Biochemistry

 Volume 6, Number 2 February 11, 1967

# Antibiotics as Tools for Metabolic Studies. VIII. Effect of Nonactin Homologs on Alkali Metal Cation Transport and Rate of Respiration in Mitochondria*

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ABSTRACT: The effects of monactin and nonactin on the rate of respiration, light-scattering properties, and movements of $K^+$, $Na^+$, $Li^+$, and $H^+$ in mitochondria from rat liver have been studied. Monactin in a medium containing $K^+$ stimulated respiration, induced swelling, and stimulated the uptake of $K^+$ and ejection of $H^+$. In a medium containing 60–100 mM $Na^+$, monactin stimulated respiration and induced swelling of the mitochondria but induced little net uptake of $Na^+$. Nonactin exerted effects similar to monactin in $K^+$-containing media but was much less effective in media containing $Na^+$. Monactin had very little activity in a medium in which $Li^+$ was the only alkali metal cation. If the alkali metal cation concentration was 30 mM or less, nigericin, dianemycin, or 2,4-dinitrophenol induced release of the cation and inhibition of the respiration induced by monactin. If $K^+$ uptake was induced by adenosine diphosphate (ADP), the subsequent addition of monactin produced little further increase in the $K^+$ accumulation. In a medium containing $K^+$, nonactin stimulated respiration and induced a slight change in the light scattering of sonic particles prepared from mitochondria. Neither the change in light-scattering properties nor stimulation of respiration was reversed by nigericin. On the basis of the data presented, it was concluded that nonactin and its homologs act by altering the mechanism for uptake of alkali metal cation by mitochondria in a manner similar to gramicidin and valinomycin but with a different cation requirement for activity. It would appear that mitochondrial swelling is more related to cation turnover than to cation gradient or net accumulation. The data do not support the osmotic theory as the sole mechanism involved in mitochondrial swelling.

The antibiotics nonactin, monactin, dinactin, and trinactin have been shown to stimulate ATPase,[1] induce swelling, stimulate respiration, and uncouple oxidative phosphorylation in rat liver mitochondria (Graven *et al.*, 1966a,b). Three of the homologs, monactin, dinactin, and trinactin, were qualitatively and quantitatively similar in activity and had similar requirements for alkali metal cations. These observations prompted an investigation of the influence of nonactin and its homologs on alkali metal cation transport by mitochondria. We have reported (Graven, 1966; Graven *et al.*, 1966c) incidental to other findings that monactin induces rat liver mitochondria to take up $K^+$ in much the same manner as does valinomycin (Moore and Pressman, 1964). The present paper is a more detailed report of the induction by monactin and nonactin of alkali metal ion translocation and a correlation with other activities of mitochondria.

Nigericin and dianemycin are agents which inhibit or reverse the cation uptake induced by valinomycin, monactin, or gramicidin (Graven *et al.*, 1966c; S. N. Graven, S. Estrada, and H. A. Lardy, in preparation). The effect of these agents on the activity of monactin and nonactin is also presented.

* From the Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin. *Received September 30, 1966.* Supported by grants from the National Science Foundation, the National Institutes of Health, and the Life Insurance Medical Research Fund. Paper VII of this series is Graven *et al.* (1966c).

[1] Abbreviations used: ATPase, adenosine triphosphatase; ADP, adenosine diphosphate.

