brought forward by Roux, at once put this method in a secure position from the practical standpoint.

Preparation of Antitoxin.

For the preparation of antitoxins on a large scale the horse is the animal now almost exclusively used. The horse can be suitably immunised against tetanus, diphtheria, and other diseases, and the sera obtained are capable of attaining to a very high antitoxic value.

The horses are selected by a skilled veterinary surgeon. They must be free from all signs and symptoms of disease. Every horse is tested for latent glanders with mallein, and even a suspicion of a reaction is regarded as a sufficient basis for the rejection of the animal. If the animal has satisfactorily passed the test, he is received into the establishment, but is isolated and kept under observation for some time. Under certain circumstances a second mallein test is carried out. Having passed through this period of probation the animal is admitted into the stables, and the process of immunisation is commenced. During the process the animal is kept under the strictest observation, his temperature is duly registered, and the greatest possible care is taken in regard to his comfort and feeding.

The general principle is that the animal is treated with increasing doses of the particular poison. The toxins, which have been previously tested on small animals, such as rabbits and guinea-pigs, are injected subcutaneously, intramuscularly, or intravenously.

At first either very minute doses of weak toxins, or toxins which have been modified by chemical agents, or in other ways, are employed. In the case of tetanus, in the early stages the toxin is usually modified by being treated with iodine. The injection of the toxin may be followed by swelling at the site of inoculation, loss of appetite, general *malaise*, and rise of temperature. When these have passed off, the animal receives a second, rather larger, injection, and in this way the quantity of toxin is increased, until within a few months the horse is capable of tolerating many thousand multiples of what would be a lethal dose if given as a first injection. When the serum has reached the strength suitable for clinical use, blood is withdrawn from time to time by venesection.

All the processes connected with immunisation are carried out under the strictest antisepsis. Syringes, needles, and instruments are boiled, and the blood is received into vessels which have been sterilised in the autoclave at a temperature of 120° C. The skin of the horse is shaved and thoroughly cleansed with soap and water. The shaved area is then washed with a 2 per cent. solution of lysol, which is especially suitable for the removal of the oily substances from the horse's skin. It is finally washed with 1 in 1,000 corrosive sublimate solution.

Precautions.

The vessels containing the sterile blood are removed into a small isolated building used for this purpose only. The serum, when it has separated from the clot, is decanted into sterilised vessels, the usual bacteriological precautions being taken to prevent the admission of extraneous germs. In certain cases the blood is received into a special apparatus in which the fibrin is removed by whipping, the blood corpuscles being separated either by sedimentation or by the use of a centrifuge. To the serum thus obtained 0·2 to 0·3 per cent. carbolic acid is added. As a final precaution the serum is passed through a Berkefeld filter. It is then in a condition to be put into the small phials in which it is sent out for use. Before this is done, however, it is subjected to the following tests:—

1. For sterility. It must be germ-free.
2. For absence of toxicity.
3. For antitoxic value. This must reach a required standard.

The last two tests are carried out on small animals. The serum is also kept for a certain time, during which the horse remains under observation; if during this time the animal shows any signs of illness the serum is thrown away.

Testing.

Several methods of testing the strength of antitoxins have been employed. The first methods consisted in ascertaining the exact dose of toxin, or of living culture, required to kill an animal, such as a guinea-pig, of a certain weight, and pitting against this dose varying quantities of antitoxin.

With a single minimal lethal dose the results were irregular, and it was soon found more satisfactory to employ as the test dose ten times this quantity. By Ehrlich's older method of testing, ten times the amount of serum which protected a guinea-pig of 250 grammes weight against a ten-fold lethal dose was called one unit, *i.e.*, an amount of serum capable of completely neutralising about 100 fatal doses of this toxin solution for a guinea-pig of 250 grammes.

This method of standardising the serum, however, presented difficulties, for it was found that different solutions of toxin which were equally poisonous required quite different amounts of serum to neutralise them. Ehrlich afterwards discovered this to be due to the fact that a solution of diphtheria toxin undergoes certain changes by which it loses its lethal power without losing the power of neutralising antitoxin. On the other hand, diphtheria antitoxin, especially in the dried state and *in vacuo*, remains stable. Ehrlich, therefore, made his standard an antitoxin one. The unit he fixed on, while derived from the older method of testing, was to some extent an arbitrary one.

The bottles of diphtheria serum supplied by the Institute contain at least 2,000 of Ehrlich's units.

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