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Comparative Effectiveness of Various Chemical Sprays in Protecting Monkeys against Nasally Instilled Poliomyelitis Virus.

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We reported[1] the protective action of sodium alum and tannic acid instillations against nasally instilled poliomyelitis virus in *Rhesus* monkeys. It was pointed out that the action of these chemicals was dependent on their concentration and was exerted on the nasal mucosa of the host rather than on the virus, although it appeared from one experiment that the potency of the test dose of virus influenced the results. Upon resuming this work at a later date we were unable to repeat our original results with sodium alum. Investigation of this failure disclosed the participation of the following factors: (1) the sodium alum which we used in the original experiments was assumed to have 24 molecules of water of hydration (as indicated by the manufacturers' label) while actually it possessed only 2 molecules and contained some insoluble Al_2O_3; (2) the sodium alum used in subsequent experiments really had approximately 24 molecules of water of hydration, had no Al_2O_3 and gave a clear solution, so that the actual amount of $Na_2SO_4 . Al_2(SO_4)_3$ in a 4% solution of this preparation was in the ineffective range; (3) that the virus used in the subsequent experiments had become for some unknown reason considerably more potent; while originally only about 80% of control monkeys developed poliomyelitis, all untreated animals now succumbed even when the dose of virus was reduced.

In an attempt to elucidate further the mechanism of the protective action of these chemicals as well as to find the most effective substance, experiments were performed with a number of astringents which are used in human beings for one purpose or another. Rather than reduce the test dose of virus to a point where only a majority of untreated monkeys would develop poliomyelitis and have a larger number of agents exhibit protective properties, the more highly infective amount of virus was used (regularly paralyzing all untreated monkeys) to discover whether under these conditions any of the substances would still be effective. It was furthermore realized

[1] Sabin, A. B., Olitsky, P. K., and Cox, H. R., *J. Bact.*, 1936, **31**, 35; *J. Exp. Med.*, 1936, **63**, 877.

that a reliable comparison of the effectiveness of various chemicals can be made only when all the substances to be compared are used in a single experiment under identical conditions of treatment and infection—for even with 100% infectivity the potency of various virus preparations can vary in different experiments.

The astringent solutions were sprayed into the nostrils by hand with a DeVilbiss atomizer, delivering approximately 1.5 cc. of the solution in each side of the nose by means of 10 to 15 bulb compressions. The monkeys were thus treated once a day for 7 days, and 2 days after the last spraying poliomyelitis virus was instilled into the nose. The monkeys were then observed for a month, and those which survived and failed to develop paralysis were, without any additional chemical treatment, again instilled with virus, the processes being repeated until practically all monkeys became paralyzed, thus indicating the duration of chemical protection. The virus consisted of pooled glycerolated bits of spinal cords from 4 to 6 paralyzed monkeys, ground up to a 5% suspension in physiological saline solution and centrifuged only enough to eliminate the alundum and large tissue particles. One cc. of this suspension was dropped into each nostril in the morning and then again in the afternoon of the same day.

The following substances were studied: (1) 4% tannic acid; (2) 4% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$ including suspended Al_2O_3; (3) 7% $Na_2SO_4 . Al_2(SO_4)_3 . 24H_2O$; (4) 7% $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$; (5) 0.5% picric acid —0.5% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$; (6) 1% $ZnSO_4 . 7H_2O$ (granular and crystalline); (7) 5%

TABLE I.

Chemical used	Days after last chemical treatment poliomyelitis virus instilled in nose							
	2		30		64		93	
	Polio	No Polio	Polio	No Polio	Polio	No Polio	Polio	No Polio
Untreated controls	8	0	4	0	3	0	–	–
4% tannic acid	0	4	2	2	2	0	–	–
1% $ZnSO_4 . 7H_2O$ (granular)	0	4*	0	1	1	0	–	–
5% $FeSO_4 . 7H_2O$	1	3†	0	2	1	1	0	1‡
0.5% picric acid— 0.5% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$	1	3	3	0	–	–	–	–
4% $Na_2SO_4 . Al_2(SO_4)_3 . 2H_2O$ (including suspended Al_2O_3)	2	2	2	0	–	–	–	–
7% $Na_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	1	2	2	0	–	–	–	–
7% $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	1	3	0	3	1	2	0	2‡

* Three of these 4 monkeys and one of the 3 marked † died of tuberculosis at the end of one month and showed no microscopical evidence of poliomyelitis.

