "
468	0.4	10	" 9	10:1	10	11	Infected, killed
311	0.3	298	Control, no serum	—	6	14	Poliomyelitis
312	0.15	298	" " "	—	6	14	"
279	0.3	267	" " "	—	6	7	"
282	0.15	267	" " "	—	7	9	"
475	0.3	10	" " "	—	8	11	Infected, killed
458	0.15	10	" " "	—	8	—	Lived, paralyzed
460	0.3	457	" " "	—	11	—	" "
461	0.15	457	" " "	—	11	—	" "
210	0.5	89	" " "	—	8	9	Poliomyelitis
231	0.3	226	" " "	—	8	9	"
252	0.3	248	" " "	—	7	10	"
261	0.15	248	" " "	—	9	10	"
215	0.5	89	Virus emulsion + saline + tricresol	10:1	9	11	"

effect a neutralization in a ratio of 1:10, but usually fail in 1:15. According to our observations four out of nine were effective in 1:10.

This knowledge is of practical value in that it is not necessary to search for convalescent cases for a supply of effective serum.

TABLE II

Progress of Horse 4

Monkey No.	Mixture inoculated	Virus No.	Serum tested	Ratio virus to serum	Paralysis appeared	Death	Result
	cc.				*days*	*days*	
250	0.4	145	Poliomyelitis, Horse 4, initial bleeding, 9/13/27	5:1	7	7	Poliomyelitis
30	0.4	145		10:1	7	8	"
79	0.4	145		20:1	7	8	"
214	0.4	145	Poliomyelitis, Horse 4, bleeding of 1/14/29	10:1	—	—	Remained well
34	0.4	145		20:1	9	10	Poliomyelitis
253	0.4	145		20:1	20	23	"
254	0.4	145		25:1	14	16	"
228	0.4	145	Control	—	7	9	"
259	0.3	145	"	—	4	4	"
268	0.4	248	Poliomyelitis, Horse 4, bleeding of 2/21/29	20:1	9	10	"
269	0.4	248		25:1	7	8	"
252	0.3	248	Control	—	7	10	"
261	0.15	248	"	—	9	10	"
314	0.4	298	Poliomyelitis, Horse 4, bleeding of 3/20/29	20:1	—	—	Remained well
315	0.4	298		10:1	—	—	" "
316	0.4	298	Poliomyelitis, Horse 4, bleeding of 5/8/29	20:1	17	21	Poliomyelitis
317	0.4	298		10:1	—	—	Remained well
311	0.3	298	Control	—	6	14	Poliomyelitis
312	0.15	298	"	—	6	14	"
285	0.4	284	Poliomyelitis, Horse 4, bleeding of 7/5/29	66:1	9	10	"
329	0.4	285		50:1	13	15	"
328	0.4	320		20:1	—	—	Remained well
315	0.4	320		10:1	—	—	" "
314	0.3	320	Control	—	7	13	Poliomyelitis
317	0.15	320	"	—	6	19	"
327	0.3	284	"	—	6	6	"
313	0.15	284	"	—	6	6	"
330	0.4	285	Poliomyelitis, Horse 4, bleeding of 8/26/29	25:1	—	—	Remained well
335	0.3	285	Control	—	8	11	Poliomyelitis
336	0.15	285	"	—	7	Killed	"

