Stimulation of Hepatic Biogenesis of Sterols on Administration of Adenosine Compounds

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1. Re-feeding starved rats increased the biogenesis of sterols in livers, with highest activity at 6h after the start of food intake. 2. Complete deficiency of protein or fat and partial deficiency of carbohydrate in the diet had no effect on sterol biogenesis. 3. Glucose, citrate or pyruvate, when administered intraperitoneally to starved rats, stimulated the biogenesis of sterols only at high concentrations. 4. ATP given intraperitoneally at low concentrations (10mg/rat) stimulated biogenesis of sterols, but not of fatty acids, from [1-¹⁴C]acetate. This effect was also obtained with other adenosine compounds, but not with adenine or guanosine. 5. Administration of adenosine compounds to starved rats also increased the incorporation of [1-¹⁴C]acetate into sterols in liver slices and also the activity of microsomal 3-hydroxy-3-methylglutaryl-CoA reductase. The results suggest a regulatory role for adenosine compounds in the hepatic biogenesis of isoprenoid compounds.

Biogenesis of cholesterol in the liver is known to be altered significantly under a variety of conditions. It was shown to be suppressed during cholesterol feeding (Langdon & Bloch, 1953; Tomkins *et al.*, 1953; Frantz *et al.*, 1954) and starvation (Tomkins & Chaikoff, 1952; Bucher *et al.*, 1959; Migicovsky, 1964). Administration of Triton WR-1339, and X-irradiation increased it severalfold (Bucher *et al.*, 1959). It exhibits circadian variation, with the highest activity at midnight and the lowest at noon (Back *et al.*, 1969; Subba Rao & Ramasarma, 1971). These changes were demonstrated by studying the incorporation of [1-¹⁴C]acetate into sterols either *in vivo*, by injecting the tracer intraperitoneally, or *in vitro*, by incubating the tracer with liver slices. Since similar changes were not observed in the incorporation of [2-¹⁴C]mevalonate into sterols, it was proposed that a site before the formation of mevalonate was affected (Gould & Popjak, 1957; Bucher *et al.*, 1959). It was shown that under conditions in which biogenesis of cholesterol was altered, corresponding changes also occurred in the activity of the microsomal enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.34), the rate-limiting step in the pathway (Linn, 1967; Hamprecht *et al.*, 1969; White & Rudney, 1970; Shapiro & Rodwell, 1971). Alterations in the activity of this enzyme therefore appear to influence the incorporation of [1-¹⁴C]acetate into sterols [see Ramasarma (1972) for a review].

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Complete deprivation of food for even short periods suppresses biogenesis of sterols. On re-feeding, the activity is restored. Protein, carbohydrate or fat, given alone, stimulates the activity in starved rats (Tomkins & Chaikoff, 1952), indicating that any type of calorie source is capable of restoring the biogenic activity. The changes that occur during starvation–re-feeding therefore may not be due to the absence from or presence in the diet of any particular component. It is possible that a metabolite is modulated in parallel with the food intake, and this may bring about these changes. The present paper describes studies on the stimulation of the incorporation of [1-¹⁴C]acetate into sterols and the activity of hydroxymethylglutaryl-CoA reductase in starved rats by glucose, ATP and adenosine compounds.

Materials and Methods

Chemicals

ATP and other nucleotides and nucleosides, and hydroxymethylglutarate were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Glucose, citrate and pyruvate were of AnalaR grade. [1-¹⁴C]-Acetate (specific radioactivity, 49 mCi/mmol) was obtained from Bhabha Atomic Research Centre, Bombay, India. Hydroxymethyl[3-¹⁴C]glutarate (specific radioactivity, 7.7 mCi/mmol) was purchased from New England Nuclear Corp., Boston, MA, U.S.A. After suitable dilution with non-radioactive hydroxymethylglutarate, it was converted into its anhydride, and then into the CoA derivative by the method of Louw *et al.* (1969). The specific radio-

activity of the hydroxymethyl[3-¹⁴C]glutaryl-CoA used was 365 c.p.m./nmol. Glucose 6-phosphate and glucose 6-phosphate dehydrogenase were purchased from Calbiochem, Los Angeles, CA, U.S.A.

