Influence of Ribonuclease on Respiration and Mevalonic Acid Utilization in Liver Homogenates.* (27218)

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The preincubation of rat liver homogenates with ribonuclease (RNAase) is associated with a marked reduction in the capacity of the homogenates to convert mevalonic acid (MVA) into non-saponifiable material (NSF, largely cholesterol)(1). The loss of this synthetic capacity is a consequence of failure to maintain a level of ATP required in utilization of MVA(2,3). RNAase inactivation of homogenates may be prevented or reversed by certain anionic polymers such as DNA, polyethylene sulfonate (PES), or heparin(4). In the homogenates employed, phosphorylations accompanying the terminal respiratory chain are the primary source of ATP, but it has not been determined whether preincubation with RNAase precipitates only an uncoupling of phosphorylation or a failure in respiration as well. The data reported here provide evidence that in homogenates preincubated with RNAase phosphorylation is impaired first, followed by a decreased rate of oxygen uptake. It has also been demonstrated that polyanions not only prevent the uncoupling of phosphorylation associated with preincubation with RNAase, but prevent the eventual respiratory decline as well.

Methods and materials. The experimental procedures involved in biosynthesis of NSF from MVA by rat liver homogenates have been described(5). In brief, cooled liver (1 volume) from young rats was homogenized quickly in a "loose" Potter-Elvehjem homogenizer (1 mm clearance) with pH 7.0 phosphate (0.1 M)-magnesium (0.006 M)-nicotinamide (0.03 M) buffer (2 volumes) and centrifuged (200 x *g* 3 min) to remove connective tissue. This essentially whole homogenate was dispensed (5 ml aliquots) into flasks (125 ml Erlenmeyer flasks fitted with centered wells) containing ATP (1 mg), DPN (10 mg), sodium succinate (160 mg), and RNAase (10 mg) where indicated as well as polyethylene sulfonate (PES, 0.2 mg), polyaspartic acid (PAA, 10 mg), or polyglutamic acid (PGA, 10 mg) or combinations of materials (5 ml) to be studied with respect to biosynthesis of NSF. Total volume (including homogenate) was 10 ml. The flasks were aerated with a stream of oxygen (15 sec), stoppered, and preincubated with agitation (30 min, 37°, 100 oscillations per min). Following preincubation MVA-2-C^{14} (0.5 mg DL MVA in 1 ml, 60,000 cpm) was added to each flask, aeration was repeated and the flasks reincubated (3 hr). Following the biosynthetic period the contents of the flasks were saponified (10 ml of 2 N 80% alcoholic KOH, 100°), extracted with petroleum ether (30-60°, 4x with 50 ml amounts), the combined extracts dried with sodium sulphate (30 g), filtered, evaporated to dryness, taken up in scintillation mixture (10 ml, 4 g PPO (2,5-diphenyloxazole) and 30 mg POPOP (1,4-di-2(5-phenyloxazoyl)-benzene) per liter of toluene), and counted. The results given are in terms of cpm per incubation flask.

Respiration of the liver homogenates was measured with a Warburg respirometer in the conventional manner using NaOH (1 ml, 10%) in the center wells of the 125 ml modified Erlenmeyer flasks to absorb the CO_2 liberated(6). Determinations of biosynthetic capacity and of respiratory rate were ordinarily carried out in a single flask simultaneously. Fresh rat liver was considered to have a dry matter content of 15% in the calculation of respiratory rates.

The polyethylene sulfonate (PES) used had a mean molecular weight of 12,900 and was obtained from Dr. J. H. Flokstra, Upjohn Laboratories, to whom we are indebted. The polyaspartic acid used had a molecular weight range of 7000-8500. Two preparations of polyglutamic acid were used. One had a

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TABLE I. Influence of Ribonuclease and Polyanions on Respiration and Mevalonic Acid Utilization by Liver Homogenates.

Exp.	Preincubation time, min.	Supplement	Oxygen uptake, $\mu l \times 10^{-3}$/g dry wt/30 min.	NSF bio synthesis, cpm
1	20	None	4.4	4795
	20	RNAase	4.5	46
2	30	None	6.4	4323
	30	RNAase	4.4	19
3	30	None	5.6	4564
	30	RNAase	4.8	34
4	30	None	4.8	2915
	30	RNAase	4.5	10
	30	RNAase + PES	7.6	3758
5	30	None	3.4	3626
	30	RNAase	4.0	994
	30	RNAase + PAA	3.1	3893
	30	RNAase + PGA	3.5	3674

PES, polyethylene sulfonate; PAA, polyaspartic acid; PGA, polyglutamic acid.

molecular weight range of 15,000-20,000; the other had a mean molecular weight of 80,000. All of the polyamino acids were purchased from Schwarz BioResearch. Ribonuclease was purchased from Worthington Biochemical Corp.

Results. The data of Table I and Fig. 1 show that oxygen uptake of liver homogenates usually is not adversely influenced by preincubation with RNAase for 30 min. On the other hand, conversion of MVA to NSF is markedly inhibited by this preincubation. The inhibition of NSF biosynthesis accomplished by preincubation with RNAase is completely prevented by the presence of polyanions, in this instance with PES, PAA, or PGA. Eventually the rate of oxygen uptake by liver homogenates is decreased by the action of RNAase to a rate of about one-half that of controls. Here again the decrease in rate of respiration accomplished with RNAase is completely prevented by polyanions. In the present studies the capacity to utilize MVA was not tested following preincubation periods greater than 30 min. It is known from previous studies, however, that control homogenates of liver frequently maintain normal synthetic capacity after preincubation at 37° for an hour or more.

