Regulation of 3-Hydroxy-3-methylglutarate and Mevalonate Biosynthesis by Rat Liver Homogenates. Effects of Fasting, Cholesterol Feeding, and Triton Administration*

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ABSTRACT: Regulation of biosynthesis of 3-hydroxy-3-methylglutarate (HMG) and mevalonate has been studied in fractionated rat liver homogenates using methods that permit evaluation of possible control points between acetate and mevalonate. Effects of fasting, cholesterol feeding, and Triton WR 1339 administration, known to influence incorporation of [^{14}C]acetate into hepatic cholesterol, have been examined. Fasting results in depression of HMG-CoA reductase activity and decreased activation of acetate. Triton WR 1339 administration is associated with increased acetate activation and HMG-CoA reductase activity. By contrast cholesterol feeding is associated with inhibition of both HMG-CoA condensing enzyme and HMG-CoA reductase; however the major effect is on the reductase and the inhibition is generally greater than 90%, thereby accounting for the major part of the decrease in cholesterol synthesis. The condensing enzyme is inhibited only partially (*ca.* 50%) and this effect is apparent only when the microsomes are removed and HMG is the product of the reaction. It is suggested that studies on the control of the enzymes involved in mevalonate synthesis require the adoption of special conditions to demonstrate inhibitory or stimulatory effect. Under each condition, substrate incorporation into and rates of synthesis of HMG and mevalonate were determined; changes in ^{14}C incorporation were found to reflect changes in product synthesis. The validity of assumptions made in earlier studies on control of hepatic cholesterol synthesis and the significance of the work reported here are discussed.

Many workers have shown that a variety of physiological alterations influence hepatic incroporation of [^{14}C]acetate[1] into cholesterol. Rates of [^{14}C]Ac incorporation into cholesterol are increased by radiation and Triton WR 1339 administration, and decreased by fasting and cholesterol feeding (Bucher *et al.*, 1959). Because similar changes in incorporation of [^{14}C]mevalonic acid into cholesterol are not observed, it has been proposed that physiological regulation of cholesterol biosynthesis is exerted primarily at a step in the pre-mevalonate segment of the pathway (Gould and Popjak, 1957; Bucher *et al.*, 1959). Recently it has been reported that dietary cholesterol and fasting depress hepatic cholesterol synthesis by a negative feedback system acting on the conversion of HMG-CoA into mevalonic acid, a step catalyzed by HMG-CoA reductase (mevalonate-NADP oxidoreductase, E.C. 1.1.1.34) (Siperstein and Fagan, 1966; Regen *et al.*, 1966; Linn, 1967). Inhibition or repression of an early step in the synthetic pathway suggests that cholesterol biosynthesis may be regulated by homeostatic mechansims analogous to those described in other metabolic pathways and in certain systems of microorganisms (Bucher *et al.*, 1959; Siperstein and Fagan, 1966; Stadtman, 1966).

As previously outlined (White and Rudney, 1970), conclusions relating to the site of feedback control of cholesterol synthesis have been based on several assumptions that may not always be valid. Thus, possible regulation at the step catalyzed by HMG-CoA condensing enzyme (HMG-CoA AcAc-CoA lyase, E.C. 4.1.3.5.) must also be considered. In addition, conclusions regarding rates of synthesis cannot simply be based on altered rates of incorporation of labeled precursors, since these changes may be secondary to altered specific activity of these precursors or labeled intermediates through dilution from unlabeled endogenous sources. In a previous publication (White and Rudney, 1970), appropriate systems for studying regulation of mevalonic acid formation were developed in order to fully assess all factors involved in the exertion of control. These approaches were applied to an evaluation of the effect of fasting, dietary cholesterol, and Triton WR 1339 administration, on control of hepatic cholesterol biosynthesis, and the results are reported in this communication.

