ATP and the Conversion of Mevalonic Acid to Cholesterol and Precursors By Liver Homogenates.* (25311)

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Inability of whole rat liver homogenates preincubated with ribonuclease (RNAse) to convert mevalonic acid (MVA) into non-saponifiable material (NSF) is associated with absence of ATP in these homogenates(1,2). Control homogenates preincubated without RNAse actively convert MVA into NSF and contain relatively large amounts of ATP. Homogenates preincubated with RNAse and heated liver extract from which ATP was removed by treatment with anion exchange resin contain ATP following preincubation and similarly convert the precursor into NSF. It has been concluded that the factor of liver extract essential for utilization of MVA by RNAse-treated homogenates is concerned with maintenance in these preparations of a functional level of ATP(3,4). Under conditions studied the conversion of MVA to NSF appears to be a direct function of ATP level existing in a homogenate at the time it is supplemented with MVA. This communication is concerned with the effect of ATP added to homogenates either before or after preincubation with or without RNAse under conditions of an aerobic or anaerobic environment.

Methods and materials. Experimental procedures involved in biosynthetic studies have been described in detail(5). In brief, liver from young rats is homogenized with a loose homogenizer and centrifuged at 200 xg for 3 min. to remove connective tissue. The top layer together with a loosely packed intermediate layer are decanted to yield a "200 xg supernatant fraction." An aliquot of this fraction is centrifuged at 9000 xg for 10 min. to yield a "9000 xg supernatant fraction" considered free of tissue fragments. 5 ml aliquots of the 200 xg or 9000 xg supernatant fractions fortified with DPN (1 mg) are preincubated (30 min.) in 125 ml Erlenmeyer flasks with and without ribonuclease (10 mg) in total volume of 10 ml. The present experiments differ from those previously described in that all flasks are supplemented with sodium succinate at 0.05 M. Supplementation with this substrate reduces the incidence of experiments where no incorporation of MVA occurs even in control flasks. ATP (10 mg unless otherwise indicated) where studied was added prior to or following the preincubation period. Following preincubation 1 ml of MVA-2-C^{14} solution was added to each flask and the flasks reincubated (3 hr.) for a determination of the capacity of treated homogenates to synthesize NSF from labelled precursor. Flasks were flushed with either oxygen or nitrogen prior to preincubation and again at time of MVA addition. Following incubation of flasks containing added MVA-2-C^{14} the contents were saponified, extracted with petroleum ether, the extract dried with sodium sulfate, filtered, evaporated, taken up in scintillation mixture and counted(5). The data presented are in terms of total counts recorded/incubation flask.

Results. Fig. 1 shows the effect on biosynthesis of labelled NSF of varying amounts of ATP added to homogenates following a preincubation period with and without RNAse in an atmosphere of oxygen. Under these conditions ATP is inhibitory to MVA utilization in aliquots of the homogenate preincubated without RNAse but it is somewhat stimulatory to MVA utilization in similar aliquots preincubated with RNAse. Experiments involving addition of ATP to homogenates prior to preincubation period yielded essentially similar results except that the magnitude of "stimulation or inhibition" was generally less.

Fig. 2 A shows the effect of ATP, RNAse, and aerobic *vs.* anaerobic environment on biosynthesis of labelled NSF from MVA-2-C^{14} by a 200 xg and a 9000 xg supernatant fraction of liver homogenate. In this experiment, A, ATP was added to flasks prior to preincubation period. As reported previously, in an

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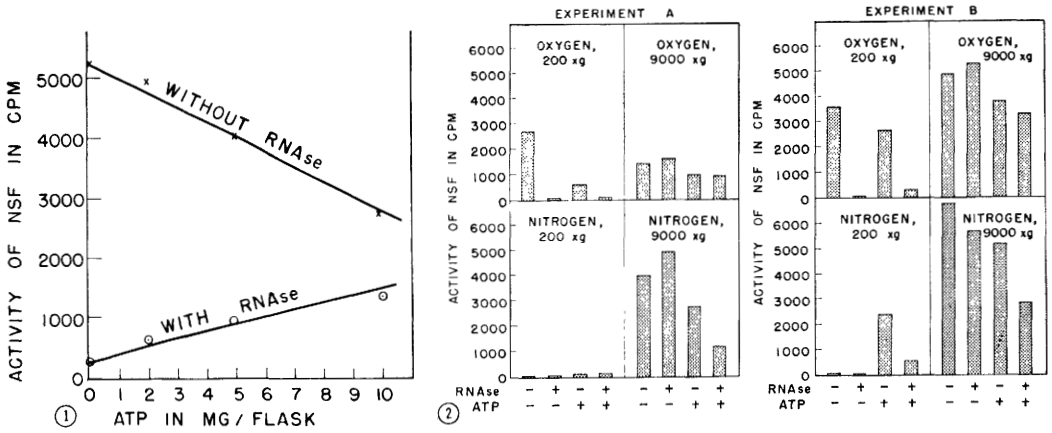

FIG. 1. Effect of ATP on biosynthesis of NSF from MVA by a 200 xg supernatant fraction of rat liver following preincubation with or without RNAse.

