

EFFECTIVENESS OF ANTIMYCIN A, OLIGOMYCIN, AND SODIUM CYANIDE AS INHIBITORS OF RAT BONE MARROW OXYGEN UTILIZATION¹

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Abstract. The inhibition of oxygen consumption was studied *in vitro* using the metabolic inhibitors antimycin A, oligomycin, and sodium cyanide. Primary concern was to determine the rate and extent of oxidative inhibition upon intact rat bone marrow cells. At concentrations of 5×10^{-2} sodium cyanide, or higher, inhibition was total and immediate. At a concentration of 5×10^{-3} it was found that sodium cyanide was not significantly inhibitory during the first ten minutes of incubation. Antimycin A and oligomycin were found to be significantly inhibitory for oxygen consumption within two minutes at the 5×10^{-2} and 5×10^{-3} concentrations. At concentrations higher than 5×10^{-2} antimycin A and oligomycin were equally effective inhibitors of oxygen consumption. Use of lower concentrations of the two antibiotics showed that oligomycin was slightly more inhibitory to bone marrow oxygen consumption than antimycin A.

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The inhibitory qualities of antimycin A (Dickson *et al.*, 1971; Georg *et al.*, 1971; Kanivga *et al.*, 1969; Perry and Williams, 1971; Rieseke, 1971) and oligomycin (Fairhurst and Elison, 1964; Yates, 1966; Pinna, 1967; Hackenbrock *et al.*, 1971; Sabadie-Pialoux and Gautheron, 1971) have received considerable attention. Most of this work, however, was concerned with isolated cellular fractions, or specific biochemical reactions. It was of interest to determine the sensitivity of intact bone marrow cells to these oxidative inhibitors by measuring oxygen utilization rates. Sodium cyanide inhibition was used as a reference point, because of its historical use as an oxidative inhibitor.

MATERIALS AND METHODS

Male Holtzman rats 42 ± 2 days age were killed by cervical dislocation and the two femora and tibiae removed. The bones were split lengthwise and the marrow teased apart in Tyrode's solution adjusted to 310 milliosmoles/liter and a pH of 7.35. Cell suspensions were obtained by passing the bone marrow cells successively through two 100-mesh stainless steel screens. Determinations of

cell counts were made on aliquots of the bone marrow suspension.

After taking a sample of the bone marrow suspension for counting, an aliquot was diluted to contain 8×10^7 cells per 5 ml of Tyrode's solution. Five milliliters of this suspension was placed into each of two sample chambers of a YSI model 53 biologic oxygen monitor equilibrated, and measured with an oxygen electrode for 20 minutes at 37°C to determine oxygen consumption. Only one chamber had inhibitor added providing each experimental determination with a paired control. Antimycin A and oligomycin (consisted of 15 percent A and 85 percent B) were obtained from The Sigma Chemical Co. Ten trials were performed at each of three concentrations for each metabolic inhibitor, antimycin A, oligomycin, and sodium cyanide.

RESULTS

It can be seen that a significant decrease in cellular oxygen consumption occurred when marrow cells were incubated in media containing 5×10^{-2} or 5×10^{-3} M concentrations of antimycin A (fig. 1). At the lowest concentration (5×10^{-4}) the inhibition of cellular oxygen consumption was almost insignificant. The inhibition of cellular oxygen consumption occurred within two minutes but this is not evident from figure 1, since the first point plotted was at the 5 minute interval.

