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Effects of Monensin on ATP Levels and Cell Functions in Rat Liver and Lung in Vitro

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Summary. Effects of the proton-alkali cation-exchanging ionophore, monensin, on aspects of cellular metabolism and ionic exchanges have been studied in rat tissues in vitro. Incubation of liver slices at 38°C with 0.1 μM monensin induced time-dependent vesiculation, initially in the Golgi region, reduction of ATP content and of protein synthesis. At 1 μM, monensin also reduced net, active movements of K^+, Na^+, Cl^- and water in liver slices and inhibited state 3 respiration in isolated mitochondria. The respiratory inhibitor, amytal, similarly reduced ATP content and protein synthesis at concentrations lower than those inhibiting ion transport in slices. Low concentrations of monensin (0.1–1.0 μM) had similar effects on ATP and ion transport in slices of adult lung. By contrast, late-fetal liver and lung were much less sensitive to monensin; in these tissues, glycolysis sustained substantial levels of ATP. Monensin also induced vesiculation of the Golgi apparatus in fetal lung cells. It is concluded that by lowering ATP levels, monensin can markedly alter various metabolic activities in those cells which depend primarily on oxidative phosphorylation for their metabolic energy.

Key Words monensin · cell ATP · cell ions, water · liver · lung · fetal liver · fetal lung

Introduction

The ionophore, monensin, is used to study Golgi function in intact cells, for it causes swelling of vesicles in the Golgi region of the cytoplasm while secretory activity is either reduced [4, 13, 18, 30, 34, 36] or increased [5, 32, 38]. In common with other intracellular organelles [16, 37], Golgi membranes actively accumulate H^+ accompanied by Cl^- [9, 26, 39]. Griffing and Ray [10] suggested that H^+ uptake would induce electro-neutral exchange for alkali cations present in the Golgi cisternae and so result in a loss of water. However, the entry of Cl^- with the protons would maintain electrical neutrality and a subsequent exchange of accumulated H^+ for external alkali cations would lead to a net uptake of solute, which could induce swelling. Since monensin facilitates transmembrane exchanges of H^+ for Na^+ or K^+ [8], this mechanism could account for the observed Golgi vesiculation.

Previous workers have used monensin over a very wide concentration range (0.01–100 μM). Although monensin can reduce respiration in isolated mitochondria, it is believed that cellular ATP levels are little affected [33] and that protein synthesis is normal [30, 34, 36]. However, preliminary experiments with slices of rat liver indicated a marked reduction of cellular ATP contents at monensin concentrations as low as 1 μM or less, while the ATP-requiring processes, protein synthesis and ion transport, were also reduced [15]. The following work pursues these observations.

Materials and Methods

Tissues

Albino rats of a Wistar strain were purchased from Zivic-Miller (Allison Park, PA). Experiments on adult tissues used males (300–500 g), which were killed by decapitation. For experiments on fetal tissues, pregnant females were killed by decapitation at 20 days gestation. The fetuses were quickly removed from the uterus, decapitated before breathing and the lungs and liver removed. The organs were immediately cooled in ice-cold Ringer solution (*see below*) and slices (0.2–0.3 mm thick) were cut with a blade guided by a glass slide. The organs from a single adult, or the pooled organs of an entire litter, provided enough material for a single experiment. Slicing was completed within 5 min (adults) or 15 min (fetuses) of death.

Experiments with Slices

The general design of the experiments was dictated by the requirement for measuring net movements of ions and water. The slices were preincubated for 90 min at 1°C, during which the reduction of metabolism-dependent transport activity resulted in

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net loss of K^+ and gain of Na^+ and Cl^- accompanied by water; upon rewarming to 38°C, the movements of ions and water were reversed. Further details of these procedures can be found elsewhere [24, 28].

The Ringer medium contained (in mM): Na^+ 149.0, K^+ 5.0, Ca^{2+} 1.3, Mg^{2+} 1.0, Cl^- 163.5, SO_4^{2-} 1.0, phosphate 2.0, Tris 10.0 (pH 7.4). Erlenmeyer flasks (25 ml) used for incubation contained approximately 100 mg (wet wt) slices in 3 ml medium; they were gassed at 1°C with O_2 after introduction of the slices, stoppered and transferred to a shaking water bath at 38°C. Duplicate or triplicate incubation flasks were prepared for each treatment in an experiment, and at least three experiments were carried out for each of the series illustrated. Inhibitors were added to the slices 60 min before the end of the preincubation at 1°C, to permit penetration into the tissue before restoration of metabolic activity. Monensin was added from a stock, ethanolic solution (0.3 mM); a final concentration of 0.3% (vol/vol) ethanol was present in all incubation flasks. For studies of amino acid incorporation into slice protein, 5 mM ^{14}C-leucine or ^{14}C-glycine (0.5 μCi/ml medium) was added 30 min after onset of incubation at 38°C. The purpose of the delay was to permit cell K^+ to recover, for protein synthesis is reduced when cell K^+ is low [7].

Assay Methods

After incubation, slices were recovered by vacuum filtration through filter paper (Whatman, no. 54) supported on a sintered glass funnel and were then gently blotted. Further details of the collection procedures and of the extraction and analysis of water, ions, ATP and unlabeled protein have been published previously [24, 28]. Slice proteins labeled with ^{14}C were precipitated from homogenates (Polytron homogenizer, Kinematica Gmbh., Luzern) with 8% (vol/vol) $HClO_4$. They were washed with organic solvents [19] and were dissolved in 5 N NaOH before being counted by liquid scintillation spectrometry.

Electron Microscopy

Slices were removed directly from the incubation medium with a wooden stick and dropped into ice-cold glutaraldehyde (2% vol/vol) in 0.1 M phosphate buffer (pH 7.0) with 0.2 mM $CaCl_2$. Further treatment has been described previously [27]. Sections cut with a diamond knife were examined in a Philips EM300 microscope.

Experiments with Mitochondria

Mitochondria were isolated from adult rat liver as described previously [25]. Oxygen consumption was measured with a Clark-type O_2 electrode and amplifier (Instech Laboratories, Glenn Mills, PA).

