POLIOVIRUS AS AN ANTIGEN

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(With 1 Figure in the Text)

The use of killed poliovirus as an antigen has produced considerable speculation as to whether sufficient virus protein is injected to account for the observed antibody responses. Three possible interpretations have been offered at one time or another: (*a*) the vaccine contained sufficient antigen to induce the observed response; (*b*) the vaccine contained a small amount of live virus which replicated forming more antigen without producing central nervous system symptoms; (*c*) the vaccine contained incomplete forms which can replicate for one or two cycles forming more antigen but without producing central nervous system symptoms. It would appear that sufficient data are now on hand to decide which of these hypotheses is correct.

The antigenicity of an antigen may be expressed in one of two ways:

(i) By calculation of the ratio of antibody (*Ab*) produced to antigen (*Ag*) given using appropriate units; this has the disadvantage of being dependent upon the dose of *Ag* used, since the dose-response is not usually linear (Stevens, 1956).

(ii) A more satisfactory method is by calculation of K_1 from the equation

$$K_1 c^{1/n} = Ab \tag{1}$$

where K_1 is a constant, c the concentration of antigen given in moles/kg., n is a constant dependent upon the chemical constitution of the *Ag*, and *Ab* is the maximum concentration of serum antibody in moles/l. (Stevens, 1956).

We may consider diphtheria toxoid as an example. It has been shown that the value for n for protein antigens is about 1·9 (Stevens, 1956). When men having low titres of antitoxin were given 1 Lf of purified fluid toxoid subcutaneously, the average booster or secondary response was 56 units/ml. of neutralizing *Ab* in the serum (Edsall, Banton & Wheeler, 1951). The conversion data for diphtheria calculations are taken from Kabat & Mayer (1948). To compute the *Ag* dose, 1 Lf = $0{\cdot}46 \times 10^{-6}$ g. N = 3×10^{-6} g. protein. Dividing by body weight of 70 kg., we secure $4{\cdot}3 \times 10^{-8}$ g./kg. The molecular weight (MW) of diphtheria toxin is about 70,000. Since the toxin has the same sedimentation constant (Pillemer & Robbins, 1949) as the toxoid (Pillemer, Toll & Badger, 1947), the MW is probably the same. This MW would give an antigen dose of $6{\cdot}1 \times 10^{-13}$ moles/kg. For *Ab* calculations, 1 unit of *Ab* = $1{\cdot}6 \times 10^{-6}$ g. N or 1×10^{-5} g. protein. Hence 56 units/ml. = 56×10^{-2} g. protein/l. The MW of antibody is 160,000, giving an antibody value of $3{\cdot}5 \times 10^{-6}$ moles/l. We may now solve for K_1.

$$\log K_1 = \log Ab - 1/n \log c \tag{2}$$

$$K_1 = 10{\cdot}4$$

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For the primary response to diphtheria toxoid we may use the data of Jensen (1933). Nine children with an average age of 5 years were given 150 Lf of toxoid and developed geometric mean maximum titres of $8{\cdot}3 \times 10^{-3}$ units/ml. Hence K_1 for the primary response is $5{\cdot}2 \times 10^{-5}$.

The direct Ab/Ag ratios are calculated as follows. The antibody response for the secondary was 56×10^{-2} g./l. Dividing this by the antigen dose of $4{\cdot}3 \times 10^{-8}$ g./kg. gives a ratio of $1{\cdot}3 \times 10^{7}$. For the primary response a ratio of 3·4 is found.

