V. HISTOPATHOLOGICAL STUDIES
RESULTS

Effect of HMG on Different Tissues of Normocholesterolemic Rats - Normal five young male Albino rats weighing about 100 g, were orally given 20 mg HMG/Kg/day for 4 weeks. Liver, heart, kidney, adrenals, spleen, aorta, brain and lungs of HMG-treated and untreated animals were taken out and preserved in 10% formalin. Histological study was carried out by conventional paraffin embedding. Sections of four micron thickness were cut on a rotary microtome, stained with haematoxylin and eosin. On microscopical examination the cellular architecture of HMG-treated tissues was normal and similar to untreated animals.

These studies showed that HMG treatment did not induce any toxic or abnormal changes at the microscopic level of the tissues examined.

Effect of HMG on Fatty Livers of Hypercholesterolemic Rats - The livers of rats, used for Table V, were taken out and preserved in 10% formalin. The histopathologic studies were carried out by conventional paraffin embedding after
dehydration of the tissue in alcohol. Sections of four micron thickness were cut on a rotary microtome, stained with the routine haematoxylin and eosin stain.

A histopathological study on livers from normal rats (525 ± 29 mg%), revealed the cellular architecture of the normal liver. The parenchymatous cells are normal (Fig. 7). When compared to the hypercholesterolemic liver, (1783 ± 36 mg%), the section shows intracellular fatty deposits as evidenced by areas of empty looking cells (Fig. 8). At frequent places whole liver tissue was found converted into islands of fat cells.

Histological studies were then extended towards the livers of HMG-treated hypercholesterolemic rats. Figures 9 to 11 show sections of livers of hypercholesterolemic control rats and HMG-fed rats on 4th and 6th day of HMG treatment. It is observed that on 4th day the liver cells of hypercholesterolemic control group animals, were still foamy (Fig. 9) where as those of HMG-fed rats did not show any foamy appearance (Fig. 10), though an increase in reticuloendothelial cells (RE cells) was seen in both the groups as compared to normal (Fig. 7). It is interesting to mention that cholesterol content of livers of HMG-treated
Fig. 7. Normal architecture of rat liver (H & EX 288). Separation bonds in between cells are artifacts. See text for details.

Fig. 8. Advanced fatty metamorphosis as evidenced by islands of empty looking liver cells (H & EX 288). Broad empty bands are artifacts produced by undue hardening during fixation. See text for details.
Fig. 9. Moderate degree of fatty metamorphosis (H & EX 288). See text for details.

Fig. 10. Normal liver parenchyma with increase in reticuloendothelial cells (H & EX 288). See text for details.
rats touched the normal level (656 ± 8 mg%) whereas that of hypercholesterolemic control rats remained significantly elevated (1078 ± 40 mg%; p < 0.001). On 6th day of treatment, the sections of hypercholesterolemic control liver (608 ± 18 mg%), still showed mild lipid deposition (Fig. 11) although physiological levels of hepatic cholesterol were normal. However, in case of HMG-fed rats, (626 ± 11 mg%) the liver cells show no evidence of fatty metamorphosis though RE cells remain prominent in number as in (Fig. 10). In cholesterol plus HMG-fed group, the livers section on 4th day (962 ± 34 mg%) were compared with that of parallel cholesterol-fed group (1258 ± 40 mg%). The liver cells of cholesterol plus HMG-fed group rats showed no evidence of fatty metamorphosis (Fig. 12) whereas the liver cells of cholesterol-fed group rats showed moderate degree of fatty infiltration similar to those observed (Fig. 8).
Fig. 11. Mild degree of fatty metamorphosis (H & Ex 288). See text for details.

Fig. 12. Normal liver cells, fatty changes being reversed under HMG (H & Ex 288). See text for details.
DISCUSSION

Several cholesterol decreasing agents, e.g., SKF 525-A and SKF 3301-A are capable of inducing a reversible fatty changes of the liver. On the basis of histological and histopathological studies of HMG-treated tissues of normocholesterolemic and hypercholesterolemic rats, (Figs. 7 to 12) the following conclusions are made:

1. HMG-feeding did not induce any toxic effects or untoward changes within the cellular architecture of liver, heart, kidney, adrenals, spleen, aorta, brain and lungs.

2. Feeding of fat-rich cholesterol diet to animals produced fatty metamorphosis of livers which is in agreement to earlier findings (Swell and Flick, 1953) (Fig. 8).

3. As compared to hypercholesterolemic control rats (Fig. 9), administration of HMG to hypercholesterolemic animals caused quicker reversal of fatty
changes towards normalcy (Fig. 10).

4. The physiological lowering of hepatic cholesterol levels in hypercholesterolemic control rats was slower as compared to HMG-treated hypercholesterolemic animals on the 4th day of treatment (Table V). The same was found true at the microscopic level also, as evidenced by the absence of foamy cells in the HMG-fed group as compared to foamy cells in hypercholesterolemic group (Figs. 10 and 11).

5. Administration of HMG along with cholesterol did not induce any fatty deposition (Fig. 12).

The general rise in the number of hepatic RE cells of hypercholesterolemic rats and an appreciable increase in the sections of HMG-fed rats is due to operation of a simple defensive mechanism, because the breakdown of cholesterol is known to involve metabolism by reticulo-endothelial system (RES) (Friedman and Byers, 1954; St. George et al., 1954; Neven et al., 1956; DiLuzio, 1960). Therefore agents known to stimulate RES function may be effective to lower the incidence of atherogenesis associated with hypercholesterolemia. Estrogens have been demonstrated to enhance the RES activity (Heller et al.,
and, in fact, any protective effect of estrogens against coronary atherogenesis had been found to depend on an intact proliferative capacity of RES (Pick et al., 1962). Similarly the hypocholesterolemic action of hexestrol has been associated with increase in RES activity (Boyd, 1962). Therefore, it is quite likely that protective effect of HMG against hepatic hypercholesterolemia and fatty infiltration may be attributed to increase in RES cells.