large or small numbers of bacteria. As shown in Fig. 1, plaque formation was revealed to be independent of phenotypic alterations in colonial morphology, but dependent upon the rate of growth occurring subsequent to phage exposure. Thus, on media inoculated with a large number of bacteria the logarithmic phase of growth terminated sooner in the presence of penicillin than in its absence (see upper portion of Fig. 1) and a correspondingly earlier termination of the period leading to plaque formation resulted. Smaller bacterial inocula led to longer periods of logarithmic growth and consequently to no differences in plaque formation between cells grown on penicillin-free and penicillin-containing media during 48 hr incubation at 37 C (see lower portion of Fig. 1). These results were identical regardless of whether the tests were conducted with penicillin-modified organisms first grown for several transfers on penicillin-containing media, or with organisms that had been transferred from media free of penicillin to media containing from 20 to 200 units of penicillin per ml.

These findings indicate that with regard to colonial morphology and acriflavine reactivity penicillin-induced phenotypic variants of smooth *B. abortus* represent true phenocopies (Braun, *Bacterial Genetics*, W. B. Saunders, 1953, p. 3) of genotypically nonsmooth mutants; however, with regard to receptor sites for brucellaphage, there appears to be a basic difference between genotypically nonsmooth mutants and penicillin-induced phenotypically, but not genotypically, nonsmooth variants.

# MICROBIAL TRANSFORMATION OF RAUWOLFIA ALKALOIDS

## II. 10-HYDROXYYOHIMBINE

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Pan and Weisenborn (J. Am. Chem. Soc., **80,** 4749, 1958) reported on the microbial hydroxylation of yohimbine at carbon 18, and we now wish to report the prevalence of microorganisms that effect the conversion of the same alkaloid to a product with the properties described for 10-hydroxyyohimbine. This transformation was noted by Godtfredsen et al. (Experientia, **14,** 88, 1958), using *Cunninghamella blakesleeana* Lendner with three analogues of yohimbine: apoyohimbine, $\beta$-yohimbine methyl ether and 3-epi-apoyohimbine. Loo and Reidenberg discovered the 10-hydroxylation of yohimbine by *Streptomyces platensis* (Arch. Biochem. Biophys., **79,** 257, 1959). The purpose of this note is to extend the number and diversity of microorganisms shown to be capable of effecting this transformation.

The fermentation was carried out in a medium containing: soy-bean meal, 15 g; glucose, 25 g; $CaCO_3$, 2.5 g; yohimbine hydrochloride, 0.5 g; water, 1 liter; pH 7.2. The fermentation was allowed to proceed for 10 days before harvesting. Details of the fermentation and chromatography were previously described (Pan and Weisenborn, J. Am. Chem. Soc., **80,** 4749, 1958). A chromatographic system using *n*-butanol-water-glacial acetic acid (8:2:1) was used in addition to the *i*-amyl alcohol-carbon tetrachloride-propionic acid system described by Pan and Weisenborn.

The fermentation product was compared with a sample of authentic 10-hydroxyyohimbine kindly supplied by Dr. Loo of the Lilly Research Laboratories. Only one spot was observed when the product was cochromatographed with the authentic 10-hydroxyyohimbine in both of the chromatographic systems. In the *i*-amyl alcohol system, the material had an $R_F$ of 0.07; in the *n*-butanol system, it had an $R_F$ of 0.63. A positive test for phenols was obtained with both acid ferric ferricyanide and diazotized sulfanilic acid spray reagents. The nitrosonaphthol test of Udenfriend et al. (J. Biol. Chem., **215,** 337, 1955) showed the presence of a 5-hydroxyindole structure, i.e., hydroxylation at carbon 10 of yohimbine. The ultraviolet absorption spectrum of the product agrees with that of the authentic material, as reported by Loo and Reidenberg (Arch. Biochem. Biophys., **79,** 257, 1959).

