transport is enhanced in an oxygen-saturated hemoglobin solution, and within the limits of experimental error is equal to the net transport which was found when one side of the membrane was kept at zero oxygen tension.

EDVARD HEMMINGSEN
Scripps Institution of Oceanography, La Jolla, California

References and Notes

1. P. F. Scholander, *Science* **131**, 585 (1960).
2. E. Hemmingsen and P. F. Scholander, *ibid.* **132**, 1379 (1960).
3. R. E. Collins, *ibid.* **133**, 1593 (1961).
4. J. H. Wang, *ibid.* **133**, 1770 (1961).
5. I. Fatt and R. C. La Force, *ibid.* **133**, 1919 (1961).
6. This system was suggested by T. Enns.
7. A. Mauro, *Science* **126**, 252 (1957).
8. This investigation was supported by a research grant (No. RG-5979) from the U.S. Department of Health, Education, and Welfare. I wish to thank P. F. Scholander and T. Enns for stimulating discussions and advice.

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Myo-inositol in the Biosynthesis of Streptomycin by Streptomyces griseus

Abstract. When myo-inositol is present in a growing culture of *Streptomyces griseus* the yield of streptomycin is increased, and the amount of carbon-14—from uniformly labeled glucose—incorporated into the streptidine and streptobiosamine moieties of streptomycin is lowered by 41 percent and 21.7 percent, respectively. These results present strong indirect evidence for the participation of myo-inositol or its metabolic products in streptidine biosynthesis.

Karow *et al.* (*1*) were the first to show that labeled carbon of C^{14}-glucose is incorporated into the streptomycin molecule. Later Hunter and Hockenhull (*2*) demonstrated that the carbon-14 of uniformly labeled glucose is equally distributed among the three constituents of the streptomycin molecule—namely, streptidine, streptose, and N-methyl-L-glucosamine. Only the carbon atoms of the guanidine side chains of streptidine appeared to be derived from CO_2, arginine being a possible intermediate (*3*). A cyclitol formed by ring closure of the glucose supplied in the medium was postulated as an intermediate in the synthesis of the streptidine ring and of the L-glucosamine (*2*). However, no intermediate between glucose and these units has yet been demonstrated. The observation that myo-inositol, especially in combination with arginine, stimulates streptomycin formation (*4*) prompted us to undertake the investigation reported here. Since labeled inositol was not available, its incorporation into streptomycin had to be studied with the help of the isotope dilution technique.

The organism used in these studies was *Streptomyces griseus*, strain L118 (*5*). The fermentation of 50-ml broth portions placed in 250-ml erlenmeyer flasks was carried out on a reciprocal shaker at 28°C. Two flasks contained a medium of the following composition (in grams per liter): glucose, 10.0; yeast extract, 10.0; arginine 0.2; NaCl, 2.5; $MgSO_4 \cdot 7H_2O$, 0.25; $FeSO_4 \cdot 7H_2O$, 0.01. The *p*H of the medium before sterilization was 7.2. Two other flasks contained the same medium supplemented with myo-inositol (0.5 g/lit.). After 31 hours of growth, 50 μc of uniformly labeled C^{14}-glucose (1.08×10^{-2} mmole) (*6*) was added to each medium. The cultures were harvested 103 hours after inoculation. Streptomycin was assayed by the paper disk method of Loo *et al.* (*7*). The isolation of the antibiotic and its chemical degradation to streptidine and streptobiosamine were carried out by the method of Hunter *et al.* (*3*). All radioactivity measurements were made with a Geiger-Müller gasflow counter (Superscaler, Tracerlab, Inc.).

The yield of streptomycin from the medium without myo-inositol was 260 μg/ml; with myo-inositol there was an increase to 350 μg/ml. As shown in Table 1, the streptomycin isolated from the control medium exhibited a specific activity of 7288 count/min per μmole, whereas the streptomycin from the same medium supplemented with myo-inositol showed a specific activity of 5127 count/min per μmole. It is also evident from the data that less of the carbon-14 from the supplied glucose was incorporated into the streptidine and streptobiosamine moieties of the streptomycin isolated from the myo-inositol-supplemented broth; the decrease of specific activity was 41 and 21.7 percent, respectively. The values obtained for streptidine strongly suggest that myo-inositol serves as a precursor for this part of the streptomycin molecule. The role of myo-inositol in the formation of streptose and/or L-glucosamine, which appears to be less significant, cannot be evaluated from the available data. There is a possibility that opening of the inositol ring between the C-3 and C-4 positions may lead to the formation of the L-sugar. Such a mechanism has been suggested by Charalampous *et al.* (*8*) to explain the formation of L-glucuronic acid from myo-inositol by enzymes of rat kidney. Inconsistent with such an assumption, however, are the results of Silverman and Rieder (*9*).

Table 1. Influence of unlabeled myo-inositol on the incorporation of radioactivity from C^{14}-glucose into various parts of the streptomycin molecule, given in counts per minute (cpm).

Specific activity of control		Specific activity with myo-inositol added	
cpm/μmole	cpm/mg carbon ($\times 10^3$)	cpm/μmole	cpm/mg carbon ($\times 10^3$)
Streptomycin			
7288	28.9	5127	20.3
Streptidine			
2980	31.0	1755	18.3
Streptobiosamine (by difference)			
4523	29.0	3541	22.7

S. K. MAJUMDAR*
H. J. KUTZNER†
Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

References and Notes

1. E. O. Karow *et al.*, *J. Am. Chem. Soc.* **74**, 3056 (1952).
2. G. D. Hunter and D. J. D. Hockenhull, *Biochem. J.* **59**, 268 (1955).
3. G. D. Hunter *et al.*, *ibid.* **58**, 249 (1954).
4. N. S. Egorov, *Antibiotiki* **4**, 265 (1959); L. A. Galanina and P. A. Agatov, *Doklady Akad. Nauk S.S.S.R. (Biol. Sci. Sect.)* **127**, 670 (1959); S. K. Majumdar and H. J. Kutzner, *Appl. Microbiol.*, in press; K. L. Temple, thesis, Rutgers Univ. (1948).
5. The *Streptomyces griseus*, strain L118, was obtained from H. B. Woodruff of Merck, Sharp and Dohme Research Laboratories, Rahway, N.J.
6. The C^{14}-glucose was obtained from New England Nuclear Corp., Boston, Mass.
7. Y. H. Loo *et al.*, *J. Bacteriol.* **50**, 701 (1945).
8. F. C. Charalampous *et al.*, *J. Am. Chem. Soc.* **80**, 2022 (1958).
9. M. Silverman and S. V. Rieder, *J. Biol. Chem.* **235**, 1251 (1960).

* Chas. Pfizer postdoctoral fellow in microbiology. Present address: Department of Biochemistry, Calcutta University, Calcutta, India.

† Supported in part by National Science Foundation grant G-13950. Present address: Institut für Bakteriologie, Weihenstephan, Germany.

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