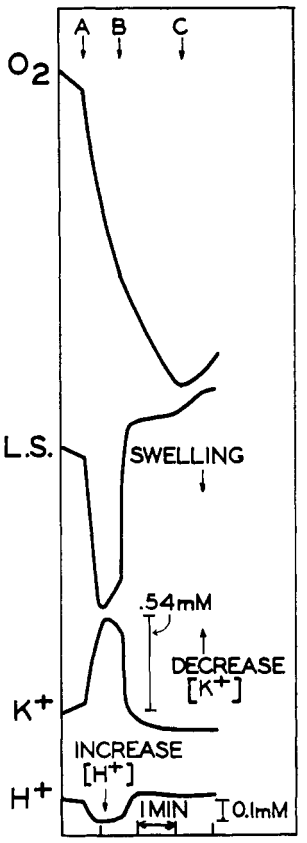


FIGURE 1: The effect of monactin on rate of respiration, light scattering, and ion movement in mitochondria. The medium for the experiment contained 15 mM KCl, 8 mM acetate, 3 mM $MgCl_2$, 0.2 M sucrose, 12 mM glutamate, and mitochondria equivalent to 1.2 mg of N in a final volume of 5 ml, at 28° and pH 7.2. The additions to the media were as follows: (A) monactin, $1.5 \times 10^{-3}$ μmole; (B) nigericin, $2.8 \times 10^{-3}$ μmole (final concentration $5.6 \times 10^{-7}$ M); and (C) antimycin A, 5 μg (final concentration $1.8 \times 10^{-6}$ M). An upward deflection of $K^+$ or $H^+$ tracing represents a decrease in the concentration of $K^+$ or $H^+$ in the medium or uptake of the ion by the mitochondria. The upward deflection of the oxygen trace reflects oxygen absorption from air by the medium in the open cuvet when oxygen tension has been greatly reduced by prior respiration.

## Experimental Material and Methods

*Preparation of Mitochondria.* Mitochondria were obtained from the livers of male, white rats of the Sprague–Dawley strain by standard procedures (Johnson and Lardy, 1966) except that the homogenization medium contained 0.25 M mannitol, 0.08 M sucrose, and 0.001 M EDTA. The mitochondria were washed three times and resuspended to a concentration of 1 ml/g of original liver in a medium of 0.25 M mannitol and 0.08 M sucrose. The experiments were begun as soon as the mitochondria were prepared and were completed within 4 hr.

For the preparation of sonic particles, mitochondria were recovered in the manner described. The final mitochondrial pellet was homogenized in cold, redistilled water by hand in a Teflon glass homogenizer. The mitochondria were diluted in redistilled water to a volume of 2 ml/g of original liver. The suspension (12 ml) was placed in a 30-ml beaker in ice and sonicated with a Branson Model LS-75 sonifier at setting no. 6, approximately 2 amp, for 30 sec. The sonicated suspension was centrifuged at 25,000*g* for 10 min. The supernatant fraction was removed with a bulb-capped pipet using extreme care to avoid any of the loosely packed pellet. The supernatant fraction was then centrifuged at 100,000*g* for 45 min to collect the particles. The gelatinous pellet was suspended in cold, redistilled water and diluted to a volume of 1 ml/g of original liver.

The experiments were performed using an apparatus which simultaneously monitors and records concentrations of $O_2$, $K^+$ or $Na^+$, $H^+$, and light scatter (Rasmussen *et al.*, 1965; Pressman, 1965; Graven *et al.*, 1966c). It was designed, developed, and constructed by B. Chance, D. Mayer, and B. Pressman at the Johnson Foundation, University of Pennsylvania. Light (520 mμ) scatter was monitored at 180°. The concentrations of $K^+$ and $Na^+$ were monitored with Beckman cation-sensitive electrodes No. 39047 and 39046, respectively. The concentration of $H^+$ was measured with a Beckman 39030 combination pH electrode and a Radiometer Model 22 pH meter. Oxygen concentration was determined with a collodion-coated, vibrating platinum electrode. After suitable amplification the data were recorded using a six-channel Honeywell 1508 visicorder. The cuvet (5 ml volume) was maintained at 28° with a constant-temperature jacket. The sources of the antibiotics used were documented in a previous paper (Graven *et al.*, 1966c) except for dianemycin which was obtained from Dr. R. Q. Thompson, Eli Lilly and Co., Indianapolis.

## Results

Monactin, a homolog of nonactin (Gerlach and Prelog, 1963), was used for most of the experiments presented below. Monactin was found to have maximal influences on ATPase induction and mitochondrial swelling at concentrations between $1 \times 10^{-6}$ and $1 \times 10^{-7}$ M (Graven *et al.*, 1966a). Consequently it was used at a concentration of $3 \times 10^{-7}$ M for most of the experiments reported.