TABLE III

Serum Preparations from Horse 4 Refined by Banzhaf

Monkey No.	Mixture inoculated	Virus No.	Serum tested	Ratio virus to serum	Paralysis appeared	Death	Result
	cc.				*days*	*days*	
275	0.4	252	Globulin preparation 1	100:1	5	6	Poliomyelitis
276	0.4	252	" " 1	66:1	—	—	Remained well
313	0.4	298	" " 3	40:1	—	—	" "
308	0.4	320	" " 3	50:1	10	13	Poliomyelitis
321	0.4	320	" " 3	70:1	—	—	Remained well
322	0.5	320	" " 4	40:1	—	—	" "
323	0.5	320	" " 5	40:1	—	—	" "
345	0.4	285	" " 5	25:1	—	—	" "
330	0.4	285	" " 5	50:1	21	26	Poliomyelitis
357	0.4	285	" " 5	66:1	12	14	"
336	0.4	285	" " 6	66:1	—	—	Remained well
334	0.4	285	" " 6	100:1	8	11	Poliomyelitis
365	0.4	402	" " 6	66:1	—	—	Remained well
398	0.4	402	" " 6	100:1	—	—	" "
367	0.4	402	" " 7	66:1	—	—	" "
368	0.4	402	" " 7	100:1	—	—	" "
444	0.4	9	" " 7	125:1	17	20	Poliomyelitis
443	0.4	9	" " 7	156:1	11	14	"
277	0.4	252	Control	—	5	8	"
311	0.3	298	"	—	6	14	"
312	0.15	298	"	—	6	14	"
314	0.3	320	"	—	7	13	"
317	0.15	320	"	—	6	19	"
354	0.3	285	"	—	7	7	"
355	0.3	285	"	—	9	10	"
356	0.15	285	"	—	9	14	"
417	0.3	402	"	—	7	7	"
418	0.1	402	"	—	10	10	"
376	0.6	402	Normal horse serum Control A	1:1	9	9	"
377	0.6	402	" " " " B	1:1	9	10	"
385	0.6	402	" " " " C	1:1	10	12	"
442	0.6	9	" " " " D	1:1	7	12	"

The Results of Immunization

Referring now to the horses, the first animal, Horse 4, had previously been injected subcutaneously by one of us (Banzhaf) over a period of

16 months with virus of very dubious potency. Bleedings, during and subsequent to this treatment failed to show any specific antibody.

The inoculations with emulsions of known virulence, were resumed in October, 1928, this time intravenously. This work was begun on a more elaborate scale because of the fund given by Mr. Jeremiah

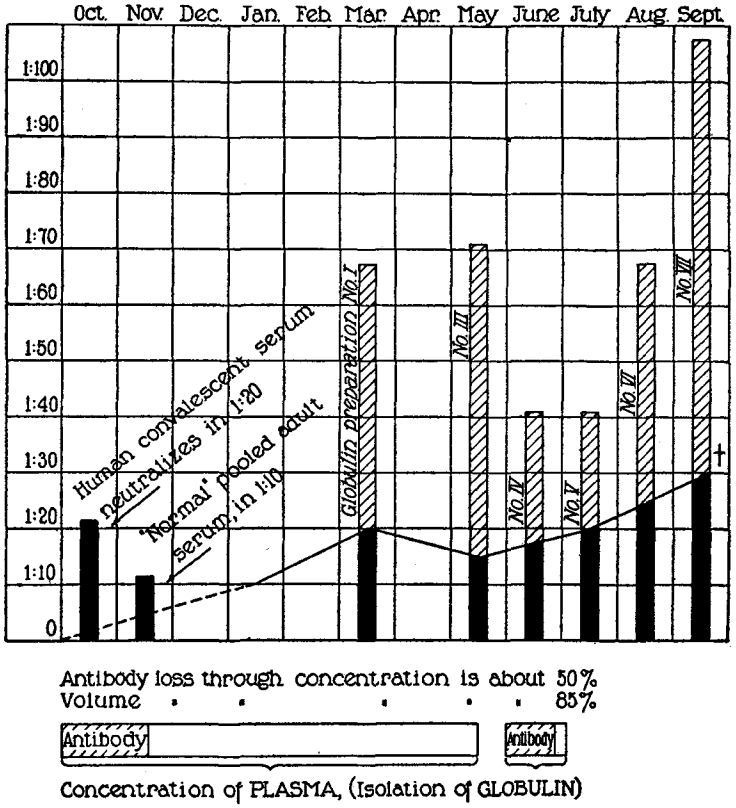

CHART 1

Milbank. Encouraging results were obtained and in the following January the serum was capable of neutralization in the ratio of 10:1. The titre continued to mount and bulk bleedings were made from time to time when late in the summer the animal died from an obscure infection which resulted in the death of several other horses in the same stable. A preliminary report dealing with the successful immunization

of this horse was presented by Park before the American Association of Pathologists and Bacteriologists at Chicago, March 28th, 1929 (8, 9).