Methods

Preparation of tissues, separation and purification of intermediates, and analytical methods have been previously detailed (White and Rudney, 1970). Control animals were fed Purina rat pellets and water *ad libitum*. Where indicated, animals were fasted for variable periods. Cholesterol-fed rats received 1% cholesterol in the diet, prepared by adding a solution of cholesterol dissolved in ether to rat pellets and allowing the solvent to evaporate. Triton-treated rats re-

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[1] The following abbreviations are used throughout the text: HMG and HMG-CoA for 3-hydroxy-3-methylgutaric acid and its CoA ester; Ac and Ac-CoA for acetate and its CoA ester; AcAc and AcAc-CoA for acetoacetate and its CoA ester.

TABLE I: Effect of Fasting.[a]

Dietary Treatment	A Ac → HMG	B Ac-CoA → HMG Microsomes	C Ac-CoA → HMG Soluble	D Ac → HMG + Mevalonic Acid		E Ac-CoA → HMG + Mevalonic Acid		F HMG-CoA → Mevalonic Acid
				HMG	Mevalonic Acid	HMG	Mevalonic Acid	
Normal	36.7			47.5	4.9			
Fasted	59.0			0.25	0.25			
Ratio: fasted:normal	1.61			0.01	0.05			
Normal		10.0						0.72
Fasted		35.4						0.49
Ratio: fasted:normal		3.54						0.68
Normal	19.0			22.0	60.0			
Fasted	38.0			0.9	0.6			
Ratio: fasted:normal	2.00			0.04	0.01			
Normal		33.8	57.0			27.8	18.7	6.76
Fasted		40.2	59.0			33.0	4.2	0.16
Ratio: fasted:normal		1.22	1.03			1.18	0.22	0.02
Normal	30.5	35.3	62.5	27.5	9.5	31.2	5.9	14.4
Fasted	20.0	24.9	64.6	16.5	0.9	24.4	1.6	2.52
Ratio: fasted:normal	0.65	0.70	1.03	0.59	0.09	0.78	0.27	0.17
Normal	8.9		24.9	19.8	99.0	6.2	88.0	25.6
Fasted	7.6		20.5	18.0	2.3	14.0	7.9	1.3
Ratio: fasted:normal	0.85		0.82	0.91	0.23	2.34	0.09	0.05
Average ratio	1.28	1.82	0.96	0.39	0.09	1.43	0.19	0.23

[a] Incubations were performed in air at 37° for 2 hr. Contents of each incubation flask, in a total volume of 7 ml, were as follows: (A) soluble fraction of 105,000*g* supernatant, 5 μmoles of [1-^{14}C]Ac, 0.1 μmole of CoA, 10 μmoles of ATP, 2 μmoles of EDTA, and 300 μmoles of potassium phosphate buffer (pH 7.0); (B) microsomal fraction, 1 μmole of [^{14}C]Ac-CoA, 10 μmoles of EDTA, 7 μmoles of dithiothreitol, and 300 μmoles of potassium phosphate buffer (pH 7.0); (C) soluble fraction, 1 μmole of [^{14}C]Ac-CoA, 2 μmoles of EDTA, and 300 μmoles of potassium phosphate buffer (pH 7.0); (D) soluble and microsomal fractions, 5 μmoles of [1-^{14}C]Ac, 0.1 μmole of CoA, 10 μmoles of ATP, 10 μmoles of EDTA, 12 μmoles of $MgCl_2$, 50 μmoles of mevalonic acid (potassium salt), 20 μmoles of glucose 6-phosphate (dipotassium salt), 10 μmoles of TPN^+, 0.5 unit of glucose 6-phosphate dehydrogenase, and 250 μmoles of potassium phosphate buffer (pH 7.0); (E) microsomal fraction, 1 μmole of [^{14}C]Ac-CoA, 10 μmoles of EDTA, 7 μmoles of dithiothreitol, 50 μmoles of mevalonic acid, 20 μmoles of glucose 6-phosphate, 10 μmoles of TPN^+, 0.5 unit of glucose 6-phosphate dehydrogenase, and 300 μmoles of potassium phosphate buffer (pH 7.0); (D) microsomal fraction, 400 mμmoles of [^{14}C]HMG-CoA, 10 μmoles of EDTA, 70 μmoles of dithiothreitol, 50 μmoles of mevalonic acid, 20 μmoles of glucose 6-phosphate, 10 μmoles of TPN^+, 0.5 unit of glucose 6-phosphate dehydrogenase, and 300 μmoles of potassium phosphate buffer (pH 7.0). Results expressed as mμmoles of substrate incorporated into product.

