Some Observations on the Use of Alum Precipitated Diphtheria Toxoid*

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SINCE Glenny and Barr,[1] in 1931, first described a method for the precipitation of diphtheria toxoid with potassium aluminum sulphate and showed its exceptional antigenic efficiency in animals, evidence has been rapidly accumulating that a single dose will change well over 90 per cent of Schick positive children to Schick negative in 8 weeks. Wells, Graham, and Havens,[2] in 1932, confirmed the observations of Glenny and Barr in guinea pigs and gave the single dose injection to 98 children, all with strongly positive Schick reactions. At the end of 8 weeks all but 6 had been rendered Schick negative. Graham, Murphree, and Gill [3] obtained more than 92 per cent negative Schick reactions in 185 Schick positive children, within 2 to 4 months after a single injection. Baker and Gill [4] in 197 Schick positive children (22 of whom were older than 13 years) obtained negative Schick reactions in 100 per cent after a single dose. McGinnis and Stebbins,[5] using 1 dose of precipitated toxoid and working with a representative number of children, all of whom were Schick tested before and after immunization, concluded that—

One dose of alum precipitated toxoid is as effective as 2 doses of toxoid containing 10 units with 0.2 per cent alum added.

They obtained with 2 doses of toxoid containing 0.2 per cent alum and 1 dose of alum precipitated toxoid, 95.4 and 95.9 per cent Schick negatives, respectively, in 295 and 266 children in the age group 5 to 9 years. Thus there does not seem to be any question as to the efficiency of alum precipitated toxoid as an antigen—it is without doubt the most effective agent yet devised for immunization against diphtheria, but it has certain distinct disadvantages which will be mentioned later.

Most workers have indicated that the probable reason for the extraordinary activity of precipitated toxoid lies in the slow rate of absorption of the comparatively insoluble precipitate, the single injection acting as both the primary and secondary stimulus defined by Glenny and Sudmersen.[6] To test this theory, an experiment was planned wherein the nodule which persists at the site of injection was dissected from the tissues of guinea pigs at weekly intervals, and reinjected into guinea pigs in which the serum had previously been titrated at the 1/250 unit level to insure the absence of antitoxin. Nodules were dissected from 2 to 4 pigs each week up to the 7th, ground in a mortar with salt solution, and reinjected into

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2 test pigs. These test pigs were bled after 6 weeks, the serum of each pair pooled and tested for antitoxin. Antitoxin was present in the serum of all, those reinjected at the 7th week showing 1/5 unit per c.c. Thus it is apparent that 1 dose in the tissues of the guinea pig exerts its antigenic effect for at least 7 weeks. Since induration following injection in children may be elicited for as long as 6 to 8 weeks, it seems reasonable to assume that the stimulation of antitoxin production continues for this period.

This long continued antigenic action not only serves as a constant stimulant to the mechanism of antitoxin production but also seems to produce in a few persons hypersensitiveness to the protein contained in a subsequent Schick test. McGinnes [7] has reported a number of immediate allergic reactions following the Schick test applied 8 weeks after precipitated toxoid, in at least one of which adrenalin was required. Preliminary Schick test in the same children was not followed by unusual reaction. The writer [8] has described the antigenic action of alum precipitated pollen extract as compared to liquid extract in the guinea pig and has shown that the precipitated extract is a much better sensitizing agent than the same extract before precipitation. It is probable that the explanation for the allergic reactions observed by McGinnes lies in this increased sensitizing action of the precipitated toxoid over the fluid toxoid from which it was prepared. It is of considerable interest that the same immunizing procedure is so highly effective in the production of both antitoxic immunity and protein hypersensitiveness.

In a small number of observations it has been noted that vaccination against smallpox, if done as early as 11 days after injection of precipitated toxoid, may be followed by a localization of the virus around the indurated nodule. This localization is shown by an aerola equal in size and character to that surrounding the vaccine pustule, and fades at the same time. Vaccination 21 days after injection of toxoid in 11 subjects showed normal vaccinia in 9, and in 2 there seemed a slight tendency of the virus to localize at the site of toxoid injection. Further studies on this tendency of localization of vaccine virus should be made, the site of toxoid injection being carefully examined from the 9th to the 12th day after vaccination. Simultaneous immunizations offer an excellent opportunity for study of these reactions.

The immediate local or general reactions following precipitated toxoid are no more noticeable than those following crude toxoid. Most workers believe that they are less so. The very slow rate of absorption as evidenced by the persistence of an indurated nodule at the site of injection should decrease the allergic reaction due to hypersensitiveness to diphtheria protein. Toxins intended for the preparation of precipitated toxoid should be grown for as short a period as is consistent with the production of a strong toxin in order that the concentration of autolytic products may be kept as low as possible. Good toxins may be produced in 7 to 8 days; and longer cultivation, while increasing flocculating value, probably adds materially to the number and severity of reactions in sensitive subjects.

During the past few months an increasing number of reports of the occurrence of sterile abscesses following precipitated toxoid have been received. These develop in from 6 to 10 days after injection, are very slightly painful, sterile, and heal readily after incision. Some may open spontaneously while many indurated areas develop small points of fluctuation which go on to resolution without surgical interference. To date, all products reported as hav-

ing caused abscesses have either been manufactured from toxins grown for longer periods than 8 days or have contained an excess of aluminum, or both factors have been found to be present. Manifestly, the injection into the tissues of a comparatively insoluble precipitate will of itself set up some degree of local irritation, and any additional irritating factor will necessarily add to the probability of abscess formation, hence the necessity for keeping the content of aluminum and of bacillary protein at the lowest possible figures. While approximately 1.5 per cent potassium alum which is present in toxoids precipitated with 2 per cent alum seems to cause a degree of induration which is not particularly objectionable, the preparation of toxoids which require less alum for precipitation, reducing the aluminum to a still lower percentage, will represent a distinct improvement in the product. The possibility of a very slight residual toxicity in crude toxoids from which the precipitates are prepared must also be considered. Such toxicity would necessarily be so slight as to escape detection by the routine 5 c.c. doses in guinea pigs observed for 30 days, but might conceivably be sufficient to produce unpleasant reactions during the extended period that the precipitate is in contact with the tissues. This point is being further investigated.

Children under 6 years give a minimum of local and practically no general reactions to precipitated toxoid, and since a large proportion of this age group is susceptible to diphtheria, they may be immunized without prior Schick test. Older children, however, in addition to furnishing a lower percentage of susceptibles are more prone to show unpleasant reactions and therefore only susceptibles should be injected. Precipitated toxoid is so dependable in its antigenic activity that for practical purposes in mass immunization, a post-Schick test may be omitted, not more than 5 or 6 per cent of children who receive a single dose showing a positive Schick after 8 weeks. The post-Schick test is more difficult to interpret after precipitated toxoid, probably due to increased sensitivity to the protein in the Schick material which is induced by the long continued stimulation during the immunizing process. A heated control is necessary to avoid confusion in doubtful cases.

The enormous advantage of immunization by a single injection needs no comment and the rapidity with which precipitated toxoid is supplanting the older agents is evidence of its popularity with the profession. However, the objection of unpleasant reactions must be kept in mind. There is increasing evidence that these reactions, frequently resulting in abscess formation, are more common than reports indicate, and in order that the product may be steadily improved, field experiences should be reported to central authorities for transmission to the manufacturers.

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