of clubs and fairs, the continuation of the projects award, the extension of the lantern slide loan program and the continuation of the "discussion meeting" for high-school teachers at the time and place of the academy meeting. The academy appropriated additional funds for lantern slides.

The high-school projects award of $20.00 was voted to Mr. Don Fisher, of the Salisbury High School, and Mr. Clyde Vaugh, of the Charlotte High School, was recommended for the junior membership in the American Association for the Advancement of Science.

The research grants committee for the American Association research grant recommended that the grant be divided between S. B. Knight and H. W. Jensen.

Resolutions upon the death of Dr. A. A. Dixon, of State College, were adopted by a rising vote.

The report of the conservation committee dealt with the problems of making the great Dismal Swamp a national preserve, governmental purchase of certain wooded mountain areas and the commercialization of the venus fly trap and its possible extinction.

Action was taken supporting the report of the subcommittee of the academy conference on the "Relations of the American Institute to the Junior Academy."

The Poteat Award for the most noteworthy paper presented before the academy was given to Dr. N. F. Conant, of the Duke Medical School.

The report of the treasurer was ordered printed in the *Proceedings* after being properly audited.

The following officers were elected:

President: J. L. Stuckey, State College.

Vice-President: O. J. Thies, Davidson College.

New member of the Executive Committee: A. S. Pearse, Duke University.

New members of the Research Grants Committee: O. C. Bradbury, Wake Forest and H. D. Crockford, University of North Carolina.

The following were elected as officers of sections:

Botany: Chairman, J. N. Couch; *Secretary,* Earl H. Hall.

Geology: Chairman, J. W. Huddle; *Secretary,* Willard Berry.

Physics: Chairman, R. H. Lyddane; *Secretary,* F. W. Lancaster.

Psychology: Chairman, O. H. Lundholm; *Secretary,* J. F. Dashiell.

Zoology: Chairman, H. F. Prytherch; *Secretary,* Z. P. Metcalf.

High School Science Teachers: Chairman, Thomas Baldwin; *Secretary,* John W. Wood.

Davidson College entertained the academy in excellent style; administration, faculty and students cooperated to make the meeting pleasant and successful. The college entertained at dinner on Friday evening, and following the retiring presidental address by Dr. H. L. Blomquist on "Grasses and Man," provided a social hour where old acquaintances could be renewed and new ones made.

BERT CUNNINGHAM,
Secretary

DUKE UNIVERSITY

SPECIAL ARTICLES

BIOTIN (BIOS II_B, VITAMIN H)—AN ESSENTIAL GROWTH FACTOR FOR CERTAIN STAPHYLOCOCCI

THE basic nutritional requirements of *Staphylococcus aureus* have only recently been elucidated. In a medium composed of amino acids, glucose and inorganic salts, it has been necessary to add a minute amount of an unidentified supplement before aerobic growth will occur. Knight[1] first resolved the supplement into three parts, two being the pyrimidine and thiazole components of vitamin B_1, and the third being nicotinamide, a component of Warburg's coenzymes. For the anaerobic growth of this bacterium, Richardson[2] has demonstrated that uracil must be added to the medium, in addition to the above compounds.

A study was undertaken in our laboratory to determine whether the quantity of growth produced by several strains of *Staphylococcus aureus* in a chemically-defined medium (Gladstone[3]) was equal to that obtainable in standard glucose meat-infusion broth. The quantity of growth was determined by measuring the bacterial nitrogen with a micro-Kjeldahl technique. Preliminary results revealed that the chemically-defined medium was inferior to glucose meat-infusion broth. More interesting, however, was the fact that certain strains could not initiate growth in the synthetic medium on continued subculture. In an attempt to ascertain the substance or substances required for growth by these more fastidious strains, the chemically-defined medium of Gladstone was supplemented with several other compounds which have been demonstrated to be of importance in bacterial nutrition. These included other amino acids (norleucine, asparagine, α-amino valeric acid, β-amino valeric acid, taurine, threonine, β-alanine, diiodotyrosine and ornithine) in addition to the sixteen present in the basic medium, as well as the growth factors: riboflavin,[4] ino-

[1] B. C. J. G. Knight, *Biochem. Jour.*, 31: 731, 966, 1937.
[2] G. M. Richardson, *Biochem. Jour.*, 30: 2184, 1936.
[3] G. P. Gladstone, *Brit. Jour. Exp. Path.*, 18: 322, 1937.
[4] For these compounds we wish to thank the Research Department of Merck and Company, Dr. Henry Tauber and Professor Roger J. Williams.

sitol, pimelic acid, glutamine, glutathione, vitamin B_6,[4] pantothenic acid,[4] cocarboxylase,[4] cozymase,[4] uracil, guanine, adenine, adenylic acid[4] and adenosine triphosphate.[4] Repeated attempts to cultivate these staphylococci in media containing various concentrations of the above compounds met with failure. It thus became apparent that some other nutrient or vitamin-like substance was needed.

