Acute Changes in Liver Lipids during Myocardial Infarction Induced by Isoproterenol* (33778)

JOSEPH T. JUDD AND BERNARD C. WEXLER[1]
(With the technical assistance of David D. Shaw)
May Institute for Medical Research of the Jewish Hospital and Department of Pathology, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45229

The potent catecholamine, isoproterenol, will induce myocardial infarcts in rats (1, 2). The severity of the infarct may be regulated on a dose:body weight basis. We have found that serum transaminase levels (SGOT, SGPT) and lactic dehydrogenase undergo rise and fall with the acute onset and repair of the myocardial infarct in a pattern similar to man following a heart attack (2). Changes in adrenal histology (2) and *in vitro* adrenal steroidogenic patterns indicate that there is maximal stimulation of the adrenal cortex during the acute onset of myocardial necrosis (3). Concomitant with the appearance and gradual resolution of severe congestive heart failure in these rats there is a significant increase in aldosterone production accompanied by diuresis as the increased aldosterone production subsides (3). During the acute onset of the myocardial infarct we have observed striking fatty infiltration of the liver, hyperlipidemia, accompanied by disappearance of periadrenal and body adipose tissue (4). During the repair phase the fatty liver and hyperlipidemia disappear. Further investigations demonstrated that during the acute myocardial necrosis phase there is also marked hyperglycemia and hyperlipidemia accompanied by alterations in serum total protein and changes in the normal alpha:beta lipoprotein ratio (5). Again, all of these metabolic changes subside and return toward normal during the repair phase.

We believe that the simultaneous disappearance of body adipose tissue and accumulation of fat in the liver is due to the mobilization of lipid from adipose tissue sites to the liver during the acute duress of myocardial infarction. Because of the temporary lack of sufficient protein or lipoprotein lipid-carrying agents the mobilized lipid accumulates as triglyceride and remains in the liver. During the myocardial repair phase when lipoprotein again becomes available (5) the fatty liver condition is resolved. In order to investigate these dynamic hepatic lipid changes further we made a daily analysis of hepatic lipids of rats during the acute development and repair phases of myocardial infarction.

Methods. Adult, male virgin rats (Blue Spruce Farms) of the Long-Evans strain averaging 375 ± 10 g were given two subcutaneous injections of isoproterenol (HCl) dissolved in distilled water at 24-hr intervals. A dose of 20 mg/100 g of body wt. was used because at this dose level massive myocardial infarction may be produced with approximately 60% of the subjects surviving. Groups of five animals each were sacrificed at 4 and 28 hr after the first injection (the 28-hr group being 4 hr after the second injection) and at 2, 3, 4, 5, 6, and 7 days.

At autopsy, all of the animals were sacrificed by decapitation to avoid the stress of anesthesia. The entire liver was removed and weighed as quickly as possible. Each liver was flushed with isotonic saline to remove as much blood as possible. The livers were placed in a large tube and then frozen by immersion in a dry ice–acetone bath and stored at −10° until analyzed.

Lipid extraction. The entire liver was homogenized in a Ten-Broeck homogenizer, diluted to a given volume, and aliquots were taken for analysis. Lipid extracts were prepared by the method of Folch *et al.* (6). Lipid phosphorous was determined by a

