ESTIMATE OF MOLECULAR EQUIVALENTS OF ANTIBODY REQUIRED FOR PROPHYLAXIS AND THERAPY OF POLIOMYELITIS

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Since the ultimate goal of quantitative biology is to express biological quantities in units capable of use in chemical and physical terms, an attempt has been made to express the concentration of protective antibody (*Ab*) in molar equivalents. In a recent publication from this laboratory (Stevens, 1957), it was shown that neutralizing titres of *Ab* to poliovirus can be converted to molar equivalents of antibody. Data are now available which permit a rough calculation of the moles of *Ab* required for the prevention of poliomyelitis in man and for the treatment of experimental poliomyelitis in monkeys.

Hammon, Coriell, Wehrle & Stokes (1953) have shown that 0·31 ml./kg. of normal human gamma-globulin given intramuscularly to children produced a statistically significant decrease in the incidence of paralytic poliomyelitis for about 5 weeks during exposure to natural infection. In these studies, a child weighing 24 kg. would have received about 7 ml. of gamma-globulin, having an anti-poliovirus neutralizing antibody titre for each of the three types of about 1:3400/ml. when tested against thirty-two tissue culture infective doses 50% ($TCID_{50}$) of virus (Youngner, 1953).

Using the method of calculation referred to above (Stevens, 1957), 1:3400 is computed to represent approximately 1×10^8 anti-poliovirus molecules/ml. of serum for any given type and 7 ml. of such material contains around 7×10^8 anti-poliovirus molecules. Since the extracellular fluid (E.C.F.) comprises about 16% of the body weight (Bland, 1956), a child weighing 24 kg. would have about 3·8 l. of E.C.F. After equilibration, the final concentration of *Ab* would be about 2×10^5 molecules/ml. in the E.C.F. The half-life of gamma-globulin in man is not definitively established, but about 3 weeks appears likely, except in babies in whom it is longer (Dixon, Talmage, Maurer & Deichmiller, 1952; Good & Zak, 1956). The 5-week protective period therefore represents slightly less than twice the half-life. Thus, a serum concentration of 6×10^4 molecules/ml. appears to be protective clinically in some children at least. By the use of Avogadro's number (6×10^{23}) and the molecular weight of antibody ($1{\cdot}6 \times 10^5$), this figure can be converted to mole/l. (1×10^{-16}) or to mg./ml. ($1{\cdot}6 \times 10^{-11}$). As judged by chemical standards, this is an extremely small amount of antibody. Even such biologically active substances as atropine, colchicine and penicillin require from 10^{-8} to 10^{-6} mole/l. for demonstrable activity in man, i.e. well over a million times more molecules.

It may be noted here that the minimal dilution of serum ordinarily assayed in routine tests for neutralizing antibodies against poliovirus is 1:4 when 100 $TCID_{50}$

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of virus are employed and values < 1:4 are considered negative. Yet, a titre of 1:4 would represent considerable antibody, viz. 7×10^{-16} mole/l., which is seven times greater than the clinically protective level.

Only a very small fraction of commercial gamma-globulin is anti-poliovirus *Ab*. The total amount of poliovirus *Ab* against all three poliovirus types is 5×10^{-13} mole/l. in gamma-globulin having a titre of 1:3400 against each of the agents. Commercial poliomyelitis immune globulin contains 165 mg./ml. or 1×10^{3} mole/l. of globulin. Hence, the anti-poliovirus *Ab* present would be less than one part in 10^{9}.

Liu, Carter, De Sanctis, Geating & Hampil (1958), Liu, Carter, Sanders, Smith & Hampil (1959) have shown recently that large doses of hyperimmune rabbit serum (10 ml./kg.) given intravenously to monkeys halted the progress of poliomyelitis in the central nervous system (C.N.S.) when administered 2 days after intraspinal inoculation of 10 $TCID_{50}$ of Mahoney type I poliovirus, at a time when peripheral paralysis was already present. Ten minutes following administration of rabbit antiserum, the serum of the monkey had a neutralizing antibody titre of 1:35,000/ml. when tested with 30 $TCID_{50}$ of Mahoney type I poliovirus (Liu *et al.* 1959). This represents 2×10^{-12} mole/l. of *Ab* in the monkey's serum.

As early as 1900, Ransom found that the distribution ratio of tetanus antitoxin in a horse was one part in the cerebrospinal fluid (C.S.F.) to 400 parts in the serum. Freund (1930) found a mean distribution ratio of 1:300 for typhoid agglutinins in rabbits. Although the distribution of *Ab* between C.S.F. and serum has not been determined in man, the concentration of gamma-globulin in normal human C.S.F. is about 0·02 mg./ml. (Burtin & Pocidalo, 1954; Roboz, 1953) and in plasma about 8 mg./ml. (White, Handler, Smith & Stetten, 1954), giving a distribution ratio of 1:400. Since the distribution ratio of two types of *Ab* in two species of animals is essentially the same as the distribution ratio of gamma-globulin in man, it appears reasonable to assume that poliovirus *Ab* will also have a distribution ratio of about 1:400 in monkeys and man.

