solvent of higher CF_3COOH concentrations is a better solvating agent. In particular it can form solvated complexes with the small anion, $CF_3COOH \cdot {}^-OOCCF_3$. This solvation of anions would increase the dissociation of ion pairs and hence produce a rise in electrical conductance.

Thus it is apparent that conductance data confirm infrared observations in regard to the formation of protonated peptide groups in solvents containing the strong acid CF_3COOH.

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Antibiotics As Tools for Metabolic Studies. V. Effect of Nonactin, Monactin, Dinactin, and Trinactin on Oxidative Phosphorylation and Adenosine Triphosphatase Induction*

Stanley N. Graven, Henry A. Lardy, Diane Johnson, and Anthony Rutter

ABSTRACT: Four homologous antibiotics, nonactin, monactin, dinactin, and trinactin, were studied for their effect on oxidative phosphorylation and adenosine triphosphatase (ATPase) induction. Nonactin, monactin, dinactin, and trinactin uncoupled oxidative phosphorylation at a concentration of 1×10^{-7} M and partially uncoupled at 1×10^{-8} M. All four homologs induced ATP hydrolysis. Monactin, dinactin, and trinactin were more potent inducers of ATPase activity than nonactin.

A monovalent cation, Na^+, K^+, Rb^+, or Cs^+, was required for the action of the antibiotics. None of the nonactin homologs was active in the presence of Li^+ as the cation. At low antibiotic concentrations (1×10^{-7} M and below), less ATPase activity was induced in the presence of Na^+ than in the presence of K^+, Rb^+, or Cs^+. At low monovalent cation concentrations (7.5 mM), greatest activity was observed in the presence of Rb^+. The antibiotics are potent uncouplers of oxidative phosphorylation and inducers of ATPase activity with their activity possibly mediated through an effect on monovalent cation transport. The nonactin homologs were comparable in activity to valinomycin and less active than gramicidins A–D as measured by ATPase induction. The monovalent cation requirement of the nonactin homologs differentiated their activity from that of valinomycin, tyrocidine, and gramicidins A–D.

Corbaz *et al.* (1955) reported the isolation of an Actinomycete metabolite which they named nonactin because it lacked antimicrobial activity toward their test organisms. A similar product was isolated independently by Dutcher (1962) and designated SQ 15859. The compounds were noted to be cytotoxic for tumor and certain bacterial cells (Arnow *et al.*, 1962; Meyers *et al.*, 1965) and to uncouple oxidative phosphorylation (Lardy, 1961). In addition to nonactin, three homologs named monactin, dinactin, and trinactin were subsequently isolated from the same fermentation (Beck *et al.*, 1962; Dominguez *et al.*, 1962). The basic structure is that of a cyclic macrotetralide (see Figure 1) composed of four nonactinic or homononactinic acid subunits. The homologs differ in that methyl substituents (R = H in Figure 1) are replaced by one, two, or three ethyl groups, and the stereoconfiguration of the constituent acids varies as well. The structure and absolute configuration of the nonactin and its homologs were determined by Gerlach and Prelog (1963). This report presents data on the effects of nonactin, monactin, dinactin, and trinactin on oxidative phosphorylation and the induction of ATPase in rat liver mitochondria.

Materials and Methods

Mitochondria were prepared from livers of male, 150–220-g rats according to the method of Lardy and

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[1] Abbreviation used: ATPase, adenosine triphosphatase.

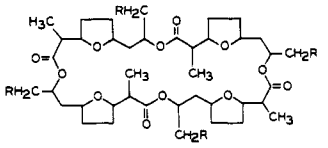

FIGURE 1: The structure of nonactin (Gerlach and Prelog, 1963). The homologs differ in the presence of one or more CH_3 groups at the sites labeled R.