ceived 200 mg of Triton WR 1339, obtained from Rohm and Haas Co., in 2 ml of water by intraperitoneal injection 16–24 hr before sacrifice.

The following systems were assessed with respect to alterations of fasting, cholesterol-feeding, and Triton WR 1339 administration: (A) incorporation of [1-^{14}C]Ac into HMG in the presence of the 105,000*g* soluble supernatant; (B) incorporation of [^{14}C]Ac-CoA into HMG in the presence of microsomes; (C) incorporation of [^{14}C]Ac-CoA into HMG in the presence of soluble fraction; (D) incorporation of [^{14}C]Ac into HMG and mevalonic acid in the presence of soluble fraction and microsomes; (E) incorporation of [^{14}C]-Ac-CoA into HMG and mevalonic acid in the presence of microsomes; (F) incorporation of [^{14}C]-HMG-CoA into mevalonic acid in the presence of microsomes. Contents of each incubation flask were as previously outlined (White and Rudney, 1970). The material isolated as HMG includes HMG-CoA, free HMG, and possibly other ester forms.

In the case of each manipulation, experiments were performed in which the specific activity of AcAc was measured as previously described (White and Rudney, 1970), thereby enabling rates of HMG and mevalonic acid synthesis to be determined.

In preliminary experiments it was shown that quadruplicate assays from the same animal gave essentially identical results. In addition, several experiments were performed in

TABLE II: Effect of Dietary Cholesterol.[a]

Dietary Treatment	A Acetate → HMG	B Ac–CoA → HMG Microsomes	C Ac–CoA → HMG Soluble	D Ac → HMG + Mevalonic Acid: HMG	D Ac → HMG + Mevalonic Acid: Mevalonic Acid	E Ac–CoA → HMG + Mevalonic Acid: HMG	E Ac–CoA → HMG + Mevalonic Acid: Mevalonic Acid	F HMG–CoA → Mevalonic Acid
Control	99			44	92			
Cholesterol fed, 3 days	57			16	2.5			
Ratio: treated:control	0.57			0.36	0.027			
Control	57							
Cholesterol fed, 24 hr	36.5							
Ratio: treated:control	0.63							
Control	49.5			12.5	36.5			
Cholesterol fed, 20 hr	23.5			13.8	3.3			
Ratio: treated:control	0.45			1.1	0.090			
Control	17.0							
Cholesterol fed, 18 hr	9.4							
Ratio: treated:control	0.55							
Control		67.4	111.			48.2	10.9	3.28
Cholesterol fed, 6 days		33.2	68.6			22.6	1.1	0.73
Ratio: treated:control		0.49	0.62			0.47	0.10	0.22
Control	31.0	6.8	5.7	29.5	65.5		12.5	124.0
Cholesterol fed, 6 days	14.0	2.3	2.3	14.0	2.5		2.6	6.3
Ratio: treated:control	0.45	0.34	0.40	0.47	0.038		0.21	0.05
Control	30.0	4.8	12.6	31.0	107.0	6.7	11.5	13.5
Cholesterol fed, 2 days	15.5	2.0	5.3	17.7	3.8	2.3	3.4	1.7
Ratio: treated:chotrol	0.52	0.42	0.42	0.57	0.036	0.34	0.30	0.13
Average ratio	0.53	0.42	0.48	0.60	0.048	0.50	0.17	0.13

[a] Conditions of incubation were as in Table I. Results expressed as mμmoles of substrate incorporated into product.

which four control animals were sacrificed at the same time and assays run simultaneously. Under these conditions, the variation in results between animals never exceeded 10%.