The induction of $K^+$ uptake by monactin supported by different substrates and the release of $K^+$ by nigericin or dinitrophenol are shown in Figures 1–3. In a medium containing $K^+$, acetate or phosphate, $Mg^{2+}$, and substrate, monactin ($3 \times 10^{-7}$ M) stimulated respiration, induced swelling of the mitochondria, $K^+$ uptake, and $H^+$ ejection (Figure 1). Nigericin ($5.6 \times 10^{-7}$ M) induced contraction of the mitochondria, $K^+$ ejection, and $H^+$ uptake but did not inhibit the oxidation of succinate. The $K^+$ uptake was consistently greater than the $H^+$

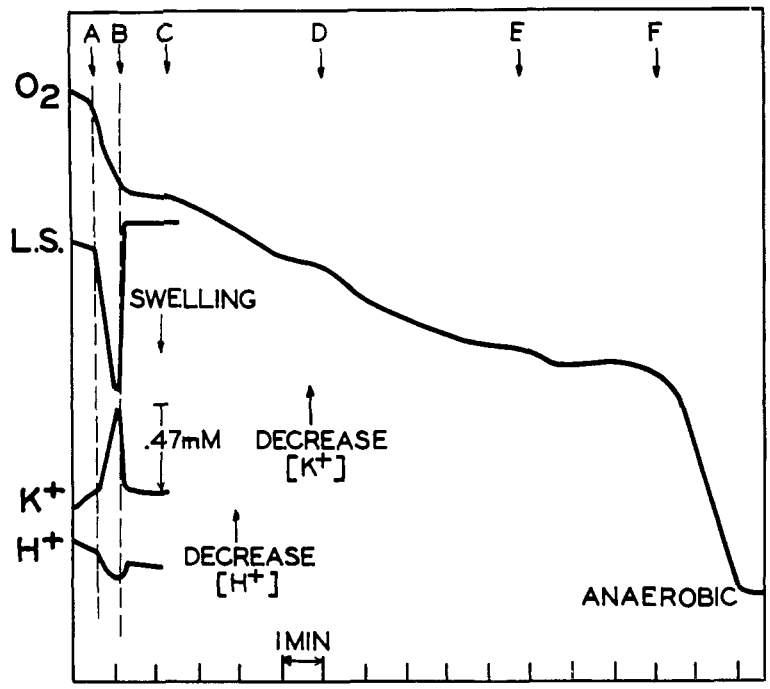


FIGURE 2: The effect of monactin on the rate of respiration, light scattering, and ion movement in mitochondria with the addition of different substrates. The medium contained 15 mM KCl, 10 mM triethanolamine acetate, 3 mM $MgCl_2$, 0.2 M sucrose, 12 mM citrate, and mitochondria equivalent to 1.2 mg of N in a 5-ml final volume at 28°. The additions were as follows: (A) monactin, $1.5 \times 10^{-3}$ μmole; (B) nigericin, $2.8 \times 10^{-3}$ μmole; (C) L-malate, 24 μmoles; (D) isocitrate, 12 μmoles; (E) succinate, 24 μmoles; and (F) β-hydroxybutyrate, 36 μmoles. The tracings are as in Figure 1.

ejection. A similar experiment with citrate as the substrate is depicted in Figure 2. The effects of monactin on respiration, swelling, $K^+$ uptake, and $H^+$ ejection are apparently not influenced by the nature of the substrate. Nigericin ($5.6 \times 10^{-7}$ M) inhibited respiration in addition to inducing contraction of the mitochondria, $K^+$ ejection, and $H^+$ uptake. The addition of L-malate partially released the inhibition of respiration, whereas β-hydroxybutyrate restored the initial rapid rate of respiration. The role of L-malate in the uptake and oxidation of citrate and isocitrate has been previously reported (Chappell, 1964; Ferguson and Williams, 1966). In the experiment presented in Figure 2, succinate failed to release the respiratory inhibition induced by nigericin. Succinate oxidation, previously shown not to be inhibited by nigericin (Graven *et al.*, 1966c), is inhibited by nigericin when L-malate is present in the medium prior to the addition of succinate. These interrelations will be discussed in a future publication.

With glutamate as the substrate the respiratory stimulation, swelling, $K^+$ uptake, and $H^+$ ejection induced by monactin can be reversed by 2,4-dinitrophenol as well as by nigericin (Figure 3). Dinitrophenol reversed the effects induced by monactin with glutamate as substrate. With β-hydroxybutyrate or succinate as substrate, dinitrophenol did not inhibit respiration but still induced contraction of the swollen mitochondria, $K^+$ ejection, and $H^+$ uptake. The inhibition of glutamate oxidation by nigericin or dinitrophenol can be prevented by increasing the concentration of $K^+$ to 60–90 mM or $P_i$ to 40–80 mM (Graven *et al.*, 1966c; S. N. Graven, S. Estrada, and H. A. Lardy, in preparation; Graven, 1966).