Results

Adult Liver

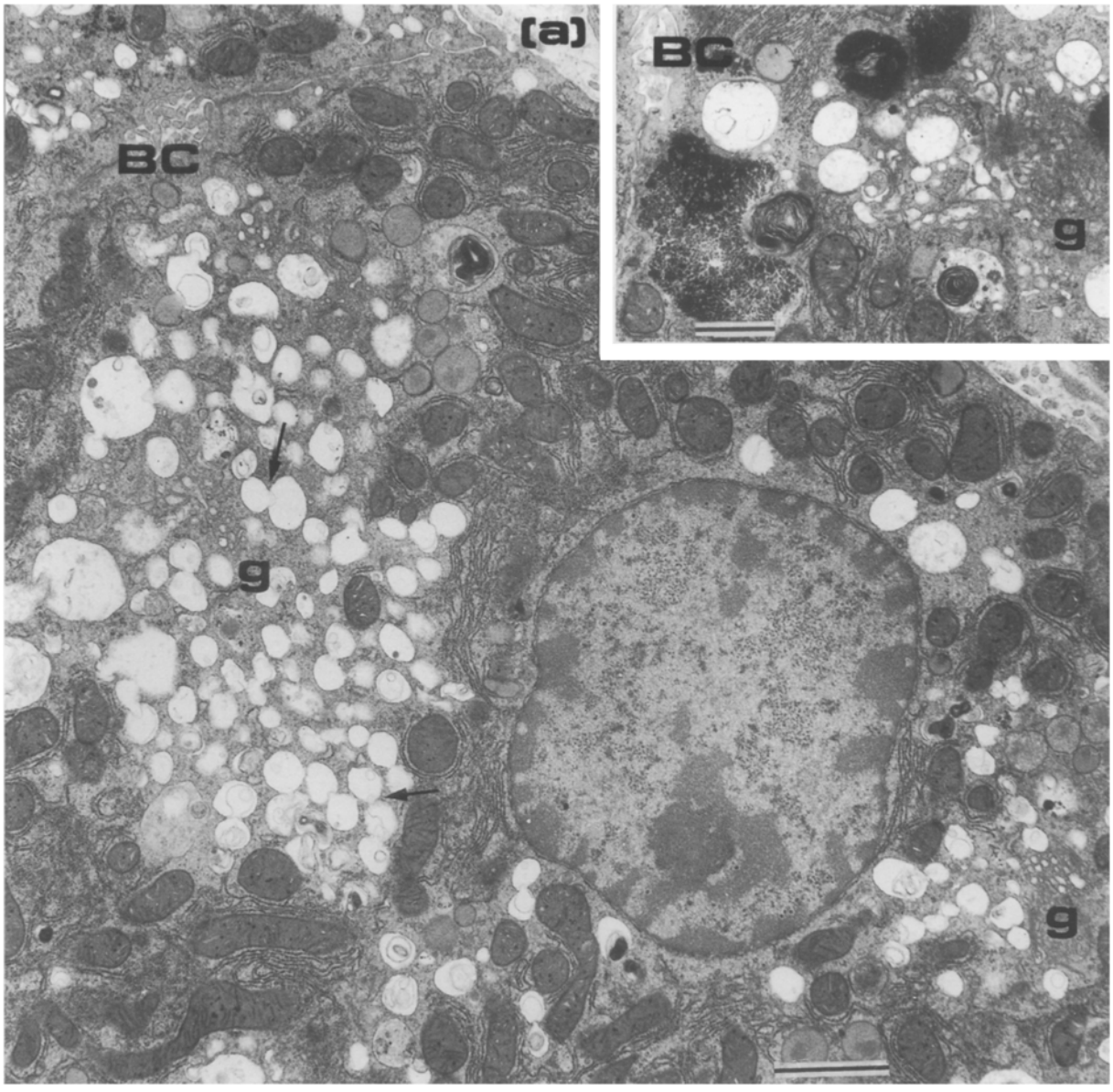
Electron Microscopy

After 15–60 min incubation at 38°C, control liver slices showed complete recovery from the disorganization, including swelling of endoplasmic reticulum and mitochondria, which occurred during preincubation at 1°C. There was little or no vesiculation of Golgi apparatus (Fig. 1*b*) and the general appearance was very similar to that of liver fixed immediately *post mortem* [29]. In slices incubated with 0.1 μM monensin, a slight vesiculation of Golgi membranes appeared in some cells after 15 min (Fig. 1*a*, inset); the vesicles were largely restricted to the immediate region of the Golgi apparatus. By 60 min, small, clear vesicles were more numerous and widespread, although still centered around Golgi elements (Fig. 1*a*). Many of the vesicles were fusing and a few larger vesicles could also be seen, mainly towards the periphery of the vesiculated zone. Mitochondria were in orthodox to condensed forms (compared to orthodox in controls; terminology of Hackenbrock [11]) and the endoplasmic reticulum formed parallel arrays.

At 1 μM monensin, a concentration often used by other workers, [4, 13, 18, 22, 31, 34, 35], very few vesicles were seen after 15 min at 38°C (Fig. 2) and Golgi elements could be recognized only occasionally (Fig. 2, inset); mitochondria were condensed and endoplasmic reticulum was in good condition. After 60 min, however, vesiculation was extensive (Fig. 3) and many of the vesicles now contained granular material and, occasionally, myelin-like bodies (*cf.* results of Morré et al. [18] after 30 min). However, the endoplasmic reticulum was less well organized and the mitochondria less condensed, than after 15 min (Fig. 2) or 30 min [18].

Biochemical Effects

After incubation for 60 min at 38°C, 0.1 μM monensin caused a significant reduction of the incorporation of ^{14}C-leucine into slice proteins (44% inhibition) and of the final ATP content of the slices (35% reduction), but only minimal reduction (10%) of the final K^+ content (Fig. 4). Considerable further reduction of each of these factors was given by 1 and 5 μM monensin. The major part of the fall of ATP occurred during the first 15 min at 38°C, even at 0.1 μM monensin (Fig. 5*a*). This was also true of the effects of 1 and 5 μM monensin on K^+ reaccumulation and Na^+ extrusion (Fig. 5*b*), but 0.1 μM monensin did not significantly affect these ionic movements (*not shown*). Water and Cl^- extrusion during incubation at 38°C, and their inhibition by monensin, both followed a similar pattern to Na^+ extrusion (*not shown*). When expressed as net moles transported, monensin had a larger inhibitory effect on Na^+ than on K^+ movements. For example, after 15 min at 38°C (Fig. 5*b*), net Na^+ extrusion from slices treated with 1 μM monensin was a mean of 205 mmol/kg dry wt less than extrusion from control