Wellman (1952). The mitochondria were suspended in 0.25 M sucrose to a volume of 1 ml/g of original liver, stored at 0°, and used within 1 hr of preparation. Respiration and phosphorylation were measured manometrically by the method of Lardy and Wellman (1952) and ATPase by the method of Lardy and Wellman (1953). Phosphate was determined by the method of Sumner (1944). Doubly distilled water and chemicals of the highest purity available were used in the work described. The antibiotics were generous gifts from the following: nonactin, monactin, dinactin, and trinactin, Professor V. Prelog, Eidgenössischen Technischen Hochschule, Zurich; Rutamycin, Dr. R. L. Mann, Eli Lilly and Co., Indianapolis; Aurovertin, Dr. H. A. Nash and C. L. Baldwin, Pitman Moore Division, Dow Chemical Co.; SQ 15859, Dr. D. Perlman, The Squibb Institute; Peliomycin, Dr. H. Schmitz, Bristol Laboratories, Syracuse, N. Y.; gramicidins A–C, Dr. Bernard Witkop, National Institutes of Health; gramicidin S was an international reference standard prepared by Professor J. Gause, Institute of New Antibiotics, Moscow, and supplied through the World Health Organization; Na^+ and K^+ salts of ATP were obtained from Pabst Laboratories, Milwaukee, Wis. The Tris salts of ATP and tyrocidine were obtained from Sigma Chemical Co., St. Louis, Mo.

Results

The activity of all of the nonactin homologs decreased during storage at 2–4° in 60% 1,2-propanediol. A decrease in activity of *ca.* 50% was observed on storage for 6–12 weeks. Monactin, dinactin, and trinactin had similar solubilities in alcohol or 1,2-propanediol. Nonactin was less soluble than the other three homologs with a solubility of 75–100 μg/ml at 2°.

Mitochondrial Oxidation and Phosphorylation. The effects of nonactin, monactin, dinactin, and trinactin on glutamate, succinate and ascorbate oxidation are presented in Table I. Monactin, dinactin, and trinactin nearly completely uncoupled oxidative phosphorylation at a concentration of 1×10^{-7} M with the three substrates and uncoupled partially at a concentration of 1.7×10^{-8} M. Nonactin was slightly less active. A 20–38% increase in $Q_{O_2}(N)$ with glutamate was observed at an antibiotic concentration of 1×10^{-7} M. There was slight inhibition of succinate respiration and no change in ascorbate respiration at the same antibiotic concentration. The experiments were performed using glucose–hexokinase in nonlimiting amounts as the phosphate acceptor.

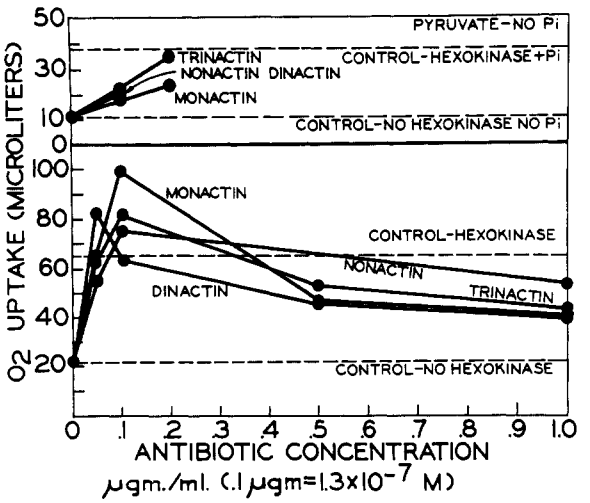

FIGURE 2: The effect of nonactin and its homologs on pyruvate oxidation by rat liver mitochondria. The media are as shown for Table I except the substrate was pyruvate 20 mmoles/3.0 ml. The upper graph is the rate of oxidation in the absence of P_i; the lower graph is the rate in the presence of P_i. The control rates of respiration with and without glucose–hexokinase phosphate acceptor are as shown. Oxygen consumption is recorded in μl consumed/10 min at 30°; 1.9 mg of mitochondrial nitrogen/flask.

