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# Effects of oligomycin on energy metabolism in isolated oxyntic cells

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The stomach is capable of translocating $H^+$ actively against an electrochemical gradient of about $10^6$-fold. This active transport requires the expenditure of large amounts of energy. The nature of the coupling of metabolic energy to $H^+$ transport is still controversial. Two models have been proposed: a redox pump and an ATP-driven mechanism. Evidence has accumulated during the past few years for an ATP-driven model. To gain more insight into the mechanism of energy coupling to acid secretion in the stomach, the effects of oligomycin on respiration and adenine nucleotide levels were studied in isolated oxyntic, or acid-producing, cells. Oligomycin is an inhibitor of oxidative phosphorylation [1, 2]; consequently, it blocks that fraction of respiration coupled to phosphorylation but has no effect on uncoupled respiration. The effects of this compound on acid secretion and respiration, reported for intact tissue, vary [3–5]. It would seem that low permeability to the compound could account for the discrepancy in the results. Thus, the effects of oligomycin on respiration and adenine nucleotide levels were re-examined in isolated oxyntic cells in which fewer permeability barriers are present. Furthermore, simultaneous measurements of respiration and nucleotides can be made with relative simplicity.

Another phosphorylative inhibitor, aurovertin, has been shown already to inhibit acid secretion and respiration in intact mucosa; however, its mode of inhibition has not been assessed [3].

Oxyntic cells were isolated from the stomachs of *Rana catesbeiana*, as reported previously [6]. The final purity of the preparation was of about 80 per cent oxyntic cells (81.3 ± 4.5 per cent: n = 14). Cells were suspended in a solution containing (in mM): 70 NaCl, 4.0 KCl, 0.8 $MhCl_2$, 1.8 $CaCl_2$, 1.0 $NaH_2PO_4$, 40 *N* Tris(hydroxymethyl)-2-aminoethane sulfonic acid (TES), and 11 glucose. In addition, the buffer solution contained bovine serum albumin (1 mg/ml) penicillin (100 units/ml), and streptomycin (100$\mu$ g/ml). Respiration was measured with Clark-type $O_2$ electrodes in a stirred glass chamber at 25° using air as the equilibrating gas phase. The system allowed simultaneous measurements in four different aliquots. Oxygen uptake was measured using control conditions; then oligomycin (10$\mu$ g/ml) was added to one of two aliquots. After an incubation period with oligomycin, dibutyryl-cyclic-AMP (db-cAMP, 5 mM) was added to both control and experimental chambers and the responses were measured. Levels of high energy phosphate compounds were measured in perchloric acid extracts of cell pellets, according to the methods of Lowry *et al.* [7].

Initial experiments demonstrated that exposure to oligomycin for 2.5 hr was necessary to inhibit completely the respiratory response to db-cAMP. Figure 1 portrays a typical experiment. During the first hour, the addition of 10 $\mu$g/ml of oligomycin brought about a rapid but small decrease in respiration, which came back to control levels at the end of 2.5 hr. Subsequent addition of db-cAMP produced an increase in respiration in the control chamber which was inhibited in the oligomycin-treated one. Inhibition of db-cAMP-stimulated respiration appears to be dependent on time: exposure to oligomycin for 1.5 hr reduced but did not abolish the response, whereas the db-cAMP increase in respiration was blocked completely when oligomycin was present for 2.5 hr. This may indicate a low permeability to the inhibitor. It has been reported [8] for kidney proximal tubule that oligomycin at equivalent concentrations takes a long time to penetrate the cell and that the presence of albumin is necessary for the uptake. It was speculated that oligomycin binds to albumin and that the complex is incorporated into the cell by pinocytosis. Albumin was present in the incubation medium in the present study but not in that of Sachs *et al.* [3], who did not obtain any inhibition by oligomycin. Whether pinocytosis is operative in the uptake of oligomycin in isolated oxyntic cells in not known. Addition of 0.1 mM 2,4-dinitrophenol (DNP) elicited a large increase in respiration in both chambers, showing that oligomycin does not block uncoupled