‡ These 3 monkeys are being studied to determine whether they developed specific immunity as a result of the repeated virus instillations which they resisted.

$FeSO_4 . 7H_2O$; (8) Iron Dialyzed (Colloidal) Merck—undiluted, and (9) diluted 1:3. Including untreated controls, 104 monkeys were used. The results are presented in 4 tables, each table containing the data of a series of simultaneous tests.

In the first series of tests (Table I) tannic acid, $ZnSO_4 . 7H_2O$, $FeSO_4 . 7H_2O$, and $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$ emerged as highly efficient agents on the basis of the 2- and 30-day tests; the sodium alum solutions and the picric acid mixture had some effect when the virus was given 2 days after the last chemical treatment but at 30 days all the monkeys succumbed. Without any change in procedure or technique except perhaps for an unknown increase in the potency of the virus, subsequent tests (Tables II, III, IV) further eliminated tannic acid, potassium alum, ferrous sulphate, and colloidal

TABLE II.

Chemical used	Days after last chemical treatment poliomyelitis virus instilled in nose			
	2		49	
	Polio	No polio	Polio	No polio
Untreated controls	6	0	4	0
4% tannic acid	6	0	–	–
7% $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	5	0	–	–
5% $FeSO_4 . 7H_2O$	5	1	1	0
1% $ZnSO_4 . 7H_2O$ (granular)	1	5	3	2

TABLE III.

Chemical used	Days after last treatment poliomyelitis virus instilled in nose			
	2		30	
	Polio	No polio	Polio	No polio
Untreated controls	3	0	4	0
7% $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$	2	1	–	–
1% $ZnSO_4 . 7H_2O$ (granular)	0	3	0	3

TABLE IV.

Chemical used	Virus 2 days after last chemical treatment	
	Polio	No polio
Untreated controls	4	0
1% $ZnSO_4 . 7H_2O$ (crystalline)	0	4
4% tannic acid (Baker and Adamson)	2	1
4% tannic acid (Mallinckrodt)	2	1
Dialyzed (colloidal) iron (Merck) undiluted	3	1
Colloidal iron—1 part + Distilled water—2 parts	4	1

iron, leaving zinc sulphate as the most effective protective agent of all the substances investigated. This high efficiency of zinc sulphate is in agreement with recent observations of Schultz and Gebhardt.[2] With this very potent test dose of virus zinc sulphate protected 16 of 17 monkeys tested at 2 days, all of 4 at 30 days and 2 of 5 at 49 days after the last chemical treatment.

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P-Aminobenzenesulfonamide and Antipneumococcal Serum Therapy in Type I Pneumococcal Infections of Rats.

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Although Hörlein[1] claimed that Prontosil was effective against Type III pneumococcal infections, experimental proof for this assertion was lacking until Rosenthal[2] and Cooper, Gross, and Mellon[3] independently investigated this problem.

They demonstrated that *p*-aminobenzenesulfonamide gave mice a certain degree of protection against lethal doses of the particular strains of Type III pneumococcus employed. Buttle, Parish, McLeod, and Stephenson,[4] however, were unable to demonstrate any significant protection in mice infected with Types I and II pneumococci, whereas Rosenthal[2] obtained protection against all 3 fixed types.

The lack of parallelism between pneumococcal septicemia in mice and pneumonia in man led to the choice of the experimental pneumococcal pneumonia in the rat[5, 6] as a closer approximation to human pneumonia. The encouraging results obtained by treating such experimental Type III pneumonia with *p*-aminobenzenesulfonamide[7, 8]

[2] Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 524.

[1] Hörlein, H., *Proc. Royal Soc. Med.*, 1936, **29**, 321.

[2] Rosenthal, S. M., *Public Health Reports*, 1937, **52**, 48.

[3] Cooper, F. B., Gross, P., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 148.

[4] Buttle, G. A. H., Parish, H. J., McLeod, M., and Stephenson, D., *Lancet*, 1937, **1**, 681.

[5] Nungester, W. J., and Jourdonais, L. F., *J. Bact.*, 1935, **29**, 34.

[6] Gunn, F. D., and Nungester, W. J., *Arch. Path.*, 1936, **21**, 813.

[7] Gross, P., and Cooper, F. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 225.

[8] Cooper, F. B., and Gross, P., to be published.