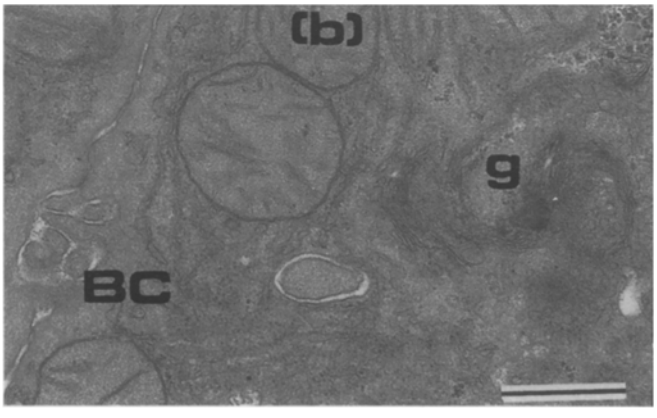


Fig. 1. Electron micrograph of liver slices incubated at 38°C after preincubation at 1°C. (*a*) Slice incubated with 0.1 μM monensin for 60 min at 38°C. Small vesicles are seen, especially surrounding two groups of Golgi elements (*g*). *BC*, bile canaliculus. Several instances of vesicles apparently fusing are to be seen (e.g., arrows). Bar, 2 μm. *Inset*: Detail of part of a cell in a slice incubated for 15 min at 38°C with 0.1 μM monensin to show rather limited vesiculation around Golgi elements. Other regions of this cell were practically free of vesicles. Bar, 1 μm. (*b*) Detail of a Golgi apparatus in control slice incubated for 60 min at 38°C. The Golgi membrane system shows no vesiculation. This is most frequently found to be the case. A small amount of vesiculation is seen on occasion, but not as much as in the inset, above. Bar, 0.5 μm

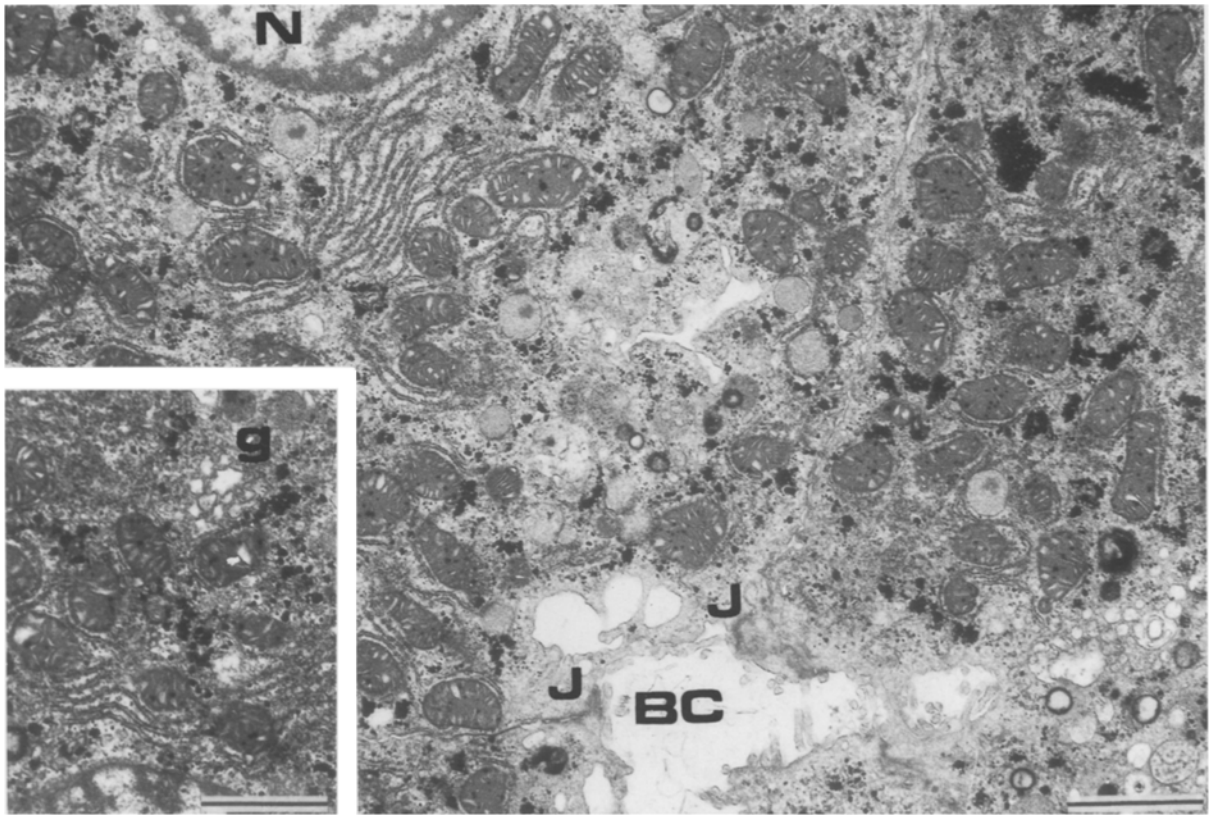


Fig. 2. Electron micrograph of liver slice after incubation with 1.0 μM monensin for 15 min at 38°C. Very few vesicles are present in the cytoplasm and Golgi elements are difficult to recognize. Mitochondria are in condensed configurations (contrast Figs. 1 and 3). Endoplasmic reticulum, cellular boundaries and the junctional complexes (*J*) delimiting canaliculi (*BC*) are well organized; *N* = nucleus. Bar, 2 μm. *Inset:* Detail of one of the few examples, under these conditions, of slightly expanded Golgi cisternae (*g*). Bar, 2 μm

slices, while K^+ accumulation was only reduced by 86 mmol/kg. At the same time, Cl^- extrusion was reduced by 197 mmol/kg. At least after this lengthy time, the net changes of these ions are not explicable solely by a monensin-induced exchange of H^+ for Na^+ or K^+ at the plasma membrane.

To test the importance of the reduced ATP in inhibiting energy-requiring reactions, the ATP content of liver slices was reduced by varying the concentration of a known inhibitor of electron transport, amytal. Amino-acid incorporation into proteins was drastically reduced at an ATP content and rate of O_2 consumption at which K^+ content was still unaffected (Fig. 6). Analogous results have been obtained with CN^- and oligomycin [24, 25]. These results suggest that the fall of ATP induced by monensin could underly the inhibition of protein synthesis and ion transport.

A possible explanation of the monensin-induced loss of ATP is that the altered ionic content of the cells increases the activity of the Na^+-K^+-stimulated adenosine triphosphatase, so consuming ATP more rapidly. In the absence of monensin, 25–40% of cell respiration appears to be coupled to ATP utilization by Na-K transport [1, 6]. This possibility is excluded by the failure of ouabain (2 mM) to protect ATP levels at any of the monensin concentrations or incubation times used in Fig. 5*a*. For example, the ATP content of slices incubated for 60 min with 1.0 μM monensin was 1.2 ± 0.2 mmol/kg protein without, and 0.6 ± 0.1 mmol/kg with ouabain.