The stimulation of glutamate and succinate oxidation by 3.3×10^{-9} M di- or trinactin, and the negligible effect on P/O, resulted in a greater net uptake of P_i than in the control experiments without antibiotic. Similar stimulation of net phosphate uptake was reported (Johnson *et al.*, 1950) to occur in the presence of the antibiotic usnic acid at 1×10^{-6} M.[1]

The effects of the nonactin homologs on the rate of pyruvate oxidation in the presence and absence of phosphate and glucose–hexokinase phosphate acceptor are presented in Figure 2. All of these antibiotics, at concentrations between 0.05 and 0.2 μg/ml, stimulated respiration in the presence or absence of P_i. In the presence of P_i, the maximal respiration achieved in the presence of optimal concentrations of the antibiotics exceeded the rate of respiration achieved with the glucose–hexokinase acceptor system. There was less stimulation of respiration in the absence of P_i and, in this condition, there was usually a lag period before the increase in rate of respiration occurred. No significant inhibition of respiration was observed with any of the antibiotics at concentrations up to 1 μg/ml. The replacement of P_i with acetate did not change the degree of respiratory stimulation observed with antibiotic concentration of 0.05 and 0.1 μg/ml. In the absence of

[1] Similar enhancement of phosphate uptake by low concentrations of valinomycin have been reported by Hofer, Cockrell, and Pressman (1966), *Federation Proc. 25*, 414.

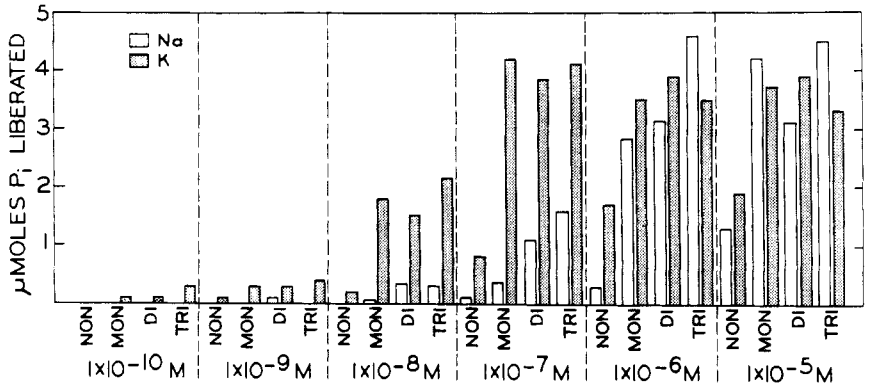

FIGURE 3: Induction of ATPase activity by non-, mon-, di-, and trinactin. The reaction mixture contained: 10 mmoles of TEA, 6 mmoles of (Tris) ATP, 60 mmoles of NaCl or KCl, 120 mmoles of sucrose, and 0.3 ml of mitochondria containing 0.25-0.35 mg of N in a total volume of 1 ml. The antibiotics were in 40–50% 1,2-propanediol solution with 0.02 ml added to each tube. The reaction was started with the addition of the mitochondria and stopped with the addition of cold 10% trichloracetic acid. The P_i liberated is expressed as μmoles/0.2 mg of N per 10 min.

TABLE I: Effect of Nonactin, Monactin, Dinactin, and Trinactin on Oxidation and Phosphorylation.[a]

		Glutamate		Succinate		Ascorbate + TMPD	
	Concn (M)	$Q_{O_2}(N)$	P/O	$Q_{O_2}(N)$	P/O	$Q_{O_2}(N)$	P/O
Control		198	3.5	334	2.0	485	0.65
Nonactin	3.3×10^{-7}	256	0	187	0	498	0
	1×10^{-7}	267	1.4	216	0.64	510	0.36
	1×10^{-8}	264	3.1	212	2.7	536	0.52
Control		400	3.10	315	1.84	425	0.70
Monactin	1×10^{-7}	480	0.61	280	0	445	0.05
	3.3×10^{-8}	455	1.80	290	0.16	415	0.12
	1.7×10^{-8}	455	2.90	290	1.20	440	0.53
Dinactin	1×10^{-7}	520	0.12	245	0.12	240	0.13
	3.3×10^{-8}	475	1.10	295	0.65	310	0.06
	1.7×10^{-8}	425	2.60	295	1.10	290	0.65
	3.3×10^{-9}	440	3.10	320	1.90	345	0.63
Trinactin	1×10^{-7}	550	0.37	240	0.12	465	0.02
	3.3×10^{-8}	490	1.00	310	0.56	450	0.41
	1.7×10^{-8}	470	2.00	290	1.70	445	0.33
	3.3×10^{-9}	440	3.20	345	1.80	435	0.74

