A NEW FACTOR ESSENTIAL FOR THE UTILIZATION OF MEVALONIC ACID

Sir:

We have observed that the preincubation of a rat liver homogenate with crystalline ribonuclease (RNAse) abolishes the capacity of the system to utilize mevalonic acid (MVA) for the biosynthesis of non-saponifiable material (NSF) or cholesterol (CHL). The technique has been employed to show accumulation of MVA from mevaldic acid in such treated homogenates.[1] Further study of the mechanism of RNAse inhibition has established the following new facts.

RNAse inhibition of MVA utilization is readily demonstrable with homogenates[2,3] that have been subjected to a brief 200 × *g* centrifugation. On the other hand, homogenates that have been subjected to more extensive centrifugation prior to RNAse treatment do not yield inactivated preparations (see Table I). Other experiments have also provided evidence for the participation of material sedimentable between 200 and 9,000 × *g* in the phenomenon of RNAse inactivation. For example, when a 200 × *g* supernatant preparation is preincubated with RNAse and then centrifuged at 9,000 × *g*, inactivation of MVA utilization is obtained. When material sedimentable between 200 and 9,000 × *g* is preincubated separately with RNAse, however, and then added back to the 9,000 × *g* supernatant fraction, no inactivation of MVA utilization results.

TABLE I

BIOSYNTHESIS OF NSF AND CHL BY VARIOUS FRACTIONS OF RAT LIVER HOMOGENATE AS INFLUENCED BY RNAse

Each experimental flask contained 1 mg. of ATP, 1 mg. of DPN and 5 mg. of crystalline RNAse where indicated. Rat liver homogenized and centrifuged at 200 × *g* for 3 min. Aliquots of the 200 × *g* supernatant material removed and the remainder centrifuged at 500 × *g* for 10 min. Aliquots of the 500 × *g* supernatant material removed and the remainder centrifuged at 9,000 × *g* for 10 min. The appropriate fractions added to the flasks as indicated and all flasks initially aerated with a stream of oxygen, stoppered, and incubated with agitation (50 oscillations per min.) at 37° for 30 min. 0.5 mg. 2-C^{14}-MVA (calculated as the DL-dibenzylethylenediammonium salt, 15,000 c.p.m.) then added to each flask followed by additional flushings with oxygen. Incubation continued for a total of 4.5 hours. The preparation and counting of the NSF and CHL digitonide were carried out as previously described.[1]

Liver fraction tested supernatant	Activity of fraction, c.p.m./mg. NSF Without RNAse	NSF With RNAse	CHL Without RNAse	CHL With RNAse
200 × *g*	1086	8	1744	17
500 × *g*	842	996	2887	4020
9,000 × *g*	2330	2910	3946	4299

TABLE II

BIOSYNTHESIS OF NSF BY HOMOGENATES AS INFLUENCED BY RNAse AND AUTOCLAVED TISSUE EXTRACT

Each experimental flask contained 1 mg. of ATP, 1 mg. of DPN, 5 ml. of rat liver homogenate (supernatant layers after centrifugation at 200 × *g* for 3 min.) and 5 mg. of crystalline RNAse where indicated. The flasks were initially aerated with a stream of oxygen, stoppered, and incubated with agitation at 37° for 30 min. The autoclaved liver homogenate then added as indicated to the appropriate flasks. 0.5 mg. 2-C^{14}-MVA added to all flasks which were then flushed with oxygen, stoppered, and reincubated for a total of 4.5 hours.

Synthesizing system	Activity of NSF fraction, c.p.m./mg.
Homogenate	745
Homogenate, RNAse inactivated	2
Homogenate, RNAse inactivated + 2 ml. autoclaved tissue extract	390
Homogenate, RNAse inactivated + 5 ml. autoclaved tissue extract	760

A system inactivated with respect to MVA utilization may be restored to full capacity for NSF synthesis by a supplement of autoclaved liver homogenate (see Table II). Dialyzed autoclaved liver homogenate is inactive and the factor is not directly inactivated with RNAse. Coenzyme A or DPN is inactive in lieu of the autoclaved liver homogenate. Fractionation of natural material is in progress. Subsequent manuscripts will be concerned with the mechanism of RNAse inactivation.

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