Effect of 3-Hydroxy-3-methylglutaric Acid on Hypercholesterolemic Liver

In an earlier communication, we have shown that oral administration of 3-hydroxy-3-methylglutaric acid (HMG) to normal as well as hypercholesterolemic rats resulted in significant reduction of serum cholesterol[1]. Now we wish to report the effect of HMG on the microscopic anatomy and cholesterol content of the liver, which not only acts as the site of cholesterol biosynthesis[2] but also regulates the flow of cholesterol to blood[3].

Materials and methods. To study the effect of HMG on hepatic cholesterol, young male albino rats weighing 100 g were maintained for 2 weeks on an experimental diet. The liver cholesterol level of such hypercholesterolemic rats was found significantly elevated (1183 ± 36 mg %; $p < 0.001$) as compared to normal rats kept on basal diet only (525 ± 29 mg %). The rats were then divided into groups of 5 each. The cholesterol-fed and cholesterol-plus-HMG-fed rats continued receiving experimental diet. For the hypercholesterolemic control groups and HMG-fed groups, the experimental diet was replaced by basal diet. The experimental conditions were exactly the same as described earlier[1]. At varying periods of HMG treatment (as indicated), the total liver cholesterol was determined by the modified method of REINHOLD and SHIEL[4] (Table).

A histopathological study of the liver was carried out by conventional paraffin embedding after dehydration of tissue in alcohol. Sections of 4 μ thickness, cut on a rotary microtome, were stained with the routine hematoxylin and eosin and VAN GIESEN's differential stains. Figures 1–6 show sections of liver obtained from rats in various nutritional states.

Results. It appears from the Table that HMG administration to hypercholesterolemic rats significantly lowers the total cholesterol as compared to hypercholesterolemic control groups. It is also evident that as compared to cholesterol-fed groups, combined feeding of cholesterol along with HMG significantly depresses the hepatic cholesterol levels.

As compared to normal rats (Figure 1), sections obtained from livers of hypercholesterolemic rats (Figure 2) show intracellular fatty deposits as evidenced by areas of empty looking cells. At frequent places the whole liver tissue has been found converted into islands of fat cells. Figures 3–5 show sections of livers of hypercholesterolemic control rats and HMG-fed rats on fourth and sixth day of HMG treatment. It is observed that on the fourth day the liver cells of hypercholesterolemic control animals were still foamy (Figure 3), whereas those of HMG-fed hypercholesterolemic rats did not show any foamy appearance (i.e. lipid deposition) (Figure 4), though an increase in reticuloendothelial cells was seen in both the groups as compared to normal. On the sixth day of treatment, the sections of hypercholesterolemic liver still showed mild lipid deposition (Figure 5) although physiological levels of hepatic cholesterol were normal. However, in the case of HMG-fed rats, the liver cells show no evidence of fatty metamorphosis although reticuloendothelial cells remain

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Effect of HMG and cholesterol plus HMG feeding on liver cholesterol of hypercholesterolemic rats (average ± S.E.)

HMG treatment (day)	Total cholesterol content, mg/100 g liver			
	Cholesterol-fed group	Cholesterol + HMG-fed group	Hypercholesterolemic control group	HMG-fed group
1	1167 ± 52	994 ± 6[a]	1376 ± 38	912 ± 33[b]
2	1366 ± 31	789 ± 47[b]	1084 ± 42	633 ± 11[b]
3	1279 ± 25	958 ± 52[b]	934 ± 40	586 ± 28[b]
4	1258 ± 40	962 ± 34[b]	1078 ± 40	656 ± 8[b]
5	1566 ± 63	1004 ± 24[b]	896 ± 23	775 ± 36[c]
6	–	–	608 ± 18	626 ± 11

[a] Difference as compared to respective control group statistically significant $p = 0.001$; [b] $p < 0.001$; [c] $p < 0.02$.

