The linearity of this function in the range of degree of polymerization $DP = 100$–1000 can be seen in Fig. 1. The conditions of the viscosity measurements were chosen because of the following considerations. In Fig. 2 the concentration dependence of a number of polylysine·HBr samples in salt-free aqueous solution is given. It has been demonstrated that extrapolation of curves of this kind to $c = 0$ in order to obtain intrinsic viscosities is not feasible[8]. The concentration of 1% was chosen so as to avoid working in the steep region of the curve, and in the absence of added salt viscosity values of reasonable magnitude are obtained even for polymers of rather low molecular weight ($DP = 100$).

The most serious drawback of the second method lies in the following peculiar behavior of poly-L-lysine·HBr, which is as yet not understood. The polymer when kept at −15° shows complete stability of its viscosity properties. However, at room temperature certain changes take place which result in viscosity increases. Thus the value of $\eta_{sp\,(c = 1\%)} = 2.5$ in water of a freshly prepared sample rose to 4.6 after keeping it in a desiccator at room temperature for four months. This change is not due to an increase in molecular weight of the sample, as can be seen from the fact that the polycarbobenzoxylysine preparations made from the fresh and from the aged material had the same viscosity. It is therefore clear that if a polylysine sample has been kept under unfavorable or unknown conditions for a considerable length of time, the first procedure (recarbobenzoxylation) for the determination of its degree of polymerization must be employed.

This work was supported by a research grant (FG-Is-104-60) from the U.S. Department of Agriculture.

Department of Biophysics, The Weizmann Institute of Science, Rehovoth (Israel) ARIEH YARON
ARIEH BERGER

[1] M. SELA, E. KATCHALSKI AND M. GEHATIA, *J. Am. Chem. Soc.*, 78 (1956) 746.
[2] A. BERGER AND A. YARON, in M. A. STAHMANN, *Polyamino Acids, Polypeptides and Proteins*, The University of Wisconsin Press, Madison, 1962, p. 13.
[3] E. KATCHALSKI AND M. SELA, *Advan. Protein Chem.*, 13 (1958) 243.
[4] M. SELA AND E. KATCHALSKI, *Advan. Protein Chem.*, 14 (1959) 391.
[5] G. D. FASMAN AND E. R. BLOUT, *J. Am. Chem. Soc.*, 83 (1961) 709.
[6] E. DANIEL AND E. KATCHALSKI, in M. A. STAHMANN, *Polyamino Acids, Polypeptides and Proteins*, The University of Wisconsin Press, Madison, 1962, p. 183.
[7] D. BEN-ISHAI AND A. BERGER, *J. Org. Chem.*, 17 (1952) 1564.
[8] H. EISENBERG AND J. POUYET, *J. Polymer Sci.*, 13 (1954) 85.
[9] A. KATCHALSKY AND H. EISENBERG, *J. Polymer Sci.*, 6 (1951) 145.

Received June 13th, 1962

Biochim. Biophys. Acta, 69 (1963) 397–399

SC 2193

Mitochondrial formation and hydrolysis of adenosine triphosphate in the presence of some rare-earth ions

The intravenous injection of relatively small amounts of soluble salts of lanthanum, cerium, praseodymium, neodymium, and samarium causes in some species of experimental animals, after a lag period of 2 or 3 days, an extensive accumulation of lipids in the liver[1–5]. It is remarkable that these lanthanide ions produce a large increase in triglycerides and a smaller rise of phospholipids of the mitochondria, in

contrast to other fatty-liver-producing agents, which do not significantly change the mitochondrial lipid content[5]. In addition the metabolic changes brought about by rare earths have become of increased interest since some of these substances have been detected as fission products of nuclear reactions.

Earlier studies of oxidative phosphorylation in mitochondria isolated from fatty livers after La^{3+} or Pr^{3+} administration have shown essentially unaltered P/O ratios under some conditions but in other cases a definite uncoupling effect[6]. It became of interest therefore to investigate in more detail whether the toxic effect of rare earths and the changes in liver lipid metabolism are due to an alteration in ATP metabolism in mitochondria.

Experiments illustrated in Table I show that Pr^{3+} or La^{3+} ions up to a concentration of $1 \cdot 10^{-4}$ M added directly *in vitro* to normal rat-liver mitochondria have no effect on respiration or phosphorylation, with either β-hydroxybutyrate or α-ketoglutarate as substrate. Even after a 30-min preincubation of the mitochondria with the salts of these rare earths (at 2°) oxidative phosphorylation was not affected. There was some uncoupling with the highest La^{3+} concentration tested but the significance of this finding is questionable because the lanthanide ions precipitate from the medium at these concentrations, presumably as the phosphates. These experiments were carried out in the presence as well as in the absence of EDTA with no essential difference in the findings. The distribution of the EDTA complexes of the rare earths within the body differs from that of the simple salts[7] and it appeared possible that the state of ionization of the rare earths could also affect their penetration into mito-

TABLE I

OXIDATIVE PHOSPHORYLATION IN THE PRESENCE OF RARE-EARTH IONS

The same mitochondrial suspension was used in Expt. 1. Oxidative phosphorylation was determined as reported earlier[6]. The incubation medium was somewhat turbid with the highest concentrations of the rare earths. Their molar concentrations were therefore smaller than indicated because of precipitation of rare-earth phosphates. Experiments with and without EDTA gave the same results. In Expt. 2 the rare earths were given intravenously to female rats 6 h before isolation of mitochondria. All data give typical examples out of a number of similar experiments.