FIG. 2. Influence of ATP on biosynthesis of NSF from MVA by 200 xg and 9000 xg supernatant fractions of rat liver homogenate under aerobic and anaerobic conditions following preincubation with or without added RNAse. A. ATP added prior to preincubation. B. ATP added following preincubation.

oxygen atmosphere and with an essentially whole homogenate preincubation with RNAse abolishes the capacity of the preparation to convert MVA to NSF. ATP is inhibitory in the absence of RNAse. With a 9000 xg supernatant fraction of liver homogenate incubated under oxygen no inhibition of MVA incorporation is obtained by preincubation with RNAse as has been reported previously. ATP is somewhat inhibitory and its effect is not influenced by the presence of RNAse. Under nitrogen there is essentially no incorporation of MVA-2-C^{14} into labelled NSF by a 200 xg supernatant preparation of liver homogenate and in this experiment there was no effect of added ATP although in other experiments involving ATP added prior to preincubation some stimulation of incorporation has been observed. In contrast, a 9000 xg supernatant fraction readily incorporates MVA into NSF under nitrogen. Preincubation with RNAse was without effect on the system. ATP was inhibitory and this inhibition seemed to be greater in the presence of RNAse.

Fig. 2 B summarizes the results of similar experiments described in Fig. 2 A except that here ATP was added to the homogenates after the preincubation period at time of MVA-2-C^{14} addition. The results are essentially the same except that under nitrogen ATP is definitely stimulatory to incorporation with a 200 xg preparation.

Discussion. The data are readily interpretable in terms of extent to which a source of ATP is available for phosphorylation of MVA. The amount of ATP existing at any particular time in a homogenate may be considered as the steady state level of that originating largely from oxidative phosphorylation against that undergoing hydrolysis by various ATPases. Thus with a 200 xg fraction incorporation of MVA into NSF takes place in an atmosphere of oxygen because oxidative phosphorylation is able to keep ahead of the ATPases. On the other hand, with a 200 xg fraction incorporation of MVA into NSF does not take place in an atmosphere of nitrogen because in absence of oxidative phosphorylation a positive balance of ATP is not maintained in the face of the same level of ATPases. It is under these latter conditions that stimulation of biosynthesis by added ATP is sometimes observed particularly when ATP is added along with the MVA after the preincubation period.

A 9000 xg fraction incorporates MVA into NSF under either aerobic or anaerobic conditions because in the relative absence of ATPases which are largely sedimented in going from a 200 xg to a 9000 xg fraction the demands for ATP can be met by that present in the homogenate as well as by that formed by anaerobic substrate phosphorylation.

RNAse inhibition of MVA utilization occurs

only with the essentially whole homogenate incubating under oxygen. Such RNAse-treated homogenates are devoid of ATP and evidence has been presented that the factor of liver extract that prevents or reverses RNAse inhibition is concerned with the maintenance of an adequate level of ATP. It would appear that the liver extract factor is involved either as a cofactor in the generation of ATP or as an inhibitor of ATP hydrolysis.

Summary. The influence of ATP on biosynthesis of cholesterol and precursors from mevalonic acid has been studied with relatively crude (200 xg supernatant) and more refined (9000 xg supernatant) fractions of rat liver homogenate under aerobic and anaerobic conditions following preincubation with or without added ribonuclease. Mevalonic acid incorporation occurred under conditions favorable for oxidative phosphorylation or under conditions where the ATPase of tissue fragments had been removed by centrifugation (9000 xg). Ribonuclease inhibition of mevalonic acid utilization occurred only under oxygen and in the presence of tissue fragments relatively rich in ATPase.

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Serum, Bile and Liver Total Cholesterol of Laboratory Animals, Toads and Frogs.* (25312)

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There is much information in the literature on serum and tissue cholesterol concentrations of laboratory animals. Values reported vary a great deal. The differences can be attributed in part to the various methods of extraction and estimation procedures used. During the past few years a saponification-extraction procedure has been used in this laboratory to determine serum and tissue total cholesterol. The color is developed with the ferric chloride reaction. This procedure is adapted to determine an amount as small as a few μg of total cholesterol per sample(1). This communication reports serum, bile, and liver total cholesterol levels of 9 species of laboratory animals most commonly used in this laboratory and of 2 species of toads and 2 species of frogs.

Material and methods. The animal and amphibian species, body weight, sex, source of blood, and anesthetics used are compiled in Table I. All animals were from our animal stock house. They were kept on ordinary laboratory chow made by Purina for the respective species. The toads and frogs had been kept in the refrigerator. Bile samples were collected from the gall bladder of monkeys, dogs, cats, rabbits, guinea pigs, toads and frogs. Hepatic bile was collected from rats through cannulation of the common bile duct with polyethylene tubing. Total cholesterol was determined on an aliquot of undiluted serum, bile, and liver digested with potassium hydroxide(1). Samples were usually collected and kept in the refrigerator and determinations were made within a few days. Storage of samples in the refrigerator for a few days did not seem to affect total cholesterol concentrations.

Results. The serum, bile, and liver total cholesterol concentrations are summarized in Table II. There is no direct relationship between total cholesterol concentrations and body weight in the various species studied, nor is there a close relationship between se-

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