Since, as previously reported (Graven *et al.*, 1966b), monactin induced swelling and cyclic oscillations in light scattering in a medium containing NaCl in place of KCl, the effect of monactin on the transport of $Na^+$ and $Li^+$ was investigated. When the $Na^+$ concentration was below 30 mM, the monactin homologs induced a little swelling of the mitochondria. It was not possible conclusively to demonstrate uptake of $Na^+$ by mitochondria induced by monactin. In the $Na^+$ concentration range (50–100 mM) in which monactin has significant activity, it was difficult to measure small changes (*i.e.*, <0.5 mM) in $Na^+$ concentration reproducibly. The comparative effect of monactin, valinomycin, and gramicidin on the rate of respiration by mitochondria at different $Na^+$ concentrations is presented in Figure 4. Gramicidin stimulates respiration of mitochondria in $Na^+$ concentrations below 9 mM and is active at all concentrations of $Na^+$ up to 135 mM. Monactin induced little stimulation of respiration in a medium containing $Na^+$ at 30 mM but was effective when $Na^+$ concentration was >60 mM. Valinomycin had no effect on respiration when $Na^+$ was 75 mM or less but was slightly effective at very high concentrations of $Na^+$ (>90 mM).

In media containing $Li^+$ at 15–30 mM, monactin

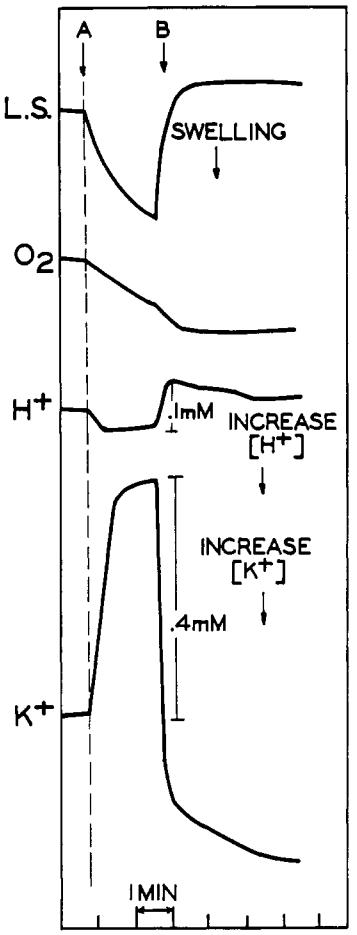


FIGURE 3: The effect of 2,4-dinitrophenol on the respiratory stimulation, swelling, and $K^+$ uptake induced by monactin in mitochondria. The medium contained 6 mM KCl, 8 mM phosphate, 3 mM $MgCl_2$, 0.2 M sucrose, 12 mM glutamate, and mitochondria equivalent to 1.2 mg of N in a 5-ml final volume, at 28°. The additions were as follows: (A) monactin, $1.5 \times 10^{-3}$ μmole; and (B) 2,4-dinitrophenol, 0.5 μmole.