The larger bleedings from this horse were drawn into citrate solution and concentrations were made according to the following scheme:

The method of fractional concentration of antisera with ammonium sulfate (Banzhaf (9)), without the heating process, is followed. We have not up to the present time determined the heat resisting point of the neutralizing antisubstances. After the method mentioned has been carried through to dialyzing free from salts, it will be noted that some precipitation has formed which contains some of the antisubstances. A gentle rotation of the concentrated fluid in the dialyzing bags will resuspend the precipitate. The fluid is measured and three volumes of distilled water added. Sufficient sodium chloride is added to bring the whole to one-twentieth normal (50 cc. normal sodium chloride per litre) and adjusted to pH 5.0–5.1 and placed into a cold room 5–10°C. overnight. The resulting precipitate consists of further inert substances and practically all the chill producing substances usually present in all sera. These substances are removed by filtration through paper pulp. The clear filtrate adjusted to pH 6.8 and allowed to stand overnight for a further inert fibrin like precipitate which may form, if present it is removed by filtration. To the clear filtrate a half volume of saturated ammonium sulfate solution is added to reprecipitate the globulins and antisubstances. The precipitate is recovered by filtration, pressed free from fluid and dialyzed free from salts. To the dialyzed concentrated fluid 1 per cent sodium chloride and 0.5 per cent phenol are added, it is clarified by filtering through paper pulp and passed through a Berkefeld filter to sterilize.

Referring to Chart 1, it will be seen that the increase in globulin concentration from this procedure is three to four-fold and that the preparation of maximum potency is approximately five times as potent as average human convalescent serum.

Following the loss of Horse 4, immunization was commenced upon two other horses, Nos. 5 and 6. The jugular vein was selected as the route of injection in view of the success obtained by that method in Horse 4. Neither horse had any demonstrable "native" antibody to begin with, nor was any acquired though they received the total substance of approximately 25 virulent spinal cords and brains over a period of a year. During this period the route of inoculation was varied and the sequence of injection changed but with the appearance of no neutralizing value, even in a ratio of 1:1, these horses were abandoned in May, 1930.

TABLE IV

Protocols of Horses 5 and 6, Failure to Respond

Monkey No.	Mixture inoculated	Virus No.	Serum tested	Ratio virus to serum	Paralysis appeared	Death	Result
	cc.				*days*	*days*	
4/2/29							
265	0.4	277	Horse 5, initial bleeding	5:1	7	10	Poliomyelitis
263	0.4	277	" 6, " "	5:1	7	10	"
5/13/29							
281	0.4	267	" 5, bled 5/7	20:1	7	8	"
286	0.4	267	" 5, " 5/7	10:1	7	8	"
288	0.4	267	" 6, " 5/7	20:1	5	7	"
289	0.4	267	" 6, " 5/7	10:1	9	10	"
6/27/29							
318	0.4	298	" 5, " 6/24	5:1	10	17	"
319	0.4	298	" 6, " 6/24	5:1	11	18	"
320	0.3	298	Control	—	8	11	"
7/17/29							
324	0.4	320	Horse 5, bled 7/15	5:1	7	9	"
325	0.4	320	" 6, " 7/15	5:1	12	17	"
314	0.3	320	Control	—	7	13	"
317	0.15	320	"	—	6	19	"
8/21/29							
323	0.4	314	Horse 5, bled 8/21	2.5:1	8	11	"
326	0.4	314	" 6, " 8/21	2.5:1	8	11	"
310	0.15	314	Control	—	8	11	"
9/9/29							
321	0.6	267	Horse 5, bled 9/9/29	1:1	7	10	"
315	0.6	267	" 6, " 9/9/29	1:1	6	10	"
327	0.3	267	Control	—	6	6	"
313	0.15	267	"	—	6	6	"
11/11/29							
364	0.6	285	Horse 5, + Horse 6, bled 11/13/30	1:1	8	9	"
366	0.3	285	Control	—	6	6	"
2/11/30							
377	0.3	402	Horse 5, bled 2/11/30	1:1	9	9	"
376	0.3	402	" 6, " 2/11/30	1:1	9	10	"
417	0.3	402	Control	—	7	7	"
418	0.1	402	"	—	7	10	"
5/20/30							
368	0.5	445	Horse 5, bled 5/20/30	2.5:1	7	11	"