Animals

Male albino rats, obtained from the Institute colony and weighing 100–150 g, were used. They were fed on a pellet diet obtained from Hindustan Lever, Bombay, India. For starvation experiments, the food was removed 48 h before the animals were killed. A synthetic diet, composed of 20% (w/w) casein, 60% (w/w) starch, 10% (w/w) sucrose, 5% (w/w) fat (peanut oil) and 5% (w/w) mineral salt mixture (U.S. Pharmacopoeia, 1947), was prepared. For the protein-deficient and fat-deficient diets, the specified component was replaced by starch. A diet composed of 65% (w/w) casein, 25% (w/w) starch, 5% (w/w) fat and 5% (w/w) mineral salt mixture was used as the carbohydrate-insufficient diet; complete removal of carbohydrate was not attempted, since the consumption of such a diet was very low. All the animals were given supplements of water-soluble and fat-soluble vitamins (U.S. Pharmacopoeia, 1947), and water was given *ad libitum*.

Incorporation of [1-¹⁴C]acetate into sterols *in vivo*

[1-¹⁴C]Acetate (10 μ Ci) was injected intraperitoneally into each rat. After 30 min the rats were killed by cervical dislocation, and the livers were removed, homogenized in aq. 80% (v/v) ethanol, and saponified with 10M-NaOH (1 ml/g of fresh liver). The non-saponifiable lipids were extracted with light petroleum (b.p. 40–60°C), and fractionated on 5% deactivated alumina as described by Ramasarma & Jayaraman (1971). Since the effects observed were at the early step in the isoprene pathway of mevalonate formation, the amount of radioactivity incorporated into the total sterol fraction in different groups was compared. In the preliminary experiments, it was found that the distribution pattern in the compounds of the non-saponifiable fraction remained unchanged in control and experimental animals. Similar results obtained on comparing incorporation into the sterol fraction and into the digitonin-precipitable sterols.

Assay of hydroxymethylglutaryl-CoA reductase activity

Rats were killed by cervical dislocation, and the livers were excised and placed in an ice-cold solution consisting of 30 mM-EDTA, 70 mM-NaCl, 1 mM-dithiothreitol and 50 mM-potassium phosphate buffer (pH 7.4); they were minced, and homogenized (5 ml/g of liver) in a Potter–Elvehjem-type glass homogenizer. Microsomal fractions were prepared by the method of Shapiro & Rodwell (1971). The microsomal pellet was resuspended in the above solution, and the suspension was used as the enzyme source. The enzyme was assayed by the micro method of

Shapiro *et al.* (1974). It (about 0.3 mg of microsomal protein) was preincubated for 5 min at 37°C with 4.5 μ mol of glucose 6-phosphate, 450 nmol of NADP⁺ and 0.5 unit of glucose 6-phosphate dehydrogenase in a total volume of 90 μ l. The reaction was started by the addition of 50 nmol of hydroxymethyl[3-¹⁴C]glutaryl-CoA (specific radioactivity, 365 c.p.m./nmol). After 15 min at 37°C, 10 μ l of 4M-NaOH was added to stop the reaction and also to hydrolyse the remaining thioester. After 10 min at 37°C, 20 μ l of 10M-HCl was added followed by unlabelled mevalonolactone, and the mixture was further incubated at 37°C for 30 min for the lactonization of mevalonic acid. The precipitated protein was removed by centrifugation, and a portion of the supernatant was spotted on silica gel G t.l.c. plates (20 cm $\times$ 20 cm; 0.5 mm thick), which were developed with acetone/benzene (3:2, v/v). Silica gel in the area between R_f 0.6 and 0.9, containing mevalonolactone, was scraped off and transferred to scintillation vials for determination of radioactivity.

Protein was determined by the method of Gornall *et al.* (1949) with bovine serum albumin as standard.

Measurement of radioactivity

Radioactivity measurements were carried out in 0.5% 2,5-diphenyloxazole in toluene in a Beckman LS-100 liquid-scintillation spectrometer.

Results

Effect of starvation–re-feeding

In the first set of experiments biogenesis of sterols was measured in starved rats during re-feeding *ad libitum*. Incorporation of [1-¹⁴C]acetate into sterols *in vivo* increased simultaneously with the increase in food intake for a period of 6 h (Fig. 1). In a second set of experiments the starved rats were given only a small amount of food (5 g/rat). A twofold stimulation of the incorporation of [1-¹⁴C]acetate into sterols was obtained 6 h after the start of feeding, with no change up to the 4 h interval (results not shown). It appears that the small amount of the composite diet was unable to restore the cholesterol-biogenic activity to the normal value.