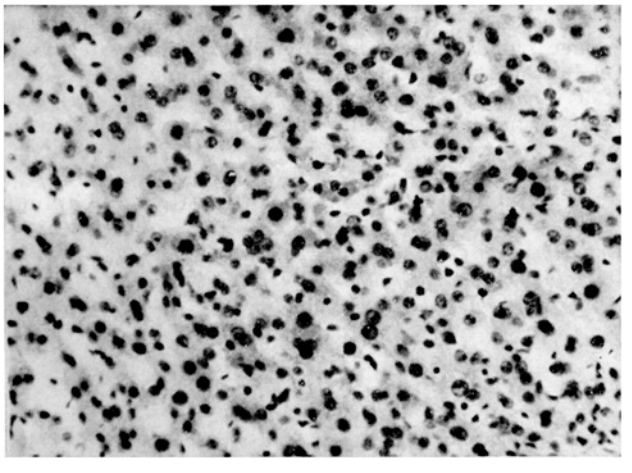

Fig. 1. Normal architecture of rat liver. Light separation bands in between cells are artifacts. H & E × 288.

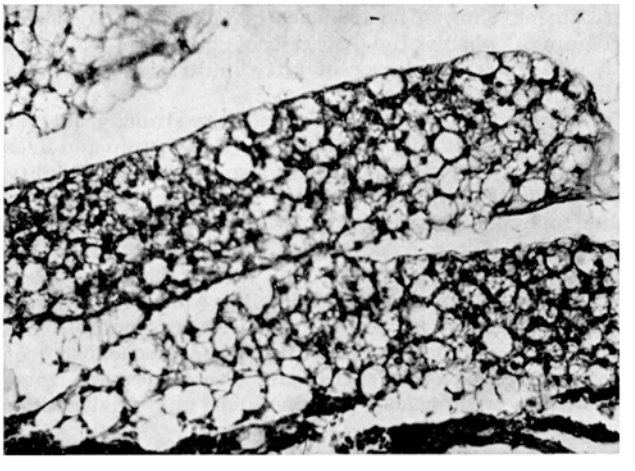

Fig. 2. Advanced fatty metamorphosis as evidenced by islands of empty looking liver cells. Broad empty bands are artifacts produced by undue hardening during fixation. H & E × 288.

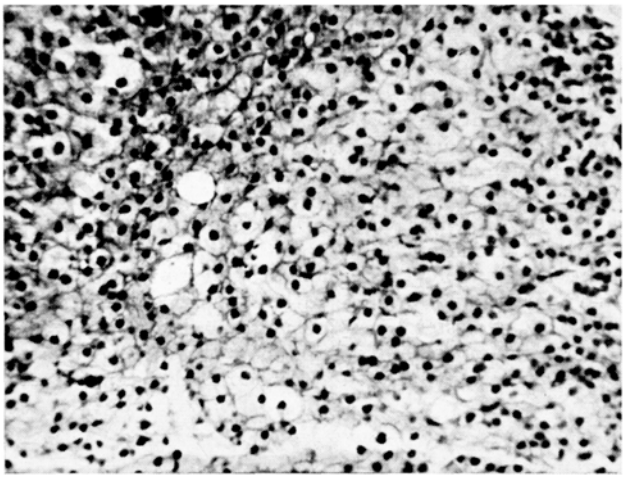

Fig. 3. Moderate degree of fatty metamorphosis. H & E ×288.

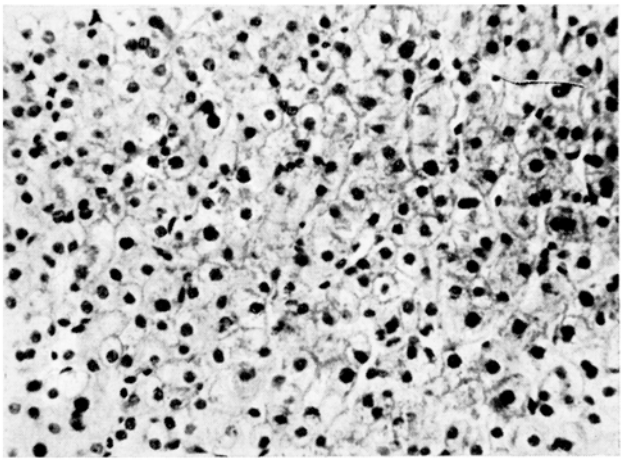

Fig. 5. Mild degree of fatty metamorphosis. H & E × 288.