Two more horses were started, June, 1930. The injections were given intradermally in one case and subcutaneously in the other. As will be seen from the protocols, antibody content of the serums rose from an apparent zero value to the vicinity of 25.1 in 5 months in each case.

TABLE V

Monkey No.	Mixture inoculated	Virus No.	Serum tested	Ratio virus to serum	Paralysis appeared	Death	Result
	cc.				*days*	*days*	
383	0.5	402	Horse 7, serum before treatment	1:1	10	13	Poliomyelitis
417	0.3	402	Control	—	7	7	"
418	0.1	402	"	—	7	10	"
391	0.5	445	Horse 7, after 7 wks.	2.5:1	—	—	No infection
454	0.15	445	Control	—	6	6	Poliomyelitis
452	0.05	445	"	—	7	11	"
448	0.4	9	Horse 7, after 11 wks.	5:1	—	—	No infection
375	0.4	9	" 7, " 11 "	10:1	—	—	" "
447	0.3	9	Control	—	10	12	Poliomyelitis
455	0.15	9	"	—	10	12	"
457	0.5	9	Horse 7, after 17 wks.	20:1	—	—	No infection
463	0.15	9	Control	—	11	14	Poliomyelitis
469	0.4	457	Horse 7, after 25 wks.	30:1	9	11	"
460	0.3	457	Control	—	11	40	"
461	0.15	457	"	—	11	23	"
442	0.4	9	Horse 8, before treatment	1:1	7	12	"
447	0.3	9	Control	—	10	12	"
455	0.15	9	"	—	10	12	"
456	0.5	9	Horse 8, bled after 9 wks.	5:1	—	—	No infection
458	0.5	9	" 8, " " 9 "	20:1	—	—	" "
463	0.15	9	Control	—	11	14	Poliomyelitis
470	0.4	10	Horse 8, after 9 wks.	30:1	9	17	"
475	0.3	10	Control	—	8	11	"
458	0.15	10	"	—	8	40	"

In the case of these two animals, over a portion of the immunizing period, 0.1 per cent of alum was incorporated in the saline used in preparing the emulsions.

Again, in May, 1930, three additional horses were put on the immunizing treatment. By August, two of these horses were found to have responded. The injections were given subcutaneously, intra-

muscularly and intravenously in each animal. The third horse failed to respond satisfactorily and was discarded. Concentrated preparations from the two producing animals showed complete neutralization in 100:1 or better.

That the mechanism of virus neutralization by the horse serums might be explained by some non-specific phenomenon has been advanced. It is true that the immunization treatment brings forth a decided response with respect to the nerve tissue necessarily contained in the inoculum. This is readily demonstrated by complement fixation or simple flocculation procedures. However, it is hardly conceivable that this flocculation of emulsified cord by the antiserum could inactivate the virus by entrainment or otherwise in view of proof that serum preparations are still potent after removal therefrom of the antinerve tissue bodies by absorption. A monkey was intracranially injected with an incubated mixture composed of virulent cord emulsion and a serum preparation which had previously been incubated for 2 hours with a heavy (20 per cent) emulsion of normal monkey cord and subsequent centrifugalization. Furthermore, horses which have made no antivirus response have acquired anticord properties.

Pettit (6) and others have advocated that in the absence of suitable antipoliomyelitis serum other serums or indeed any protein substance, *e.g.* milk, may be used intraspinally with amelioration of symptoms effected by the non-specific protein shock resulting therefrom. Our results gleaned from the treatment of infected monkeys tend to indicate that the chance for recovery is in direct ratio to the virucidal potency of the serum used. In addition, the results obtained when the serum preparations were used intraspinally were far better than similar treatment introduced through any other route, even though the amount of serum injected were 3 to 5 times as much. If the spinal cord is chosen as the most advantageous route for treatment, it follows that concentration of antibodies is of prime importance in view of the limited capacity of the canal. (1 to 2 cc. in small monkeys and 5 to 20 cc. in children.)

