IMMUNOGLOBULIN STANDARD PREPARATIONS
IN REPLACEMENT OF HYPERIMMUNE ANIMAL SERA

With special reference to
anti-rabies, anti-tetanus and anti-diphtheria
antibody preparations

by

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The WHO Expert Committee on Biological Standardization established the International Standard for Rabies Immunoglobulin and assigned an activity of 59 International Units of Rabies Immunoglobulin to the contents of each ampoule of the standard preparation (WHO, TRS, in press; WHO/BS/84.1433). This immunoglobulin preparation replaced the International Standard for Anti-Rabies Serum, Equine, and the unitage assigned to the new standard was based on the results obtained in comparative assays using the so-called rapid fluorescent focus inhibition test (RFFIT). RFFIT is a test based on the neutralization of the effect of rabies virus on a cell culture.

In a recent paper (J. Biol. Stand. (1985) 13, 123) scientists from the Paul-Ehrlich-Institut in Frankfurt, Federal Republic of Germany reported on assays of human rabies immunoglobulin preparations and equine serum preparations against a common equine reference preparation by means of RFFIT and also by means of a virus neutralization test in mice. For eight out of nine immunoglobulin preparations, the potencies obtained by means of the two methods were significantly different. In all nine cases the potencies found by the RFFIT method were lower. The ratio between the potencies obtained by the two methods varied from 1.22 to 10.14. For the equine sera, the potencies obtained by the two methods were not significantly different and the RFFIT results were not systematically lower.

The authors ascribe the observations to avidity differences between equine sera and human immunoglobulins. It is reasonable to assume that such avidity differences exist, since the horses have probably been much more intensively immunized than the human donors.

On the other hand both methods, the RFFIT and the neutralization test in mice, are based on the incubation of rabies virus with dilutions of serum. For both methods the serum dilution range is about the same. To be able to demonstrate avidity effects, it is necessary to titrate in most systems on widely different antigen dilution levels. However, in the test in mice, the incubated serum-virus mixtures are injected into the brains of the mice with-

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out further dilution, whereas in the RFFIT the mixtures are diluted by a mixture of an equal volume of a cell suspension and then further incubated in this state. The differences between these two methods might give the dilution effect.

These observations raise a number of questions:

**International Standard for Rabies Immunoglobulin**

It will be necessary to investigate whether the potency assigned to the standard (59 IU per ampoule) would have been the same if another assay method had been used. Perhaps such studies are already on the way, otherwise they will have to be arranged.

**Equine or Human serum?**

If different assay methods give strongly deviating potencies, as indicated in the paper referred to above, then the whole idea of replacing the equine serum standard with a human immunoglobulin standard should be re-evaluated.

**Two standard preparations?**

Since many developing countries will continue to use equine sera, it is most important to be able to control the potency of these sera in a reliable way. To this end, it might be necessary to continue having an equine serum standard. However, the stocks of the first equine standard serum are very low and a number of ampoules are needed to calibrate a replacement preparation and perhaps also to assay the immunoglobulin standard in other test systems besides the RFFIT. Therefore the procurement of a replacement preparation may be very urgent.

**Two units?**

If it is decided to have a human immunoglobulin standard as well as an equine serum standard for rabies antibodies, this will be the first time that two standard preparations are established for the potency control of the same type of antibodies. It would therefore seem worthwhile to consider the consequences very carefully. For instance, what would be the relationship between the IU for rabies immunoglobulin and the IU for rabies serum?

**Avidity**

A more complicated question is the importance of the avidity of the antibodies used prophylactically after exposure. If differences in avidity are the reasons for the observed potency differences, and if it can be as high as indicated by the results referred to above, then it seems likely that avidity differences might also have consequences for the success of a prophylactic administration.

The advantage of homologous serum-prophylaxis is a longer persistence of antibodies in the organism and the absence of the risk of sensitization. However, it is conceivable that in certain cases an antibody preparation of high avidity could be life-saving, whereas a less avid one would not be so.
Other antibody preparations

This problem is not confined only to rabies. For other antibody preparations used for treatment or prophylaxis, the avidity of the preparations, from a clinical point of view, might in some cases be more important than the species homology. Another obvious example is tetanus antitoxin of animal origin as opposed to human tetanus immunoglobulin.

In the case of diphtheria similar problems arise. After many years without toxic diptheria, a few cases occurred recently in Sweden and Denmark. Treatment with equine antitoxin was not attempted but a few serious cases were unsuccessfully treated by means of specific human immunoglobulin in high doses.

Clearly, the avidity of hyperimmune animal tetanus antitoxins are much higher than the avidity of human antitetanus immunoglobulins. This might cause problems in the potency assay of sera and immunoglobulins, and again it has to be discussed whether we need two standard preparations and two unit definitions or whether one will suffice. What is the importance of the avidity for a prophylactic effect and for treatment?

Avidity differences are also observed among different lots of animal sera. The control of such differences could be important, e.g. for botulinus antitoxins or anti-sera for the treatment of snake bites.