[a] The reaction vessel contained 6 mmoles of ATP, 50 mmoles of potassium phosphate, 20 mmoles of Tris buffer, 15 mmoles of $MgSO_4$, 50 mmoles of glucose, yeast hexokinase (type IV), and 0.5 ml of rat liver mitochondria containing 1.8–2.0 mg of N, in a total volume of 3.0 ml at pH 7.4. Substrates were present in the following amounts: glutamate 30 mmoles, succinate 20 mmoles, ascorbate 30 mmoles, and TMPD (tetramethylphenylenediamine) 5 mmoles/3.0 ml. After 10 min at 30°, the reaction was stopped with cold 10% trichloroacetic acid. The antibiotics were dissolved in 30–50% 1,2-propanediol and added in volumes of 0.02–0.1 ml/flask. $Q_{O_2}(N)$ = cmm of O_2 consumed/mg of N per hr.

glucose–hexokinase as phosphate acceptor, the stimulation of respiration in the media containing acetate was the same or greater than in the media containing P_i at an antibiotic concentration of 0.1 μg/ml.

Adenosinetriphosphatase Induction. The amount of ATPase activity induced by non-, mon-, di-, and trinactin in the presence of sodium or potassium is presented in Figure 3. In media containing K^+, ATP hydrolysis was detectable at 1×10^{-10} M concentration of mon-, di-, and trinactin and 1×10^{-9} M nonactin;

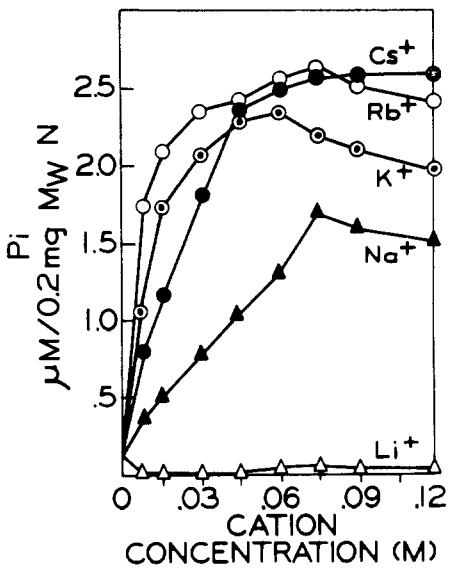

FIGURE 4: Induction of ATPase activity by trinactin as related to the monovalent cation in the media. The reaction mixture was the same as used in Figure 3 except the cation concentration is as shown and the antibiotic was trinactin at 1×10^{-7} M.

maximal induction in the presence of K^+ was obtained at 1×10^{-7} M of the first three antibiotics. In the presence of Na^+ and in the absence of exogenous K^+, ATP hydrolysis was detectable at antibiotic concentrations of 1×10^{-8} M with maximal activity at a concentration of 1×10^{-6} M. The hydrolysis of ATP induced by nonactin homologs did not require exogenous Mg^+.

The effect of different monovalent cations on the induction of ATP hydrolysis by trinactin is presented in Figure 4. The maximal extent of ATPase induction by trinactin is similar in the presence of K^+, Rb^+, and Cs^+. For each cation there appears to be an optimal concentration.

Maximum ATP hydrolysis was observed at concentrations of 0.045–0.06 M K^+ while in Cs^+ and Rb^+ maximum hydrolysis was observed at higher concentrations. At an antibiotic concentration of 1×10^{-7} M, less ATP hydrolysis was observed in the presence of Na^+. At the lowest cation concentration (0.0075 M), ATP hydrolysis consistently was greatest in the presence of Rb^+. Consistent differences in extent of ATP hydrolysis in the presence of Na^+, K^+, Rb^+, and Cs^+ were not observed when higher concentrations ($>5 \times 10^{-6}$ M) of antibiotic were used (see Figure 3).