Expt.			Substrate	−ΔO (μatoms)	P/O	Inhibition of P/O	Inhibition of O_2 uptake
	Control		α-ketoglutarate + malonate*	4.4	3.2		
			β-hydroxybutyrate	3.7	2.6		
1	*Additions*	*Concn. (mM)*					
	KNO_3	0.5	α-ketoglutarate + malonate	4.3	3.2	0	0
	$Pr(NO_3)_3$	0.5	α-ketoglutarate + malonate	4.4	3.0	6	0
		0.1	α-ketoglutarate + malonate	4.4	3.2	0	0
	$LaCl_3$	0.5	α-ketoglutarate + malonate	4.6	2.6	20	0
		0.1	α-ketoglutarate + malonate	4.4	3.2	0	0
		0.2	β-hydroxybutyrate	3.3	2.8	0	10
				3.7	2.6	0	0
2	*Injections*	*mg/kg*					
	$Pr(NO_3)_3$	10	α-ketoglutarate + malonate	5.3	3.0	0	
				5.1	3.3	0	
	$LaCl_3$	10	α-ketoglutarate + malonate	5.3	2.9	0	
				4.3	3.0	0	

* See ref. 6.

chondria. Furthermore an especially pronounced liver damage has been reported in some experiments with the EDTA complex of Nd^{3+} (see ref. 8).

In order to test the possibility that oxidative phosphorylation is impaired by the parenteral administration of lanthanide metal ions prior to manifestation of fatty infiltration of the liver, the metabolism of rat-liver mitochondria was studied 6 h after the intravenous injection of $LaCl_3$ or $Pr(NO_3)_3$. LASZLO *et al.*[7] have demonstrated that after intravenous injection the maximal concentration of rare earths in the liver is reached by 4–6 h. However, as shown in Table I, the P/O ratios of the liver mitochondria isolated after such pretreatment were found to be normal.

BAMANN and coworkers[9,10] have suggested that the toxic effects of rare-earth ions are due to their ability to catalyze non-enzymic hydrolysis of physiologically important phosphate esters and anhydrides such as ATP, since lanthanum hydroxide is known to catalyze hydrolysis of phosphate esters at higher pH values. The effects of the rare-earth ions on non-enzymic hydrolysis of ATP at pH 7.3–7.5 were therefore tested. As shown in Table II, La^{3+} has insignificant activity in catalyzing non-enzymic hydrolysis of ATP at physiological pH. The data in Table II also show that the trivalent ions of the rare earths tested do not stimulate the enzymic splitting of ATP by mitochondrial digitonin fragments. This finding is in agreement with work of COOPER AND LEHNINGER[11], who showed that only divalent ions such as Mg^{2+}, Mn^{2+}, Co^{2+} and Fe^{2+} stimulate this ATPase activity. Of the metals tested in this study only samarium forms a divalent ion.

The uncoupling activity *in vitro* of certain substances such as thyroxine or Ca^{2+} is probably primarily due to an alteration of mitochondrial structure rather than a

TABLE II

RATE OF NON-ENZYMIC AND ENZYMIC HYDROLYSIS OF ATP IN THE PRESENCE OF RARE-EARTH IONS

Mitochondrial ATPases were tested with digitonin fragments of rat-liver mitochondria[11], at a level of 50 μg total N/ml.

Rare earth	*Temp. (°)*	*Time (min)*	*pH*	*Digitonin fragments*	*Activator*	*ATP (mM)*	*P_i liberated (μmoles)*
	22	30	7.5	+	—	10	0.1
	37	30–180	7.3	−	0.2 mM Mg^{2+}	60	0
	22	30	7.5	−	0.3 mM Mg^{2+}	50	0
	22	30	7.5	−	3.0 mM Mg^{2+}	50	0
	22	30	7.5	+	0.1 mM Mg^{2+}	10	0.6
	22	30	7.5	+	0.2 mM Mg^{2+}	10	0.9
	22	30	7.5	+	0.1 mM Mg^{2+}	10	2.8
	22	30	7.5	+	0.1 2,4-dinitrophenol	10	0.5
0.04 mM La^{3+}	22	30	7.5	−	—	50	0
0.2 mM La^{3+}	22	30	7.5	−	—	50	0
0.2 mM La^{3+}	37	30–180	7.3	−	—	60	0
1.0 mM La^{3+}	22	30	7.5	−	—	50	0
0.2 mM Pr^{3+}	37	30–180	7.3	−	—	60	0
0.2 mM Nd^{3+}	37	30–180	7.3	−	—	60	0
0.1 mM La^{3+}	22	30	7.5	+	—	10	0
1.0 mM La^{3+}	22	30	7.5	+	—	10	0.65
1.0 mM La^{3+}	22	30	7.5	+	0.2 mM Mg^{2+}	10	0.97
1.0 mM La^{3+}	22	30	7.5	+	0.1 mM 2,4-dinitrophenol	10	0.4

