A Visit to an Antitoxin Laboratory.

By E. Virginia Reinoehl.

At Berkeley, California, for more than three years, an analytical laboratory has been producing antitoxin, vaccine virus, and antistreptococcin serum for mankind; also, a number of veterinary serums. Hearing of this laboratory, some nurses recently paid a visit to Berkeley to learn something of the methods followed. Mr. M. Trowbridge, Ph.C., gave us several hours of very interesting instruction.

This is the only commercial laboratory west of Detroit which engages strictly in the manufacture of serums and vaccines. It works under Government licence No. 8.

Diphtheria bacillus was first described by Klebs in 1883, and first cultivated by Loeffler in 1884. About ten years later treatment by antitoxin was begun. The discovery of antitoxin was the result of many years of research observation and experiment. There is at present some prejudice concerning the use of antitoxin. Those opposing it claim that heart failure, paralysis, and rheumatism are the results of its use. The objections do not hold good, as statistics show that diphtheria patients have better chance for recovery when the antitoxin is used. It should be given early to get the best results. It has, however, been used in cases as a last resort, and patients have recovered who had been thought hopeless.

Since the antitoxin the death-rate has been reduced ninety per cent. Formerly ninety-three per cent. of the cases died; now the mortality is only three per cent., which is in itself a very strong argument in favour of its administration.

The production of antitoxin is the most important one in this laboratory, and new cultures of diphtheria are made daily. The first step in making antitoxin is to obtain the diphtheria bacilli, either from a laboratory or from a diphtheria patient. Then bouillon is prepared by boiling finely chopped meat for thirty minutes, adding salt and pepton. This is placed in wide flasks which afford a broad surface for placing the germs. Very careful handling is required to prevent these germs from going beneath the surface and drowning or suffocating, as they require much oxygen. They are taken from the culture tube with a platinum wire loop and placed on the surface of the bouillon, then put in an incubator and kept at body temperature. In a day or two these germs multiply and cover the entire surface of the bouillon.

The bacilli during development absorb the bouillon as food and give off products that are poisonous. The bouillon becomes saturated with this poison, and is toxic. At the end of seven days it has become a heavy white tenacious, membrane-like substance, such as patches that appear in the throat of a diphtheria patient.

This toxin is then ready for injection into horses, for the production of antitoxin serum. The horse is chosen for this important work because of its freedom from disease, its large body, and the separating property of its blood. The medium-bred horse produces a finer serum than the high-bred one. The animals are not injured by the use made of them for this work.

The injections are given under antiseptic precautions. The field surrounding the jugular vein is shaved, scrubbed with a five per cent. solution of carbolic acid, and aseptic methods used throughout the whole procedure, and the horse receives as careful attention as would a human patient. The first dose may make him feel ill, but he seems to feel no bad effects from succeeding doses.

A record is kept of each case (horse), the temperature taken morning and evening, and note made of the quantity of toxin given. This is administered in increasing doses until the animal can tolerate one pint of the poison—enough to kill a thousand men! The horse's tolerance of the poison is due to the fact that certain cells in the organism of the animal are, through the effects of the toxin, stimulated to produce a secretion which neutralises the toxin and preserves the animal's life. The secretion thus produced is, as the result demonstrates, an antitoxin. Three or four months' treatment of the horse is required to make him immune, and one week after the final injection of toxin has been administered the horse is bled, for at this period he yields the largest amount of anti-toxin serum. A large trocar is then introduced into the vein, a rubber tube is attached to the cannula, and about two and a quarter gallons of blood drawn directly into large tubes, similar in shape to a culture-tube, but with a capacity of 1,000 cc. Twelve of these tubes are filled about three quarters full, and set away in a rack for four days, and at the end of that time the clotted blood, containing both red and white corpuscles, has settled to the bottom, leaving the clear yellowish serum on top. This is the antitoxin serum. It is siphoned off from the tubes to the storage bottle, and a solution of four-tenths of one per cent. of tricresol added as a preservative. It is then filtered, tested, and placed in the syringes ready for the market.

The horse seems none the worse for the loss of blood, and after clamping the vein for a few

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