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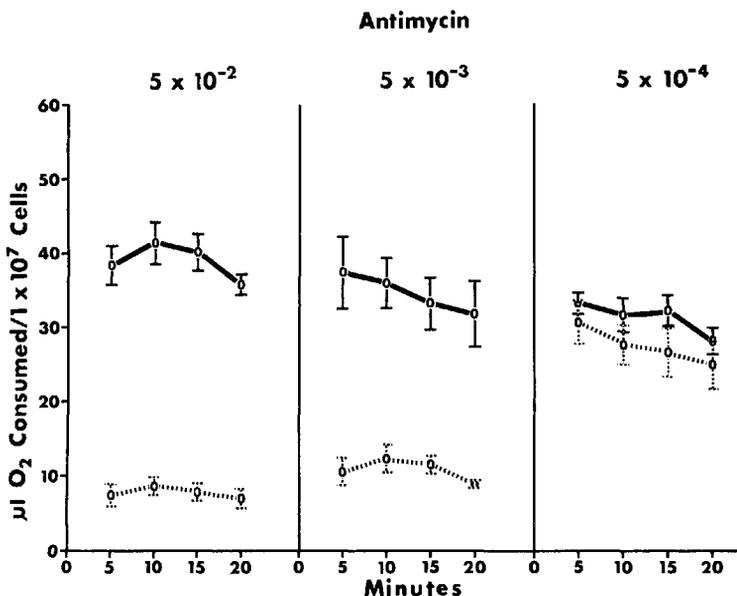


FIGURE 1. Effect of antimycin A on rat bone marrow oxygen consumption with three different concentrations of inhibitor. Total cell concentration was $8 \times 10^7/5$ ml incubation medium. Each point represents the mean of ten experiments \pm the standard error of the mean. Solid line represents control, and dotted line experimental samples.

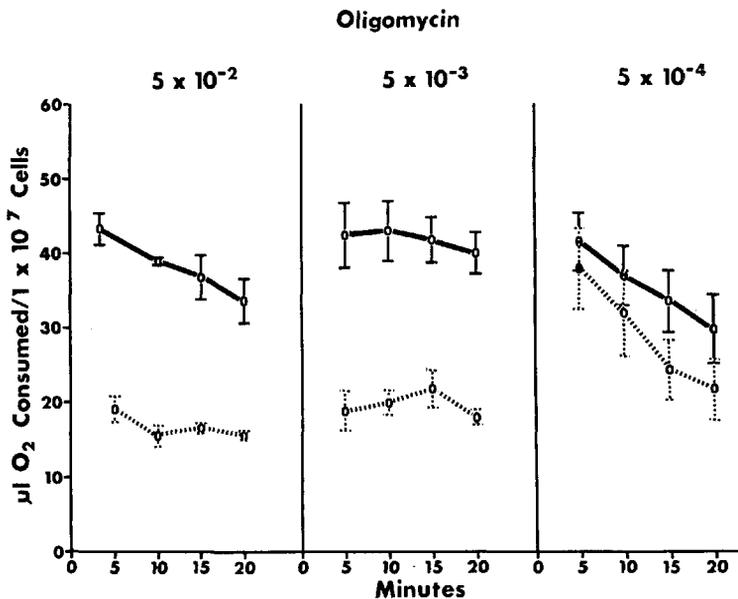


FIGURE 2. Effect of oligomycin on rat bone marrow oxygen consumption with three different concentrations of inhibitor. Total cell concentration was $8 \times 10^7/5$ ml incubation medium. Each point represents the mean of ten experiments \pm the standard error of the mean. Solid line represents control, and dotted line experimental samples.

A comparison of cellular oxygen consumption, with oligomycin added to the media, between the 5×10^{-2} and 5×10^{-3} M concentrations showed a significant decrease of oxygen consumption by bone marrow cells but about 50% less than with antimycin. With the lowest concentration of oligomycin (5×10^{-4}) there was a slightly more effective inhibition of oxygen consumption with control cells than with antimycin A (fig. 1, 2).

Sodium cyanide was found to be completely inhibitory to bone marrow cellular oxygen consumption within the first minute when the NaCN concentration was 5×10^{-2} M (fig. 3). Cellular oxygen consumption was not significantly inhibited for the first ten minutes when the NaCN concentration was 5×10^{-3} . With the lowest concentration (5×10^{-4}) NaCN, there was some escape from the total inhibition of oxygen consumption in the 15 to 20 minute time interval. It was found that at a concentration of 5×10^{-6} NaCN had little or no measurable influence on bone marrow cellular oxygen consumption during a 20 minute incubation period.