We may now calculate values for the secondary response to poliovirus. Since poliovirus is a nucleoprotein (Schwerdt & Schaffer, 1955), we may expect n to be around 1·9. However, we have available experimental data to support this for the range of Ag dosage used clinically. Salk (1955) gave children varying doses of vaccine and we may graph a dose-response curve to determine n. When the geometric mean titres (GMT) are used, for Type 1, $n = 2{\cdot}0$; Type 2, $n = 1{\cdot}4$; Type 3, $n = 2{\cdot}0$. From this it would appear that Type 2 acted differently from both other Types and other proteins. For a uniform series, the GMT and the median should agree closely. If we compute the values for n based on medians rather than means, we secure, Type 1, $n = 2{\cdot}0$; Type 2, $n = 1{\cdot}8$; Type 3, $n = 2{\cdot}0$. Hence it appears justified to use the value of 1·9 for n for all three types.

Since the response to Type 2 gave the highest Ab titres, we shall start with it. Salk (1955) gave eight children a booster injection of reference vaccine A, 19 months after the first injection. This was the fourth injection, 1 year after the third and should have elicited a very adequate secondary response. This vaccine contained 2×10^{7} $TCID_{50}$/ml. before inactivation (Salk, Bazeley & Rotundo, 1955). Tissue culture fluids with a titre of 5×10^{6} $TCID_{50}$/ml. have been shown to contain approximately $0{\cdot}3 \times 10^{-6}$ g. virus/ml. (Schwerdt & Schaffer, 1955; Charney, 1956), after correction for losses in purification. Hence the Salk preparation contained about $1{\cdot}2 \times 10^{-6}$ g. virus/dose. The average weight of the children (6–8 years old) would be 24 kg. (Nelson, 1950), giving a dose of 5×10^{-8} g./kg. The weight of the virus is stated to be $1{\cdot}4 \times 10^{-17}$ g. (Schwerdt & Schaffer, 1955) which gives a molecular weight of $8{\cdot}6 \times 10^{6}$. The dose would be $5{\cdot}8 \times 10^{-15}$ moles of virus/kg. If the antigen is actually a subunit, the moles of antigen will be increased and K_1 will become smaller.

This amount of antigen given intramuscularly produced a geometric mean Ab titre of 6000/ml. *v.* 100 $TCID_{50}$ of virus. Dulbecco, Vogt & Strickland (1956) have found that when Ab is dilute, one molecule of Ab neutralized one infectious poliovirus particle, although in the presence of excess Ab, up to 15 molecules of Ab can be adsorbed. Since the neutralization test involves limit dilution of Ab, it is not in excess. Using the upper figure of 15 will only alter the result by an order of magnitude. The ratio of physical particles to plaques is at most 1000 (Schwerdt & Schaffer, 1955) and may be as low as thirty (Schwerdt & Schaffer, 1956). Since the higher figure was secured by the use of systems more comparable to those employed in antibody determinations, this value will be used. If there are only thirty particles per infectious unit, the calculations derived will be about 33-fold too high. Hence 6000 titre/ml. $\times$ 100 $TCID_{50} \times 1$ molecule Ab/particle $\times$ 1000

particles/plaque × 1000 ml./l. gives 6×10^{11} molecules of *Ab*/l. or 1×10^{-12} moles *Ab*/l. Solving for K_1,

$$\log K_1 = (\log 1 + \log 10^{-12}) - 1/1{\cdot}9\ (\log 5{\cdot}8 + \log 10^{-15})$$

$$K_1 = 3{\cdot}5 \times 10^{-5}.$$

For direct ratio calculations, *Ab* = 6000 titre/ml. × 100 $TCID_{50}$ × 1 *Ab* molecule/virus particle × 1000 ml./l. = 6×10^8 neutralizing *Ab* units/l. The vaccine had an initial titre of 2×10^7 divided by 24 kg. gives $8{\cdot}4 \times 10^5$ infectious units of virus/kg. This gives a ratio of $7{\cdot}2 \times 10^2$.