Based on a distribution ratio of 1:400, the 2×10^{-12} mole/l. of anti-poliovirus *Ab* in the monkey's serum should produce a C.S.F. *Ab* concentration of 5×10^{-15} mole/l. However, the serum *Ab* level is constantly falling, and Liu (personal communication) found that the level of gamma-globulin at 2 days was 30 % of the 10 min. value. Hence, a more realistic maximum may be $1{\cdot}5 \times 10^{-15}$ mole/l. This amount of *Ab* in the C.S.F. is about 15-fold greater than the concentration of *Ab* required in the blood to protect against the clinical disease in the epidemics studied by Hammon *et al.* (1953). Hence, this intravenously administered antiserum should offer some protection against virus introduced directly into the C.N.S. This amount of antibody did indeed protect against 10 $TCID_{50}$ given intraspinally or 500,000 $TCID_{50}$ given intracerebrally (Liu *et al.* 1959). However, when half this dose of antiserum or less was given, the reduction in protection was disproportionally large. Liu *et al.* (1958) attributed this to a threshold between plasma and C.S.F. which must be exceeded for *Ab* to enter the C.S.F. There are very few examples of true threshold phenomena relating to the distribution of a substance in the animal body. It has been shown that within 40 min. after the intravenous administration

of a few mg. of labelled human albumin into dogs, activity was detectable in the C.S.F. (Fishman, 1953). This indicates either no threshold or an extremely low threshold for human albumin and supports the view that a distribution ratio, and not a threshold, is the limiting factor. In this light, the results of Liu and his colleagues are more compatible with the view that the concentration of antiserum and virus were so related that even a twofold decrease in antiserum provided insufficient *Ab* to neutralize the virus. It is postulated that since the virus was a replicating system while the passively administered *Ab* was not, the critical ratio of virus to antibody was passed yielding very little protection.

In summary, it appears that specific *Ab*, against poliovirus at least, is very much more active on a molar basis than any other type of therapeutic agent currently used in the treatment of any disease. When prophylaxis against poliovirus viraemia is desired, as in the studies of Hammon *et al.* (1953), small quantities of *Ab* given intramuscularly suffice. When therapy of C.N.S. infection is wanted, a greater amount of antibody is required. This appears related to the fact that there is usually more virus present to be neutralized. Much of this virus is intracellular and not immediately accessible to *Ab*, and only a fraction of the antibody introduced reaches the C.N.S. In addition, most cells already infected with virus at the time of globulin administration will be destroyed or damaged irrespective of the amount of antibody administered.

The intravenous route of antibody administration has the obvious disadvantage of wasting most of the *Ab* since only one part in 400 enters the C.S.F., and the volume of the C.S.F. is small in relation to the extracellular volume. Additionally gamma-globulin given intravenously tends to produce some vascular collapse (Liu *et al.* 1959). Because of greater safety, the intramuscular route has been used almost exclusively for the administration of gamma-globulin. However, large volumes cannot be given by this route, and the peak blood levels attained after intramuscular administration of a protein can never equal the levels attained after intravenous injection of the same amount. The futility of the intramuscular administration of antibody is clearly shown in a report by Colio, Criley & Coriell (1958). These investigators gave a patient 10 ml. I.M. of rabbit gamma-globulin having extremely high neutralizing titres to polioviruses and were unable to detect antibody in the C.S.F. When calculations are made to determine the maximum antibody which could be present in the C.S.F., it is found that the titre could not exceed 1:4. Hence, the negative results they obtained would be expected. Theoretically, the most satisfactory method of antibody administration would be by direct injection into the C.S.F. The relatively low potency of currently available gamma-globulin preparations which contain only one part of specific *Ab* in 10^8 to 10^9 parts of globulin necessitates the introduction of such a large mass of globulin as to constitute a hazard. The hope for the future lies, therefore, in development of methods for preparing more highly purified specific antibody which could be administered safely into the C.N.S. Such should have therapeutic interest not only for poliomyelitis but for other diseases of the C.N.S. as well.

SUMMARY

Calculations have been carried out which suggest that as few as 6×10^4 molecules of anti-poliovirus antibody per millilitre of serum may be sufficient to prevent the clinical disease under certain epidemic conditions. Other calculations have been made involving therapy of the experimental disease in monkeys and certain factors required for successful serum therapy have been discussed.

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