on spores, but by L-alanine and perhaps by other amino-acids which are probably released by the proteolytic action of the enzyme during its incubation.

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[1] Gould, G. W., and Hitchins, A. D., *J. Gen. Microbiol.*, **33**, 413 (1963).
[2] Gould, G. W., and Hitchins, A. D., *Spores III*, 213, edit. by Campbell, L. L., and Halvorson, H. O. (American Soc. Microbiol., Ann Arbor, Michigan, 1965).
[3] Gould, G. W., Georgala, D. L., and Hitchins, A. D., *Proc. Third Intern. Symp. Fleming's Lysozyme*, **1b**, 61 (Scuola Arti Grafiche O.S.F., Milan, 1964).
[4] Vinter, V., *Folia Microbiol.*, **5**, 217 (1960).
[5] Sierra, G., *Canad. J. Microbiol.*, **10**, 929 (1964).
[6] Hills, G. M., *Biochem. J.*, **45**, 363 (1949).
[7] Fey, G., Gould, G. W., and Hitchins, A. D., *J. Gen. Microbiol.*, **35**, 229 (1964).

VIROLOGY

Marker Studies of Poliovirus

VARIATION in the chemical properties of the protein coat of different strains has been used as a marker test in investigations of the genetics of polioviruses. Bengtsson, Philipson, Persson and Laurent[1] recently showed that a group of these tests had a common basis. Small differences in the electrical charge of the protein of various strains of the same serotype resulted in different patterns of binding to charged adsorbents.

The *d* (ref. 2) and *m* (ref. 3) markers and the inhibition of plaque formation by dextran sulphate[4] measure depression of growth in the presence of polyanions, while the aluminium adsorption[5] and elution tests[6] and elution from DEAE cellulose[7] depend on the physical separation of infectious particles by differential adsorption.

For application on an epidemiological scale, a test of this property which is simple to perform and which conserves both materials and labour is desirable. The possibility that tube titration in the presence of a low concentration of dextran sulphate might meet these requirements was therefore investigated.

Primary rhesus monkey kidney cell cultures were used, with the control series maintained on medium 199 alone and the test series on medium 199 containing dextran sulphate 2000 (Pharmacia, molecular weight 2×10^6) in the concentrations shown in Tables 1 and 2. Virus dilutions were made at $\log_{10}$ intervals and two tissue culture tubes in each series were inoculated per dilution. The viruses tested are shown in Tables 1 and 2, and the results, calculated by the Kärber method, are based on readings for day 6.

Table 1 shows that the poliovirus type 1 vaccine strain (*LSc2ab*) can readily be differentiated from the type 1 Mahoney strain by titration in 0·05 per cent dextran sulphate. The poliovirus type 2 and type 3 prototype strains are not, however, clearly separated under any of the conditions tested by such a minimal technique. This is reminiscent of the findings of Wallis, Melnick and Wimberley[5], who showed large differences in the adsorption of poliovirus type 1 strains to aluminium salts but less satisfactory results for types 2 and 3.

Table 2 shows the results of tests on two groups of poliovirus type 1 strains: (*a*) strains isolated from patients with clinical poliomyelitis in the prevaccine years; (*b*) strains isolated from subjects given oral poliomyelitis vaccine.

The groups are clearly separated by the test, although the small range of values may limit its usefulness when applied to an individual strain. The titrations were carried out using minimum numbers of tubes. Greater accuracy would be obtained by increasing the number of tubes inoculated per dilution but this would mean corresponding increase in the labour and materials used.

Table 1. REDUCTION IN VIRUS TITRE OF PROTOTYPE POLIOVIRUS STRAINS IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF DEXTRAN SULPHATE