No ATPase activity was induced by trinactin in the presence of Li^+ or NH_4^+. Allowing the mitochondria to become depleted of endogenous K^+ (suspended in sucrose at 2–4° for 60 min, followed by centrifugation and resuspension) did not alter significantly the extent of ATP hydrolysis in the presence of the various cations shown in Figure 4. At cation concentrations below 0.045 M, the controls (no antibiotic) were similar for all monovalent cations tested. At concentrations of cation over 0.06 M, spontaneous ATP hydrolysis in the control tubes was consistently highest in the presence of Na^+ (0.5 ± 0.1 *vs.* 0.3 ± 0.1 μmole of P_i/10 min). The values recorded in Figure 4 for the P_i liberated are the differences between the experimental and the control tubes at comparable cation concentrations.

The induction of ATPase activity by nonactin and monactin compared with that induced by several other antibiotics in the presence of different monovalent cations is presented in Table II. The activity of monactin in K^+ or Rb^+ was similar to that of valinomycin and slightly less than that of gramicidins. Nonactin was less active than monactin as previously noted in Figure 3. The monovalent cation requirements were different for each of the groups of antibiotics. The gramicidins were active in the presence of any of the alkali metal ions and at higher antibiotic concentrations ($>2 \times 10^{-8}$ M) were active in the absence of added cation. Tyrocidin had little activity in sucrose but was active in the presence of any of the alkali metal ions. The nonactin homologs had little activity in Li^+ but were active in Na^+, K^+, Rb^+, and Cs^+. Valinomycin was the most selective having significant activity only in the presence of K^+, Rb^+, and Cs^+, as reported by Moore and Pressman (1964). Gramicidin S had essentially no activity at the concentrations tested.

Oligomycin D and peliomycin at 2 μg/ml completely inhibited (>90%) the ATPase inducing activity of all of the nonactin homologs in the presence of Na^+ and/or K^+. Aurovertin inhibited ATPase activity 50–70% in either Na^+ or K^+.

Discussion

The nonactin homologs partially uncouple oxidative phosphorylation at concentrations as low as 2×10^{-8} M with complete uncoupling at 10^{-7} M. The four homologs have similar activities with nonactin being the least active. In addition to uncoupling oxidative phosphorylation, these antibiotics stimulate the oxidation of pyruvate and glutamate above the rate with a phosphoryl acceptor. Optimal stimulation of respiration is observed at the lowest antibiotic concentration which completely uncoupled oxidative phosphorylation. P_i is not required for respiratory stimulation although a greater stimulation is observed when P_i is present. However, acetate can replace P_i with no change in the extent of respiratory stimulation. Since the respiratory stimulation occurs at antibiotic concentrations that block phosphate uptake, the effect of P_i or acetate is presumably due to its role as an anion and not related to any phosphorylated intermediate or phosphorylation process. Moore and Pressman (1964) reported stimulation of respiration by valinomycin only in the presence of P_i or arsenate. In later studies Pressman (1965b) observed respiratory stimulation with valinomycin in the absence of P_i. There was, however, a time lag between the addition of valinomycin and the increase in the rate of respiration. The uptake of K^+ from the media occurred with or without P_i in the media. In the same experiments gramicidin B stimulated respiration in the presence or absence of P_i. However, the stimulation of respiration in the presence of P_i was greater than when

TABLE II: The Effect of Different Monovalent Cations on the ATPase Induced by Different Concentrations of Nonactin, Monactin, Gramicidins A, B, C, D, and S, Tyrocidine, and Valinomycin.[a]