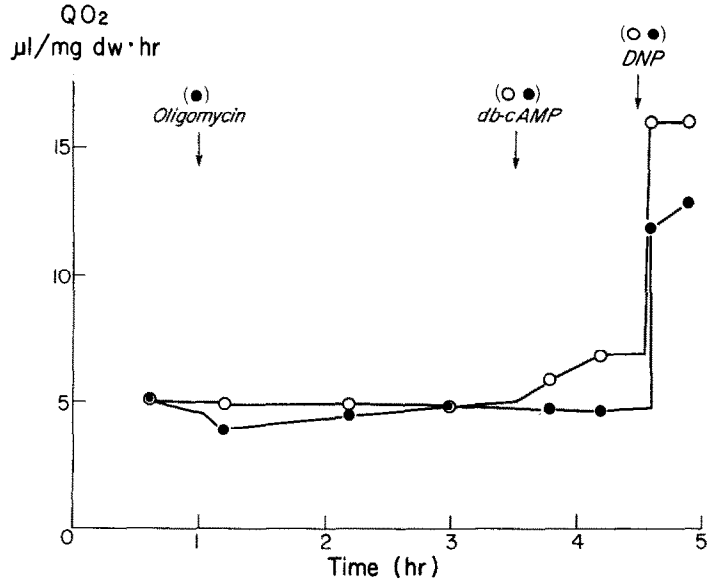


Fig. 1. Effect of oligomycin on respiration and the subsequent response to db-cAMP and DNP. Cells were divided in two aliquots. One was treated with oligomycin (10$\mu$ g/ml) for 2.5 hr (closed circles), whereas the other was left as a control (open circles). At the end of this period, db-cAMP (5 mM) was added to both aliquots. DNP was added 1 hr after addition of db-cAMP.

Table 1. Effect of oliomycin (10 μg/ml) on respiratory response to db-cAMP by isolated oxyntic cells*

| | $QO_2$ (μl/mg dry wt hr) | | | | |
|---|---|---|---|---|---|
| | Control | 1 hr | 2.5 hr | db-cAMP | % |
| Control | 4.95 ± 0.15 | 4.83 ± 0.09 | 4.75 ± 0.18 | 6.30 ± 0.28 | 33.0 ± 6.0 |
| Oligomycin | 4.87 ± 0.17 | 4.24 ± 0.09 | 4.54 ± 0.09 | 4.57 ± 0.09 | 1.2 ± 1.3 |
| P | NS | < 0.02 | NS | < 0.001 | < 0.001 |

* Cells were pooled from six mucosae in each experiment; n = 5. Experiment conditions were as in Fig. 1. Per cent represents the percentage stimulation of $QO_2$, by db-cAMP in relation to the 2.5 hr value. NS = not significant.

respiration. Table 1 shows the pooled results in a series of experiments. These results indicate that db-cAMP-stimulated respiration is entirely coupled to phosphorylation, since it is abolished by oligomycin. On the other hand, cAMP elicits a large increase in acid secretion and respiration in both the intact tissue [9] and in isolated oxyntic cells [6, 10]. Since this respiration is coupled to phosphorylation, it is reasonable to assume that ATP is linked closely to the secretory process. In addition to the inhibition of coupled respiration and reduction in ATP levels in some systems, oligomycin has been reported to inhibit ATPases and ion trasport by virtue of this action [11, 12]. Thus, it was necessary to examine the effects of this compound on the levels of high energy phosphated in isolated oxyntic cells. Table 2 shows the results. Addition of oligomycin induced a fall in ATP concentration to less than half of control values. This was accompanied by a large increase in ADP and AMP levels. Consequently, both the ATP/AMP and ATP/ADP ratios were largely depressed. Phosphocreatine (PCr) levels fell to values almost twenty times lower than those of controls. This was matched by an equally large increase in creatine (Cr) concentration. These changes effect a very large decrease in the PCr/Cr ratio. In this way these effects are similar to those induced by anoxia [13, 14], indicating that oligomycin is an effective inhibitor of phosphorylation. Addition of db-cAMP to oligomycin-treated cells resulted in a further decrease in ATP and PCr levels with an increase in ADP and creatine concentration. As has been reported for intact mucosa [14, 15], a substantial fraction of ATP is resistant to the action of inhibitors.