The ability to bring about this change is not limited to a definite taxonomic group, but rather

is widespread. We have found that representatives of the Actinomycetes, Phycomycetes, Ascomycetes and Fungi Imperfecti can cause this transformation. This diversity is paralleled in the field of steroid transformations (Vischer and Wettstein, Advances in Enzymol., **20,** 237, 1958). The following list of organisms that can transform yohimbine to 10-hydroxyyohimbine illustrates this point:

*Streptomyces scabies, S. rimosus, S. venezuelae, S. chrysomallus, S. griseus, S. aureofaciens, Synchephalis nodosum, Cunninghamella blakesleeana, C. baineri, Sordaria fimetaria, Stysanus microsporus,* and *Trichoderma viride.* The ability is strain-specific as not all strains of any one species were capable of effecting the change. Interestingly, none of the bacteria or yeasts tested gave evidence of carrying out this oxidation. However, this apparent restriction might be a reflection of the conditions employed, rather than of the inherent abilities of these microorganisms.

## RELATION OF BIOTIN TO INORGANIC PHOSPHATE: ADENOSINE TRIPHOSPHATE EXCHANGE

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In a study of the enzymatically catalyzed exchange of inorganic phosphate with adenosine triphosphate (ATP), we have found that this exchange is a linear function of the biotin content of the enzyme preparations (Fig. 1). This provides independent support for the concept of Lynen et al. (Angew. Chem., **15/16,** 481, 1959) that there exists a biotin-containing enzyme reacting with ATP as follows: (1) ATP + biotin-enzyme $\rightleftharpoons$ biotin-enzyme-ADP + inorganic P. For the study of this exchange, we used a method essentially that of DeMoss and Novelli (Biochim. et Biophys. Acta, **22,** 49, 1956) using the charcoal adsorption method of Crane and Lipmann (J. Biol. Chem., **201,** 235, 1953) for the separation of the adenine nucleotides from the inorganic $P^{32}$ used as a tracer. For the enzyme source we used acetone-dried powders of *Saccharomyces cerevisiae* strain 139, grown for 96 hr in defined biotin-free media (Snell et al., J. Am. Chem. Soc., **62,** 175, 1940) supplemented with 100 $\mu$g of niacin and 20 $\mu$g of *p*-aminobenzoic acid per liter. This organism is capable of a modest growth in this medium and a modest synthesis of biotin, but its growth is markedly enhanced by the addition of aspartate (1 mg/ml) without concomitant increase in biotin synthesis. As such, it was possible to prepare a series of enzyme preparations containing different quantities of biotin which was estimated in the preparations by the method of Chang et al. (J. Bacteriol., **58,** 33, 1949). The activity of the ATP$\rightleftharpoons$phosphate exchange enzyme was directly proportional to the biotin content of the enzyme preparations.

The activity of the exchange system could be neither inhibited nor enhanced by the addition of biotin to the measurement system. The addition of $CO_2$, which would be expected by Lynen's hypothesis (Angew. Chem., **15/16,** 481, 1959) to lower the ATP exchange, showed a markedly decreased exchange in some experiments, but in others there was only a small decrease. Whether or not the $CO_2$ was capable of decreasing the ATP exchange would depend on the presence of active systems capable of exchanging $CO_2$ with the biotin enzyme-adenosine diphosphate (ADP) complex, which enzyme may not have been present in all preparations.

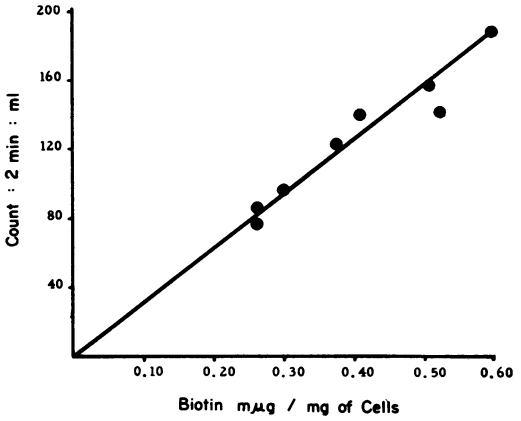


*Fig. 1.* Relationship of biotin content to $P^{32}$ $\rightleftharpoons$ ATP exchange.

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