Results

Fasting. The effects of fasting on each system is shown in Table I. No consistent effect on incorporation of [^{14}C]-substrate into HMG was observed when only soluble supernatant was present (A and C). Incorporation of [^{14}C]Ac, [^{14}C]Ac–CoA and [^{14}C]HMG–CoA into mevalonic acid (D, E, F) were all depressed by fasting, indicating an effect on HMG–CoA reductase. When microsomes were present and [^{14}C]Ac was substrate (D), marked depressed of incorporation into HMG was present. This did not occur with [^{14}C]Ac–CoA as substrate (B and E), indicating that HMG–CoA condensing enzyme was not affected. Depressed incorporation of [^{14}C]Ac into HMG occurred only in the presence of microsomes (contrast D with A), indicating that microsomes, but not soluble fraction, from fasted rats depressed acetate activation.

Cholesterol Feeding. Effects of dietary cholesterol are shown in Table II. In contrast to the situation with fasting, incorporation of both [^{14}C]Ac and [^{14}C]Ac–CoA into HMG was consistently inhibited, suggesting an effect on HMG–CoA condensing enzyme. This depression did not depend on the presence of microsomes. As with fasted animals, incorporation of [^{14}C]Ac, [^{14}C]Ac–CoA, and [^{14}C]HMG–CoA into mevalonic acid were all depressed, indicating an effect on HMG–CoA reductase. *In the presence of microsomes,* incorporation of [^{14}C]Ac into HMG was not consistently depressed (D), in contrast to the situation in the absence of microsomal fraction and a TPNH generating system (A, B, C). We have previously shown that no consistent pattern in incorporation of [^{14}C]Ac into HMG is seen in the presence of microsomes, and have pointed out that it is invalid to draw conclusions regarding HMG formation in the presence of HMG–CoA reductase and a TPNH generating system (White and Rudney, 1970). Thus, in the experiments reported here with microsomes present, HMG and HMG–CoA are not end products, and simultaneous effects of cholesterol feeding on HMG–CoA formation and removal *via* microsomal reduction to mevalonic acid

TABLE III: Effect of Triton WR 1339 Administration.[a]

Treatment	A Acetate → HMG	B Ac-CoA → HMG Microsomes	C Ac-CoA → HMG Soluble	D Ac → HMG + Mevalonic Acid: HMG	D Ac → HMG + Mevalonic Acid: Mevalonic Acid	E Ac-CoA → HMG + Mevalonic Acid: HMG	E Ac-CoA → HMG + Mevalonic Acid: Mevalonic Acid	F HMG-CoA → MVA
Normal	105			54.0	51.0			
Triton	127			18.0	85.0			
Ratio: Triton:normal	1.21			0.33	1.66			
Normal	36.9			18.5	86			
Triton	70.5			45.7	192			
Ratio: Triton:normal	1.91			2.48	2.24			
Normal	179	11.6						
Triton	209	15.3						
Ratio: Triton:normal	1.17	1.32						
Normal	27.5			25.0	22.5			
Triton	69.5			8.0	155			
Ratio: Triton:normal	2.49			0.32	6.89			
Normal		48.8	94.0			30.5	6.0	2.12
Triton		61.6	97.0			38.2	20.1	3.28
Ratio: Triton:normal		1.26	1.03			1.22	3.34	1.63
Normal	23.0	87.0	129	66.0	41.0	50.4	66.2	36.0
Triton	33.0	78.0	152	56.0	48.0	29.5	70.8	63.2
Ratio: Triton:normal	1.43	0.90	1.18	0.85	1.17	0.56	1.07	1.75
Normal	19.3	13.2	22.1	11.8	24.0	10.8	10.3	15.6
Triton	31.8	14.9	27.0	19.0	87.0	8.2	27.5	44.4
Ratio: Triton:normal	1.65	1.13	1.22	1.61	3.62	0.76	2.66	3.52
Average ratio	1.64	1.15	1.14	1.12	3.12	0.85	2.36	2.30

[a] Conditions of incubation were as in Table I. Results expressed as mμmoles of substrate incorporated into product.

would be expected to result in variable changes in net HMG-CoA formation.