As an alternative explanation, the possibility of a direct effect of monensin on mitochondrial respiration was investigated. Mitochondria isolated from rat liver underwent a sixfold stimulation of O_2 consumption upon addition of ADP (transition from state 4 to state 3, as defined by Chance and Williams [2]), with glutamate *plus* L-malate as substrates. Neither ouabain nor 0.1 μM monensin affected respiration in state 3, but 1 μM monensin rapidly inhibited it (Fig. 7*b*). It should be noted that the ADP initially added was sufficient to maintain state 3 respiration until the onset of state 5 (anaerobiosis). Subsequent addition of valinomycin released the inhibition by monensin, implying that monensin did

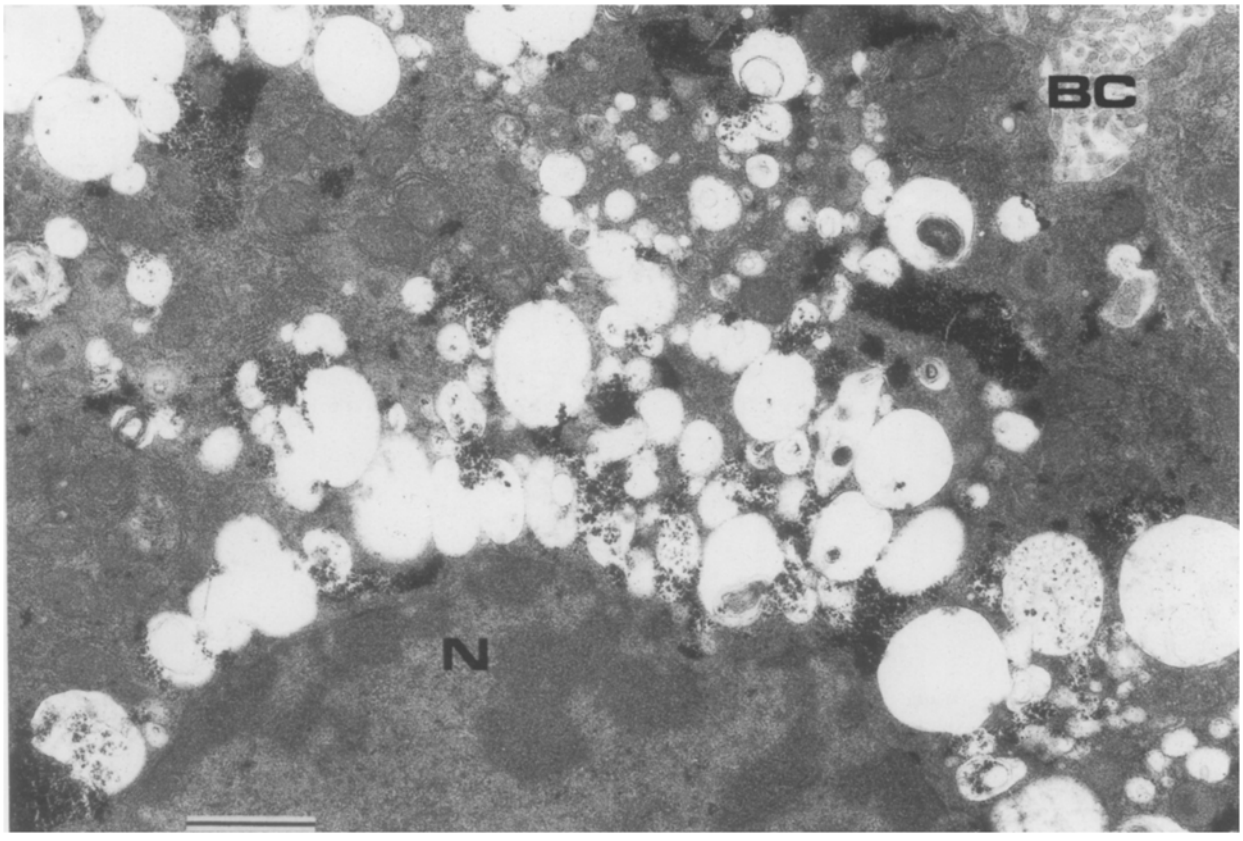


Fig. 3. Electron micrograph of liver slice after incubation with 1.0 μM monensin for 60 min at 38°C. Description in text. Bar, 2 μm

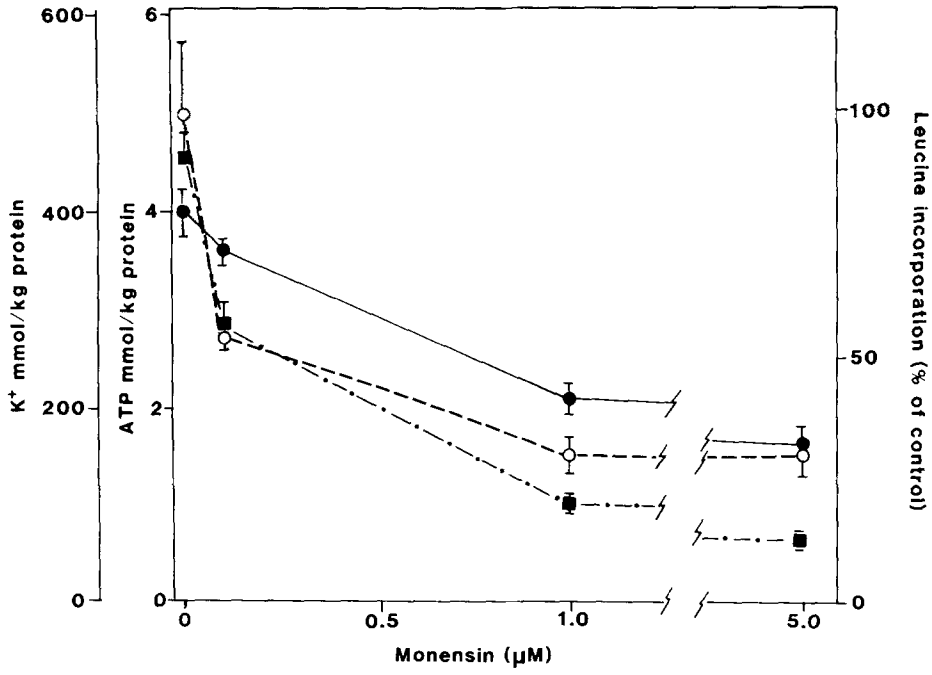


Fig. 4. Effects of monensin concentrations on net accumulation of K^+ (●), ATP content (■) and leucine incorporation into protein (○) by liver slices. Slices were preincubated for 90 min at 1° followed by 30 min at 38°C, at which time ^{14}C-leucine (5 mM; 0.5 μCi/ml) was added and incubation continued for an additional 60 min. Accumulation of K^+ is calculated from the difference in content of slices assayed after 90 min at 1°C and after further incubation at 38°C. Other details are as in Materials and Methods. Each point is the mean ± SEM of seven observations

