Relationship Between Mevalonic Acid Utilization and ATP Content in Liver Homogenates Pretreated with Ribonuclease.* (25122)

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Preincubation of whole rat liver homogenates with crystalline ribonuclease abolishes the capacity of these homogenates to convert mevalonic acid (MVA) into non-saponifiable material or cholesterol(1). Homogenates that have been inactivated by ribonuclease may be restored to full synthetic capacity by autoclaved extract of fresh liver and existence in such heated liver extracts of an unknown factor essential directly or indirectly for MVA utilization was postulated(2). It was suggested that the factor may be concerned with phosphorylation of MVA since in homogenates pretreated with ribonuclease added MVA remains in a microbiologically determinable form(1,3). Evidence is here presented that liver homogenates pretreated with ribonuclease are devoid of ATP while homogenates suitably fortified with liver extract contain ATP. The factor contained in liver extract appears to be concerned with a functional level of ATP which, in turn, is required for phosphorylation of MVA(4,5,6).

Methods and materials. The experimental procedures involved in biosynthetic studies have been described(7). In brief, liver from young rats is homogenized with a loose homogenizer and centrifuged briefly to remove connective tissue. Aliquots of the essentially whole homogenate fortified with DPN but not with ATP are preincubated with and without ribonuclease and autoclaved liver extract alone or in various combinations. Perchloric acid extracts are prepared after preincubation for ATP determinations. Paired flasks are then supplemented with labelled MVA and reincubated for a determination of capacity of treated homogenates to synthesize non-saponifiable material from the labelled precursor. Flasks are aerated with oxygen prior to preincubation and again at the time of MVA addition. Perchloric acid extracts were prepared by treating the homogenates with an equal volume of 4% perchloric acid. Excess perchloric acid was removed as the insoluble potassium salt. ATP was determined spectrophotometrically following adsorption and gradient elution of the extracts on Dowex-1-formate. Details involved in the preparation of extracts and chromatographic separation were as described by Allfrey and Mirsky(8). Following incubation of flasks containing added MVA-2-C^{14} the contents were saponified, extracted with petroleum ether, the extracts dried with sodium sulfate, filtered, evaporated, taken up in scintillation mixture and counted (7). Autoclaved liver extract was prepared by treating fresh guinea pig liver in the cold with twice its weight of water in a Waring Blendor for about a minute. The brei was then autoclaved at 105-110° for 20 minutes and the mixture cooled and centrifuged at 10,000 x g for 20 minutes. The coagulum was discarded. Dowex-1-treated liver extract was prepared by passing a liver extract solution over a large excess of Dowex-1 in the chloride form.

Results. Optical densities at 260 mμ of the fractions obtained by ion exchange chromatography of perchloric acid extracts of a control and a ribonuclease-treated homogenate of liver are presented in Fig. 1. The curves are typical of quite a group and show that at the time of MVA addition to preincubated homogenates ribonuclease-treated preparations in contrast to controls contain no ATP and do not incorporate MVA into non-saponifiable material. In Table I are presented similar data from a number of experiments. Included are the results from a few experiments where inactivation of MVA utilization as evidenced by counts found in the non-saponifiable fraction was not complete (See Exps. 1, 2 and 5). Such incomplete inactivation is sometimes encountered and is probably attributable to biological variability in the homogenates. Where counts in the ribonuclease-treated flasks were

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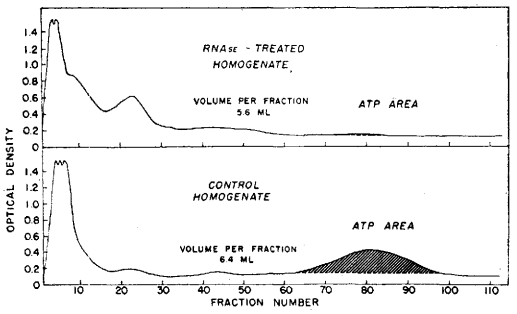

FIG. 1. ATP content of rat liver homogenates incubated with and without ribonuclease. Activity of the non-saponifiable material isolated from a duplicate control flask to which MVA-2-C^{14} was added at the time the first flask was treated with perchloric acid in preparation for chromatography was 5,450 cpm. Activity of the non-saponifiable material isolated from a duplicate ribonuclease-treated flask similarly treated was 17 cpm.

only slightly reduced ATP was present in amounts comparable to that found in control homogenates.