DISCUSSION

At higher concentrations antimycin A and oligomycin (5×10^{-2}) were equally effective inhibitors of oxidative metabolism. At lower concentrations of the two inhibitors (5×10^{-4}) oligomycin was slightly more inhibitory than antimycin A. At the 5×10^{-3} and 5×10^{-4} concentrations used in this study, antimycin A and oligomycin produced a more rapid inhibition of oxidative metabolism than sodium cyanide, but sodium cyanide had a more pronounced and profound effect after the initial static affect. The delay in inhibition of oxygen consumption by cells after exposure to cyanide at concentrations of 5×10^{-3} M or 5×10^{-4} M may be explained by an initial binding of cyanide to methemoglobin in many of the cells. When the initial intracellular concentration of cyanide is not too high, this binding would cause a delay in the build-up of the intracellular concentration of cyanide in cells with mitochondria, and hence delay the inhibition of oxygen utilization. The relative molecular sizes of antimycin A and CN^- therefore need not necessarily be considered. Since anti-

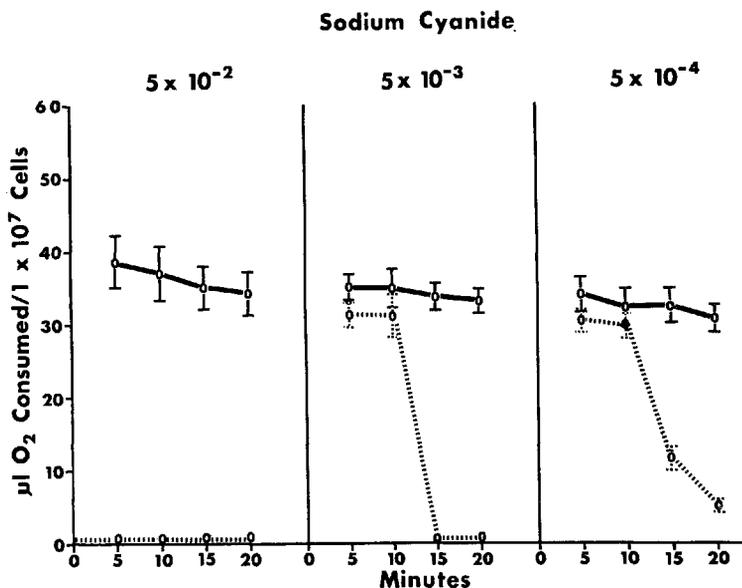


FIGURE 3. Effect of sodium cyanide on rat bone marrow oxygen consumption with three different concentrations of inhibitor. Total cell concentration was $8 \times 10^7/5$ ml incubation medium. Each point represents the mean of ten experiments \pm the standard error of the mean. Solid line represents control, and dotted line experimental samples.

mycin A and sodium cyanide both have their influence on the electron transport chain (Wilson, 1971), a meaningful comparison can be made as to their relative inhibitory effectiveness. It was surprising to find that antimycin A had a more rapid inhibitory effect on bone marrow cellular oxygen consumption than did sodium cyanide in spite of its being a larger molecule. It appears, that antimycin A can penetrate bone marrow cellular membranes more rapidly, or overcome the homeostatic cellular mechanisms more readily, than sodium cyanide or both may occur. The work reported in our paper does not offer the opportunity to differentiate between these two events. It is evident, however, that antimycin A at low concentrations does have a rapid inhibitory affect upon oxygen utilization of bone marrow cells.

The results obtained with oligomycin are not so readily explained. Oligomycin is inhibitory on the utilization of ATP at the site of active transport (Robinson, 1971). ATP utilization itself is not oxygen dependent, hence the rapid inhibition of oxygen utilization cannot be readily explained. One would have to surmise that some other cellular mechanism is involved in the inhibition of oxygen utilization. A mechanism other than inhibition of ATP synthesis would seem appropriate since it appears unlikely that bone marrow cells would not have adequate stores of ATP, and have to utilize newly synthesized ATP immediately or suffer a metabolic crisis.

What is evident from this work is that sodium cyanide at higher concentrations (5×10^{-2} or greater) has a rapid and complete inhibitory affect on bone marrow oxygen consumption. Higher concentrations of antimycin A and oligomycin could not be tested because the solubility limit of each was reached at 5×10^{-2} . With low concentrations (5×10^{-3} or less) sodium cyanide does not inhibit oxygen

utilization as rapidly as does antimycin A or oligomycin.

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