Having acquired a particularly potent strain of monkey virus which proved sufficiently invasive to produce typical poliomyelitis when the emulsion was dropped into the noses of the monkeys on 3 successive days, experiments represented in the following charts were conducted,

with a view to the determination of the relative merits of human convalescent serum and the concentrated horse product when used intravenously and intraspinally. The virus is doubtless transmitted by way of the nasal mucosa in human contacts so these results should prove of some value from the standpoint of human therapeutics. So

TABLE VI

Monkey No.	Mixture inoculated	Virus No.	Serum tested	Ratio virus to serum	Paralysis appeared	Death	Result
	cc.				*days*	*days*	
448	0.5	445	Horse 9, after 12 wks.	2.5:1	—	—	No infection
440	0.4	9	" 9, " 12 "	5:1	—	—	" "
441	0.4	9	" 9, " 12 "	10:1	—	—	" "
454	0.15	445	Control	—	6	6	Poliomyelitis
452	0.05	445	"	—	7	11	"
448	0.4	9	Horse 9, after 20 wks.	20:1	10	20	"
375	0.3	9	Control	—	10	11	" —killed
413	0.1	9	"	—	12	15	" "
471	0.4	10	Horse 9, after 32 wks.	20:1	—	—	No infection
474	0.4	487	" 9, " 32 "	25:1	—	—	" "
475	0.3	10	Control	—	8	11	Poliomyelitis
458	0.15	10	"	—	8	40	"
494	0.3	487	"	—	7	7	"
495	0.15	487	"	—	9	9	"
451	0.5	445	Horse 10, after 10 wks.	2.5:1	7	11	"
454	0.15	445	Control	—	6	6	"
452	0.05	445	"	—	7	11	"
439	0.5	9	Horse 10, after 18 wks.	2.5:1	—	—	No infection
439	0.4	9	" 10, " 18 "	10:1	8	14	Poliomyelitis
463	0.15	9	Control	—	11	14	"
463	0.15	9	"	—	11	14	"
413	0.1	9	"	—	12	15	"

far, we have not found curative measures to be effective when the monkey is inoculated intracranially.

These data were presented by Weyer in a preliminary report read before the American Association of Immunologists on April 16th, 1930, and confirmation of the results with our serum concentrates has been more recently presented by Rhoads (10).

				8 controls														
Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	⊕	⊕	⊕						1	4	2	1		-	All	died	-	
No.	Human convalescent serum, intraspinally (S), about 1.0 cc																	
394	⊕	⊕	⊕			S				Paral.				Dying, K.				
395	⊕	⊕	⊕					S		Paral.				Dying, K.				
409	⊕	⊕	⊕			S							Paral.				Died	
412	⊕	⊕	⊕					S									Survived	
	Horse globulin, intravenously (V), 5.0 cc																	
377	⊕V	⊕	⊕V														Survived	
380	⊕	⊕	⊕		V		V							Paral.				Died
383	⊕	⊕	⊕				V										Survived	
413	⊕	⊕	⊕			V											Survived	
407	⊕	⊕	⊕				V				Paral.			Died				
411	⊕	⊕	⊕					V									Survived	
	Horse globulin, intraspinally (S), about 1.0 cc																	
382	⊕	⊕	⊕S		S												Survived	
375	⊕	⊕	⊕		S		S										Survived	
406	⊕	⊕	⊕			S											Survived	
376	⊕	⊕	⊕				S										Survived	
404	⊕	⊕	⊕				S										Survived	
403	⊕	⊕	⊕					S									Survived	
430	⊕	⊕	⊕						S								Survived	
428	⊕	⊕	⊕							S							Survived	
432	⊕	⊕	⊕								S				Paral.			Died

CHART 2

The results indicated in Chart 2 tend to show that the horse antibody preparations are more effective when used intraspinally than even greater amounts directed into the blood stream. Four monkeys out of six, treated intravenously survived as contrasted with the eight animals out of nine which failed to come down after intraspinous treatment even though instituted later in two cases.