Antibiotic	Concn (M)	μmoles of P_i Liberated (30°, 10 min/0.2 mg of M_w N)					
		Sucrose	Li^+	Na^+	K^+	Rb^+	Cs^+
Nonactin	2×10^{-6}	0.3	0.3	1.9	2.5	2.3	2.4
Monactin	2×10^{-6}	0.3	0.3	1.8	2.0	2.0	1.8
Gramicidin D	2×10^{-6}	0.5	1.0	1.4	1.8	1.6	1.6
Gramicidin S	2×10^{-6}	0	0	0	0.2	0.2	0.2
Tyrocidine	2×10^{-6}	0.2	1.7	1.6	1.8	1.9	1.8
Nonactin	2×10^{-7}	0.1	0	0.3	3.0	2.8	1.6
Monactin	2×10^{-7}	0	0.2	0.5	2.9	2.7	2.2
Gramicidin A	2×10^{-7}	0.3	2.2	1.9	2.4	2.5	2.1
Gramicidin B	2×10^{-7}	0.1	1.6	1.6	2.3	2.4	2.3
Gramicidin C	2×10^{-7}	0.7	2.4	2.0	2.3	2.3	2.1
Gramicidin D	2×10^{-7}	0.7	2.4	2.2	2.7	2.3	2.3
Gramicidin S	2×10^{-7}	0	0	0	0.13	0	0
Tyrocidine	2×10^{-7}	0	0.2	0.4	0.8	0.8	0.9
Valinomycin	2×10^{-7}	0	0.1	0.1	2.8	2.8	2.4
Nonactin	2×10^{-8}	0	0	0	0.6	0.3	0.1
Monactin	2×10^{-8}	0	0.1	0.2	1.5	1.1	0.7
Gramicidin A	2×10^{-8}	0.2	1.1	1.0	2.1	1.9	2.0
Gramicidin B	2×10^{-8}	0.1	1.3	0.8	1.1	1.5	1.5
Gramicidin C	2×10^{-8}	0.2	1.9	1.7	2.2	2.2	2.1
Gramicidin D	2×10^{-8}	0.2	1.4	1.2	2.1	2.2	1.9
Gramicidin S	2×10^{-8}	0	0	0	0	0	0
Tyrocidine	2×10^{-8}	0	0.1	0.2	0.3	0.3	0.5
Valinomycin	2×10^{-8}	0	0.1	0.1	1.4	1.2	1.2
Nonactin	2×10^{-9}	0	0	0	0	0.1	0.1
Monactin	2×10^{-9}	0	0	0	0.1	0.2	0.2
Gramicidin A	2×10^{-9}	0	0.5	0.4	0.7	1.1	1.2
Gramicidin B	2×10^{-9}	0	0.2	0.4	0.7	0.9	1.2
Gramicidin C	2×10^{-9}	0	0.6	0.7	1.1	1.6	1.7
Gramicidin D	2×10^{-9}	0	0.4	0.5	0.8	0.7	0.9
Tyrocidine	2×10^{-9}	0	0.1	0.3	0.2	0.1	0.2
Valinomycin	2×10^{-9}	0	0.1	0.1	0.2	0.2	0.2
Nonactin	2×10^{-10}	0	0	0	0	0	0
Monactin	2×10^{-10}	0	0	0.1	0.1	0.2	0.2
Gramicidin A	2×10^{-10}	0	0.1	0.5	0.1	0.7	0.6
Gramicidin B	2×10^{-10}	0	0.1	0.5	0.2	0.8	0.5
Gramicidin C	2×10^{-10}	0	0.1	0.5	0.6	0.6	0.6
Gramicidin D	2×10^{-10}	0	0.1	0.3	0.2	0.2	0.2
Tyrocidine	2×10^{-10}	0	0	0.1	0.1	0.2	0.2
Valinomycin	2×10^{-10}	0	0	0	0.1	0.2	0.1

[a] Media contained monovalent cation 60 mmoles, sucrose 25 mmoles with cation or 120 mmoles without cation, Tris–ATP 6 mmoles, TEA 10 mmoles, and 0.3 ml of mitochondria in sucrose containing 0.25–0.4 mg of nitrogen in a total volume of 1 ml. The antibiotics were in 40–70% ethyl alcohol with 1–15% dimethylformamide and added in 0.02-ml volume. The reaction was run for 10 min at 30° and stopped with the addition of cold 10% trichloracetic acid (TEA = triethanolamine).

