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.

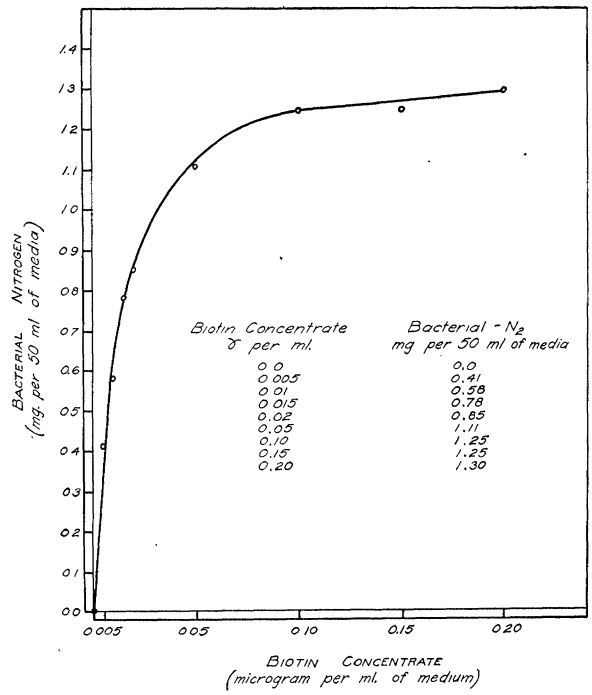

FIG. 1. Effect of increasing the concentration of biotin on the growth of *Staphylococcus aureus* (X 3 strain).

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.

J. R. PORTER
MICHAEL J. PELCZAR, JR.
STATE UNIVERSITY OF IOWA

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.

fixed in 10 per cent. formalin and stained by Gömöri's[4] method for the demonstration of reticulum and collagen. This procedure stains reticulum black and collagen old-rose.

These studies support the view that reticulum is a precollagenous type of connective tissue and may be transformed into collagen. A transitional phase was found between the two. The uterine connective tissue of the 15-day rat was chiefly reticulum. In the endometrium a fine reticular network extended from beneath the lining epithelium, where it was condensed to form a basement membrane, to or almost to the circular smooth muscle (C.S.M.). A narrow zone of collagen usually bordered the C.S.M. A network of reticulum was found about the muscle cells in both the circular and longitudinal smooth muscle (L.S.M.) layers. Peripheral to the L.S.M. and underlying the serosa a condensation of narrow collagen fibers formed a subserosal layer. Numerous trabeculae extended inward and divided the L.S.M. layer into irregular columns of cells. The connective tissue of the vaginal mucosa was definitely collagenous, although immediately below the epithelium a narrow line of argyrophilic material was found.

In increasing older rats there was in the uterus a gradual transformation of reticulum into collagen and a definite increase in the amounts of collagen which was most marked in the very old animals. Such changes occurred earliest and most markedly in the endometrium. In rats over six months of age collagen extended from the C.S.M. practically to the lining epithelium, although a reticular basement membrane almost invariably persisted even in the oldest rats. Here there was a gradual thickening of the collagen fiber bundles which often measured as much as 12 micra. As age increased there was a tendency for the reticulum of the C.S.M. and the L.S.M. to be transformed into collagen. These changes, however, were extensive only in rats over eighteen months of age. The subserosal layer and the trabeculae penetrating the L.S.M. became markedly thickened. Throughout life, and especially in the very old animals, there was an increase in the size and density of the collagenous fibers of the vaginal mucosa.

J. M. WOLFE
ETHEL BURACK
WINIFRED LANSING
A. W. WRIGHT

ALBANY MEDICAL COLLEGE,
UNION UNIVERSITY

RADIOACTIVE NITROGEN IN THE STUDY OF N_2 FIXATION BY NON-LEGUMINOUS PLANTS

THERE has been considerable controversy regarding the ability of non-leguminous plants to fix atmospheric nitrogen. Lipman and Taylor[1,2] and many others have presented positive evidence for N_2 fixation by wheat, barley, etc. In the experiments of Lipman and Taylor plants were grown both in the presence and absence of nitrogenous salts and were analyzed by a modified Kjeldahl method. The results showed that the plants contained more N than could have been obtained from the growth media. However, their conclusions have not been generally accepted.

It should be possible, using radioactive nitrogen, to obtain additional information regarding this problem. Despite the 10.5 minute half-life of N^{13} the yields obtainable in the Berkeley cyclotron are such as to make this feasible. The nuclear reactions are:

$$ {}_1D^2 + {}_6C^{12} \rightarrow {}_7N^{13} + {}_0n^1 \text{ and } {}_7N^{13} \rightarrow e^+ + {}_6C^{13}. $$

Carbon (charcoal) was bombarded in a gas-tight chamber incorporating many features found to be of value in the Radiation Laboratory for the handling of high intensity beams. The active gas in the target holder was passed through a heated combustion tube containing cupric oxide into a pyrex desiccator containing the barley plants. Only the tops were used; the plants were cut well above the roots to eliminate any bacteria clinging to the roots. The charcoal which contained the major fraction of the N^{13} was introduced into the combustion tube and burned in a stream of O_2. As a control an equal weight of barley killed by immersion in boiling water was present in the desiccator.

After the plants were exposed to the N_2^* for ~ 20 minutes they were removed and extracted with boiling 80 per cent. ethanol. After filtration the extract was boiled vigorously in a stream of air. The method of counting has been described elsewhere.[3] In the first experiments both the live and dead plants were found to contain N^*. This was due to the presence of radioactive combined nitrogen (*i.e.*, CN, NH_3, NO, etc.) produced by the recoiling N^{13} atoms during the bombardment with the energetic (8 MEV) deuterons. The N_2^* was purified of combined nitrogen by slowly passing the active gases over heated CuO and then through a series of five traps and spirals immersed in liquid air. Before entering the trap system the gas stream was passed through solutions of HNO_3 and NH_4OH. The effectiveness of the purification was shown by the fact that no traces of NH_3 could be detected with Nessler's reagent, which is extremely sensitive for small amounts of NH_3.

Observing these precautions the live plants were found to contain N^*, while no activity (< 1 per cent.) could be detected in the control plants. The results of a typical experiment are shown in Table 1. The activ-

[4] G. Gömöri, *Am. Jour. Path.*, 13: 993–1001, 1937.

[1] Lipman and Taylor, SCIENCE, 56: 605, 1922.
[2] Lipman and Taylor, *Jour. Frank. Inst.*, 198: 475, 1924.
[3] Ruben, Hassid and Kamen, *Jour. Am. Chem. Soc.*, 61: 661, 1939.