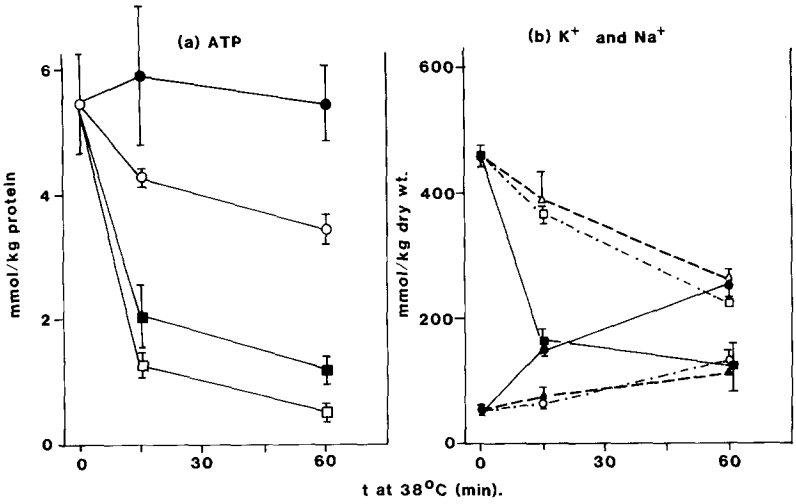


Fig. 5. Effects of monensin on ATP and ionic content of liver slices after 15 and 60 min at 38°C. Values at zero time show the content of slices after preincubation for 90 min at 1°C. (*a*) ATP content (n = 8 for each point). ● control slices, ○ 0.1 μM monensin, ■ 1.0 μM monensin, □ 5.0 μM monensin. (*b*) Cation content (n = 6). K^+ content: ● control, ○ 1 μM monensin, ▲ 5 μM; Na^+ content: ■ control, □ 1 μM monensin, △ 5 μM. Monensin had no effect on Na^+ or K^+ contents at 0.1 μM. Each point is the mean ± SEM

Table 1. Effects of monensin on water and ionic content of slices of adult lung[a]

Incubation	Tissue composition				(n)
	Water (kg/kg dry wt)	Na^+	Cl^- (mmol/kg dry wt)	K^+	
90 min at 1°C	6.6 ± 0.1	861 ± 104	792 ± 43	125 ± 13	(5)
Then 60 min at 38°C with:					
Control	5.5 ± 0.1	527 ± 25	675 ± 20	240 ± 11	(8)
Monensin 0.1 μM	5.9 ± 0.2	683 ± 22	720 ± 34	177 ± 10	(11)
Monensin 1.0 μM	6.3 ± 0.2	790 ± 32	762 ± 30	133 ± 8	(11)

[a] Slices were preincubated for 90 min at 1°C followed by experimental incubation for 60 min at 38°C. For other experimental details, *see* Materials and Methods. Samples at 1°C were incubated with and without 1 μM monensin in each experiment, but since monensin did not affect the composition at this temperature, the results have been pooled. Values are the mean ± SEM (n = number of incubation flasks).

not inhibit electron transfer directly. Figure 7*a* confirms that 1 μM monensin alone had little or no effect on state 4 respiration, but prevented stimulation by ADP.

Adult Lung

The effects of monensin seen above were not peculiar to the liver, for lung slices from adult rats were more sensitive than liver slices to low concentrations. Thus, 0.05 μM monensin reduced both the ATP content and K^+ accumulation by 55–60%. Over a wider concentration range, there was a close parallel between the declining ATP and K^+ (Fig. 8). The net extrusions of Na^+, Cl^- and water were also significantly reduced by monensin (Table 1). As with the liver slices, the overall effects of monensin on the movement of ions across the plasma membrane are too extensive to be explained solely by the ionophore-facilitated, 1 : 1 exchange of Na^+ or K^+ for protons. Either secondary movements of ions, including Cl^-, follow the initial exchange, or the primary cause of the inhibition of net active transport is quite different, such as a lack of ATP.

Fetal Lung and Liver

Biochemical Effects

In contrast to the results with adult lung, Fig. 8 shows that the ATP of lung slices from late-fetal rats was reduced only 25% by 1 μM monensin and 50% by 5 μM. As a consequence, the net amount of K^+ reaccumulated in the presence of 1.0 μM monensin was only 28% less than in control slices

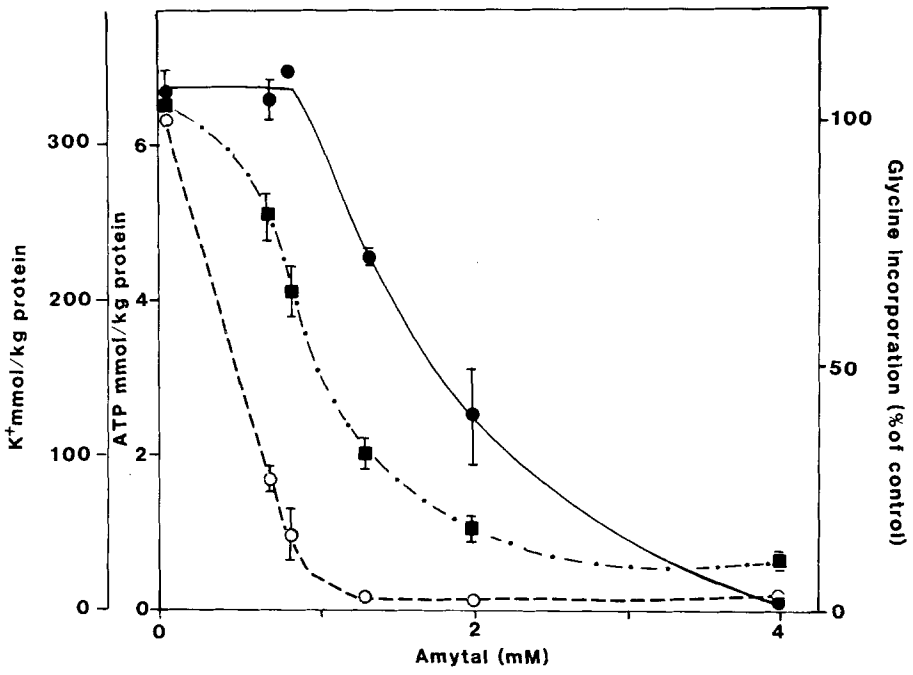


Fig. 6. Effects of amytal concentration on net accumulation of K^+ (●), ATP content (■) and glycine incorporation into protein (○) by liver slices. Details as for Fig. 4, except that ^{14}C-glycine replaced leucine. Each point is the mean of six observations; in some cases, standard errors fell within the area of the symbols

