

weeks, it has not been possible to perform experiments designed to bring out the immunological relationship between the agent described and that of the common cold.

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A Simply Prepared Broth for Producing Hemolytic Streptococcal Hematoxin (Streptolysin).*

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Todd¹ has demonstrated recently that the sera of patients suffering from various hemolytic streptococcal infections contain antibodies that neutralize streptococcal hematoxin, and he, collaborating with Coburn^{1, 2} has shown that these antibodies are almost constantly present in abnormal concentrations in the serum of patients suffering from rheumatic fever. Obviously a new technique has been developed for the study of infections of this type. Todd's³ original medium for the production of streptolysin consisted of horse meat infusion, peptone and yeast extract, which after mixing was sterilized by filtering, first through a coarse earth filter and then through Chamberland "F" candles. Later Todd and Hewitt⁴ found that a more suitable medium could be prepared by substituting for the yeast extract the following substances: sodium bicarbonate as a buffer, inorganic phosphates and glucose as growth stimulating materials and sodium chloride to supply suitable isotonicity.

We have had little success with the broth containing yeast extract, but have obtained a satisfactory yield of streptolysin from the buffered horse meat broth, and a still better yield when fresh beef heart was substituted for the horse meat. The tedious and time-consuming filtration of the broth, through 2 different filters, makes the production of this medium very difficult unless special apparatus is available. It is possible, on the other hand, to prepare

* The generic term *hematoxin* is used for bacterially produced substances that lyse red blood cells, and the specific term *streptolysin* for such substances elaborated by hemolytic streptococci.

¹ Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

² Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

³ Todd, E. W., *J. Exp. Med.*, 1932, **55**, 267.

⁴ Todd, E. W., and Hewitt, L. F., *J. Path. and Bact.*, 1932, **35**, 973.

a medium highly satisfactory for the production of streptolysin without filtering the broth. This is done by sterilizing the mixture of meat infusion and peptone in the Arnold apparatus, and subsequently adding the solution of salts and glucose which has been sterilized previously by filtering through a Berkefeld candle. The medium, prepared by the appended method, is usually clear, and not infrequently contains a slight granular sediment. The disadvantage of contamination while adding the salts and glucose is far outbalanced by the constant yield of streptolysin and the heavy growth of bacteria. A 15-hour growth of both streptococci and pneumococci has given nearly 4 times the volume of bacterial sediment that was obtained from the same quantity of good beef heart infusion broth. This medium is, therefore, very useful for obtaining heavy bacterial growths, and this effects a saving in both time and material. This broth passes through a Chamberland filter much more readily after streptococci have grown in it than before inoculation. The beef heart infusion 2% proteose peptone, reinforced with 0.2% dextrose, has furnished practically as good a hemolysin-yielding medium, but the amount of streptococci obtained from a unit of this broth has been smaller than that obtained from the medium described below. The detailed procedure for the manufacture of the broth and the production of streptolysin is as follows:

Beef heart, obtained the day of slaughtering, is freed of fat; the muscle and fibrous tissue are finely minced, and weighed. To each pound of mince 1 liter of tap water is added. The mixture, in an enamel container, is infused in the ice-box for 16 hours, then placed over a low flame and brought slowly to 85°C., where it is maintained for 30 minutes. The infusion is filtered through Prat Dumas filter paper and the meaty pulp squeezed very dry. The resulting infusion should equal the original volume of water. Twenty gm. of Difco proteose peptone is dissolved in each liter of infusion; the reaction is adjusted to pH 8 (about 30 cc. of N/1 NaOH per liter). It is filtered through No. 12 Whatman filter paper into flasks, and sterilized in an Arnold apparatus on 3 successive days, allowing 30 minutes for each liter of broth or less in the container.

Multiples of the following salt-dextrose solution are prepared:

Dextrose	2 gm.	} All chemicals of Reagent Purity.
NaHCO ₃	2 "	
NaCl	2 "	
Na ₂ HPO ₄ :12 H ₂ O	1 "	
Distilled water	50 cc.	

This solution is filtered through a Berkefeld candle, tested for ster-

ility, and then aseptically pipetted into the broth in the proportion of 50 cc. to 1 liter.

As a precautionary measure the completed broth should be incubated 2 days before inoculating to insure sterility.