Effect of feeding modified diets

Feeding diets deficient in protein or fat and insufficient in carbohydrate to the rats did not have any effect on the incorporation of [1-¹⁴C]acetate into sterols *in vivo* (Table 1). Therefore the decrease in cholesterol biogenesis during starvation is not due to the depletion of any particular component, but due to the lack of calories, which has already been indicated by the work of Tomkins & Chaikoff (1952). Although the rats fed on a protein-deficient diet consumed less food than those fed on a control diet, the incor-

Table 1. *Effect of feeding modified diets*

The animals were fed on the specified diets for 10 days, and the incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols was measured after administration of $10\text{ }\mu\text{Ci}$ of the tracer intraperitoneally 30 min before the rats were killed. The composition of the diets and experimental methods are described in the text. The radioactivity values are means $\pm$ S.E.M.

Diet	No. of animals	Diet consumed (g/day per rat)	Approx. calorie intake (cal/day per rat)	Radioactivity in sterol fraction (c.p.m./g of liver)
Control (standard diet)	6	18	73	1560 ± 265
Protein-deficient	6	10	41	1560 ± 360
Carbohydrate-insufficient	4	14	57	1480 ± 205
Fat-deficient	4	18	68	1785 ± 410

Fig. 1. *Incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols in livers, and diet intake in rats fed ad lib after starvation for 48 h*

[$1\text{-}^{14}\text{C}$]Acetate ($10\text{ }\mu\text{Ci}$) was injected intraperitoneally to each rat 30 min before the rats were killed. The points represent means $\pm$ S.E.M. obtained by independent analysis of livers from seven animals in each group. —, Radioactivity incorporated into sterols (c.p.m./g of liver); ----, food consumed (g).

poration of [$1\text{-}^{14}\text{C}$]acetate into sterols remained unaffected. From this it appears that about half the normal calorie intake of the control animals is sufficient for the maintenance of cholesterol biogenesis.

Effect of administration of glucose, citrate and pyruvate

Since any calorie source could maintain the level of cholesterol biogenesis in the liver, it was decided to test whether intraperitoneally administered glucose, citrate or pyruvate would show any stimulation. A single dose of high concentration of glucose, citrate or pyruvate stimulated the incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols, but not a dose of low concentration (Table 2).

Table 2. *Effect of administration of glucose, citrate and pyruvate on the incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols in starved rats*

The animals were starved for 48 h. The compounds were administered as solutions in 0.9% NaCl 2 h before the animals were killed. Sodium salts of citrate and pyruvate were used, and the control rats were given 0.9% NaCl. [$1\text{-}^{14}\text{C}$]Acetate ($10\text{ }\mu\text{Ci}$) was injected 30 min before the animals were killed. Other details are given in the Materials and Methods section. The radioactivity values are given as means $\pm$ S.E.M.

Compound administered	No. of animals	Concentration (mg/rat)	Radioactivity in sterol fraction (c.p.m./g of liver)
Control (starved)	6		740 ± 195
Glucose	4	20	1100 ± 300
	4	400	5370 ± 470
Citrate	4	20	840 ± 215
	4	150	1300 ± 250
Pyruvate	4	20	810 ± 175
	4	200	2140 ± 610

Effect of ATP

Administration of ATP, the mobile intracellular energy source of the body, was found effectively to stimulate the biogenesis of cholesterol in the liver (Subba Rao & Ramasarma, 1972). When a single dose of ATP ($18\text{ }\mu\text{mol}$ or 10 mg of the neutralized disodium salt per rat) was administered intraperitoneally to starved rats there was a threefold stimulation of incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols, but not into fatty acids (Table 3). Administration of ATP to the rats fed *ad libitum* resulted in only a small increase in the incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols, showing that the stimulatory effect of ATP at the concentrations used is virtually limited to starved animals, with low initial activity.

Effects of different concentrations and time after administration

Incorporation of [$1\text{-}^{14}\text{C}$]acetate into sterols in starved rats after the administration of different

Table 3. *Effect of ATP on the incorporation of [1-¹⁴C]acetate into hepatic sterols and fatty acids*

The animals were starved for 48 h, and 18 μ mol of ATP was injected 2 h before the animals were killed. [1-¹⁴C]Acetate (10 μ Ci) was injected 30 min before the animals were killed. The radioactivity values are means $\pm$ S.E.M.

