## MISCELLANEOUS.

membrane in the throat of a child, suffering from the disease. The swab is then transferred to a tube of culture media containing Loefflers blood serum mixture (which is made by mixing blood serum with beef bouillon and a small amount of glucose and sterilizing.) This tube and swab is then placed in the incubator which is kept at 37° Centigrade and allowed to develop from twelve to eighteen hours when it is taken out and examined microscopically. If found with other bacteria present as is usually the case, it is purified by Plating on gelatin media from which the diphtheria colonies are picked with a sterilized platinum wire, and transferred to flasks containing beef bouillon (made from prime lean beef with peptone and salt added and made alkaline by the addition of Sodium Hydrate).

These flasks are also put in the incubator at  $37^{\circ}$  C. and remain for seven to fifteen days. At the end of this time they are removed and examined to insure the purity of the culture. If found pure a small amount of Trikresol is added as a preservative.

The bouillon which contains the toxin is now tested for strength on guinea pigs to determine the minimum fatal dose for a guinea pig of known weight. After this is complete the toxin is ready to be injected in the horse.

The horse is another important factor in making serum. Animals five or six years old are selected by a Veterinarian to insure their healthy condition, but to be doubly certain, they are injected with Tuberculin and Mallein to see that they are free from any latent form of Tuberculosis or Glanders.

After all these precautions have been taken they are given an initial injection of the toxin which usually causes a rise of temperature and other symptoms which subside in a few days. When the animal has completely recovered he is again injected with an increased amount of toxin. This process is repeated again and again until at the end of five or six months the horse is immunized, taking many hundreds of times the amount of toxin given at the first injection.

Now the horse is bled from the jugular vein by inserting a trocar and allowing the blood to flow into sterile containers. These containers are placed in the refrigerator where the serum separates—and is syphoned off into sterile bottles and Trikresol added as a preservative.

It is now ready for the test for Antitoxic strength and bacteriological and physiological tests. The first is made by giving guinea pigs one hundred fatal doses of toxin and a small amount of the serum from an immunized horse. Depending on the amount of serum necessary to protect the guinea pig from death the number of antitoxic units is calculated.

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