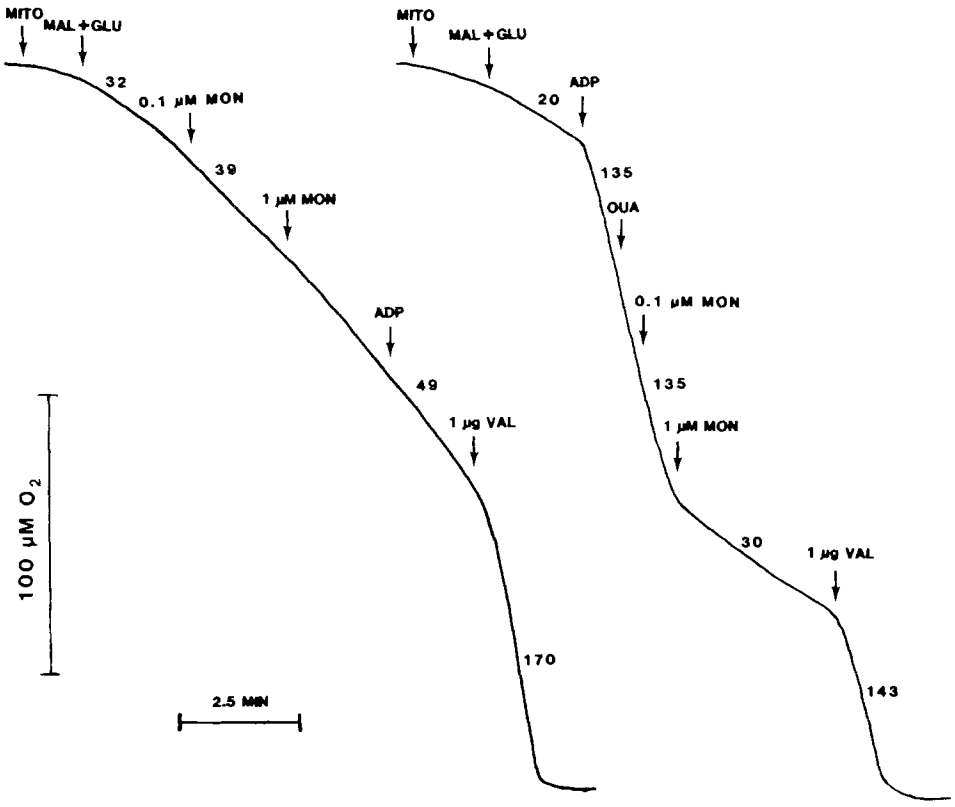


Fig. 7. Record of O_2 consumption by mitochondria isolated from rat liver, and effects of monensin. Incubation medium contained 250 mM mannitol, 75 mM sucrose, 2.5 mM Tris · HCl, 0.1 mM ethylenediamine tetra-acetic acid, 5 mM $MgCl_2$, 5 mM potassium phosphate, 1% (wt/vol) bovine serum albumin ("essentially fatty-acid free," Sigma Chemical); pH was 7.4. Additions were: *MITO*, mitochondria at 1.0 mg protein/ml; *MAL + GLU*, L-malate (1 mM final concentration) *plus* glutamate (10 mM); *MON*, monensin at concentrations indicated; *ADP*, final concentration of 0.5 mM; *VAL*, valinomycin (2 µg/ml). Incubation was at 25°C. The mitochondrial preparation illustrated was representative of five. Numbers on the traces are rates of O_2 consumption in mM/mg protein/min. In the right-hand trace, the respiratory control ratio upon addition of ADP was 6.7 and the ratio, ADP/O, was 2.75

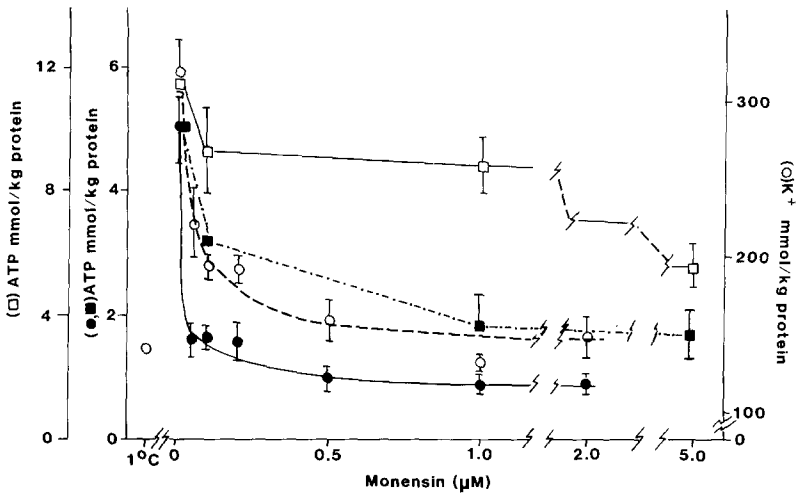


Fig. 8. Effect of monensin concentration on K^+ (○) and ATP (●) content of adult lung slices and ATP content of 21 day-old fetal lung (□) and liver (■) slices. Slices were preincubated for 90 min at 1°C (contents shown at left of figure, as *1°C*) followed by 60 min at 38°C. Each point represents the mean ± SEM of three to nine observations for adult and of six observations for fetal slices

Table 2. Effect of incubation and inhibitors on the final ATP and K^+ contents of lung slices from fetal rats 1 day pre-partum[a]

Incubation conditions	ATP content (mmol/kg protein)	K^+ content (mmol/kg dry wt)	(*n*)
90 min at 1°C	ND[b]	170 ± 9	(6)
Then 60 min at 38°C with:			
No additions (control)	11.9 ± 0.2	492 ± 14	(10)
Azide (1 mM)	3.3 ± 0.4	322 ± 30	(6)
Antimycin A (10 μg/ml)	3.4 ± 0.5	327 ± 26	(10)
Iodoacetate (1 mM)	0.6 ± 0.2	88 ± 10	(10)
Antimycin A + iodoacetate	0.4 ± 0.1	74 ± 6	(10)

[a] The slices were preincubated for 90 min at 1°C followed by 60 min at 38°C. Incubation conditions and analytical techniques are noted in Materials and Methods. Values are mean ± SEM (*n* = number of replicate incubation flasks).
[b] ND = not determined.

