fluoride excretion systems are either associated or are the same system and that subjecting a fish to concentrations of chloride which are nontoxic in low concentrations can elicit a response in fish for fluoride excretion which is toxic in low concentrations (*4*).

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Accelerated Exchange of Oxygen-18 Through a Membrane Containing Oxygen-Saturated Hemoglobin

Abstract. Membranes containing hemoglobin solution were subjected to identical oxygen pressures on both sides, which were high enough to fully saturate the hemoglobin. The exchange of oxygen through the membrane was studied by means of oxygen-18, and it grossly exceeded that obtained with membranes containing methemoglobin or only water.

Previous works have shown that when a porous membrane is filled with hemoglobin solution and subjected to a vacuum on one side and various oxygen tensions higher than the saturation value on the other, the steady-state transport of oxygen through the membrane is enhanced (*1*, *2*). The increase in rate appears to be a constant which is added to a straight Fick's diffusion through the solvent. The effect decreases with viscosity and is abolished by a slight back-pressure of oxygen.

This transport has recently been treated theoretically by various authors (*3–5*). From two of the proposed equations it may be inferred that the oxygen transport enhancement depends upon a hemoglobin saturation gradient (*3*, *5*). This is not borne out by the published data, however, for oxygen pressures were used which surpassed the saturation value by as much as 5 times without diminishing the enhancement. With such high oxygen pressure, it seemed evident that full steady-state transport took place through a saturated layer.

In the present investigation, the passage of O^{18}-labeled oxygen through membranes containing fully oxygen-saturated hemoglobin has been studied. A Millipore filter saturated with hemoglobin solution (*1*, *2*), was placed between two chambers 4 cm in diameter and 1 cm deep. Each chamber was furnished with an inlet and an outlet and kept moist by a wet filter paper. The total gas pressure was kept at 1 atm, but the oxygen-nitrogen ratio was varied. The two sides were always maintained at identical oxygen pressures, but at different O^{18} concentrations.

The two chambers could be separately connected to a single collector mass spectrometer for determination of total oxygen content and O^{18}/O^{16} ratio. The mass spectrometer inlet system consisted of a piece of stainless steel tubing (6 feet long, 0.010 inch inside diameter) going directly from the diffusion compartment to the ion source. Pressure in the ion source could be adjusted to a proper value by the length of a fine steel wire, 0.009 inch thick, inserted into the tube for almost the entire length. By such an arrangement, the samples could be introduced at atmospheric pressure (*6*).

After the membrane was placed in position, the two chambers were flushed with pure nitrogen. With the mass spectrometer connected to the upper chamber, O^{18}-labeled oxygen was injected into the upper chamber, and the oxygen pressure was simultaneously determined by the O^{16} peak on the mass spectrometer. When the desired partial pressure of oxygen had been reached, the tube connecting the chamber to the atmosphere was closed. The mass spectrometer inlet tube was switched to the lower chamber, and the same procedure repeated, but this time with regular tank oxygen slowly introduced until the O^{16} peak reached the same value as in the upper chamber. At the start of the experiments, the upper chamber contained 1.66 atom percent or slightly less O^{18}; the lower one O^{18} in natural abundance, that is, 0.20 atom percent. Membranes were run for 5 to 10 minutes to obtain a steady state, and then for another 40 to 45 minutes while recordings of O^{18} concentration were made on the mass spectrometer. The technique was applied to membranes containing oxyhemoglobin, methemoglobin, and water.

Figure 1 illustrates the nature of the data obtained from the mass spectrometer, namely, the increase of O^{18}/O^{16} in the lower chamber versus time. From these isotope data one may calculate the one-way flux of total oxygen (*7*). The results of such calculations are shown in Fig. 2. Seventeen membranes were run at four pressures, all above saturation pressure of hemoglobin. The solid lines represent the present observations and give the one-way flux with oxy- and methemoglobin. The dotted line *A* indicates the net flux of oxygen in a system where vacuum was maintained on one side of the membrane, and dotted line *B*, the net flux where an oxygen back-pressure of 20 mm-Hg was applied (*1*, *2*).

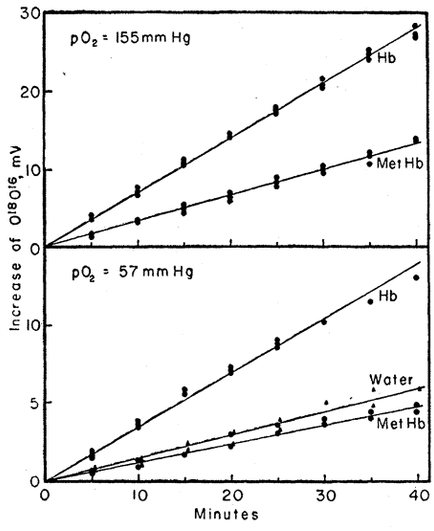

Fig. 1. Increase of O^{18} in diffusion chamber at 155 and 57 mm-oxygen.

The data show that the unidirectional

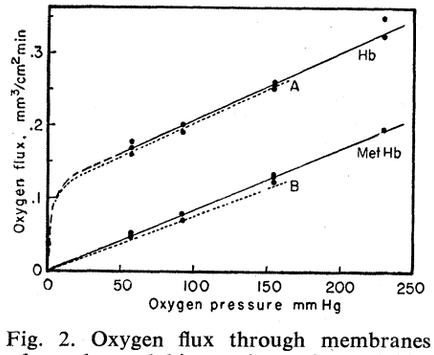

Fig. 2. Oxygen flux through membranes of oxyhemoglobin and methemoglobin. Solid lines are calculated from present O^{18} measurements and represent the total one-way exchange of oxygen in oxy- and methemoglobin solutions. Dotted lines represent net flux of oxygen through hemoglobin solutions with a gradient applied across the membranes; *A*, with no opposing oxygen pressure (*1*); *B*, with 20-mm oxygen back-pressure (*2*).

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.

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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

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