Triton Administration. Effects of administration of Triton WR 1339 are shown in Table III. In the presence of soluble fraction alone, increased incorporation of [^{14}C]Ac into HMG was observed (A); this did not occur with [^{14}C]Ac-CoA as substrate (C). In contrast with the findings with soluble fraction alone, effects on incorporation of substrate into HMG in the presence of microsomes were extremely variable (D, E), probably related to the fact that both HMG formation and removal were influenced. Incorporation of [^{14}C]Ac, [^{14}C]Ac-CoA, or [^{14}C]HMG-CoA into mevalonic acid was consistently increased (D, E, F). No effect on incorporation of [^{14}C]Ac into HMG or mevalonic acid was observed 4 hr after Triton injection; increased incorporation consistently occurred 16–24 hr following Triton administration.

Measurement of Synthesis. HMG and mevalonic acid syntheses were calculated in selected experiments as shown in Table IV. It can be seen that alterations in incorporation were paralleled by alterations in the same direction in synthesis; consequently changes in incorporation noted with fasting, cholesterol feeding, or Triton administration were not the consequence of altered specific activity of intermediate pools. It was noted that specific activity of AcAc fell with fasting, while no significant change occurred with cholesterol feeding or Triton administration.

Discussion

Plasma cholesterol concentrations do not change significantly when dietary cholesterol ingestion is markedly varied over short periods (Gould, 1951). Demonstration of altered incorporation of [^{14}C]Ac into cholesterol in intact animals and liver slices has suggested that hepatic cholesterol synthesis varies inversely with the level of dietary cholesterol (Gould, 1951; Tomkins *et al.*, 1953; Frantz *et al.*, 1954; Siperstein and Guest, 1960).

While incorporation of [^{14}C]Ac into cholesterol is impaired by dietary cholesterol, incorporation of [^{14}C]mevalonic acid into cholesterol is not similarly influenced (Gould and Popjak, 1957; Bucher *et al.*, 1959), indicating an effect on early steps in cholesterol synthesis prior to mevalonic acid formation. Furthermore, since dietary cholesterol has no effect

TABLE IV: Synthesis of HMG and Mevalonic Acid.[a]

Treatment	(A) [^{14}C]Acetate → HMG		(B) [^{14}C]Acetate → Mevalonic Acid	
	Incorporation (mμmoles of Ac)	Synthesis (mμmoles of HMG)	Incorporation (mμmoles of Ac)	Synthesis (mμmoles of Mevalonic Acid)
Control	19.0	29.4	60	142
Fasted	38.0	63.0	0.6	4.4
Control	49.5	35.8	36.5	50.4
Cholesterol fed	23.5	20.0	3.3	7.0
Control	17.0	8.1		
Cholesterol fed	9.4	4.6		
Control	36.9	43.2	86	194
Triton administration	70.5	82.4	192	248
Control			41.5	223
Triton administration			48.5	240
Control	19.3	6.1	24.0	8.0
Triton administration	31.8	9.6	87.0	24.0