Strain	Titre in 199	$\log_{10}$ reduction in the presence of dextran sulphate 0·005%		0·05%	0·5%
			Type 1		
Mahoney	$10^{6\cdot0}$	−1·0		0	−0·5
	$10^{6\cdot0}$	−0·5		−0·5	−0
	$10^{6\cdot0}$			+0·5	
	$10^{6\cdot5}$			0	
	$10^{6\cdot5}$			−0·5	
			Average	−0·1	
			Standard deviation	0·31	
Sabin 1	$10^{6\cdot5}$	−0·5		−3·0	−1·0
	$10^{6\cdot0}$	−1·5		−1·5	−2·5
	$10^{6\cdot0}$			−2·5	
	$10^{5\cdot5}$			−3·0	
	$10^{6\cdot0}$			−2·5	
			Average	−2·5	
			Standard deviation	0·55	
			Type 2		
YSK	$10^{5\cdot5}$	0		−2·0	−1·5
	$10^{5\cdot5}$	0		−0·5	−0·5
	$10^{5\cdot5}$			−1·5	
	$10^{5\cdot5}$	−1·0		−3·0	−1·0
Sabin 2	$10^{5\cdot5}$	−0·5		−2·0	−1·5
	$10^{5\cdot5}$			−2·0	
			Type 3		
Saukett	$10^{6\cdot5}$	+0·5		−1·0	−0·5
	$10^{6\cdot0}$	−0·5		0	0
	$10^{6\cdot5}$			0	
Sabin 3	$10^{5\cdot5}$	−1·0		0	0
	$10^{6\cdot0}$	−0·5		−1·0	−1·0
	$10^{6\cdot0}$			−1·5	

Table 2. COMPARISON OF NATURALLY DERIVED AND VACCINE STRAINS OF POLIOVIRUS TYPE 1

Virus reference lab. No. 3	Year	Clinical	Passage	Titre in 199	$\log_{10}$ reduction in 0·05% dextran sulphate
4657	1953	Paralytic polio	*MK*2	$10^{5\cdot5}$	+0·5
4862	1953	,,	,,	$10^{7\cdot0}$	+0·5
4863	1953	,,	,,	$10^{6\cdot5}$	0
5037	1953	,,	,,	$10^{6\cdot0}$	+1·0
5038	1953	,,	,,	$10^{7\cdot5}$	0
15	1957	,,	,,	$10^{7\cdot5}$	−1·0
17	1957	,,	,,	$10^{7\cdot5}$	0
20	1957	,,	,,	$10^{6\cdot5}$	+0·5
55	1957	,,	,,	$10^{6\cdot5}$	0
73	1957	,,	,,	$10^{7\cdot0}$	+0·5
				Average	+0·2
				Standard deviation	0·42
		Weeks post vaccination			
568	1962	1	*MK*1	$10^{5\cdot0}$	−2·0
569	1962	2	,,	$10^{5\cdot5}$	−1·5
570	1962	2	,,	$10^{6\cdot5}$	−2·0
183	1962	3	,,	$10^{6\cdot0}$	−2·0
474	1962	2	,,	$10^{6\cdot5}$	−2·5
496	1962	1	,,	$10^{7\cdot0}$	−2·0
498	1962	2	,,	$10^{7\cdot0}$	−2·0
551	1962	1	,,	$10^{6\cdot5}$	−1·0
751	1965	2	,,	$10^{7\cdot0}$	−2·0
812	1965	1	,,	$10^{5\cdot5}$	−1·0
				Average	−1·8
				Standard deviation	0·46

*MK*1 = first passage in rhesus monkey kidney.
*MK*2 = second ,, ,, ,, ,, ,,

The test should be applicable to the epidemiological study of type 1 polioviruses. This is the type mainly associated in the past with paralytic poliomyelitis. It has also been the cause of the limited outbreaks of poliomyelitis which have occurred in Britain since vaccination became widespread.

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[1] Bengtsson, S., Philipson, L., Persson, H., and Laurent, T. C., *Virology*, **24**, 617 (1964).
[2] Vogt, M., Dulbecco, R., and Wenner, H. A., *Virology*, **4**, 141 (1957).
[3] Agol, V. I., and Chumakova, M. Y., *Acta Virol. (Prague)*, **7**, 97 (1963).
[4] Takemoto, K. K., and Kirschstein, R. L., *J. Immunol.*, **92**, 329 (1964).
[5] Wallis, C., Melnick, J., and Wimberley, J., *Amer. J. Hyg.*, **77**, 250 (1963).
[6] Woods, W. W., and Robbins, F. C., *Proc. U.S. Nat. Acad. Sci.*, **74**, 150 (1961).
[7] Hodes, H. L., Zepp, H. D., and Ainbender, E., *Virology*, **11**, 306 (1960).