Diet status	No. of animals	ATP supplement (μ mol)	Radioactivity incorporated from [1- ¹⁴ C]acetate (c.p.m./g of liver)	
			Sterols	Fatty acids
Starved (48 h)	12	Nil	990 $\pm$ 160	3720 $\pm$ 265
Starved (48 h)	12	18	3390 $\pm$ 545 ($P < 0.005$)	4310 $\pm$ 505 ($P > 0.1$)
Fed <i>ad lib.</i>	8	Nil	4440 $\pm$ 665 ($P < 0.005$)	36630 $\pm$ 4470 ($P < 0.005$)
Fed <i>ad lib.</i>	5	Nil	3750 $\pm$ 1225	Not estimated
Fed <i>ad lib.</i>	5	18	4870 $\pm$ 440 ($P > 0.1$)	Not estimated

Fig. 2. *Effect of different concentrations of ATP on the incorporation of [1-¹⁴C]acetate into sterols in rats starved for 48 h*

ATP was injected intraperitoneally 2 h before the rats were killed, and 10 μ Ci of [1-¹⁴C]acetate was injected 30 min before killing. Means $\pm$ S.E.M. obtained by independent analysis of livers from four animals in each group are given.

Table 4. *Time-course of stimulation of sterol biogenesis by ATP*

The incorporation of [1-¹⁴C]acetate into sterols was determined at specified time-intervals after the administration of 20 μ mol of ATP. The animals were starved for 48 h before the experiment, and the [1-¹⁴C]acetate was injected 30 min before the animals were killed. The radioactivity values are means $\pm$ S.E.M.

Time (h)	No. of animals	Radioactivity in sterol fraction (c.p.m./g of liver)
0	4	500 $\pm$ 80
1	4	1190 $\pm$ 145
2	4	3670 $\pm$ 370
4	4	1810 $\pm$ 200
6	4	1710 $\pm$ 450

concentrations of ATP is shown in Fig. 2. Increases in the incorporation were obtained at concentrations of 20 μ mol/rat, and above. Maximum stimulation was obtained 2 h after administration of ATP (Table 4). It should be pointed out that the extent of stimulation varied in different experiments, and this may be a reflexion of the differences in the extent of the calorie deficit and in the body weights of the animals used (Shefer *et al.*, 1972). To minimize the variations, the rats were chosen for each experiment so that the difference in their weights was within 15 g, and they were treated identically.

Stimulatory effect of ATP and adenosine in experiments with tissue slices

In this set of experiments, groups of animals were starved, or fed on cholesterol diets, two conditions known to decrease cholesterol biogenesis, and groups of such animals were treated with ATP or adenosine as indicated in Table 5. Liver slices were obtained from these animals, and their abilities to incorporate [1-¹⁴C]acetate into sterols *in vitro* were compared. Stimulations similar to those observed *in vivo* were obtained (Table 5).

Stimulation of hydroxymethylglutaryl-CoA reductase by ATP, adenosine and related compounds

The above results on the incorporation of [1-¹⁴C]acetate into sterols and fatty acids, and those previously reported on [2-¹⁴C]mevalonate incorporation into sterols (Subba Rao & Ramasarma, 1972) suggested that a site before mevalonate formation is most probably affected. Under a number of conditions in which changes in sterol biogenesis have been associated with the acetate-mevalonate stage, the enzyme activity affected has been hydroxymethylglutaryl-CoA reductase. Furthermore, the enzyme has been shown to be stimulated by ATP in both starved and cholesterol-fed animals (Subba Rao *et al.*, 1975). The present paper shows that the effect is not confined to ATP, as AMP, cyclic AMP

Table 5. Incorporation of $[1-^{14}\text{C}]$ acetate into non-saponifiable lipids in starved and cholesterol-fed animals in tissue slices

Groups of rats were fed on cholesterol or starved for 48 h, as indicated. After the specified period, the animals were killed by cervical dislocation. Liver slices were prepared from these animals, and incubated in Krebs-Ringer phosphate buffer (Krebs & Henseleit, 1932) with $[1-^{14}\text{C}]$ acetate ($5\text{ }\mu\text{Ci}/\text{flask}$) for 2 h at 37°C . Extraction and fractionation of sterols was as described in the Materials and Methods section for the liver tissue in the experiments *in vivo*. The values given are averages of triplicate determinations.