[a] Incubations were performed in air at 37° for 2 hr. Contents of each incubation flask in a total volume of 7 ml were as follows: (A) soluble fraction, 10 μmoles of [2-^{14}C]Ac, 0.2 μmole of CoA, 10 μmoles of ATP, 30 μmoles of $MgCl_2$, and 300 μmoles of potassium phosphate buffer (pH 7.0); (B) soluble and microsomal fractions, 10 μmoles of [2-^{14}C]Ac, 0.2 μmole of CoA, 10 μmoles of ATP, 30 μmoles of $MgCl_2$, 50 μmoles of mevalonic acid, 20 μmoles of glucose 6-phosphate, 10 μmoles of TPN^+, 0.5 unit of glucose 6-phosphate dehydrogenase, and 250 μmoles of potassium phosphate buffer (pH 7.0). Reactions were terminated with trichloroacetic acid, AcAc content and specific activity were determined, and rates of HMG and mevalonic acid synthesis calculated as previously described (White and Rudney, 1969).

on incorporation of [^{14}C]Ac into fatty acids or [^{14}C]CO_2 (Tomkins *et al.*, 1953; Siperstein and Guest, 1960) the site of action probably lies beyond Ac–CoA. It has also been shown that dietary cholesterol does not influence AcAc formation (Siperstein and Guest, 1960). Assuming that HMG–CoA is an obligatory intermediate in AcAc synthesis, Siperstein and Guest concluded that the point of regulation was beyond HMG–CoA formation, *i.e.*, at the step catalyzed by HMG–CoA reductase. More recently, decreased incorporation of [^{14}C]Ac into mevalonic acid, without changes in incorporation into HMG, has been demonstrated (Siperstein and Fagan, 1966), and more direct evidence that the major site of feedback inhibition is at the HMG–CoA reductase step has been offered (Linn, 1967).

Similar considerations have led to the conclusion that fasting depresses cholesterol synthesis by regulation at the same step (Bucher *et al.*, 1960), and recently direct evidence of decreased HMG–CoA reductase activity following fasting has been shown (Regen *et al.*, 1966; Linn, 1967).

However, as previously detailed, these approaches fail to take cognizance of all factors, and the validity of many of the assumptions may be challenged (White and Rudney, 1970). Using appropriate systems designed for a more comprehensive assessment of factors involved in regulation, we have shown that dietary cholesterol inhibits cholesterol synthesis at two metabolic steps prior to mevalonic acid formation. In addition to the marked inhibition of HMG–CoA reductase, suggested by other workers, HMG–CoA condensing enzyme is also inhibited, but to a lesser degree. Thus, the inhibition of the reductase is generally greater than 90%, while the condensing enzyme is *ca.* 50%. This is in agreement with and supports the concept that the major effect of cholesterol feeding is exerted at the HMG–CoA reductase level. However, there may be situations where the amount of mevalonic acid formed is limited by the quantity of substrate available for the HMG–CoA reductase; then a partial inhibition of HMG–CoA condensing enzyme might possibly be of considerable significance. In the preceding paper, White and Rudney (1970) have shown that HMG–CoA generation may be rate limiting in the formation of mevalonic acid.

The demonstration by this work and by that of Gould and Swyryd (1966) that cholesterol feeding affects more than one key enzyme involved in its synthesis may be analogous to synchronous influences on groups of key rate-limiting enzymes governing irreversible reactions in pathways of gluconeogenesis and glycolysis, described by Weber *et al.* (1966). The failure of other workers to demonstrate changes in incorporation of [^{14}C]Ac into HMG (Siperstein and Fagan, 1966) may relate to the fact that HMG–CoA reductase was also present in their experiments; *i.e.*, in the presence of microsomes, changes in the rate of HMG–CoA generation may be obscured by simultaneous changes in HMG–CoA reductase. Consequently, to demonstrate effects on HMG–CoA condensing enzyme using methods involving ^{14}C incorporation into HMG, further disposition of HMG or HMG–CoA must be prevented by removing microsomes from cell-free systems (White and Rudney, 1970). Thus,

by demonstrating an effect on incorporation into HMG in a microsome-free system, it can be concluded that HMG-CoA condensing enzyme is partially depressed by dietary cholesterol. The effect was observed with both [^{14}C]Ac and [^{14}C]Ac-CoA, ruling out an effect on acetate activation. Furthermore, changes in incorporation reflected changes in synthesis rather than altered specific activity of intermediate precursors.