(160 ± 15 compared to 222 ± 24 mmol/kg dry wt; *n* = 8). This contrasts with the complete absence of K^+ reaccumulation in slices of adult lung incubated with this concentration of monensin (Fig. 8). There was a similar resistance of the net extrusion of Na^+, Cl^- and water in the fetal slices (*not illustrated*). Fetal liver was also more resistant to monensin than was the adult tissue. At 1–5 μM monensin, fetal liver ATP was reduced by 60% after 60 min at 38°C (Fig. 8), compared to 80–90% in the adult (Fig. 5); also, the K^+ content of the fetal slices was reduced by only 30–40% (*not illustrated*).

The resistance of fetal tissues to monensin is probably due to the synthesis of ATP by glycolysis. Table 2 shows the importance of this pathway in the fetal tissue; inhibitors of electron transfer, antimycin A and N_3^-, reduced fetal ATP by 70%, a reduction sufficient to reduce net accumulation of K^+ by only 50%. The additional presence of iodoacetate was required to lower ATP sufficiently for K^+ accumulation to be inhibited completely. Iodoacetate alone caused a similar effect, suggesting that the glycogen accumulating in lung just before birth (e.g., *see* Fig. 9) is the major endogenous source of reducing equivalents for mitochondrial respiration.

Electron Microscopy

In view of the well-maintained ATP content in the fetal lungs incubated with monensin, the morphological consequences were examined. The ionophore (1 μM) induced vesiculation especially in dark, mononuclear cells and in type II epithelial cells (granular pneumocytes; Fig. 9). In some cases, the type II cells and undifferentiated epithelial cells showed swelling of the Golgi apparatus (Fig. 9). The surfactant-storage granules characteristic of the type II cells were also usually somewhat expanded,

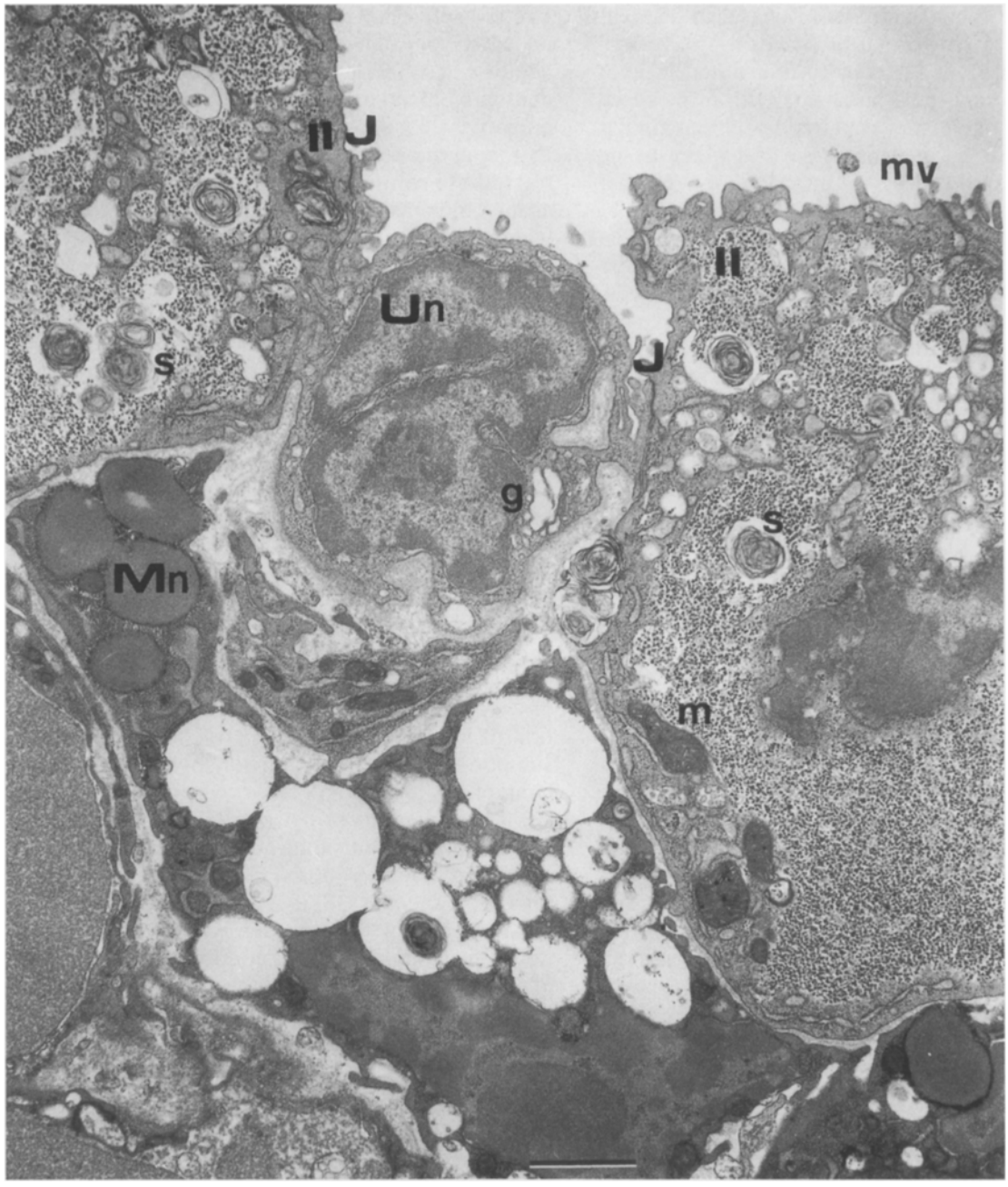


Fig. 9. Electron micrograph of slice of late-fetal lung after incubation with 1.0 μM monensin for 60 min at 38°C. Parts of two type II epithelial cells (*II*), an undifferentiated epithelial cell (*Un*) and a mononuclear cell (*Mn*) are shown. The type II cells contain many glycogen particles scattered throughout the cytoplasm, swollen surfactant granules (*S*) and many vesicles, with mainly clear contents, in the apical regions; their mitochondria (*m*) are in intermediately condensed configurations. The undifferentiated cell has an expanded Golgi system (*g*). The canalicular (future alveolar) epithelial surface bears microvilli (*mv*) and cellular junctions (*j*). The presumed macrophage, recognized by its dark cytoplasm and general form, contained many clear, large vesicles, several of which appear to be fusing; at lower magnification, most cells of this type showed similar vesiculation. Bar, 2 μm

showing a clear annulus surrounding the laminar, lipid content. In the absence of monensin (*not illustrated*), the Golgi apparatus was rarely expanded while the storage granules were always much more compact.

