for 5 minutes. On the surface of such a turbid agar plate were made pin-point inoculations of various sporogenic strains. The formation, after 24 hours of incubation, of clear transparent zones around the sporogenic colonies gave evidence of lysis, and absence of clarification of the turbid agar showed that the dead bacteria were not affected by the corresponding sporogenic strain. With living bacteria the following results were obtained: Of the 31 sporogenic strains 7 were found to be inactive both for Gram-positive and Gram-negative species. Of the remaining 24 strains, 6 were active only against some Gram-positive bacteria but failed entirely to affect the Gram-negative group, and, finally, 18 affected organisms of both groups. In the Gram-positive group: *Sarcina flava* was inhibited in its growth by 24 strains, *Staphylococcus aureus* by 21 strains, Air Coccus by 21 strains, *B. diphtheriae* by 19 strains, *Pneumococcus* Type I by 11 strains, *Streptococcus hemolyticus* by 11 strains, *Streptococcus viridans* by 9 strains; in the Gram-negative group: *B. dysenteriæ Shiga* by 14 strains, *B. prodigiosus* by 14 strains, *B. typhi* by 12 strains, *B. proteus* by 11 strains, *B. coli* by 3 strains, *B. paratyphi B* by 2 strains, *B. pyocyaneus* by no strains. In experiments with dead bacteria all the 31 sporogenic strains, including the 6 which were found to be deprived of any antagonistic properties against living bacteria, produced lysis of all 7 Gram-negative organisms, but failed to affect in any visible way the bacteria of the Gram-positive group.

Conclusion. The tested living Gram-positive organisms were more susceptible to the antagonistic action of sporogenic aerobic bacilli than the living Gram-negative. In contrast, all tested dead Gram-negative bacteria were susceptible to the action of all sporogenic strains, while dead Gram-positive remained unaffected.

12024

Biotin Synthesis by Microörganisms.

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It has been postulated that many identified growth factors, and others still unrecognized, are required by microörganisms in general, and that many types of organisms do not require these growth factors

preformed since they presumably can synthesize them from simple nutrient materials.[1]

In order to determine whether the above postulate holds true for biotin, a study was made of the synthesis of this growth factor by microörganisms. It was necessary to select for study only organisms which could be cultured satisfactorily in completely synthetic biotin-free media. A wide variety of such organisms were studied. The media used in this study varied with the particular organism investigated. All, however, were completely synthetic in composition and biotin-free.

Inocula were kept to a minimum and cultures were incubated until maximum growth had been obtained. Cultures were hydrolyzed with strong mineral acid to release any biotin in the bacterial cells.[2] To each was added 0.5 ml of concentrated HCl and they were autoclaved at 15 pounds pressure for one hour. The cultures were neutralized and assayed for biotin by the yeast growth method of Snell, Eakin and Williams.[2] This method has in our hands shown a consistent sensitivity to quantities of biotin as small as 0.1 millimicrogram. The reference standard employed in all assays was a sample of crystalline biotin methyl ester, supplied by Dr. Vincent du Vigneaud. Culture medium and toxic inhibition controls were run with all assays.

As will be noted in the examination of Tables I and II, biotin is apparently not required preformed for the organisms investigated, all of which grew in synthetic media and elaborated biotin to a greater or lesser degree. In addition it will be noted from Table II that by far the largest quantities of biotin produced was found in the cell-free medium. It was not possible to definitely ascertain whether this was due to autolysis of the bacterial cells or whether the biotin was regularly secreted into the medium. The *Azotobacter* cultures were incubated for 5 days, the others for a period of 3 days.

It is necessary to emphasize that these results are only indicative and are not to be regarded as absolute for the various organisms reported. Several variables such as culture strain, medium, inoculum, incubation time and hydrolysis or autolysis may all affect the final biotin concentration.

It would be highly improbable that the organisms tested, all of different types and all chosen haphazardly, should in every instance synthesize a substance which we at present know to be required by

[1] Koser, S. A., and Saunders, F., *Bact. Rev.*, 1938, **2**, 145.

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

TABLE I.
Biotin Synthesis by Microörganisms Grown in Amino Acid—Glucose Medium.

