area was correlated to the presence of these contractile cells which have many features of the muscular elements.

The larger quantity of 1–4 AP found in the peritubular cells of germinal aplasia, as compared with normal subjects and other pathological cases, could be explained by the fact that in germinal aplasia there is no utilization of muscular glycogen which is necessary when spermatogenesis is active[21].

Riassunto. La 1–4 amilofosforilasi è presente nel testicolo umano normale ove è localizzata nell'epitelio tubulare e in alcuni elementi peritubulari. Nella aplasia germinale la reazione è intensamente positiva solo in sede peritubulare, nell'arresto maturativo solo nel tubulo. L'enzima è probabilmente in rapporto con l'energia necessaria per la spermatogenesi e con la funzione contrattile delle cellule peritubulari.

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Effect of 3-Hydroxy-3-methylglutaric Acid on Blood Lipids in Normal and Cholesterol-Fed Rats

In view of the increasing association of elevated levels of blood cholesterol:phospholipid (C/P) ratio and triglycerides content with an increased risk of manifestation of atherosclerotic heart disease, numerous hypocholesterolemic agents have been intensively investigated for their possible hypolipemic action and prevention of atherosclerotic vascular diseases[1]. Since in this laboratory 3-hydroxy-3-methylglutaric acid (HMG) has been shown to possess hypocholesterolemic property[2,3], a systematic study on the hypolipemic role of HMG in male albino rats was undertaken.

Methods. The following methods were used for biochemical determinations: total, esterified and free cholesterol by the method of Bloor et al.[4]; phospholipids as described by Zilversmit[5]; total esterified fatty acids and triglycerides by the method of Reinhold et al.[6].

For Table I, 2 groups of young male albino rats, weighing about 110 g and each containing 5 animals were caged separately. Group I was kept as control and fed on basal diet. In addition to this diet, animals in group II were given i.p. injections of 20 mg HMG/kg/day in water for 3 or 6 days. For studies made in Table II, 5 animals weighing about 100 g (cholesterol-fed group) were kept on basal diet containing 2% cholesterol, 1% cholic acid and 5% hydrogenated vegetable oils. In addition to the above fat-rich cholesterol diet, other 5 animals (cholesterol plus HMG-fed group) were given 20 mg HMG/kg/day i.p. in water. The animals were fed the diet ad libitum for 25 days. For Table III, 10 male rats weighing about 100 g were maintained on fat-rich cholesterol diet for a period of 25 days and then divided into 2 groups of 5 each. The hyperlipemic control group was given basal diet only whereas the HMG-fed group received in addition to this diet i.p. injections of 20 mg HMG/kg/day in water for

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Table I. Effect of HMG on serum lipids of normal rats (average ± S.E.)

	Basal diet		Basal diet + HMG	
	3 days	6 days	3 days	6 days
Total cholesterol, mg %	70 ± 2	100 ± 2	60 ± 4[a]	83 ± 2[b]
Ester cholesterol, mg %	50 ± 2	74 ± 2	40 ± 4[a]	60 ± 2[b]
Free cholesterol, mg %	20 ± 3	26 ± 1	20 ± 3	23 ± 1
Total esterified fatty acids, meq/l	6 ± 0.36	5.2 ± 0.28	5.1 ± 0.44[a]	4.11 ± 0.14[b]
Phospholipids, mg %	186 ± 14	153 ± 5	187 ± 19	140 ± 8
Triglycerides, meq/l	1.45 ± 0.17	1.1 ± 0.16	0.53 ± 0.44[b]	0.4 ± 0.14[c]
C/P ratio	0.389 ± 0.001	0.660 ± 0.0004	0.339 ± 0.004	0.596 ± 0.011

[a] Difference as compared to parallel basal diet control statistically significant $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.01$.

6 days. In all the experiments, the parallel control group received injections of distilled water (1 ml) for the same duration as indicated. At the termination of studies, blood samples were obtained by cardiac puncture.

Results. It is evident from Table I that HMG administration to normal rats for 3 or 6 days significantly depresses the total cholesterol, esterified cholesterol, total esterified fatty acid and triglyceride levels. However, there is no significant effect on free cholesterol and phospholipid levels. The C/P ratio was insignificantly lowered. The data presented in Table II show that HMG treatment causes a significant decline in the levels of different lipids (except total and ester cholesterol) of hyperlipemic rats as compared with parallel cholesterol-fed group. The C/P ratio of these animals remains elevated, whereas triglycerides content is significantly depressed. In the case of Table III, where fat-rich cholesterol diet was substituted by the basal diet (though rats were hyperlipemic), C/P ratio was significantly lowered. In this experiment the decrease in various types of serum lipid contents was statistically significant.