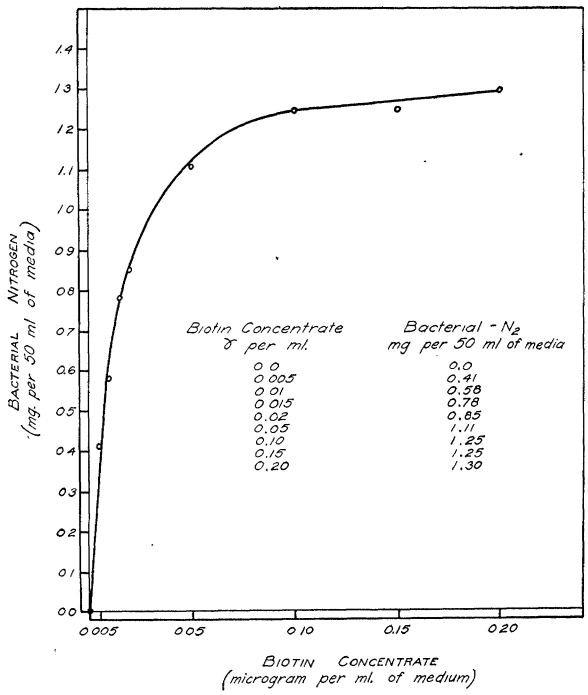

FIG. 1. Effect of increasing the concentration of biotin on the growth of *Staphylococcus aureus* (X 3 strain).

At this time extracts of both plant and animal tissues were prepared, subjected to various chemical treatments and tested for their growth-promoting properties. The results indicated that the additional growth factor required by these organisms could be readily adsorbed on charcoal and eluted with acetone containing ammonia. This suggested that we were probably dealing with biotin or some closely allied substance. Following the procedure of Kögl and Tönnis[5] for the purification of biotin, we prepared a concentrate from dried egg yolks[6] which permitted growth of these staphylococci in a concentration as low as 0.0001 microgram per milliliter, when added to Gladstone's medium. At the same time it was observed that a relationship existed between the amount of growth which took place and the concentration of biotin in the medium. This relationship was investigated quantitatively by cultivating the organisms in media containing various concentrations of the biotin preparation. After incubation at 37° C. for twenty-four hours, the amount of bacterial nitrogen was measured by a micro-Kjeldahl technique. Typical results are presented in Fig. 1, where it will be seen that the addition of a very minute amount of the biotin preparation gave a marked stimulation. A concentration of about one-tenth microgram per milliliter resulted in maximum growth under the conditions of this experiment. Without biotin, growth was neither detectable visibly nor by quantitative measurement. Similar stimulation was also observed by measurements using a photoelectric turbidimeter.

It is of interest that we have been able to replace our biotin concentrate with a preparation of bios II_B, furnished by Dr. C. N. Frey, of Fleischmann Laboratories, and a sample of vitamin H, from Dr. P. György. This fact lends further support to the recent work of György, Melville, Burk and du Vigneaud,[7] who have indicated that vitamin H, biotin and the coenzyme R factor are porbably identical.

The manner in which these particular strains of *Staphylococcus aureus* respond to the biotin, bios II_B, or vitamin H concentrates, suggests the possibility of using them for the bio-assay of these substances. Employing such strains in a technique similar to the yeast-growth test of Snell, Eakin and Williams[8] might be advantageous, since biotin (bios II_B, vitamin H) is essential before any detectable growth will occur. Evaluation of such a technique must naturally await experimental data obtained with crystalline biotin.

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CHANGES IN THE CONNECTIVE TISSUE OF THE UTERUS AND VAGINA OF THE RAT ASSOCIATED WITH ADVANCING AGE[1]

LOEB[2,3] and associates found that with advancing age there is an increase in the amount of fibrillar and hyaline connective tissue in the uterus, vagina and cervix of the mouse. Using methods which differentiate reticulum from collagen, we have carried out similar studies in the rat. Seventy-six rats varying in age from 15 to 823 days of age were used. Tissues were

[5] F. Kögl and B. Tönnis, *Ztschr. physiol. Chem.*, 242: 43, 1936.

[6] For the dried egg yolks we wish to thank V. Conquest, director of research, Armour and Company.

[7] P. György, D. B. Melville, D. Burk, V. du Vigneaud, SCIENCE, 91: 243, 1940.

[8] E. E. Snell, R. E. Eakin and R. J. Williams, *Jour. Am. Chem. Soc.*, 62: 175, 1940.

[1] These studies were aided by grants from the Josiah Macy, Jr. Foundation and from the International Cancer Research Foundation.

[2] Leo Loeb, V. Suntzeff and E. L. Burns, SCIENCE, 88: 432–433, 1938.

[3] Leo Loeb, V. Suntzeff and E. L. Burns, *Am. Jour. Cancer*, 35: 159–174, 1939.