By contrast with cholesterol feeding, fasting results in depressed activity of microsomal HMG-CoA reductase with no influence on HMG-CoA condensing enzyme. An additional effect, microsomal-dependent depression of acetate activation, may have no physiological relevance, since Ac-CoA can be provided without the obligatory participation of free acetate, as by fatty acid oxidation. Thus, inhibition of acetate activation will not necessarily influence the conversion of naturally occurring precursors to cholesterol. By analogy, phenylbutyrate interferes with acetate activation and inhibits incorporation of [^{14}C]Ac into cholesterol, but has no effect on serum or tissue cholesterol concentration (Steinberg and Frederickson, 1956).

The demonstration by Bucher *et al.* (1959) that depressed incorporation of [^{14}C]Ac into cholesterol, produced by fasting, depended more on microsomes than soluble fraction is consistent with our findings of a dual effect on both HMG-CoA reductase, a microsomal enzyme, and acetate activation, where depression occurs only in the presence of microsomes. Furthermore, their data showed that soluble fraction from cholesterol-fed rats produced considerable reduction in [^{14}C]Ac incorporation into cholesterol (58%) by contrast with soluble fraction from fasted animals (28%). This can now be accounted for by the finding that activity of HMG-CoA condensing enzyme, present in the soluble 105,000*g* supernatant fraction, is depressed by cholesterol feeding but not by fasting.

Increased hepatic AcAc production during fasting may relate to depression of HMG-CoA reductase with accumulation of HMG-CoA providing increased substrate for HMG-CoA cleavage enzyme (HMG-Co Aacetoacetate lyase, E.C. 4.1.3.4.). Alternatively, accumulation of HMG-CoA may secondarily inhibit HMG-CoA condensing enzyme leading to an accumulation of AcAc-CoA, thereby providing increased substrate for direct deacylation of AcAc-CoA. A direct effect on AcAc-CoA deacylation may also occur (Burch and Triantafillou, 1968). By contrast, the absence of increased AcAc formation with cholesterol feeding may be related to the additional inhibition of HMG-CoA condensing enzyme, preventing accumulation of HMG-CoA. Thus, if AcAc generation occurs chiefly *via* HMG-CoA cleavage, the dual site of action of dietary cholesterol will not only inhibit cholesterogenesis, but also prevent increased ketogenesis. Whether HMG-CoA cleavage, AcAc-CoA deacylation, or both, represent the mechanism for AcAc generation, the absence of altered AcAc formation indicates that cholesterol feeding has no influence prior to AcAc-CoA synthesis.

Triton WR 1339, a nonionic detergent with surface-active properties, produces hypercholesterolemia when administered parenterally (Kellner *et al.*, 1951). Increased plasma cholesterol concentration is associated with increased hepatic incorporation of [^{14}C]Ac into cholesterol, whether [^{14}C]Ac is administered *in vivo* (Frantz and Hinkelman, 1955) or added to preparations *in vitro* (Bucher *et al.*, 1959), and it has been suggested that increased cholesterol synthesis is the primary cause of the hypercholesterolemia.

With Triton administration, elevated incorporation of [^{14}C]Ac into cholesterol occurs with much less increase in [^{14}C]mevalonic acid incorporation into cholesterol (Bucher *et al.*, 1959). We have shown that this is not associated with increased activity of HMG-CoA condensing enzyme. However, increased acetate activation and, more importantly, increased HMG-CoA reductase activity were present. While it is probable that this enzyme change is responsible for increased cholesterol synthesis, it should be emphasized that these alterations do not represent the earliest effects of Triton, and are probably secondary to other phenomena. This is supported by the demonstration that increased cholesterol synthesis occurs at a later time than the initial increase in plasma cholesterol concentration (Byers *et al.*, 1963), and it has been suggested that Triton WR 1339 produces fundamental physical or chemical changes favoring mobilization and sequestration of cholesterol in circulating plasma (Friedman and Byers, 1957).