Treatment	Radioactivity incorporated into sterols (c.p.m./h per g of liver)
I Normal	86820
Cholesterol-fed (0.5%, 10 days)	21200
Cholesterol-fed (0.5%, 10 days) +ATP (18 μmol , 2 h before death)	39920
II Normal	76900
Cholesterol-fed (1%, 5 days)	28430
Cholesterol-fed (1%, 5 days) +ATP (18 μmol , 2 h before death)	75500
III Normal	32900
Starved	8940
Starved+ATP (18 μmol , 2 h before death)	13890
Starved+Adenosine (20 μmol , 2 h before death)	14500

Table 6. Effect of adenosine derivatives and related compounds on sterol biogenesis *in vivo*

ATP, ADP, GTP and theophylline were given as neutral solutions in 0.9% NaCl. Cyclic AMP, adenosine, isopentenyladenosine, adenine, xanthine, guanosine and uric acid were injected as suspensions. Each compound was administered intraperitoneally to rats starved for 48 h, at a concentration of $20\text{ }\mu\text{mol}/\text{rat}$, 2 h before the animals were killed. $[1-^{14}\text{C}]$ Acetate ($10\text{ }\mu\text{Ci}/\text{rat}$) was administered 30 min before the rats were killed, and the radioactivity incorporated into the sterol fraction was measured. The values are means $\pm$ S.E.M. from five animals in each group.

Compound administered	Radioactivity in sterols (c.p.m./g of liver)
0.9% NaCl	790 $\pm$ 110
ATP	3625 $\pm$ 617
ADP	2920 $\pm$ 250
AMP	3220 $\pm$ 600
Cyclic AMP	2930 $\pm$ 290
Adenosine	3180 $\pm$ 610
Isopentenyladenosine	2500 $\pm$ 155
0.9% NaCl	650 $\pm$ 80
Guanosine	740 $\pm$ 65
GTP	810 $\pm$ 70
0.9% NaCl	810 $\pm$ 120
Adenine	1120 $\pm$ 160
Xanthine	740 $\pm$ 90
Uric acid	840 $\pm$ 70
Theophylline	1100 $\pm$ 170

and adenosine also stimulated the incorporation of $[1-^{14}\text{C}]$ acetate into sterols *in vivo* (Table 6), and increased the activity of hydroxymethylglutaryl-CoA reductase (Table 7). Adenine, guanosine and GTP were ineffective *in vivo*, and these results indicate that biogenesis of sterols is probably sensitive to only adenosine and its derivatives.

Discussion

Although considerable work has been done on the regulation of cholesterol biogenesis, it is still not understood how the deprivation of food reversibly decreases it. The diurnal rhythm, showing a cyclic increase in cholesterol biogenesis at midnight has been shown to depend on the intake of food (Subba Rao & Ramasarma, 1971; Edwards *et al.*, 1972). The threefold increase at midnight, in fact, occurs about 5–6 h after the start of food intake. The results presented here indicate that similarly during starvation-re-feeding experiments stimulation of sterol biogenesis is highest 6 h after the start of food intake, and with small metabolites, such as glucose, citrate, pyruvate, ATP and adenosine derivatives, administered intraperitoneally, shorter periods are

Table 7. Effect of adenosine and derived nucleotides on the activity of 3-hydroxy-3-methylglutaryl-CoA reductase in starved animals

Groups of rats were starved for 48 h. AMP and ATP were given intraperitoneally as neutral solutions and adenosine and cyclic AMP as suspensions in 0.9% NaCl 2 h before the rats were killed. Starved controls and fed animals received 0.9% NaCl. Enzyme activity was measured in microsomal preparations in each animal in duplicate, and averages are taken. In each group values are means $\pm$ S.E.M., and $P < 0.01$ in each case compared with starved control.