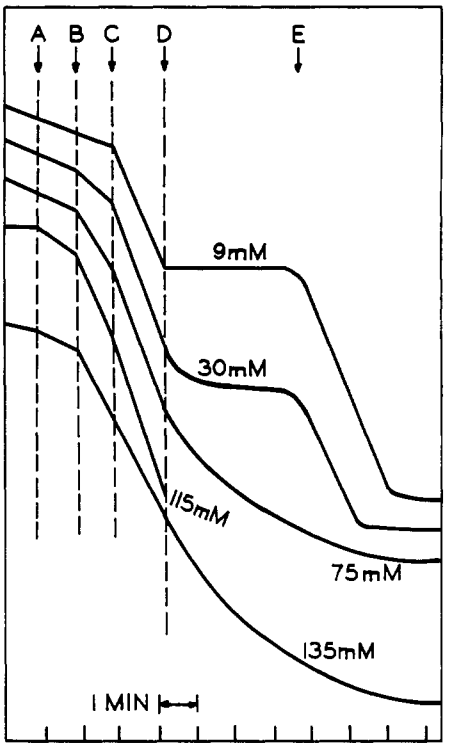


FIGURE 4: The effect of valinomycin, monactin, and gramicidin B on the rate of oxygen uptake in media containing different concentrations of NaCl. The media contained NaCl in concentrations shown in the figure, 8 mM phosphate, 3 mM $MgCl_2$, 0.2 M sucrose, 12 mM glutamate, and mitochondria equivalent to 1.0–1.5 mg of N. The other conditions were as shown in Figure 1. The additions were as follows: (A) valinomycin, $1.5 \times 10^{-3}$ μmole; (B) monactin, $1.5 \times 10^{-3}$ μmole; (C) gramicidin B, $1.5 \times 10^{-3}$ μmole; (D) nigericin, $2.8 \times 10^{-3}$ μmole; and (E) succinate, 21 μmoles. The horizontal portion of the tracings at the right-hand margin is the level when the media were anaerobic. The resting rates of $O_2$ consumption were 4–8 μmoles of $O_2$/g of protein per min. The maximum rates of $O_2$ consumption shown were 40–50 μmoles of $O_2$/g of protein per min.

induced a small amount of swelling and a slight stimulation in the rate of respiration (Figure 5). Monactin did not affect these activities when the concentration of $Li^+$ was 9 or 60 mM. In the same medium, gramicidin induced swelling, $Li^+$ uptake, and $H^+$ ejection, and stimulated respiration. The $Li^+$ uptake was measured with a $Na^+$-sensitive glass electrode with the deflection calibrated with standard $Li^+$ solutions. The downward drift of the tracing is a characteristic of the $Li^+$ tracing with the $Na^+$ electrode at $Li^+$ concentrations above 9 mM. Dianemycin, which affects $K^+$ and $Na^+$ uptake in a manner similar to nigericin, inhibited the respiration and induced contraction of the mitochondria, $H^+$ uptake, and $Li^+$ ejection. In comparable concentrations dianemycin is more effective in inducing the release of $Na^+$ and $Li^+$ than is nigericin (S. N. Graven, S. Estrada, and H. A. Lardy, in preparation).

*Effect of Monactin on Submitochondrial Particles.* From the data above and those previously presented (Graven *et al.*, 1966a,b), it was concluded that the uncoupling of oxidative phosphorylation, stimulation of state 4 respiration, induction of ATPase activity, and the swelling of mitochondria brought about by nonactin homologs were secondary to the stimulation of $K^+$ uptake similar to that reported for the action of valinomycin (Pressman, 1965; Ogata and Rasmussen, 1966). To assess the role of net $K^+$ accumulation, the effect of monactin on sonic particles of rat liver mitochondria was studied. A typical experiment is presented in Figure 6. Monactin stimulated the rate of respiration and induced a decrease in the light scatter of the particles. The apparent increase in the $K^+$ and $H^+$ concentrations in the medium is due to the solvent for the mon-

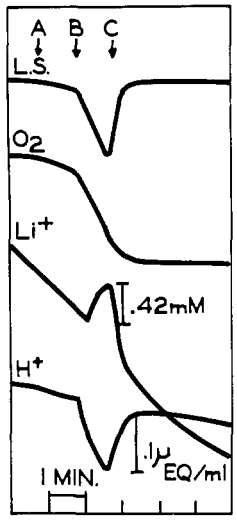


FIGURE 5: The effect of monactin and gramicidin B on light scattering, rate of respiration, and $Li^+$ and $H^+$ movements in mitochondria. The medium contained 15 mM LiCl, 8 mM phosphate, 3 mM $MgCl_2$, 0.2 M sucrose, 12 mM glutamate, and mitochondria equivalent to 1.2 mg of N. Other conditions were as described in Figure 1. The additions were as follows: (A) monactin, $1.5 \times 10^{-3}$ μmole; (B) gramicidin B, $1.5 \times 10^{-3}$ μmole; and (C) dianemycin, $2.8 \times 10^{-3}$ μmole. The $Li^+$ concentration was measured with a Beckman No. 39046 $Na^+$-sensitive electrode. The deflection was calibrated with a standard LiCl solution.