P_i was omitted from the media. These effects of gramicidin and valinomycin are similar to those observed with the nonactin homologs. A time lag in the stimulation of respiration by the nonactin homologs in the absence of P_i was also observed. Chappell and Crofts (1965) reported similar findings with gramicidin. There is little difference in the relative potencies of monactin, dinactin, and trinactin as measured by their effects on rate of respiration or oxidative phosphorylation. Nonactin is less active by a factor of *ca.* 10. Nonactin ap-

peared to be distinctly different from its homologs both in potency and in its solubility in ethyl alcohol or propanediol.

In a medium containing Na^+ or K^+, monactin, dinactin, and trinactin are nearly equally active in inducing the hydrolysis of ATP. Nonactin again is less active. All of the nonactin homologs require either Na^+, K^+, Rb^+, or Cs^+ for their activity with greater activity observed in a medium containing K^+, Rb^+, or Cs^+. The monovalent cation requirement of the nonactin homologs differs from the cation requirement of other antibiotics with similar type activity. Gramicidin is active in the presence of any of the alkali metal cations used and at higher antibiotic concentrations is active in the absence of exogenous alkali metal cation. Tyrocidine is not active in the absence of cation but had similar activities in Li^+, Na^+, K^+, Rb^+, and Cs^+. Tyrocidine was more active than gramicidin S but less active than the other antibiotics tested. The nonactin homologs had no activity in the absence of alkali metal cation or in a Li^+-containing medium. Valinomycin had no activity in either a Li^+- or Na^+-containing medium. Thus, the nonactin homologs can be differentiated from the gramicidins, tyrocidines, and valinomycin on the basis of the monovalent cations which support ATPase induction. Chappell and Crofts (1965) observed gramicidin activity with NH_4^+ as the monovalent cation. The nonactin homologs are not active in the presence of NH_4 as the cation.

The cation requirements for activity of the antibiotics studied suggest that their mechanism of action involves the transport of monovalent cations. Moore and Pressman (1964) and Pressman (1965a,b) have presented evidence that valinomycin may exert its effect through a stimulation of K^+ transport into mitochondria. A similar mechanism for the activity of the gramicidins has been proposed (Chappell and Crofts, 1965; Pressman, 1965b). Potassium ion movements induced by the nonactin homologs have been observed and are now under study in this laboratory. Because of the many similarities in the activities of the nonactin homologs and the gramicidins and/or valinomycin, it is likely that they all act *via* a similar mechanism. Chappell and Crofts (1965) and Pressman (1965b) have discussed possible means by which the peptide and depsipeptide antibiotics may bring about ion movements and the hydrolysis of ATP. The experiments reported in the present paper do not clarify the mechanisms involved but they do point up the fact that there is a continuing change of response to different types and amounts of alkali metal ions that parallels changes in structure and amount of the antibiotics used.

The nonactin homologs were more active than gramicidin S or tyrocidin, equally as active as valinomycin, and slightly less active than the gramicidins A–D. Chappell and Crofts (1965) and Pressman (1965b) have found valinomycin more active than the gramicidins or nonactin. Pressman (1965b) reported gramicidin B to be the most active of the gramicidins as measured by ion transport induction properties. As noted in Table II, gramicidin C was a slightly more potent inducer of ATPase activity than the other gramicidins. The differences were slight but consistent. The activity of gramicidin D was about as would be predicted from a combination of gramicidin A–C.

