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The Effect of Various Carbohydrates on Production of Diphtheria Toxin with Special Reference to its Flocculating Power.

E. L. HAZEN AND G. HELLER. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

Park and Williams,[1] following the work of Spronck,[2] reported that small amounts of glucose added to the broth aided in production of potent toxic filtrates from *Corynebacterium diphtheriae*. They warned at the same time that an excess of glucose sufficient to cause too great a degree of acidity would inhibit the development of the toxin. T. Smith[3] also found that small quantities of dextrose were favorable to toxin production, provided the muscle sugar had been removed from the broth by fermentation. Recently Locke and Main[4] and Ramon[5] have again called attention to the use of glucose in the production of high-titred diphtheria toxin.

We have studied the effect of a number of carbohydrates, fermentable by *C. diphtheriae*, on the production of toxic filtrates of high Lf* unitage. We are reporting here only those experiments in which glucose and maltose have been used to enrich the culture medium.

The culture filtrates (Park-Williams No. 8 strain of *C. diphtheriae*) were obtained after incubation from 48 hours to 5 days at 37.5°C., no preservative being added. The pellicles were grown in every case in one litre flasks containing 250-300 cc. of broth as used by Povitsky.[7] The initial inoculum consisted of one large loopful of an actively growing pellicle, the seed culture having been maintained previously by rapid transfer, twice daily. Apparently little harm was done to the pellicles by manipulation of the flasks in adding the sugars and adjusting the pH of the culture fluid. Toxins

[1] Park, W. H., and Williams, A. W., *J. Exp. Med.*, 1896, **1**, 164.

[2] Spronck, C. H. C., *Ann. de l'Inst. Past.*, 1895, **9**, 758.

[3] Smith, T., *J. Exp. Med.*, 1899, **4**, 373.

[4] Locke, A., and Main, E. R., *J. Inf. Dis.*, 1928, **43**, 41.

[5] Ramon, G., *Compt. Rend. Soc. de Biol.*, 1929, **101**, 718.

* The term is used in accordance with Glenny and Wallace's definition indicating "that amount of toxin (corresponding to one unit of a certain antitoxin) in that mixture which flocculates most rapidly when a series of mixtures of that toxin and antitoxin are set up in varying proportions and observed under constant conditions."[6]

[6] Topley and Wilson, *Principles of Bacteriology and Immunity*, **2**, 865.

[7] Povitsky, O., *J. Immunol.*, 1929, **16**, 421.

were obtained within 48 hours from cultures grown in this basic medium which had 6.5 Lf units per cc. and approximately 500 M.L.D. per cc.

The two sugars have been utilized as follows: (1) Fractional amounts of either glucose or maltose were added to the growing culture from one to 3 times daily for 48 to 72 hours, each addition of sugar being preceded by titration and adjustment of the culture fluid to pH 8.0. Two control flasks with the same sugar concentrations, but which remained unadjusted, were carried along at the same time. (2) A definite amount of each sugar was added to the broth before planting the pellicle with daily titration of the culture medium. (3) In another experiment a given amount of the 2 sugars in combination was added to the broth before planting the pellicle, with daily adjustment of the culture fluid to pH 8.0; control flasks of similar composition but unadjusted pH were run at the same time.

The Ramon flocculation test was carried out in the routine manner[8] on the various filtrates. In those cases where there were repeated titrations in a single day, multiple flasks were run. One sample of fresh antitoxic horse serum† (350 Ehrlich units per cc.) was employed throughout the entire series.

Only approximate M.L.D. determinations were made on the majority of the filtrates. In those instances, however, where the M.L.D. was determined, it was not uncommon to obtain frequently filtrates which would kill guinea pigs of 250-300 gm. weight within 5 days after subcutaneous injection of 1/1000 cc., 1/1400 cc., or even as little as 1/1900 cc.

Our observations may be briefly summarized. Filtrates of high Lf titre (15 units per cc.) were obtained within 48 to 72 hours by semi-daily additions of glucose alone (0.15%), provided there was an adjustment of the hydrogen ion concentration after each addition of the sugar to pH 8.0. Under such conditions the pH at no time fell below 6.7, while without adjustment values as low as 5.6 were attained. The pronounced deleterious effect of the acid reaction on the toxin is demonstrated by the fact that unadjusted filtrates yielded toxins with an Lf titre of only half the value obtained in the more alkaline medium. Very small amounts of maltose alone (0.075%), added 3 times daily, to the growing culture, were found to yield within 48 to 72 hours toxins of considerable strength (8 Lf

[8] Hazen, E. L., *J. Immunol.*, 1930, **19**, 393.

† Kindly supplied by Dr. J. F. Anderson of the Squibb & Sons Biological Laboratories.

units per cc.) although toxin production could be conspicuously enhanced by the addition of larger amounts of this sugar and prolongation of the incubation period to 5 days. In no instance was it necessary to adjust the culture fluid since the pH never fell below 6.8. Toxins of unusually high Lf value were obtained within from 3 to 5 days in a medium which contained both sugars (0.15% glucose and 0.3% maltose), added in combination before inoculation. In experiments carried on in the summer months, filtrates titrating as high as 26.3 Lf units per cc. were harvested with this particular method within 5 days. At other times the filtrates always contained 17 to 20 units per cc. Such filtrates moreover flocculated within 20 minutes, indicating high antigenicity (Schmidt[9]). Both adjusted and unadjusted culture fluids gave approximately the same values.

In conclusion, it may be stated that while either glucose or maltose definitely enhanced toxin production, filtrates of highest potency are obtained in a medium containing both sugars (0.15% glucose 0.3% maltose = 0.45% total carbohydrates). It would seem that the latter method permits of a more rapid and a more abundant toxin production than is commonly known.‡

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Passive Local Sensitization in Atopic Individuals.

MATTHEW WALZER AND KATHERINE BOWMAN.
(Introduced by M. J. Shear.)

From the Department of Bacteriology and Immunology, Cornell University, Medical College, New York Hospital, and the Jewish Hospital, Brooklyn.

The technic for studying the absorption of unaltered proteins in humans has been described.[1] A cutaneous site is passively and locally sensitized with a small amount of serum taken from an atopic patient who is extremely sensitive to the protein to be tested. On the following day, the specific protein is fed to the subject on an empty stomach. Within a few minutes to a few hours, a wheal forms at the sensitized site demonstrating roughly the rapidity and,

[9] Schmidt, S., *Ann. de l'Inst. Past.*, 1930, **45**, 357.

‡ We wish to express our thanks to Mr. Soo-Hoo of the Department of Practice of Medicine, Physicians and Surgeons, Columbia University, for his kind assistance on this work.

[1] Walzer, M., *J. Immunol.*, 1927, **14**, 159.