Discussion

Monensin induced a time- and concentration-dependent vesiculation in rat liver cells in vitro, which was initiated within 15 min, at or near the Golgi

apparatus. In its more advanced phase, as seen after 60 min at 38°C with 1 μM monensin, the vesiculation appeared very similar to that noted in liver slices from young, post-natal rats [18], in perfused liver [13] and in isolated hepatocytes [30] at similar concentrations, as well as in several other tissue preparations (references in Introduction). Similar vesiculation of Golgi elements was also noted in at least two types of cells in the fetal lung, epithelial type II cells and macrophages. In pulse-chase experiments with 1–10 μM monensin in liver slices, Morré et al. [18] associated vesiculation with a block in the passage of Golgi material from the *trans* face of the apparatus towards the cell periphery. Our results show that exposure of slices for 15 min to only 0.1 μM monensin is sufficient to cause some degree of vesiculation, together with reduction of cell ATP content and protein synthesis. Reduction in the accumulation of K^+ and extrusion of Na^+, Cl^- and water, at the plasma membrane, required rather more extensive exposure but was nevertheless marked at the concentration of 1 μM used by many other workers. Monensin thus has several effects on liver-cell activities, some of which were also noted in adult lung slices, and our experiments suggest that the lowering of cell ATP may be an important factor in the inhibition of the energy-dependent activities studied.

An ionophoric effect at the plasma membrane may contribute to the reduction by monensin (1 μM and above) of the metabolism-dependent net movements of ions at 38°C. However, our results show that the lowering of cell ATP by monensin is itself sufficient to account entirely for the inhibition. Thus, transport activity in liver slices was not significantly reduced unless exposure to monensin was sufficient to reduce ATP by more than 40%. This corresponds approximately with the point at which more specific inhibitors of mitochondrial energy-conserving reactions begin to reduce transport (Fig. 6; [24, 25]). From Fig. 6, it is also apparent that amino acid incorporation into liver-slice protein is more sensitive to reduction of ATP by amytal than K^+ uptake was, so that the inhibition of leucine incorporation by low concentrations of monensin can also be accounted for by the reduced ATP level. The alternative, that inhibition of protein synthesis is a consequence of reduced cell K^+ [7], cannot account for the marked inhibition of leucine incorporation at 0.1 μM monensin, where K^+ levels were not significantly reduced (Fig. 4).

The fall of ATP in adult liver and lung slices induced by low concentrations of monensin was unexpected in view of previous statements to the contrary [33]. The reason for this difference may reside in the relative contributions of mitochondrial oxidative phosphorylation and glycolysis to the maintenance of ATP in the cells used. Thus, in slices of adult liver, ATP levels and net, metabolism-dependent movements of K^+ and Na^+ are very low when oxidative phosphorylation is inhibited (Fig. 6; [24, 25]), and monensin markedly inhibited. By contrast, these parameters were considerably protected against monensin in fetal lung (Fig. 8) and liver [23], where substantial levels of ATP could be maintained by glycolysis in the presence of respiratory inhibitors (Table 2). Unfortunately, the statement of Tartakoff [33] does not indicate the cells in which ATP was measured.

The reduction of ATP by monensin in liver cells may be at least partly due to its direct effect on mitochondrial respiration in state 3 (Fig. 7). The requirement of intramitochondrial K^+ for optimal O_2 consumption and phosphorylation [1, 12, 21] probably underlies this effect. Respiration is reduced when isolated mitochondria are treated with nigericin at low external concentrations of K^+ [14, 20]; this is presumably associated with a loss or redistribution of endogenous K^+ [40] and monensin should induce an analogous effect. The subsequent return to the state 3 respiratory rate upon addition of valinomycin would result either from the restoration of adequate levels of matrix K^+ or from consumption of energy by an ionophore-induced cycling of K^+ [8, 17].

The reduction of cell ATP content caused by monensin may contribute to prevention of the passage of materials from the Golgi apparatus to other regions of the cell [18] if ATP-dependent processes are involved in the transfer. Under these conditions, the osmotic effect of continuing accumulation of solutes (e.g., newly synthesized substances) in the Golgi elements would predispose the cisternae to swell. However, the decline of ATP is not the only factor required for vesiculation, for lowering liver-slice ATP by, for example, inhibition of respiration, does not mimic the vesiculation caused by monensin [28, 29]. Rather, the expansion of the vesicles induced by monensin is probably a consequence of the ATP-dependent uptake of H^+ seen in isolated Golgi vesicles [9, 39], which drives an accumulation of Cl^- [9]. The uptake of Cl^- is stimulated by Na^+ or K^+ and, more strongly, by monensin [26], suggesting that exchange of cytosolic alkali cations for the previously accumulated protons leads to a net uptake of alkali chlorides, which would be accompanied by water. If that is the case, either the proton-dependent adenosine triphosphatase has a high enough affinity for ATP to be able to function when monensin lowers ATP, or a localized pool of ATP at a higher concentration remains available. The apparent swelling of the surfactant-

storage granules in the type II lung epithelial cells exposed to monensin could have a similar basis to that proposed for the Golgi vesicles, as these granules possess a proton-accumulating system [3].

In general, our results indicate that monensin can cause a wide variety of alterations in the metabolism and composition of intact cells. Its ionophoric effect is presumably primary and could underly, more or less directly, several aspects of the observations in whole cells, e.g., alterations of fluid and ion exchanges at the plasma membrane and entry of ions into intracellular vesicles. However, the action of ionophore also leads to a decline of mitochondrial respiration and ATP synthesis with the possible secondary inhibition of energy-requiring processes. Our results suggest that the reduction of ATP contributes to several effects of monensin, including inhibition of ion transport and protein synthesis, at least in cells in which oxidative phosphorylation is the major source of ATP.

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