Table 1. *Antigenicity of poliovirus*

Type	Primary or secondary	Original vaccine titre	GMT after 1 dose	K_1	*Ab*/*Ag*
1	P	$10^{7{\cdot}8}$	26	$8{\cdot}1 \times 10^{-8}$	1·0
2	P	$10^{7{\cdot}3}$	23	$1{\cdot}3 \times 10^{-7}$	2·8
3	P	$10^{7{\cdot}0}$	42	$3{\cdot}5 \times 10^{-7}$	1×10^1
1	S	$10^{7{\cdot}8}$	1500	$3{\cdot}1 \times 10^{-6}$	$5{\cdot}8 \times 10^1$
2	S	$10^{7{\cdot}3}$	6000	$3{\cdot}5 \times 10^{-5}$	$7{\cdot}1 \times 10^2$
3	S	$10^{7{\cdot}0}$	1000	$8{\cdot}1 \times 10^{-6}$	$2{\cdot}4 \times 10^2$

Column 3 from Salk, Bazeley & Rotundo (1955); column 4 from Salk (1955).

Table 2. *Comparison of antigenicity of diphtheria toxoid and poliovirus Type* 2

		K_1 ratio	*Ab*/*Ag* ratio
Primary	Diphtheria Polio	4×10^2	1·2
Secondary	Diphtheria Polio	3×10^5	$1{\cdot}8 \times 10^4$

Similar calculations can be made for the primary response. In Table 1 are shown the values of K_1 and *Ab*/*Ag* for all three Types for both primary and secondary responses. Since Type 2 gives the highest secondary, this is compared with diphtheria toxoid in Table 2. It is clear that diphtheria toxoid is a much more effective antigen than poliovirus in the secondary response when given in the same dosage as poliovirus on a molar basis, or in much smaller dosage on a weight basis.

To clarify the meaning of the constant K_1, Fig. 1 has been constructed. Since K_1 is the y intercept, it will represent the moles of *Ab*/l. produced by 1 mole of *Ag*/kg. This is obviously a theoretical figure but the actual range of *Ag* used is shown by the points on the lines. From Table 2, when one compares the primary response to diphtheria and polio by the *Ab*/*Ag* ratio, they appear to be the same. The reason for this is that much more diphtheria *Ag* than polio *Ag* was given on primary immunization: 500 times as much on a weight basis; over 50,000 times as much on a molar basis. Equation 1 compensates for these differences in amount and the diphtheria primary response is seen to be some 400-fold greater than the polio response.

Values for purified pneumococcal Type 1 polysaccharide (Heidelberger, MacLeod, Daiser & Robinson, 1946) are also shown in Fig. 1. This is another antigen of

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microbial origin which is more antigenic in man than poliovirus. The direct ratio for this system is $5{\cdot}8 \times 10^4$. The importance of n is emphasized here. K_1 values can only be directly compared if n is the same in both systems. If not, a graph such as Fig. 1 must be constructed and the Ab values read off at the same concentration of Ag.

