

accustomed to the diphtheria poison. The preparation of this poison is, therefore, the foundation of the serum therapy, and it is the more necessary to discuss it, since it is extensively used in order to immunise large animals; that is, to render their serum sufficiently effective. The most rapid process of obtaining diphtheria toxine is the cultivation of the bacilli in the presence of a moist current of air. Vessels with a flat bottom and supplied with a lateral tube are used. Alkaline bouillon, containing 2 per cent. of peptone, is placed in the vessels in such amount that the fluid reaches to a certain height. After sterilization they are inoculated with fresh, very virulent diphtheria bacilli, and heated in an oven to 37°C. The development once begun, a stream of air is admitted. After three to four weeks the culture is sufficiently rich in toxines to be employed. The ready cultures are filtered through a Chamberland's filter. The clear fluid is then preserved in well-stoppered bottles and kept in the dark at ordinary temperature. Prepared in this manner, the toxine, in doses of 0.10 ccm., is capable of killing a guinea-pig weighing 500 gme. in forty-eight to sixty-hours. It loses its effectiveness after a time, but this occurs very slowly if preserved in the manner above described.

The toxine once obtained, the next step is to immunise the animals. But in order to avoid calling forth in these too violent phenomena, it is necessary to first reduce the activity of the toxine. The best method for this purpose is that employed by Vaillard and myself in tetanus, namely, the addition of iodine. Diphtheria toxine

diluted with iodine is much less dangerous than the pure toxine. Immediately before use the toxine is diluted with one-fourth its volume of Gram's solution, and, after a few seconds, injected under the skin. A medium-sized rabbit bears a single injection of 0.5 ccm. of this solution. After a few days the injection is repeated, and so on for several weeks, whereupon the dose of iodine-toxine may be increased and the iodine content reduced. Gradually the pure toxine is reached. The animals must be carefully weighed, and the injections immediately stopped as soon a decrease of the body weight is manifested, as otherwise a fatal cachexia may be produced. Dogs rendered immune to diphtheria in this manner have supplied a very effective serum: on the other hand, sheep, and especially goats, are very susceptible to diphtheria poison; hence the immunising of such animals requires the greatest caution. The same is true of cows, the milk of which may, in addition, offer an important source of antitoxine.

Of all animals capable of supplying large quantities of anti-diphtheric serum, the horse is the most easily immunised. It bears the toxine much better than all the previously mentioned species of animals. It is not infrequent to find a horse in which the injection at one time of a 2-5 ccm. of the strong antitoxine causes but a quite transitory fever and a rapidly subsiding local œdema. If, with Behring, one assumes that an animal produces a stronger antitoxic serum the more susceptible it is to the toxine, then the selection of the horse would appear inappropriate.