That the observations reported here have relevance with respect to control of hepatic cholesterol synthesis may be inferred by the fact that alterations in enzyme activity produced by fasting, dietary cholesterol, and Triton administration are in the same direction as overall changes in cholesterol synthesis. Furthermore, both HMG-CoA condensing enzyme and HMG-CoA reductase catalyze irreversible reactions, and are located beyond branch points on the pathway to cholesterol; consequently they represent logical regulatory steps. However, optimal concentrations of substrates and cofactors that may bear no relationship to concentrations generated within the intact cell have been used, and the studies reported here, using fractionated homogenates, are limited to assessment of enzyme content and activity under optimal conditions. It is clear that other factors can influence metabolic pathways; consequently, if altered rates of cholesterol biosynthesis *in vivo* are related to differences in substrate or cofactor concentrations, these approaches will not reveal the underlying mechanisms.

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Arginine and Lysine Transport in Sugarcane Cell Suspension Cultures*

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ABSTRACT: The initial rate of transport of extracellular arginine into sugarcane cells grown in suspension culture was shown to be at least four times as great as that of lysine but the system approached saturation at an exogenous arginine concentration of about 10 μM, whereas lysine transport remained linear until the initial lysine concentration in the medium exceeded 100 μM.

The rate of lysine transport was found to be much more susceptible to the presence of arginine in the medium than was arginine transport to exogenous lysine. Preincubation of sugarcane cells with cycloheximide severely reduced the velocity of lysine uptake but the inhibition was not additive to that obtained with arginine. Under similar conditions, the interference of cycloheximide with arginine uptake was much less.

Difference of arginine and lysine in transport site affinity and in the mutual inhibition by these amino acids of their transport rates, as well as differences in response to cycloheximide treatment, suggest the existence of multiple transport sites for basic amino acids in the sugarcane cell.

Selective permeability of living cells to concentrate important nutrients against a concentration gradient has become a well-established fact in animal tissues as well as in microorganisms. The facilitation of movement of organic substances across membrane barriers by specific and active transport systems appears to be an essential feature common to all biological systems.

The precise mechanism responsible for the accumulation of amino acids in higher plant cells has not been reported, although similar processes can be tacitly assumed to function in higher plants as they do in other living organisms. Amino acids appear to be assimilated and transported by related but discrete systems and involve the mediation of agents fitting the description of permeases as originally conceived by Cohen and Monod (1957).

The concept of multiple amino acid transport systems was first proposed by Cohen and Rickenberg (1956) who measured the reversible, interdependent concentration by *Escherichia coli* of several exogenously supplied neutral amino acids and their effect on cell growth. Overlap of transport-mediating systems for neutral amino acids was demonstrated by Oxender and Christensen (1963) in Ehrlich ascites tumor cells and two sites were proposed to account for the accumulation of all amino acids in these cells. A relatively nonspecific transport system with affinity for both basic as well as a wide range of other amino acids has been shown to exist in old cultures of *Neurospora crassa* (Pall, 1969). However, most transport systems for basic amino acids appear to have specificity for these amino acids as a group (*e.g.*, Roess and DeBusk, 1968) or to have high specificity for either lysine or arginine. Specific transport systems for arginine have been reported in *Saccharomyces cerevisiae* (Grenson *et al.*, 1966) and *Escherichia coli* W (Wilson and Holden, 1969). Specific lysine transport systems were shown to exist in *Saccharomyces cerevisiae* (Grenson, 1966) and in rat kidney cortex (Segal and Smith, 1969). The interdependence of separate transport sites and their regulation are complicated (Benko *et al.*, 1967, 1968; Roess and DeBusk, 1968) and not yet well understood.

During an investigation of arginine metabolism in cell

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