The results reported here throw light on the mechanisms of energy coupling to acid secretion. The increase in oxygen consumption brought about by db-cAMP is inhibited completely by oligomycin, a well-established inhibitor of oxidative phosphorylation and coupled respiration; however, it does not have any effect on uncoupled respiration, as was shown by the addition of DNP, which elicits a large increase in respiration in the control as well as in the oligomycin-treated chamber. Basal respiration is affected only slightly by oligomycin, indicating that a large fraction of oxygen consumption, in resting conditions, is not coupled to oxidative phosphorylation. It is possible that the energy dissipated in substrate oxidation in basal conditions is utilized by other processes such as mitochondrial ion transport. On the other hand, db-cAMP-stimulated respiration is entirely coupled to phosphorylation.

It could be argued that inhibition of the transport mechamism by oligomycin, whether direct or indirect, leads to complete inhibition of secretagogue-stimulated respiration as a secondary effect. However, a number of observations indicate that this is not the case. Just, in cells inhibited by other means, such as $Cl^-$ free solutions or the presence of $SCN^-$, which presumably inhibit the transport mechanism *per se*, db-cAMP is able to induce a partial stimulation of respiration in spite of complete inhibition of transport [6, 10, *]. This is clearly not the case with oligomycin. Second, in oligomycin-inhibited cells, db-cAMP further reduces the levels of ATP and PCr; thus, oligomycin does not preclude the utilization of small amounts of ATP still available to the pump. Third, in conditions where both the pump and oxidation have been inhibited, as in the case of incubation in $K^+$-free solutions, db-cAMP fails to stimulate respiration (as seen with oligomycin); however, relatively minor changes in levels of high energy phosphate compounds are found (to be published elsewhere). Fourth, the $H^+$ translocation function of gastric, ATPase-containing microsomes is not inhibited by high concentrations of oligomycin [16, 17]. On the other hand, the concentrations which inhibited the ($Na^+$–$K^+$) ATPase *in vitro* [11, 12] are probably not attained inside whole cells. However, one should be cautious in the interpretations of these results. Since the secretagogue-stimulated respiration appears to be tightly coupled, a redox-driven pump requiring re-oxidation at complex III or IV could still be inhibited due to inhibition of the $F_1$–$F_0$ complex by oligomycin. However, inhibition of secretagogue-induced respiration and the pump, as in the case of $K^+$-free solutions, induces only relatively minor changes in organic phosphates.*

The effects of oligomycin on respiration were accompanied by the expected changes in organic phosphates. Thus, the reductions in ATP and PCr and the increases in ADP, AMP and creatine reflect the inhibition of phophorylation. A substantial fraction of ATP is not affected by oligomycin or stimulation in oligomycin-inhibited cells. This has been ob-

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Table 2. Effect of oligomycin (10μ g/ml) and db-cAMP (5 mM) on adenine mucleotides in isolated oxyntic cells*

| | ATP | ADP | AMP | PCr | Cr | ATP/ADP | ATP/AMP | PCr/Cr |
|---|---|---|---|---|---|---|---|---|
| | (μmoles/g dry wt) | | | | | | | |
| Control | 12.673 | 1.303 | 0.026 | 17.395 | 6.311 | 9.73 | 487.0 | 2.76 |
| Oligomycin | 5.564 | 4.482 | 1.003 | 0.971 | 24.212 | 1.24 | 5.6 | 0.040 |
| Oligomycin + db-cAMP | 5.100 | 5.342 | 0.925 | 0.405 | 27.818 | 0.95 | 5.5 | 0.015 |

*One aliquot was treated with oligomycin for 2.5 hr. One aliquot was treated with oligomycin for 1.5 hr, and then db-cAMP (5 mM) was added for another 60 min. One aliquot was left untreated as a control. At the end of the incubation period, cell pellets were extracted for the measurement of organic phosphates. Cells were pooled from six mucosae in each experiment; average of three experiments.