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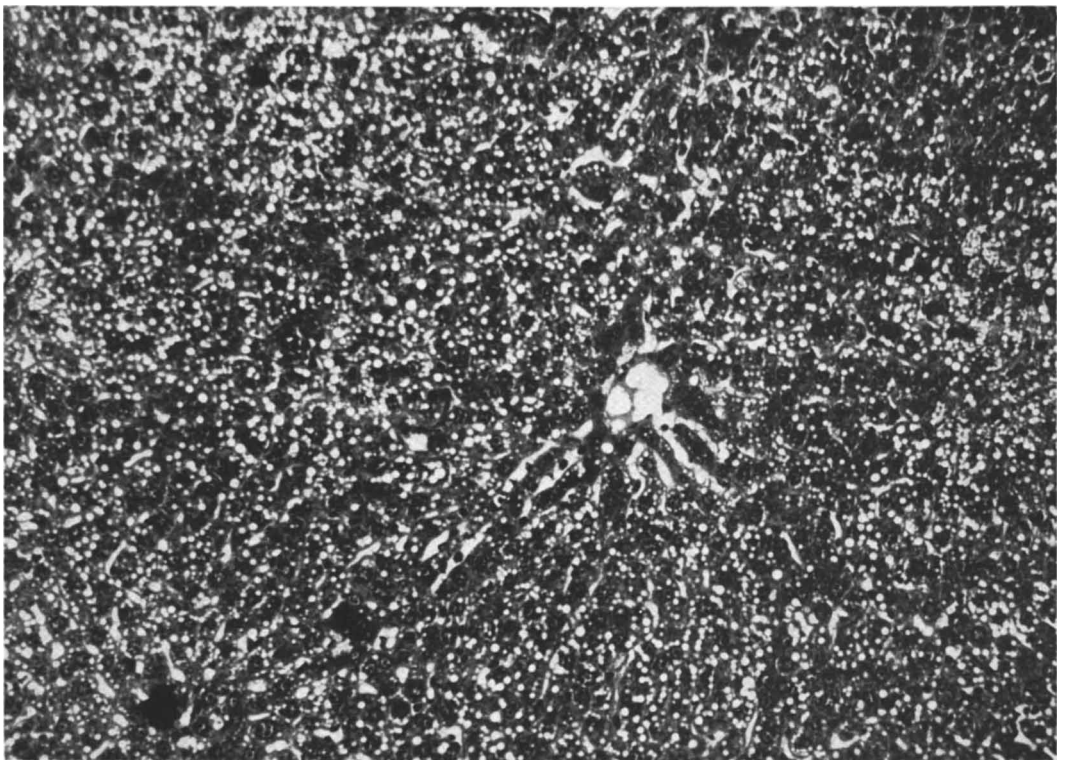

FIG. 1. Liver of an adult, male Long-Evans rat 4 hr after a second (s.c.) injection of isoproterenol. The multitude of droplets demonstrate the intense fatty infiltration of the liver which occurs coincidental with the development of acute myocardial necrosis. This condition of hepatic fatty metamorphosis clears rapidly coincidental with myocardial repair; H and E, ×100.

modification of the Fiske and Subbarow method as described by Bartlett (7). Triglycerides were determined by the method of Carlson (8) and the glyceride content estimated as triglyceride by reference to tripalmitin standard. Lipid-free dry weight was determined gravimetrically on an aliquot of liver homogenate after extraction with a mixture of chloroform:methanol (2:1). Total cholesterol was analyzed by using a commercially-prepared Lieberman-Burchard reagent (Hycel).

These experiments were designed so that an analysis of variance for data with a single classification could be used in analyzing results. The Student's *t* test was used to evaluate the statistical differences between the means of two compared groups.

Results. Massive myocardial infarction consisting of splotchy and confluent areas of necrosis was produced in these Long-Evans rats similar to the myocardial infarction in Sprague-Dawley rats as described by us previously (2–5). The adrenal glands were hypertrophied, depleted of glandular lipid and the periadrenal adipose tissue reduced to a watery tag of involuted tissue. As in Sprague-Dawley rats with isoproterenol-induced myocardial infarcts this increased adrenocortical activity (2–4) was accompanied by marked involution of the thymus gland. All of the experimental animals lost considerable amounts of body weight, especially during the acute myocardial necrosis phase (1–3 days). As in all of our previous experiments the liver underwent fatty metamorphosis which was quite apparent by gross examination within 28 hr after the injection of isoproterenol. On microscopic examination the fatty livers manifested intense, diffuse distribution of uniform lipid droplets without any apparent lobular arrangement (Fig. 1). This fatty

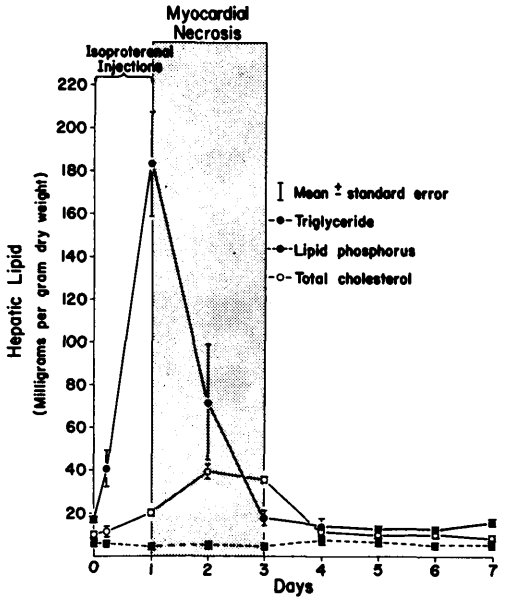

FIG. 2. Dynamic changes in hepatic triglyceride, cholesterol, and phospholipid following the injection of isoproterenol. Each point represents the average of the analyses of five livers. The shaded area indicates the time period when maximum myocardial necrosis is observed histologically.