Discussion. These studies furnish additional evidence that a polyanion is essential for aerobic homogenates to maintain the synthetic capacity to convert MVA into NSF. Previous studies have shown that the polyanion is essential for maintenance of a functional level of ATP or, in other words, for the phosphorylation phase of oxidative phosphorylation. These studies further demonstrate that the polyanion is essential for respiration or the oxidative (hydrogen and electron transport) phase of oxidative phosphorylation. There would appear to be some differential in the sensitivity of the 2 phases of oxidative phosphorylation to a reduction in polyanion level, since phosphorylation is impaired before respiration is influenced. Since in a number of experiments respiration and conversion of MVA to NSF were greater in homogenates supplemented with polyanions as compared to controls, an incipient deficiency of polyanion frequently may be the first limiting factor in the metabolic activity of aerobic homogenates.

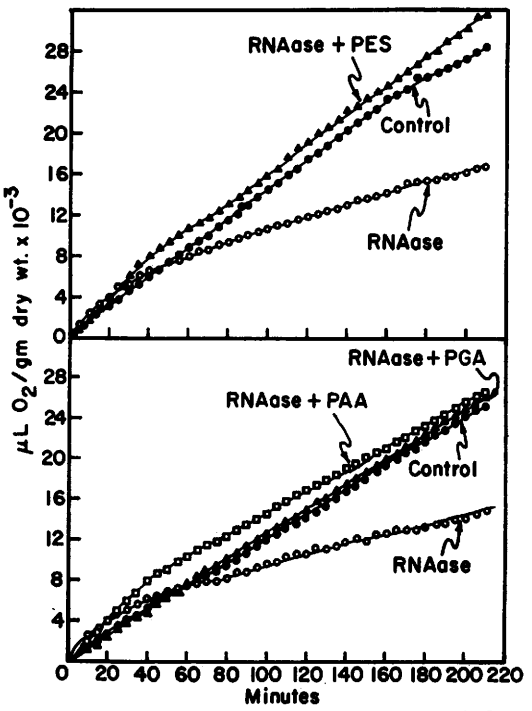

FIG. 1. Respiration of liver homogenates as influenced by RNAase or RNAase plus polyanions polyethylene sulfonate (PES), polyaspartic acid (PAA), or polyglutamic acid (PGA).

Summary. Rat liver homogenates were incubated aerobically with and without added ribonuclease. Simultaneous determinations of respiratory rate and capacity to convert mevalonic acid to non-saponifiable material were made. Loss of the synthetic capacity of the homogenates, considered as a reflection of the failure to maintain a functional level of ATP, was followed by a decline in rate of respiration. Synthetic capacity and rate of respiration were maintained by the presence of added polyanions.

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Glucose Transport by the Intestinal Mucosa of the Dogfish.* (27219)

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Few data are available in the literature on the transport of non-electrolytes across the intestine of marine fishes. In the present study we have used a simple preparation of intestinal mucosal membrane of the dogfish to study the transport of glucose, and to show the effect of temperature on transport.

Methods. Spiny dogfish (*Squalus acanthias*) used in this study were caught in the gulf of Maine during the months of July and August. They weighed 8-12 lb. and were kept in an enclosure in the ocean for one to 4 days. On the day of experiment, they were removed and immobilized by spinal transsection. The abdomen was opened immediately, the small intestine was removed and placed in an ice-chilled dogfish Ringer's solution without glucose. A duodenojejunal segment was cut open through the mesenteric line, and mounted on a cork ring, with which the tissue was submerged in ice-chilled Ringer's solution. Spiral valves of the mucosa were excised. The remaining mucosa was stripped away from serosal and muscular layers. This mucosal membrane was then cut into 3 or 4 sections. Each section was mounted over a cylindrical glass tube, 1.7 cm in diameter and 4.0 cm in length. The mucosal side was on the outside, and the submucosal side on the inside of the tube. Glucose in varying amounts was added to the dogfish Ringer's solution at the start of each experiment. Then, one ml of the fresh glucose-Ringer's solution was pipetted into the tube against the submucosal surface. Next, the tube was placed in a plastic beaker containing 5 ml of the same solution. The cylindrical tube, beaker, membrane and solutions were shaken in a Dubnoff shaker at 36°C, 26° and 16° for an hour. A gas mixture, CO_2-O_2 (5-95%) was bubbled into the outside solution throughout the experiment. Dogfish Ringer's solution was composed of: NaCl 280 mM, KCl 12 mM, $CaCl_2$ 10 mM, $NaHCO_3$ 4.5 mM, NaH_2PO_4 0.5 mM and urea 360 mM. The final solution was 960 milliosmoles/liter. After one hour of incubation, the inside or submucosal fluid was collected for measurement of volume and glucose concentration. The outside or mucosal fluid was also collected for measurement of glucose concentration. Glucose analysis was that of Nelson (1). The mucosal membrane was blotted gently with filter paper and weighed.

Results. The complete intestinal segment with intact mucosal, muscular and serosal layers, did not transport glucose. Experi-

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