The results of a typical experiment illustrating the ATP encountered when liver homogenate was preincubated with and without ribonuclease and autoclaved, Dowex-1-treated liver extract in 4 possible combinations are presented in Table II. Given also are the CPM found in paired flasks to which MVA-2-C^{14} was added following preincubation which were allowed to continue incubation for a measure of MVA utilization. It is apparent from the data that homogenates preincubated with ribonuclease and treated liver extract are active in biosynthesis of labelled non-saponifiable material and that they also contain significant amounts of ATP. This is in contrast to homogenates preincubated with ribonuclease alone where MVA utilization is negligible and no ATP is detectable. As sometimes happens control homogenates without ribonuclease are more active with respect to MVA utilization and ATP content when supplemented with liver extract (compare flasks 1 and 2). When the data of Table II are examined graphically where the counts found in the non-saponifiable fraction are plotted against ATP concentration at the time of labelled MVA addition without regard for the treatment the homogenates received a straight line with positive slope and zero intercept results. Thus in the system under study the level of ATP at time of MVA addition determines extent of MVA utilization.

TABLE I. Effect of Preincubation with Ribonuclease on ATP Content of Homogenates and Subsequent Biosynthesis of Labelled Non-Saponifiable Material (NSF) from MVA-2-C^{14}.

Exp.	Ribonuclease (mg)	Preincubation (min.)	ATP (mg)	NSF (cpm)
1	0	30	1.86	4,010
	5	"	1.38	2,980
2	0	90	.82	3,000
	10	"	.11	1,640
3	0	40	1.66	5,450
	10	"	.08	17
4	0	45	2.27	9,237
	8	"	.04	17
5	0	"	2.98	7,765
	10	"	.32	1,411

TABLE II. Effect of Preincubation with Ribonuclease and Autoclaved Liver Extract in Various Combinations on ATP Content of Homogenates and the Subsequent Biosynthesis of Labelled Non-Saponifiable Material (NSF) from MVA-2-C^{14}.

Ribonuclease (mg)	Supplement	ATP (mg)	NSF (cpm)
0	None	1.30	6,175
0	5 ml autoclaved liver extr.	2.02	9,190
10	None	.0	28
10	5 ml autoclaved liver extr.	.56	2,990

Discussion. Presumably in the aerobic homogenate system employed in these studies a steady state level of ATP exits that is a balance between the ATP originating largely from oxidative phosphorylation and the ATP undergoing phosphate transfer to various substrates or hydrolysis by ATPases. Preincubation with ribonuclease in the presence of "tissue fragments," previouly shown to be essential for the ribonuclease effect, appears to inactivate the cycle by hydrolyzing an essential polynucleotide. Thus rat liver homogenates preincubated with ribonuclease contain essentially no ATP while similar homogenates supplemented with an autoclaved extract of liver from which ATP has been removed by anion exchange chromatography prevent or reverse ribonuclease inactivation by supplying this essential polynucleotide factor. Since according to current concepts phosphorylation of MVA by mevalonic kinase in the presence of ATP is the first step in biochemical conversion of this compound to non-saponifiable material

the failure of ribonuclease-treated homogenates to utilize MVA becomes interpretable. Data presently available do not permit a decision as to the locus of action of the essential polynucleotide. Involvement of polynucleotides in ATP biosynthesis has been previously observed in thymus cell nuclei pretreated with *deoxyribonuclease* by Allfrey and Mirsky(8) and in fractionated extracts of *Alcaligenes fecalis* by Pinchot(9). A number of recent reviews have been concerned with the existence of postulated intermediates in reactions involving biosynthesis, conservation, or utilization of ATP(10,11,12).

Summary. Ribonuclease-treated homogenates of liver that do not incorporate mevalonic acid into non-saponifiable material or cholesterol were devoid of ATP. ATP was present and mevalonic acid incorporation occurred when ribonuclease pretreatment was carried out in presence of an autoclaved extract of liver. The results are interpreted as indicating existence of a polynucleotide factor essential for maintenance of a positive balance with respect to ATP in liver homogenates.

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