served with other modes of inhibition [5, 13–15, 18]. Similar results have been obtained in liver slices [19]. Oligomycin-resistant ATP levels in this system are higher that those obtained with cyanide. Substrate-level phosphorylation in the Krebs cycle accouts for the ATP synthesis remaining in liver slices after inhibition by oligomycin. Since the ATP concentration in oligomycin-inhibited cells is rather similar to that obtained with other modes of inhibition like anoxia [5, 12], arsenate [5], cyanide (unpublished observations) or amytal [18], it would seem that phosphorylation in the Krebs cycle does not play an important role in maintaining oligomycin-resistant ATP levels. This fraction of ATP may not be available to energy-consuming reactions. On the other hand, it may reflect a steady state maintained by glycolysis in either oxyntic or contaminant cells, or both. In fact, these inhibition-resistant ATP levels can be lowered further by 2-deoxy-D-glucose [5].

Thus, the inhibition of acid secretion in the intact mucosa and secretagogue-stimulated respiration in oxyntic cells by oligomycin appear to be related to the inhibition of production of ATP and not to a secondary effect of this compound. Although they do not prove it, these observations support the hypothesis that ATP plays a primary role in the coupling of energy metabolism and acid secretion in the oxyntic cell.

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## Effect of adjuvant polyarthritis on liver alcohol dehydrogenase in the rat

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The injection of *Mycobacterium* in oil (Freund's adjuvant) to induce polyarthritis has been shown to impair hepatic [1–5] as well as pulmonary [3] mixed function oxidase in rats. The cause of this depression of drug metabolism and its relationship to inflammatory polyarthritis have been the subjects of much speculation, such as the possible existence of a "toxohormone" [6] and the possible existence of drug metabolism depression in man [1–3]. These drug-metabolizing enzymes are located in the endoplasmic reticulum (microsomes) of the hepatocyte; it is also of importance, in exploring the effects of inflammatory diseases, to measure the activities of enzymes in other parts of the cell. The investigation presented here explores the effect of adjuvant-induced polyarthritis on alcohol dehydrogenase (ADH, EC 1.1.1.1), a soluble enzyme located in the cytosol of hepatocytes. This enzyme has been chosen because it is the principal enzyme responsible for ethanol elimination *in vivo* [7]. In addition, changes of the *in vivo* activity of this enzyme are of importance as it follows saturation kinetics during the metabolism of ethanol, until lower body levels are reached [7–9].

Female Sprague–Dawley rats (Charles-River) were received at 126–150 g of weight and were randomly assigned to cages. Six rats per cage were housed in an area of controlled light, temperature and humidity. Purina rat chow and tap water were provided *ad lib.* Adjuvant and control rats were randomly mixed in each cage in order to minimize the variations in ADH that might result from batch to batch of rats and from differing conditions during breeding and transportation.

Inflammatory polyarthritis in half of the rats was initiated on day 0 by the subcutaneous injection of 0.3 mg *Mycobacterium butyricum* (Difco Laboratories) suspended in mineral oil (5 mg/ml) into the right hind paw plantar surface, as described by Barbieri *et al.* [10]. The controls were injected with an equal volume of mineral oil (0.06 ml). Adjuvant arthritic and control rats were decapitated and exsanguinated on days 2, 7, 14 and 30. Livers were removed immediately and weighed, and sufficient ice-cold KCl solution (1.15%) was added to give a 20% homogenate (w/v). After homogenization at 4° with a Thomas glass homogenizer (type B) with a Teflon pestle, the homogenate was placed in a plastic centrifuge tube and centrifuged for 20 min at 12,500 *g* at 4°. The supernatant fraction was removed, kept at 4° and assayed immediately for ADH.

*Enzyme assay.* ADH was assayed as follows. The reaction mixture consisted of 1.8 ml of 0.2 M glycine buffer, pH 9.6, 0.1 ml of 2.0 M ethanol, and 1.0 ml of β-NAD (Sigma-Grade III) at a concentration of 1 mg/ml. After mixing and preincu-