THE DEVELOPMENT OF DIPHTHERIA VACCINES

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SYNOPSIS

Beginning with a discussion of the main types of toxin-antitoxin mixtures of diphtheria vaccine, the author of this article goes on to review briefly the early work done on the conversion of toxin to toxoid and the introduction of adjuvants. Among these, special attention is paid to the aluminium compounds. He also discusses the reasons advanced by different workers for the enhanced activity of vaccine under the influence of adjuvants and the difficulties met with in assessing diphtheria vaccine potency.

With the aim of producing active immunization against diphtheria, two different groups of vaccines have been developed, distinguished by the fact that in the first group the toxin produced by the diphtheria bacillus is employed as antigen without any change, while in the second a product modified by a chemical action is used, whose toxicity has been removed but whose immunizing power is retained unimpaired.

Attempts to induce active immunity with very small, gradually increasing doses of pure toxin have been confined exclusively to animals. For human vaccination on the other hand, mixtures have been prepared containing, in addition to the toxin, varying quantities of antitoxin; in this way the dangers arising from the administration of free toxin are avoided. Such vaccines were suggested by Babes¹ and von Behring,² ³ and three kinds of preparation were developed:

1. "Neutral" toxin-antitoxin mixtures
   These were prepared in such a manner as to prevent symptoms either of poisoning or local irritation at the point of injection in the experimental animals.

2. "Over-neutralized" mixtures
   These products contained an excess of antitoxin, i.e., more antitoxin than the amount necessary to overcome the toxic effects of the toxin.

3. "Under-neutralized" mixtures
   These vaccines contained a slight excess of toxin, i.e., a certain quantity of free toxin not combined with antitoxin.
Only the vaccines 1 and 3 became of any great importance for human vaccination. Those in group 3 gave good results in the course of mass vaccination campaigns, particularly in the USA.

Since the toxin and antitoxin are bound together in neutral mixtures, with flocculation, the group 1 vaccines were used especially in the form of "toxin-antitoxin floccules" and are still sometimes employed today. Nevertheless, their immunizing power is only about a fiftieth of that of the chemically detoxicated product. Furthermore, it has been found that massive doses of the floccules dissociate in the body, liberating toxins. For this reason their use cannot be considered absolutely free from danger.

As early as 1897 Ehrlich 4 showed that under the influence of a simple chemical compound (carbon disulfide, \( \text{CS}_2 \)) tetanus toxin is transformed into a non-toxic modification which produces active immunity against the real toxin in experimental animals. Ehrlich introduced the term "toxoid" for this artificially prepared toxin modification. Löwenstein 12 found in 1914 that formaldehyde (\( \text{CH}_2\text{O} \)) had the same effect as carbon disulfide. Glenny & Südmersen 9 also converted diphtheria toxin to toxoid with formaldehyde in 1921, and Ramon 18, 19 made diphtheria-formol toxoid suitable for human vaccination in 1923. The preparations thus developed are still much used today for mass vaccination, either as originally prepared or in purified form.

A further decisive advance in diphtheria vaccines was made possible by the introduction of adjuvants (activators). The treatment of toxoids with tapioca as recommended by Ramon 20 must be one of the oldest processes for increasing antigenic activity. Since tapioca powder causes abscesses, however, it could only be used for the immunization of animals.

Straus 23 then proposed that the toxoid be mixed with lanolin and olive-oil in a proportion of 100 : 200 : 75. Such admixtures may be expected, in fact, to act in a similar manner to the adjuvant subsequently proposed by Freund 8 for strengthening antigenic potency, namely, a mixture of horse serum, wool grease ("Aquaphor"), liquid paraffin, and killed tubercle bacilli. Vaccines prepared by Straus's method 23 were used in the USA for intramuscular injection of children but were not successful, since bacterial contamination in the course of manufacture is difficult to avoid.

On the other hand, Prigge 13, 14 succeeded in considerably increasing the protective power of diphtheria vaccines by the addition of soluble adjuvants, in particular the completely soluble precipitate obtained on the saturation of Pope's broth with sodium sulfate, as well as by adding other peptones.

Recently an attempt has also been made to activate diphtheria toxoid by combining it with proteins found in the mature spermatozoa of numerous kinds of fish, and consisting exclusively or predominantly of diamino-acids, known as protamines (e.g., clupeine); however, the results so far obtained do not allow any definite conclusions to be drawn.
Aluminium compounds occupy a special place among adjuvants; these compounds serve either for precipitation of the toxoid or for its adsorption. A distinction is consequently made between precipitated vaccines and adsorbed vaccines, according to the method of preparation.

