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Biotin in Elementary Bodies of Vaccinia.*

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Studies of the constituents of elementary bodies of vaccinia have shown that preparations purified by a standard technic exhibit uniformity in their chemical constitution and in their biological activity.[1, 2] The virus appears exceedingly complex and therefore the view that respiratory catalysts, which are known to play an important rôle in bacterial metabolism, may function in the organization of elementary bodies of vaccinia is not untenable. With this in mind, we have examined elementary bodies for growth-factors.

By means of a technic similar to that described by McDaniel, Woolley, and Peterson,[3] vaccine-virus has been shown to contain relatively large quantities of a substance capable of stimulating the growth of *Clostridium butylicum* in a synthetic medium. Moreover, partial hydrolysis of the virus by normal alkali or 4 normal sulfuric acid gives rise to increased quantities of this substance in active form, which, on the basis of studies similar to those made by Snell, Eakins, and Williams,[4] and others[5, 6] on liver, yeast, egg-yolk, etc., we believe to be biotin.

Two recognized technics exist for the microbiological assay of biotin. The first, employed by Snell, Eakins, and Williams[4] depends upon the growth-stimulus afforded by this substance to a standardized strain of yeast, while the second depends upon the stimulus afforded to the growth of *Clostridium butylicum* in a synthetic medium devoid of biotin.[3] Since the growth-requirements are simpler and somewhat better understood in the case of *Clostridium butylicum,* the second technic was adopted as affording the greatest guarantee against introducing with the material to be tested other physiologically active substances.

* We wish to acknowledge our indebtedness to Dr. D. W. Woolley for advice in the use of *Clostridium butylicum* in biotin-assay.

1 Hoagland, C. L., Smadel, J. E., and Rivers, T. M., *J. Exp. Med.*, 1940, **71**, 737.

2 Hoagland, C. L., Lavin, G. I., Smadel, J. E., and Rivers, T. M., *J. Exp. Med.*, 1940, **72**, 139.

3 McDaniel, L. E., Woolley, D. W., and Peterson, W. H., *J. Bact.*, 1939, **37**, 259.

4 Snell, E. E., Eakin, R. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1940, **62**, 175.

5 Weizmann, C., and Rosenfeld, B., *Biochem. J.*, 1937, **31**, 619.

6 Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1939, **61**, 3594.

Elementary bodies of vaccinia were prepared for biotin-assay in 3 ways: first, as dried virus, resuspended by physical agitation; second, by dissolving the dry virus in normal alkali with the aid of heat; and third, by refluxing the dry virus with 4 normal sulfuric acid for 30 minutes at 100°C. The hydrolysates were neutralized with normal acid and alkali, respectively, before they were added to the assay-medium.

A set of standards, consisting of biotin in graded amounts from 0.00004 to 0.0035 γ in a synthetic medium made up according to McDaniel, Woolley, and Peterson[3] which contained 2% dextrose, 0.1% asparagin, and mineral salts, was inoculated with a standard suspension of the vegetative form of *Clostridium butylicum*. The biotin used as a standard was purchased from the S.M.A. Corporation in the form of a concentrate containing 100 γ of active substance per cc when compared with crystalline biotin.

Another set of media in which biotin was replaced by virus-material to be tested was inoculated with a similar suspension of *Clostridium butylicum* and the 2 sets were incubated simultaneously at 37°C for 72 hours in an anaërobic oat jar. All other substances required for the growth of *Clostridium butylicum* were present in excess in the medium so that the degree of growth was proportional to the concentration of biotin added.

Turbidity resulting from the growth of *Clostridium butylicum* was measured by means of an Evelyn-type photocell-colorimeter[7] with the galvanometer scale adjusted to read between 0, when the tube contained media and inoculum only, and 100, when no light was admitted to the photronic cell.

Although elementary bodies alone, with no treatment except autoclaving in the synthetic media prior to inoculation with organisms, yield comparatively large amounts of the growth-factor for *Clostridium butylicum*, partial hydrolysis by normal alkali or 4 normal sulfuric acid yields a ten-fold or even greater increase of this substance. Extraction with alkali and acid serves two purposes: first, to demonstrate resistance of the growth-factor to these substances, and second, to demonstrate an increase in amount after partial hydrolysis. An increased yield of the factor by treatment of the virus with these agents is analogous to the increase in amount of biotin noted in preparations from liver following autolysis.[4]

No absolute value for the concentration of biotin in elementary bodies of vaccinia can be given at this time, but it would appear to approach that observed in egg-yolk, liver, and molasses,[4] which

[7] Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

until now have been considered the richest known sources of biotin. Preliminary studies indicate that in the successive steps of purification of virus, a marked concentration of biotin occurs, parallel with the increase in viral activity. Further studies are needed to establish the degree of hydrolysis necessary for complete release of biotin from vaccine-virus.

The fact that a bacterial growth-factor is associated with elementary bodies of vaccinia is important, but definite proof that such a substance represents an integral part of the virus constitutes a larger problem. Studies on the possible rôle of biotin and other growth-catalysts in the metabolism of vaccine-virus are under way, and will be reported in a later communication.

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Blood Volume in Cobalt Polycythemia.

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Cobalt polycythemia was first produced in rats by Waltner and Waltner.[1] Orten, *et al.*,[2] reported that cobalt polycythemia in the rat was accompanied by a definite increase in blood volume which was due to an increase of cell volume.

We have often produced polycythemia in dogs by feeding cobaltous chloride[3] but have only recently made blood volume determinations on such animals. It is the purpose of the present communication to report the results of these studies.

Procedure. Erythrocyte counts and hemoglobin percentages (Sahli) were determined regularly on 3 dogs which were fed a uniform diet of Purina dog chow. Blood volume was determined by the method of Keith, Rowntree, and Geraghty[4] using the dye brilliant vital red (Evans). Blood samples were drawn from the external saphenous vein while the dogs were lying quietly on a table,

1 Waltner, K., and Waltner, K., *Klin. Wochenschr.*, 1929, **8**, 313.

2 Orten, J. M., Underhill, F. A., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1933, **99**, 457.

3 Davis, J. E., *Am. J. Physiol.*, 1938, **122**, 397; 1939, **127**, 322; 1940, **129**, 140.

4 Keith, N. M., Rowntree, L. G., and Geraghty, J. T., *Arch. Int. Med.*, 1915, **16**, 547.