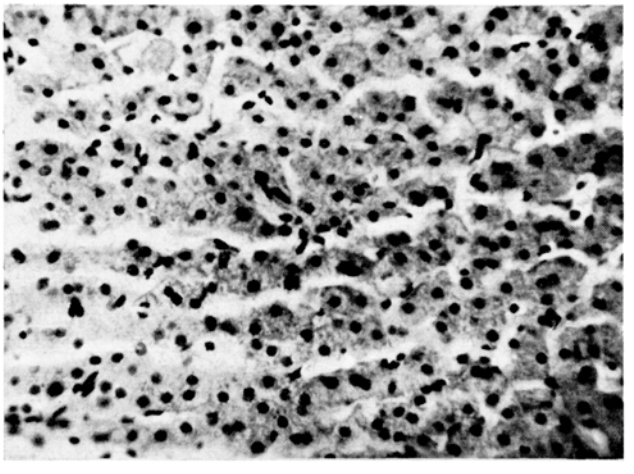

Fig. 4. Normal liver parenchyma, but an increase in reticulo-endothelial cells. Separation bands in between cells are artifacts. H & E × 288.

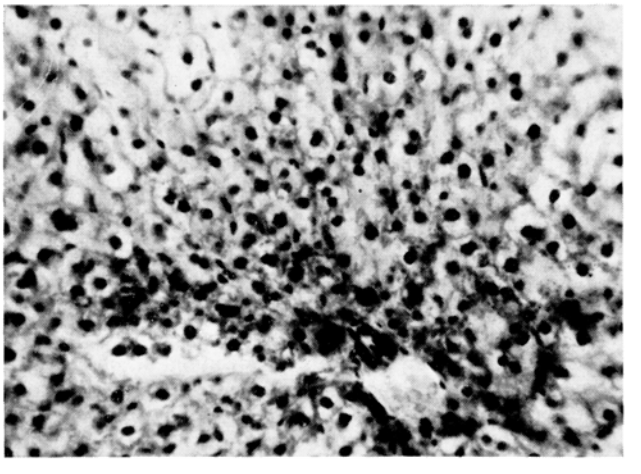

Fig. 6. Normal liver cells, fatty changes being reversed under HMG. H & E × 288.

prominent in number as in Figure 4. The liver cells of cholesterol plus HMG-fed group on the fourth day of treatment, showed no evidence of fatty metamorphosis (Figure 6), whereas the liver cells of cholesterol-fed group showed moderate degree of fatty infiltration similar to those observed in Figure 2.

Our previous findings and these data strongly suggest that HMG is not only capable of lowering serum and liver cholesterol levels of animals, but also possesses the ability to reverse the untoward changes in the liver parenchyma due to hypercholesterolemia at a quicker rate than do the body's homeostatic mechanisms. Further it has no hepato-toxic effect at microscopic level at the dosage used in the present investigations. Further work is in progress to establish how far HMG administration modifies the atheromatous changes and affects the biochemistry of blood in terms of esterified and free cholesterol, total esterified fatty acids, phospholipids and triglycerides content[5].

Zusammenfassung. Fütterung mit 3-hydroxy-3-Methylglutarsäure (HMG) führt bei hypercholesterin-ämischen Ratten zu einer bedeutenden Senkung des Leber-Cholesterinspiegels. Gleichzeitige Verabreichung von HMG und Cholesterol oder von Cholesterol allein ergab eine geringe Zunahme des Cholesterolspiegels im ersten Fall. Die histopathologische Leberuntersuchung zeigte, dass HMG-Behandlung eine schnelle Normalisierung der Fettveränderungen verursachte, ohne dass toxische Wirkung auf die Zellgewebe auftrat.

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