The development of precipitated vaccines is based on the work of Roux & Yersin.\textsuperscript{21, 22} In 1889 these authors observed that in diphtheria culture filtrates, metallic salts form insoluble, antigen-containing precipitates. In 1926 Glenny\textsuperscript{10} and his co-workers precipitated diphtheria toxoid with potassium alum (\(\text{AlK (SO}_4\text{)}_2\)) and obtained very much better results in immunization experiments with the precipitate than with the starting material. Ramon & Nélis\textsuperscript{20a} obtained similar results with calcium chloride (\(\text{CaCl}_2\)) and Holt & Bousfield\textsuperscript{11} with aluminium phosphate (\(\text{AlPO}_4\)).

The adsorbed vaccines, obtained by adsorption on aluminium hydroxide, or, to be more precise, on the C\(_7\) modification mentioned by Willstätter,\textsuperscript{24-26} are the most effective. However, in view of the obstacles in the way of the manufacture of the adsorption vehicle, they are also the most difficult to prepare. The activating power of aluminium hydroxide is characterized by an activity constant (c) which, according to investigations made by Prigge,\textsuperscript{15-17} varies over a range of about 1-10 (c = 7.1 to 66.7) even when the manufacturing processes seem to be absolutely identical.

For many years opinions on the value of the various kinds of diphtheria vaccines were extremely divided and even today we are still far from being able to carry out all vaccinations with preparations to which the maximum antigenic potency can be attributed with certainty. Similarly, opinions vary greatly as to the value of adjuvants and particularly of aluminium. For this reason the selection of the vaccines used for mass vaccination continues to be relatively arbitrary and is strongly influenced by subjective factors. In general, no attempt is made to determine precisely the immunizing power of each individual vaccine before administration, a qualitative test of its potency being regarded as adequate. Because of this, there are frequently considerable differences in prophylactic value between the preparations used in the various vaccination campaigns.

The reason for this is that at the time when the various anti-diphtheria vaccines were developed there was still no method for the exact measurement of the protective power of the different preparations. A procedure for this was only developed shortly before the Second World War, and, in view of its cost, it is used in only a small number of large laboratories. Nevertheless, by the manufacture of as large and as uniform batches of vaccine as possible it is easy to satisfy the conditions required to bring about a decisive decrease in costs.

By this method the potency of diphtheria vaccines can now be expressed in protective units ("Schutz-Einheiten"), in the same way as the potency of diphtheria sera is expressed in antitoxin units, and as a result it has been possible to determine the relationship between the antigen content and the
potency of diphtheria vaccines. If the quantity of antigen present in a given amount of vaccine is expressed in flocculation units by Lf, the weight of adjuvant (mg of peptone, aluminium hydroxide, etc.) by A, and the potency measured in protective units by PU, then

$$PU = c \sqrt{Lf A}$$

where the activity constant c is a magnitude which applies only to a given adjuvant, its value depending on the quality of the substance used as adjuvant. It was also possible to explain why opinions as to the value of adjuvants were so contradictory. By systematic measurement of the protective value of numerous aluminium preparations it was found that the action of the adjuvants was extraordinarily unreliable and under certain conditions was actually disadvantageous. Consequently, it can readily be understood why the results obtained in man with apparently equivalent vaccines at different places were so different.

By systematic use of the method of measurement it was also possible to make a selection, i.e., to exclude the preparations of lower value and only to prepare high quality vaccines for human vaccination.

Over and above this the routine measurement of the protective power of preparations obtained in the most diverse ways also reveals those processes which guarantee successful use of the adjuvants and ensure uniform results no longer dependent on chance, thus making it possible to find the most promising methods of manufacture.

Nevertheless, one difficulty still remains. The question whether the activity of all diphtheria vaccines can be expressed in the same units can probably not be answered in the affirmative. Results obtained in recent years indicate rather that diphtheria vaccines fall into two groups whose modes of action differ so greatly that they cannot be adequately covered by a uniform system of measurement. Nevertheless, it has been found that those preparations which fall into a special category are precisely those which can only be used exceptionally or not at all for purposes of vaccination. There are also theoretical considerations which seem to promise a way of overcoming the difficulties. In practice, therefore, active immunization against diphtheria will not be affected by the difficulties encountered in the further development of the system of units.

The increased activity of diphtheria vaccines under the influence of adjuvants was formerly explained by the slowing down of resorption. Investigations by Prigge,14 Faragó,7 and Eisler,5 6 however, have shown that another mechanism is of decisive importance. In particular, it was shown, taking toxin-antitoxin floccules as an example, that delayed resorption and consequent lengthening of the period of action do not lead directly to an increase in immunizing power of the vaccine, whereas on the other hand, with readily soluble adjuvants (peptone) which cause no slowing
down of resorption, a considerable increase in the potency of formol toxoids can be brought about. It may be supposed, rather, that the main factor in the activation of vaccines is change in the physico-chemical state of the active colloids. It seems possible that in adsorbed vaccines the antigen molecules are spread so far apart that every individual molecule can act, whereas in non-activated toxoids the antigen molecules group together to form large complexes (micelles). These differences are probably also one reason for the difficulties arising on applying the system of measurement to different vaccines, for the power of the body to split up the antigen micelles may well differ so considerably from individual to individual that an additional variable is added to the one represented by the reaction to the antigen.