Culture	mγ Biotin per 9 cc culture*
Mycobacterium tuberculosis	215
Proteus vulgaris	89
Alkaligenes fecalis	16
Escherichia coli	273
Bacillus anthracis	20
Aerobacter aerogenes	144
Serratia marcescens	126
Eberthella typhi	226
Pseudomonas aeruginosa	46
Staphylococcus aureus	226
Sarcina lutea	14
Klebsiella pneumoniæ	72
Bacillus subtilis	44
Monilia albicans	3
Pencillium	9
Epiphyton interdigitale	5
Mucor	5
Aspergillus niger	14
Aspergillus oryzæ	11

mγ = millimicrogram or 0.001 μg.
*Cultures hydrolyzed by autoclaving with strong acid to yield maximum values.

TABLE II.
Biotin Synthesis by Bacteria Grown in Synthetic Media in 1 Liter Volumes: Distribution of Biotin in Cells and Culture Medium.

Culture	mγ Biotin per 1000 cc culture			
	Medium	%	Cells*	%
Klebsiella pneumoniæ	12,540	97.1	375	2.9
Escherichia coli	950	82.9	163	17.1
Aerobacter aerogenes	3,382	90.1	336	9.9
Alkaligenes fecalis	338	84.0	53	16.0
Bacillus subtilis	380	96.9	12	3.1
Azotobacter vinelandii (W)	19,380	95.5	874	4.5
Azotobacter vinelandii (B-1)	15,200	88.6	1786	11.4

*Acid hydrolyzed to release bound biotin.

only a few bacterial species. Biotin is probably synthesized by many other organisms which can grow in synthetic media. Our evidence indirectly supports the postulate that biotin may be required as a growth essential by bacteria besides the *Clostridia*,[3, 4] *Rhizobia*,[5, 6] and *Staphylococci*.[7, 8]

[3] Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1939, **61**, 3594.

[4] Peterson, W. H., McDaniel, L. E., and McCoy, E., *J. Biol. Chem.*, 1940, **133**, lxxv.

[5] Nilsson, R., Bjälfve, G., and Burstrom, D., *Ann. Landw. Hochschule Schwedens*, 1939, **7**, 301.

[6] West, P. M., and Wilson, P. W., *Enzymologia*, 1940, **8**, 152.

[7] Kogl, F., and Van Wagtendonk, W. J., *Recueil des Travaux Chimiques de Pays-Bas*, 1938, **57**, 747.

[8] Porter, J. R., and Pelczar, M. J., *Science*, 1940, **91**, 576.

Summary. A wide variety of bacterial species and several molds grown in synthetic biotin-free media have been shown to synthesize biotin to a greater or lesser degree as measured by the yeast-growth biotin assay method. This evidence suggests that biotin may be of widespread importance in microbial nutrition.

12025 P

Inactivation of Testosterone Propionate by Normal Female Rats.

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The presence of androgens in normal female animals predicates a system for their inactivation and removal. The origin of these androgens is considered to be in either the ovary[1] or the adrenal.[2] The following experiment shows that one site of inactivation for these substances is the liver, just as it is for estrogens[3, 4] and androgens[3, 5] in female and male castrate rats respectively.

Pellets of testosterone propionate* produce anestrus in adult female rats when implanted in the subcutaneous tissues.[6] The duration of production of anestrus, as determined by daily vaginal smears, coincides with the presence of the pellet in the tissues. Anestrus results from inhibition of the hypophysis leading to suppression of the gonadotropic factor.[2] After the pellets are removed estrus cycles resume their normal pattern; however, occasional periods of estrus may predominate. This effect of the pellets of testosterone propionate on the estrous cycle was tested in 5 normal adult female rats for a period of 60 days. The average absorption from the pellets was .050 mg per day.

A pellet of testosterone propionate implanted in the spleen of an adult normal female rat causes no significant change in the cyclic

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2 Koch, F. C., *Physiol. Rev.*, 1937, **17**, 153.

3 Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.

4 Biskind, G. R., *Endocrinology*, in press.

5 Biskind, G. R., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 259.

* Supplied as Perandren through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, N.J.

6 Mark, J., and Biskind, G. R., *J. Clin. Endocrinol.*, in press.