It is interesting to mention that the weight of treated animals did not differ from that of respective control animals at the termination of the study. There was no significant difference between dietary intake of cholesterol-fed (11.5 ± 0.3 g/rat/day) and cholesterol plus HMG-fed rats (10.3 ± 2.3 g/rat/day).

Table II. Effect of cholesterol plus HMG feeding on serum lipids of hyperlipemic rats (average ± S.E.)

	Cholesterol-fed group	Cholesterol plus HMG-fed group
Total cholesterol, mg %	855 ± 35	780 ± 25
Ester cholesterol, mg %	673 ± 26	596 ± 44
Free cholesterol, mg %	183 ± 20	184 ± 6
Total esterified fatty acids, meq/l	27 ± 3	21 ± 2[a]
Phospholipids, mg %	341 ± 27	256 ± 23[b]
Triglycerides, meq/l	15 ± 2	11.5 ± 2[a]

[a] Difference as compared to cholesterol-fed group statistically significant $P < 0.05$; [b] $P < 0.02$.

Table III. Effect of HMG on serum lipids of hyperlipemic rats (average ± S.E.)

	Hyperlipemic control group	HMG-fed group
Total cholesterol, mg %	161 ± 10	104 ± 5[a]
Ester cholesterol, mg %	125 ± 12	67 ± 4[a]
Free cholesterol, mg %	37 ± 4	37 ± 2
Total esterified fatty acids, meq/l	9 ± 0.28	6 ± 0.5[a]
Phospholipids, mg %	182 ± 7	148 ± 8[b]
Triglycerides, meq/l	4 ± 0.28	2 ± 0.39[a]
C/P ratio	0.885 ± 0.004	0.675 ± 0.005[c]

[a] Difference as compared to hyperlipemic control animals statistically significant $P < 0.01$; [b] $P = 0.01$; [c] $P < 0.05$.

Discussion. It appears from the data that in normal animals HMG is not only capable of decreasing total cholesterol but also decreases other serum lipids (Table I). The animals receiving fat-rich cholesterol diet responded to HMG treatment and showed reduction in total cholesterol, ester cholesterol, total esterified fatty acids, phospholipids and triglycerides to the extent of 10, 12, 23, 25 and 23% respectively (Table II). However, the total and ester cholesterol are not significantly lowered for the simple reason that large quantity of cholesterol is being fed continuously; therefore the C/P ratio remains elevated although the triglycerides and phospholipids are significantly lowered. Nevertheless, treatment with HMG together with fat-rich cholesterol diet did check the rise of various lipids to a considerable extent. On withdrawal of cholesterol diet (Table III), the average C/P ratio decreased significantly to the extent of 25%. Esterified cholesterol levels were also correlated to serum phospholipid which changed from 0.68–0.456 or about 32% ($P < 0.01$). It is important to note that HMG causes maximum decrease in ester cholesterol and triglyceride levels (46 and 50% respectively). Evidently there was no effect of HMG on the free cholesterol content of normal as well as cholesterol-fed animals.

The ability of HMG to lower cholesterol, C/P ratio and triglycerides is of special importance, since elevations in these biochemical parameters have been reported as characteristic of individuals suffering from coronary heart disease and recently greater attention has been placed on the need for the evaluation of serum triglycerides than that of serum cholesterol[7–9]. It is interesting to note that HMG causes maximum reduction of triglycerides in normal as well as cholesterol-fed rats.

In comparison with other known hypocholesterolemic and hypolipemic drugs[1] which have harmful effects, HMG, in addition to its excellent hypocholesterolemic and hypolipemic actions, has been found to possess none of the harmful side effects as so far studied, common to other drugs[10].

Zusammenfassung. Intraperitonale Verabreichung von 3-Hydroxy-3-Methylglutarsäure setzt bei normalen Ratten den Serumspiegel von Cholesterin, Cholesterinestern, Esterfettsäuren und Triglyceriden signifikant herab. Bei einer cholesterinreichen Diät werden die Konzentrationen von Cholesterin und seinen Estern, bei hyperlipämischen Tieren die des Cholesterins im Serum nicht herabgesetzt. Die übrigen Lipidfraktionen verhalten sich wie im ersten Versuch.

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