The nonactin homologs, gramicidins A–D, valinomycin, and tyrocidine differ from thyroxine, selenite, and selenate in their ability to relieve the oligomycin-induced inhibition of tightly coupled respiration in mitochondria. Thyroxine and related compounds, selenite, and selenate do not release the oligomycin inhibition of tightly coupled respiration in mitochondria (Lardy *et al.*, 1964), even though they induce ATPase activity and, to varying degrees, uncouple oxidative phosphorylation in the absence of oligomycin. The cation, dependent antibiotics stimulate the oligomycin-inhibited respiration. Dinitrophenol differs from the nonactin homologs and similar antibiotics in that its alkali metal ion requirement for ATPase activity is not specific (Lardy and Wellman, 1953), does not induce cation movements, and inhibits substrate-supported swelling of mitochondria induced by the nonactin homologs (Pressman, 1963; Graven *et al.*, 1966). It is proposed that nonactin homologs, gramicidins, valinomycin, and tyrocidine, all act *via* a mechanism related to cation movements with the specific cation requirements related in some manner to the molecular configuration of the antibiotic.

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Antibiotics As Tools for Metabolic Studies. VI. Damped Oscillatory Swelling of Mitochondria Induced by Nonactin, Monactin, Dinactin, and Trinactin*

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ABSTRACT: Nonactin, monactin, dinactin, and trinactin, at concentrations of 10^{-8}–10^{-10} M, alter the light scattering properties of rat liver mitochondria. To induce swelling these antibiotics require an alkali metal cation (Na^+, K^+, Rb^+, or Cs^+), acetate or phosphate, and adenosine triphosphate (ATP) or substrate. At antibiotic concentrations between 10^{-5} and 10^{-6} M in the presence of monovalent cation, a permeant anion, and substrate or ATP, damped oscillations in the light scattering properties of the mitochondria were observed. The swelling of mitochondria induced by the nonactin homologs was dependent on an energy source and recontraction occurred when the energy source was blocked. The oscillations appeared to be the result of cyclic interruptions in the energy required to maintain the mitochondria in the swollen state. It is proposed that the antibiotics act by inducing alterations in the ion translocation system.

The cytotoxic antibiotics nonactin, monactin, dinactin, and trinactin are potent uncouplers of oxidative phosphorylation and inducers of ATP[1] hydrolysis in rat liver mitochondria (Graven *et al.*, 1966). The activity of the antibiotics in these phenomena is influenced by the species and concentration of alkali metal cations in the media. Because of the relationship between adenosine triphosphatase activity and monovalent cation composition of the media, the influence of these antibiotics on mitochondrial swelling was investigated. The following is a report of the changes in the light scattering properties of rat liver mitochondria induced by the nonactin homologs.

Materials and Methods

The mitochondria were prepared by the method of Lardy and Wellman (1952) with the exception that the homogenizing medium contained 0.07 M sucrose and 0.25 M mannitol instead of 0.25 M sucrose. Male, 150–200-g rats were used. The mitochondria were suspended in a volume of sucrose–mannitol medium to make 1 ml/g of original liver, maintained at 0°, and used within 1 hr of preparation. Light scattering by the mitochondria was measured at 180° with a Cary Model 15 recording spectrophotometer at 515 mμ using a 1-cm² cuvet. The reaction was initiated by adding 2.9 ml of reaction mixture (at 23°) to 0.07–0.1 ml of mitochondria in the cuvet. The volume of mitochondria employed was selected to produce an initial apparent optical density reading of 1.8 when suspended in 2.9 ml of 0.25 M sucrose. The reaction mixtures are shown in the figure legends. All studies were conducted at a room temperature of *ca.* 23°.

Doubly distilled water and chemicals of highest purity available were used in the work described. The sources of antibiotics were presented in the preceding paper (Graven *et al.*, 1966).

When added, acetate and formate were neutralized with triethanolamine (TEA). Sodium and potassium salts of ATP were obtained from P-L Biochemicals, Inc., Milwaukee, Wis. Tris and diethanolamine salts of ATP were obtained from the Sigma Co., St. Louis, Mo.

Results

The observations on the effect of the antibiotics on the light scattering properties of mitochondria were

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[1] Abbreviations used: ADP and ATP, adenosine di- and triphosphate; TEA, triethanolamine; DPN, 2,4-dinitrophenol.