Treatment	No. of animals	Activity of hydroxymethylglutaryl-CoA reductase (pmol of mevalonate formed/min per mg of protein)
Starved	18	27 $\pm$ 3
+adenosine (20 $\mu\text{mol}/\text{rat}$)	12	103 $\pm$ 12
+AMP (20 $\mu\text{mol}/\text{rat}$)	4	174 $\pm$ 19
+cyclic AMP (20 $\mu\text{mol}/\text{rat}$)	8	88 $\pm$ 12
+ATP (18 $\mu\text{mol}/\text{rat}$)	6	129 $\pm$ 15
Normal fed <i>ad lib.</i>	6	107 $\pm$ 8

sufficient. Since the supply of calories alone can maintain cholesterol biogenesis in fed rats or stimulate cholesterol biogenesis in starved rats, it is logical to expect that the administered compounds might have given rise to ATP, which itself stimulates sterol biogenesis. In view of its dependence on diet intake, the circadian-rhythmic change of cholesterol biogenesis may also be mediated by alterations in the intracellular concentration of ATP, or a derived metabolite. On the basis of a study of ATP concentrations in livers and brains of alert and sleeping hamsters, Jones (1971) suggested that the changes in ATP concentrations may be the primary basis for the 'biological clocks'.

Although adenine nucleotides and adenosine stimulated the biogenesis of sterols, ATP is possibly the active metabolite, for the following reasons. (1) During ethionine treatment, when the ATP concentrations in the liver are known to be depleted, biogenesis of cholesterol from acetate was inhibited (Harry *et al.*, 1973). (2) A decrease in the concentration of ATP in the liver has been reported to cause a decrease in the output of bile, indicating indirect involvement of ATP in the metabolism of cholesterol (Eakins *et al.*, 1969). (3) Administration of adenosine or ATP resulted in an increase in the concentration of ATP in the liver (Farber *et al.*, 1963). A decrease in the concentration of cyclic AMP in the livers of starved rats (Selawry *et al.*, 1973) and inhibition of cholesterol biogenesis by cyclic AMP added *in vitro* (Bricker & Levey, 1972) indicate that cyclic AMP is not the active metabolite involved in stimulating sterol biogenesis.

The question arises as to how the injected ATP enters the liver, since it is generally believed that phosphate esters do not cross cell membranes without first being hydrolysed. It is known that administration of ATP prevents the formation of fatty livers, induced by ethionine (Hyams *et al.*, 1967; Rajalakshmi *et al.*, 1964). It has also been shown that the concentration of ATP in the liver increases after the administration of exogenous ATP (Farber *et al.*, 1963; Hyams *et al.*, 1967). It is possible, therefore, that ATP is taken up directly by the liver, as is the case for NAD⁺ (Kaplan *et al.*, 1956); alternatively it is hydrolysed first, and then the nucleoside derivative, after entering the cell, is converted back into ATP.

In stimulating the biogenesis of sterols, exogenous ATP is not merely acting as a supply of energy. Neither the mevalonate → sterol nor the acetate → fatty acid pathway, both of which require ATP, is activated. It is possible that the ATP concentration acts as a signal for the regulation of biogenesis, and, by implication, the activity of hydroxymethylglutaryl-CoA reductase, which has been shown to respond in a parallel fashion under a variety of conditions, including treatment with hormones

(Nepokroeff *et al.*, 1974). It is, however, not clear how ATP modifies the activity of the enzyme, and some questions still remain to be answered. Does ATP modify the existing preformed inert protein (for example, by phosphorylation) and make it active? Recent findings show that, on the contrary, ATP decreases the activity of the enzyme *in vitro* (Chow *et al.*, 1975). Does ATP therefore modify a repressor molecule, by either binding to it or producing covalent changes, and thereby generate the enzyme protein *de novo*? These questions must await first the unequivocal proof that intracellular ATP is the controlling agent and second the setting up of experiments which can be used to investigate the regulatory mechanism. The available data, however, suggest that the role of ATP is 'supraenergetic' in modifying the activity or the concentration of the enzyme.

It should be pointed out that, in this paper, the ATP effect has been shown in starved rats. Whether it is the sole control of sterol biogenesis in the three types described (Ramasarma, 1972) is still a matter of conjecture. The present studies indicate, for the first time, a naturally occurring small molecule as a potential inducer of hydroxymethylglutaryl-CoA reductase. A similar experimental technique was used for the demonstration of the induction of the hepatic enzymes tyrosine aminotransferase and tryptophan pyrrolase on injection of cortisol, an equally complex phenomenon in which the fate and function of the inducer remains unknown. It is hoped that this discovery will aid the further understanding of the regulation of sterol biogenesis.

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