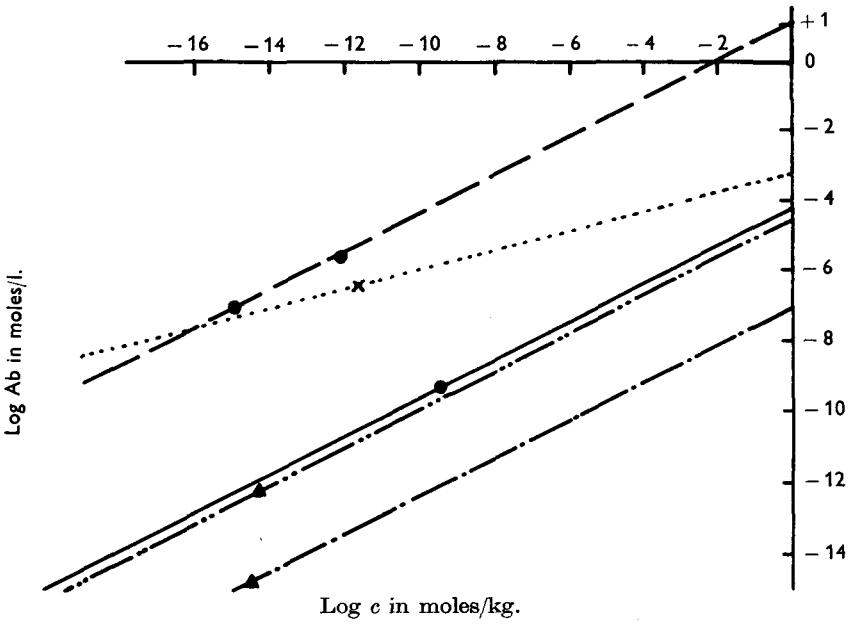

Fig. 1. Plot of Equation 2: $\log Ab = \log K_1 + 1/n \log c$. $n = 1{\cdot}9$ for diphtheria toxoid and poliovirus and 3·6 for the pneumococcus polysaccharide. ●——, Diphtheria toxoid, primary; ●— — —, Diphtheria toxoid, secondary; ×....., Pneumococcus 1, S.S.S., primary; ▲——.—— Polio virus Type 2, primary; ▲——. .——, Polio virus Type 2, secondary.

Irrespective of the possible errors involved in certain assumptions, these calculations would appear to demonstrate that the amount of antigen given in poliovirus vaccine is entirely adequate to produce the amount of antibody found, and that no replicating mechanisms need be postulated.

SUMMARY

Calculations have been carried out which indicate that poliovirus vaccine contains sufficient antigen to account for the observed antibody response.

REFERENCES

CHARNEY, J. (1956). Personal communication.

DULBECCO, R., VOGT, M. & STRICKLAND, A. G. R. (1956). A study of the basic aspects of neutralization of two animal viruses, western equine encephalitis virus and poliomyelitis virus. *Virology*, **2**, 162–205.

EDSALL, G., BANTON, H. J. & WHEELER, R. E. (1951). The antigenicity of single graded doses of purified diphtheria toxoid in man. *Amer. J. Hyg.* **53**, 283–95.

HEIDELBERGER, M., MACLEOD, C. M., DAISER, S. J. & ROBINSON, B. (1946). Antibody formation in volunteers following injection of pneumococci or their type-specific polysaccharides. *J. exp. Med.* **83**, 303–20.

https://doi.org/10.1017/S0022172400037396 Published online by Cambridge University Press

JENSEN, C. (1933). Antitoxin curves in children after active immunization with diphtheria anatoxin, with special reference to the duration of antitoxic immunity. *Acta path. microbiol. scand.* **10**, 137–58.

KABAT, E. A. & MAYER, M. M. (1948). *Experimental Immunochemistry*, Charles C. Thomas Co., Springfield, Ill.

NELSON, W. E. (1950). *Mitchell-Nelson Textbook of Pediatrics*, 5th ed., p. 62, W. B. Saunders Co., Philadelphia, Pa.

PILLEMER, L., TOLL, D. & BADGER, S. J. (1947). Immunochemistry of toxins and toxoids. III. Isolation and characterization of diphtherial toxoid. *J. biol. Chem.* **170**, 571–85.

PILLEMER, L. & ROBBINS, K. C. (1949). The chemistry of toxins. *Annu. Rev. Microbiol.* **3**, 265–88.

SALK, J. E. (1955). Considerations in the preparation and use of poliomyelitis virus vaccine. *J. Amer. med. Assoc.* **158**, 1239–48.

SALK, J. E., BAZELEY, P. L. & ROTUNDO, R. (1955). Personal communication.

SCHWERDT, C. E. & SCHAFFER, F. L. (1955). Some physical and chemical properties of purified poliomyelitis virus preparations. *Ann. N.Y. Acad. Sci.* **61**, 740–53.

SCHWERDT, C. E. & SCHAFFER, F. L. (1956). Purification of poliomyelitis viruses propagated in tissue culture. *Virology*, **2**, 665–76.

STEVENS, K. M. (1956). Some considerations of the antigen dose-antibody response relationship. *J. Immunol.* **76**, 187–91.

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