One intraspinous injection is apparently as effective as two; material removed on the 2nd day after an injection is similar in physical properties at least to the injected globulin preparation.

		1 wk. later	2 wks.	3 wks.	4 wks.	5 wks.	6 wks.
421	20 cc. human convalescent	⊕ ⊕ ⊕		Died, poliomyelitis			
422	20 cc. human convalescent			⊕ ⊕ ⊕			Died, poliomyelitis
419	10 cc. horse globulin	⊕ ⊕ ⊕			(Survived)	⊕ ⊕ ⊕	(Survived)
420	10 cc. horse globulin		⊕ ⊕ ⊕				(Survived)
411	5 cc. horse globulin				⊕ ⊕ ⊕		(Survived)
403	1 cc. horse globulin				⊕ ⊕ ⊕		Died, poliomyelitis
424	Control	⊕ ⊕ ⊕		Died, poliomyelitis			

CHART 3

The fact that four out of five of the animals treated with human convalescent serum died may be attributed to the fact that the antibody concentration was not sufficient to prevent extension of the infection.

Observations on Prophylaxis

The value of the serum as a preventative can only be judged by the results of the few experiments shown in tabular form. 10 cc. of the globulin concentrate from an immunized horse was apparently responsible for protection over a period of at least 5 weeks. Twice the amount of human convalescent serum failed to protect for 3 weeks.

The results from these experiments cause us to consider at once the possibility of utilizing an antipoliomyelitis preparation for the purpose of protecting children in a family where a case of poliomyelitis has been reported. The administration of serum would probably be of more worth from the psychological point of view in allaying the fears of parents. Statistics would indicate that a second case in one family in interepidemic times is even rarer than the normal incidence for poliomyelitis in general. Flexner has already suggested the advisability of thus using convalescent serum.

More recently, Fairbrother (11), also aided by the Jeremiah Milbank gift has obtained an antipoliomyelitic horse serum which, subsequently, yielded a potent product.

SUMMARY

1. The horse, apparently itself unsusceptible to poliomyelitis, can be stimulated in certain cases but not all to the production of virucidal antibodies.

2. The virucidal potency of such immune serum can be raised to a point comparable to that of human convalescent serum and when concentrated and refined exhibits a four-fold increase in potency.

3. Such concentrates have proved effective in the prevention of paralysis in inoculated monkeys when given intraspinously before the onset of paralysis.

4. Treatment has been found more effective when therapeutic serums are given through the spinal route.

5. Pooled serum from "normal" adult donors has proved effective in neutralizing virus but its potency is approximately one-half that of convalescent serum.

BIBLIOGRAPHY

1. Netter, A., *Bull. Acad. méd.*, Paris, 1915, **74**, 3, 403.
2. Levaditi, C., and Landsteiner, C., *Compt. rend. Soc. biol.*, 1910, **68**, 311.
3. Aycock, W. L., and Kagan, J. R., *J. Immunol.*, 1927, **14**, 85.
4. Flexner, S., *J. Am. Med. Assn.*, 1910, **55**, 1105.
5. Neustaedter, M., and Banzhaf, E. S., *J. Am. Med. Assn.*, 1917, **68**, 1531.
6. Pettit, A., *Compt. rend. Soc. biol.*, 1918, **81**, 1087.
7. Stewart, F., and Haselbauer, P., *J. Exp. Med.*, 1928, **48**, 449.
8. Weyer, E. R., Park, W. H., and Banzhaf, E. J., *Am. J. Path.*, 1929, **5**, 517.
9. *Coll. Studies Bureau Lab., Dept. Health, New York*, 1912–1913, **7**, 115.
10. Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 123.
11. Fairbrother, R. W., *Brit. J. Exp. Path.*, 1930, **11**, 43.