RÉSUMÉ

En 1897, Ehrlich montra que la toxine diphtérique — utilisée jusqu'alors en combinaison avec le sérum antiphtérique pour l'immunisation de l'homme — pouvait perdre sa toxicité sous l'action de diverses substances chimiques, sans perdre son pouvoir antigénique. Dès 1923, à la suite des travaux de Ramon, l'anatoxine obtenue par action du formol sur la toxine fut appliquée à la vaccination humaine.

Puis vint la découverte de l'action activante que certaines substances exercent sur les anatoxines. Les adjuvants solubles, tels que le précipité soluble obtenu par saturation complète du bouillon de Pope par le sulfate de sodium, certaines peptones et diaminoacides (protamines) sont parmi les produits récemment étudiés. Les composés d'aluminium occupent une place particulière, car ils peuvent être utilisés soit pour la précipitation soit pour l'adsorption des anatoxines.

La formation d'un précipité doué de propriétés antigéniques par addition de sels métalliques au filtrat de cultures de bacilles diphtériques avait été observée par Roux et Yersin en 1887 déjà. Dès 1926, l'alun potassique, le chlorure de calcium et le phosphate d'aluminium furent successivement employés avec succès.

L'adsorption des vaccins sur hydroxyde d'aluminium (modification C_y) exalte leur activité, mais rend leur préparation difficile. En effet, la constante d'activité de l'hydroxyde peut varier de 1 à 10, même si les méthodes de préparation paraissent absolument identiques.

Les opinions ont été longtemps divisées au sujet de la valeur respective des anatoxines diphtériques, et actuellement encore on ne peut être assuré de vacciner avec des préparations de valeur antigénique maximum. La valeur des adjuvants, en particulier celle de l'aluminium, est généralement controversée. Aussi le choix des vaccins diphtériques pour les vaccinations systématiques reste-t-il relativement arbitraire et subjectif.

En général, on ne tente pas de déterminer le pouvoir immunisant de chaque vaccin. On se borne à une épreuve qualitative d'activité. En conséquence, les vaccins utilisés dans les campagnes de vaccination peuvent présenter de grandes différences d'activité et de pouvoir prophylactique. Cela peut s'expliquer par le fait qu'il n'existait pas de méthode de mesure exacte du pouvoir protecteur au moment où les divers vaccins antiphtériques ont été mis au point. Ce n'est qu'après la deuxième guerre mondiale que l'on a élaboré une méthode d'évaluation. Mais, étant coûteuse, elle n'est appliquée que dans certains grands laboratoires. Il est cependant possible de réduire les frais en préparant des lots de vaccins aussi importants et aussi uniformes que possible. Cette méthode permet d'exprimer en unités protectrices l'activité des vaccins, de même que l'on exprime en
unités antitoxiques celle des sérums; elle permet de déterminer le rapport entre la teneur en antigène et l'activité du vaccin et de comparer le pouvoir protecteur de préparations obtenues par diverses techniques, afin d'adopter les meilleures.

Une difficulté subsiste encore: celle de l'établissement d'un système de notations en unités d'activité convenant à tous les types de vaccins antidiptériques. Il est peu probable que l'on puisse parvenir à un système unique; mais il semble que les difficultés rencontrées dans le choix d'un système n'affecteront pas pratiquement l'immunisation active contre la diphtérie.

L'action des adjuvants a été expliquée autrefois par un effet-retard. Il semble qu'un autre mécanisme, mis en évidence par l'emploi d'adjuvants solubles, soit déterminant. Il s'agirait d'un changement de l'état physico-chimique des colloïdes actifs. Grâce à l'adjuvant, ces colloïdes sont dispersés, de sorte que chaque molécule est active, tandis que dans les anatoxines non activées, les molécules d'antigène s'aggrègent en micelles. Ces différences dans le mode d'action sont probablement responsables, en partie, des difficultés que rencontre la recherche d'un système de notation applicable aux divers types d'anatoxines, car la capacité de scinder les micelles d'antigène peut varier d'un individu à l'autre. Cette variable vient s'ajouter à celle que représente la réaction individuelle des organismes à l'antigène.

REFERENCES

2. Behring, E. von (1913) Dtsch. med. Wschr., 39, 873