direct attack on the phosphorylating enzymes[12], since these agents cause mitochondrial swelling. Accordingly the effect of the rare-earth salts on mitochondrial swelling has also been tested. The rare-earth ions have only very weak activity in promoting water uptake by mitochondria, in comparison with thyroxine. Since rather high concentrations of the rare earths are required to give these very small effects, it appears unlikely that this type of change of mitochondrial structure can be considered the cause of fatty infiltration *in vivo*. Furthermore these slight swelling effects are probably non-specific, since they were also given by gadolinium and ytterbium salts, which do not produce fatty infiltration.

The uncoupling of oxidative phosphorylation found in earlier experiments with mitochondria isolated from fatty livers after poisoning with Pr^{3+} (see ref. 6) thus seems to represent the consequence rather than the cause of fatty infiltration. These earlier results may be explained by the well-known increase in the structural lability of mitochondria from fatty livers[6,13], which might lead to an increased damage of these mitochondria during the isolation procedure and thus to low P/O ratios. Also, an increased formation of endogenous uncoupling substances[14,15] by these lipid-rich particles could be expected.

The data presented in this paper thus do not support the view that the ions of La, Pr, Nd and Sm have a direct toxic effect on the synthesis or breakdown of ATP in mitochondria and some other basis for their action in causing fatty infiltration of liver mitochondria must be sought.

The capable technical assistance of Miss I. HOFFMEISTER in Berlin and Miss I. C. EARLING in Baltimore is gratefully acknowledged.

This work was supported in part by grants from National Institutes of Health, National Science Foundation, Nutrition Foundation, Inc., and The Whitehall Foundation.

Department of Physiological Chemistry,
The Johns Hopkins University School of Medicine,
Baltimore, Md. (U.S.A.)

DIETHER NEUBERT*

[1] G. C. KYKER, E. A. CRESS, V. M. SIVARAMAKRISHNAN, C. H. STEFFEE AND M. STEWARD, *Federation Proc.*, 16 (1957) 207.
[2] F. SNYDER, E. A. CRESS, N. STEPHENS AND G. C. KYKER, *Federation Proc.*, 18 (1959) 327.
[3] F. SNYDER, E. A. CRESS AND G. C. KYKER, *J. Lipid Research*, 1 (1959) 125.
[4] F. SNYDER, E. A. CRESS AND G. C. KYKER, *Nature*, 185 (1960) 480.
[5] D. NEUBERT AND I. HOFFMEISTER, *Naunyn-Schmiedebergs Arch. Exptl. Pathol. Pharmacol.*, 237 (1960) 519.
[6] D. NEUBERT AND I. HOFFMEISTER, *Naunyn-Schmiedebergs Arch. Exptl. Pathol. Pharmacol.*, 238 (1960) 348.
[7] D. LASZLO, D. M. EKSTEIN, R. LEWIN AND K. G. STERN, *J. Natl. Cancer Inst.*, 13 (1952) 559.
[8] E. LAAS AND E. VINCKE, *Arzneimittel Forsch.*, 7 (1957) 710.
[9] E. BAMANN, F. FISCHLER AND H. TRAPMANN, *Biochem. Z.*, 325 (1953) 413.
[10] E. BAMANN AND H. TRAPMANN, *Advan. Enzymol.*, 21 (1959) 169.
[11] C. COOPER AND A. L. LEHNINGER, *J. Biol. Chem.*, 224 (1957) 547.
[12] D. TAPLEY AND C. COOPER, *J. Biol. Chem.*, 222 (1956) 341.
[13] M. U. DIANZANI, *Biochim. Biophys. Acta*, 14 (1954) 514.
[14] L. WOJTCZAK AND A. B. WOJTCZAK, *Biochim. Biophys. Acta*, 31 (1959) 297.
[15] A. L. LEHNINGER AND L. F. REMMERT, *J. Biol. Chem.*, 234 (1959) 2459.

Received August 10th, 1962

* Present address: Pharmacological Institute, Free University, Berlin-Dahlem (Germany).