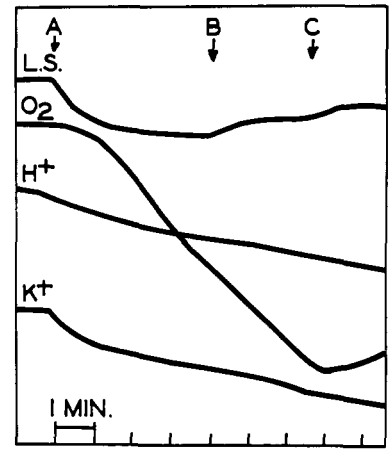


FIGURE 6: The effect of monactin on the rate of respiration and light scattering in sonic particles of mitochondria. The medium contained 6 mM KCl, 6 mM phosphate, 1.5 mM $MgCl_2$, 0.2 M sucrose, 12 mM succinate, and particles of mitochondria equivalent to 1.1 mg of N. The additions were as follows: (A) monactin, $1.5 \times 10^{-3}$ μmole; (B) nigericin, $1.4 \times 10^{-3}$ μmole; and (C) antimycin A, 2 μg. The resting rate of respiration was 4–5 μmoles of $O_2$/g of protein per min. The maximal rate after nonactin was 17–19 μmoles of $O_2$/g of protein per min. Traces are as in Figure 1.

actin and to electrode drift. Nigericin did not alter the rate of respiration but did induce a slight increase in the light scatter of the particles. Antimycin inhibited the respiration and induced a further increase in the light scattering. In the experiment depicted, the light scatter was recorded at a very high amplification and the particle suspension was very dilute compared with whole mitochondria; hence, the changes in light scatter recorded represent only minimal changes in the physical characteristics of the particles. From these experiments it was concluded that $K^+$ accumulation or net uptake against a gradient was not essential to the respiratory stimulation induced by monactin. The stimulation of respiration by monactin in mitochondrial particles could be related to the induction of an increased rate of $K^+$ turnover.

*Relation of Mitochondrial Swelling to Ion Uptake.* In the experiments presented above, the uptake of alkali metal cation appeared to be closely associated in time with the swelling of the mitochondria. However, in two types of experiments, alkali metal cation movement was not associated in time with the swelling or contracting of the mitochondria. ADP added to tightly coupled mitochondria induced the uptake of both $K^+$ and $H^+$ and stimulated respiration but did not alter the light scattering (Figure 7). The uptake of $H^+$ from the medium following the addition of ADP is most likely a reflection of the uptake of protons associated with the phosphorylation of ADP to ATP. The subsequent addition of monactin caused the mitochondria to swell but did not alter the rate of respiration. There was a slight increase in the $K^+$ uptake and a rapid release of $H^+$ which is probably a reflection of the proton release associated with ATP hydrolysis. Nigericin induced contraction of the mitochondria, loss of $K^+$, and inhibition of respiration. The respiratory inhibition was overcome by the subsequent addition of β-hydroxybutyrate. Under similar conditions, when monactin was added initially instead of ADP, the total $K^+$ movement was similar (*i.e.*, 0.3–0.5 mM; see Figures 1 and 2).

In media containing $K^+$ and phosphate with the pH at 6.4–6.8, monactin induced the uptake of $K^+$, the ejection of $H^+$, swelling of the mitochondria, and stimulated respiration (Figure 8). In the experiment depicted in Figure 8, there was considerable spontaneous uptake of $K^+$ by the mitochondria prior to the addition of monactin. This phenomenon is inconsistently observed but apparently related to the endogenous $K^+$ concentration and the condition of the mitochondria. Such uptake of $K^+$ is not associated with swelling or stimulation of respiration. When such spontaneous uptake of $K^+$ has occurred, the ejection of $K^+$ from the mitochondria results in a final concentration considerably above the initial concentration of $K^+$ in the medium. The uptake of $K^+$ was followed by spontaneous ejection of $K^+$ with no change in light scatter. The mitochondria partially recontracted when the medium became anaerobic with no specific ejection

