The testing of diphtheria toxin and antitoxin by intracutaneous injection into guinea-pigs : preliminary note / by A.T. Glenny and K. Allen.

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THE TESTING OF DIPHTHERIA TOXIN AND ANTITOXIN BY INTRACUTANEOUS INJECTION INTO GUINEA-PIGS.[1]

PRELIMINARY NOTE.

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THE intracutaneous method of Römer (1) has been adopted by us at the Wellcome Physiological Research Laboratories for all preliminary determinations of the antitoxic value of diphtheria sera. Our experience during the last eighteen months has shown that this method is of great practical value and that it deserves wider recognition.

The general method of procedure is as follows:—

A white guinea-pig is chosen, or one with one white flank. The hair is clipped off as closely as possible and the clipped area covered thickly with sulphuretted lime paste. Lime paste is made by grinding lime in a mortar, adding water gradually until a thin paste is formed (about 3 c.c. of water to each gram of lime). Sulphuretted hydrogen is then bubbled through for about twenty-four hours. Application of the lime paste for about five minutes is generally sufficient to remove all hair. All trace of the paste is then washed off with warm water and the guinea-pig dried with a towel. The skin should then be perfectly bald and white. If the paste is left on too long the skin becomes red and raw in patches, making the subsequent reading of reactions very difficult. For injections it is preferable to anæsthetise with A.C.E. to prevent movement. The number of injections that can be made depends on the area of skin available. The dilutions of toxin and antitoxin are so arranged that the amounts under test are contained in 0·2 c.c. The injections should be about an inch apart and not too near the ventral surface. Injections are made intracutaneously as near the surface as possible, using a small needle with the short bevel uppermost.

If the needle is inserted too deeply the injection becomes subcutaneous and consequently dispersed and not local. A true intracutaneous injection is distinguished by a sharply defined white raised lump.

A small fraction of diphtheria toxin produces a well-defined red area about 15 mm. in diameter at the end of thirty-six hours. With a great excess of toxin the reaction may be much greater. Readings may be taken at the end of twenty-four hours, but the reactions are not so sharply defined as at the end of thirty-six hours. After the third day the colour fades, leaving a slightly discoloured bare patch easily dis-

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tinguishable by the commencement of the growth of hair elsewhere. In cases where there was a large excess of toxin a scab is formed after four or five days which, after peeling, may leave a bare area for some weeks.

Testing Diphtheria Toxin.

Very small fractions (about 1/500th) of a fatal dose of toxin will produce a definite intracutaneous reaction. The smallest quantity of toxin which will produce a reaction is called by us "the Minimal Reacting Dose," or M.R.D. The reaction is specific and is prevented by antitoxin. The M.R.D. is probably an index of toxin content only and not of the total binding units of toxin and toxoid.

The antigenic or immunising value of a toxin can be measured by its combining value with antitoxin. The standard adopted for measuring the strength of a toxin is the limiting dose of toxin which causes a positive intracutaneous reaction when injected, together with 1/500th of a unit of antitoxin. This is termed the Ln/500 dose or "skin test dose," that is, the limiting dose causing necrosis at the 1/500th level. This level has been chosen to avoid the danger of killing the guinea-pig by excess of free toxin when six or eight tests are made at the same time on one guinea-pig. The amount of work done at these laboratories upon the method of preparation of diphtheria toxin by Dr Hartley and Miss Parnell, and the uniformity of their results, is a tribute to the utility and accuracy of the method.

Testing Diphtheria Antitoxin.

As in the subcutaneous method, the test dose of toxin to be used is determined by titration against standard antitoxin, but instead of titrating against 1 unit, 1/500 of a unit is used in the intracutaneous method.

A serum to be tested is titrated against the "skin test dose," and the quantity of serum which, when mixed with 1 Ln/500 of toxin just fails to give a positive reaction, is said to contain 1/500 of a unit of antitoxin. For instance, if negative and positive reactions are obtained when testing 1/400,000 and 1/450,000 c.c. respectively of a sample of serum against 1 Ln/500 of toxin, we infer that 1/500 of a unit of antitoxin is contained in between 1/400,000 and 1/450,000 of a c.c., or 1 unit in between 1/800 and 1/900 of a c.c. In other words, the serum contains between 800 and 900 units of antitoxin per c.c.

In practice the quantity of toxin may be varied according to the strength of the toxin used and the wideness of the limits within which the antitoxic content of the serum under test is known. As the limits within which the antitoxin value falls are narrowed, so the toxin dose may be increased to Ln/100 or Ln/50 without killing the guinea-pig. If the test toxin used has a subcutaneous Lo value as low as 0·1 c.c. it is possible to titrate against Ln/1, and our impression at present is that it

is possible to standardise to within 2 per cent., but as a general rule the margin of error in measurement is greater than the limits of susceptibility of the guinea-pig.

At frequent intervals control tests upon the "skin test dose" of toxin are made by titrating against a known standard serum.

It is our general practice to make use of the intracutaneous method for all preliminary tests of normal horses for antitoxic content before commencing immunisation with diphtheria toxin. The blood of normal horses may contain from less than 1/1000th of a unit to 5 units of antitoxin per c.c. The commencing dose of toxin which can be easily tolerated depends on this normal antitoxic content. The rapidity with which results are obtained and the economy in the number of animals required makes the intracutaneous method particularly applicable to such preliminary determinations and to subsequent tests in the immunisation of horses, the progress of which can be followed very closely by means of intracutaneous tests on sample bleedings, the subcutaneous test being used only to confirm the antitoxic content of the final bleeding.

The two values arrived at by the intracutaneous and subcutaneous methods agree satisfactorily.

The intracutaneous method has also been applied by us to the testing of cobra venom and antivenene, and to the toxins and antitoxins of *B. welchii* and *V. septique*.

REFERENCE.

1. Römer . . . . . "Zur Bestimmung sehr kleiner Mengen Diphtherie, antitoxins," *Ztschr. f. Immunitäts-forschung*, 1909, Bd. iii., S. 344.

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