At 9 a. m. a tube of 0.1 or 0.2% dextrose broth is inoculated with a suitable hemolytic streptococcus, placed in the water bath and shaken hourly, thus insuring a rapid growth. At 4 p. m. 6 cc. of this vigorously growing culture is added to 1 liter of the broth, previously warmed to 37°. This is well shaken and incubated overnight. The next morning it is filtered through an "F" Chamberland filter, by placing the candle in a long tube containing the culture and drawing the broth from the outside to the inside, and thence into a sterile flask which is connected to the house vacuum. The first 100 cc. is discarded because it contains little or no streptolysin.

As shown by Neill and Mallory⁵ streptolysin is active only in the reduced state. Todd's method for reducing it is as follows: To each liter of filtrate add 10 cc. of N/1 NaOH and 1 gm. of sodium hydrosulphite which has been freshly ground to a fine white powder; this is dropped on to the surface of the filtrate and dissolves slowly. The flask is stoppered and connected to a high vacuum pump; a high vacuum is maintained for 2 or 3 hours until only very few bubbles are given off the fluid. The reduced hemolysin is pipetted into 50 or 100 cc. flasks, covered with about 2 inches of sterile melted vaseline and stored in the ice-box. Todd recommends storing the unreduced streptolysin in the cold, and reducing the required amount the day it is to be used; but we have found that the hemolytic titer is usually higher several days after reduction than immediately after being reduced; and also that aged reduced streptolysin is more stable than that tested on the day of reduction.

The streptolysin is tested for hemolytic power in the following amounts: 0.4, 0.35, 0.3, 0.25, 0.2, 0.16, 0.14, 0.12, 0.1, 0.08, 0.06, 0.04. Then saline is added to make a total volume of 1.5 cc. Each tube is well shaken—then 0.5 cc. of 5% washed red blood cells (rabbit) is added. To obtain a very accurate end point, *i. e.*, complete hemolysis, each tube is shaken individually for about 30 seconds *immediately* after adding the red cells. An end point at about 0.12 to 0.08 is usually obtained by this method.

The streptolysin thus produced is a satisfactory reagent for testing antibodies in serum. We have not tested its antigenic ca-

⁵ Neill, J. M., and Mallory, T. B., *J. Exp. Med.*, 1926, **44**, 241.

capacity; but as Todd³ has shown that serum-free streptolysin is antigenic it seems probable that this lysin would induce antibodies when injected into animals. Only certain strains of hemolytic streptococci produce suitable streptolysin.

Summary. A very satisfactory medium for producing streptolysin and yielding heavy growths of bacteria results from adding filtered sterile salts, buffers, and glucose to beef heart, peptone broth previously sterilized at 100°C.

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Failure of a Mouse Carcinoma Material to Enhance a Mouse Sarcoma.

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It has been shown that an homologous rabbit carcinoma enhancing material^{1, 2} failed to enhance a carcinoma of the mouse.³ Haaland,⁴ Leitch,⁵ and Casey³ have demonstrated, however, that a material can be obtained from the same mouse carcinoma (No. 63) which will enhance both the incidence and the growth of the primary tumor when used in conjunction with the tumor inoculation. It seemed desirable, therefore, to determine whether homologous enhancing materials will enhance heterologous transplanted tumors within the same animal species. Since other transplantable rabbit tumors are not available, the experiments were carried out with mouse tumors. In the 2 experiments here reported the enhancing material was obtained from the Bashford mouse carcinoma (No. 63) and its effect was observed in connection with implants of mouse sarcoma (No. 180). Two different batches of preserved (enhancing) material and sarcoma emulsion were used; 60 mice of the Rockefeller Institute strain were employed; in the first experiment, there were 40 females and in the second 20 males. Thirty of these mice were injected with a saline emulsion of Bashford mouse carcinoma (No. 63) tissue which had been preserved anaerobically at 24°F. for 42 days.³ Two weeks later the entire 60

¹ Casey, A. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 816.

² Casey, A. E., *J. Exp. Med.*, 1933, in publication.

³ Casey, A. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 674.

⁴ Haaland, M., *Lancet*, 1910, **1**, 787.

⁵ Leitch, A., *Lancet*, 1910, **1**, 991.