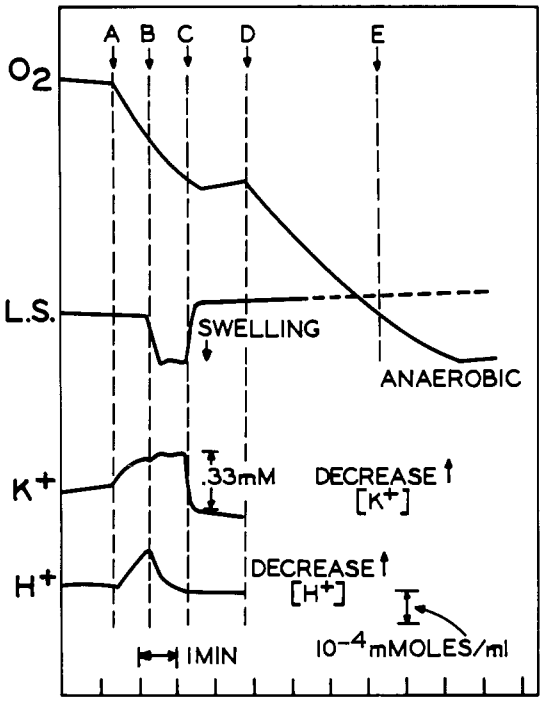


FIGURE 7: The effect of ADP, monactin, and nigericin on the rate of respiration, light scattering, and $K^+$ and $H^+$ movements in mitochondria. The medium contained 15 mM KCl, 10 mM phosphate, 3 mM $MgCl_2$, 0.2 M sucrose, 12 mM $\alpha$-ketoglutarate, and mitochondria equivalent to 1.2 mg of N. Other conditions were as shown in Figure 1. The additions were as follows: (A) TEA salt of ADP, 2.25 $\mu$moles; (B) monactin, $1.5 \times 10^{-3}$ $\mu$mole; (C) nigericin, $2.8 \times 10^{-3}$ $\mu$mole; (D) $\beta$-hydroxybutyrate, 18 $\mu$moles; and (E) succinate, 10 $\mu$moles.

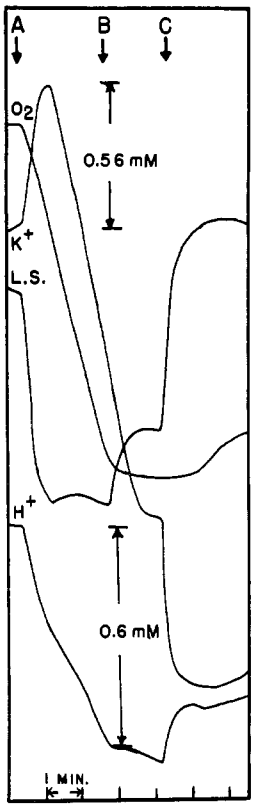


FIGURE 8: The effect of nonactin and nigericin on the rate of respiration, light scattering, and $K^+$ and $H^+$ movements in mitochondria. The medium contained 10 mM $K^+$, 5.5 mM phosphate, 1.3 mM $MgCl_2$, 0.2 M sucrose, 12 mM glutamate, and mitochondria equivalent to 1.5 mg of N. The medium was adjusted to pH 6.8 with TEA. Other conditions were as shown in Figure 1. The additions were as follows: (A) nonactin, $1.5 \times 10^{-3}$ $\mu$mole; and (C) nigericin $2.8 \times 10^{-3}$ $\mu$mole. The medium became anaerobic at B.

of $K^+$. The addition of nigericin induced contraction of the mitochondria and some further ejection of $K^+$. Thus under some conditions the changes in light scatter and $K^+$ uptake or ejection appear to be quite unrelated.

## Discussion

Valinomycin (Moore and Pressman, 1964; Pressman, 1965; Ogata and Rasmussen, 1966) and parathyroid hormone (Rasmussen *et al.*, 1964) induce the active, energy-dependent uptake of $K^+$ by mitochondria. On the basis of the data presented above, the "nactins" are another group of agents which stimulate the active uptake of $K^+$. The "nactins" induce $K^+$ uptake by mitochondria even with $K^+$ concentrations below 1 mM in the medium. At high concentrations of $K^+$ (>100 mM) "nactins" stimulate respiration and induce mitochondrial swelling. However, for technical reasons it has not been possible to demonstrate "nactin"-induced net $K^+$ uptake in media containing $K^+$ at concentrations >50 mM. Like valinomycin and gramicidins A, B, and C, monactin, dinactin, trinactin, and a mixture of "nactins" containing mostly nonactin (Sq 15859) are all active at concentrations as low as $10^{-9}$ (Graven *et al.*, 1966a; Witonsky and Johnson, 1964; S. N. Graven, S. Estrada, and H. A. Lardy, in preparation). The relative lack of activity of Sq 15859 compared with valinomycin previously reported (Pressman, 1965) may be due to differences in lots of antibiotics or failure to recognize the deterioration in activity of the "nactins" which occurs when the antibiotic is stored in solution (Graven *et al.*, 1966a).

In previous studies (Graven *et al.*, 1966b) the "nactins," like valinomycin and gramicidin, required a permeant anion, *i.e.*, phosphate, acetate, or formate, to induce maximal swelling and/or oscillations. The stimulation of respiration and $K^+$ uptake is produced by the "nactins" only in the presence of a permeant anion. Although the concentration of phosphate required for significant monactin activity is low (<1 mM), optimal or maximal activity is observed with phosphate or acetate at concentrations between 5

and 15 mM. The effect of the "nactins" as well as valinomycin and the gramicidins on swelling, cation uptake, and rate of respiration has never been studied in preparations specifically depleted of all endogenous permeant anions. In most of the experiments performed with the "nactins," phosphate or acetate was added to a concentration of between 6 and 10 mM.

Valinomycin (Moore and Pressman, 1964; Ogata and Rasmussen, 1966) and parathyroid hormone (Rasmussen *et al.* 1964) appear to require phosphate or acetate (or other permeant anion) to induce the energy-dependent uptake of $K^+$. Likewise, gramicidin appears to require phosphate or acetate to induce the uptake of $Na^+$ (Chappell and Crofts, 1965; Pressman, 1965; Graven *et al.*, 1966c) or $Li^+$ (S. N. Graven, S. Estrada, and H. A. Lardy, in preparation) by mitochondria. Further evidence that $K^+$ and phosphate or acetate move together is provided by the experiments with nigericin. Nigericin blocks the uptake of both $K^+$ and $P_i$ (S. N. Graven, S. Estrada, and H. A. Lardy, in preparation; S. Estrada, S. N. Graven, and H. A. Lardy, in preparation). By increasing the concentration of $K^+$ or $P_i$, the inhibition of respiration induced by nigericin can be prevented. This, however, occurs only when the mitochondria are exposed to the increased concentration of cation or anion prior to the addition of nigericin. Addition of $K^+$ or $P_i$ to the medium after the addition of nigericin induces only small increases in the rate of respiration (S. N. Graven, S. Estrada, and H. A. Lardy, in preparation). The uptake of $K^+$ induced by monactin, valinomycin, and other agents and the uptake of $Na^+$ and $Li^+$ induced by gramicidin are blocked by nigericin.

The $K^+$ uptake which accompanied the addition of ADP (Figure 7) had no effect on the light-scattering properties of the mitochondria (*cf.* Packer, 1961; Connelly and Lardy, 1964), while the addition of monactin induced marked swelling but little further increase in the $K^+$ uptake by the mitochondria. The spontaneous loss of $K^+$ following uptake induced by monactin at pH 6.4–6.8 (Figure 8) was not associated with recontraction of the mitochondria. On the basis of these experiments, it would seem unlikely that the swelling of mitochondria can be explained as an osmotic phenomenon associated with the uptake of $K^+$ by the mitochondria (Ogata and Rasmussen, 1966). The swelling of mitochondria, at least in the experiments shown in Figures 7 and 8, would appear to be in some manner more related to the turnover of $K^+$ than to the net accumulation of $K^+$, a concept previously proposed by others (Pressman, 1965; Harris *et al.*, 1966).

With the sonic disruption of mitochondria the capacity to accumulate $K^+$ was lost, but monactin still stimulated respiration and induced some change in light scattering. The addition of nigericin failed to inhibit the oxidation of glutamate. Nigericin would have inhibited the glutamate-supported respiration in whole mitochondria under comparable conditions. On the basis of these data, it is concluded that the stimulation of respiration induced by monactin could be related to an increased expenditure of energy by a process that in intact mitochondria results in the maintenance of a concentration gradient. However, effects of nigericin are demonstrable only when there is an intact inner compartment which contains dehydrogenases that require $K^+$ and $P_i$. By inhibiting the uptake of $K^+$ and $P_i$, nigericin inhibits the oxidation of certain substrates. Disruption of this compartment prevents the inhibition of respiration by nigericin. These data along with those previously published (Graven *et al.*, 1966c) support the concept of a functional compartmentation of the dehydrogenases (Klingenberg and Pfaff, 1965).

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