infiltration of the liver began to clear by the fourth day and the liver appeared grossly normal by the fifth day, the beginning of myocardial repair.

Chemical analysis of hepatic lipid fractions demonstrated a very prompt and striking increase in the triglyceride content of liver of the isoproterenol-injected animals. This increase was apparent within 4 hr after the first injection of isoproterenol (Fig. 2). Hepatic triglyceride content continued to rise reaching a peak, considerably above normal, 4 hr after the second injection of isoproterenol. Concomitant with establishment of maximal myocardial necrosis, the hepatic triglyceride content fell precipitously and in parallel with the grossly-observable clearance of the fatty liver condition. By the third day, when myocardial necrosis had reached its zenith and repair began, the hepatic triglyceride fraction assumed its initial level (Fig. 2). Analysis of variance indicated that these changes were highly significant ($p<.01$). Hepatic cholesterol content rose significantly, but not as dramatically or as promptly as the triglyceride fraction (Fig. 2). The peak in hepatic cholesterol occurred on the second day and remained elevated on the third day following injection of isoproterenol (Fig. 2). These progressive changes in hepatic cholesterol during the infarct-producing regime were also highly significant statistically ($p<.01$). Lipid phosphorous or hepatic phospholipid showed only relatively minor fluctuations during the course of this experiment (Fig. 2).

Discussion. These experiments demonstrate that the fatty liver which develops during the acute phase of myocardial infarction induced by the potent catecholamine, isoproterenol, is due to a profound increase in hepatic triglyceride content accompanied by a marked, but less dramatic increase in hepatic cholesterol. Whether this fatty metamorphosis of the liver is due to the myocardial infarction *per se* (2–5) or to the lipid-mobilizing effects of the adrenal cortical hormones liberated during the duress of myocardial infarction (2–4) or rather to the lipid-mobilizing effects of the catecholamine will require further investigation. In addition to the gross disappearance of periadrenal, mesenteric, and other adipose tissue depots during the acute stages of myocardial infarction we have also observed significant increase in serum cholesterol (2, 4, 5) triglyceride (5) and free fatty acids (5) concomitant with this dissolution of adipose tissue. We have also observed very marked alterations in serum protein and lipoprotein fractions coinciding with the hyperlipidemia and the appearance of a fatty liver (5). Apparently, the acute lack of available lipid-carrying protein during the onset of myocardial necrosis disrupts adequate portage of rapidly accumulating lipid from the liver to the serum. The intense mobilization of lipid from adipose depots presents lipid to the liver where it is stored largely as triglyceride. In time, as lipoprotein lipid-carrying agents become available the excess hepatic triglyceride is removed, as is cholesterol. These dynamics of hepatic lipid receipt, storage, release, and transport may well be of

vital significance since it is now known that both the normal and ischemic myocardium utilizes lipid for energy purposes (9). The adrenal steroids have also been shown to have strong lipid-mobilizing effects. Their dynamic changes during this same critical period (3) must also be considered as a significant physiological vector.

Summary. Adult, male Long-Evans rats were challenged with two subcutaneous doses of the potent catecholamine, isoproterenol. Within hours after the first injection myocardial ischemia and necrosis became apparent. On the second day, after the second injection, myocardial necrosis reached a zenith followed by myocardial repair during days 4–7 after the initial injection. During the development of myocardial ischemia and necrosis there is dramatic loss of body weight; dissolution of periadrenal, mesenteric, and other adipose tissue depots; hyperlipidemia; and grossly-visible fatty metamorphosis of the liver. During the myocardial repair phase the hyperlipidemia and the condition of fatty liver disappear rapidly. Chemical analysis of the hepatic lipid fractions demonstrate that there is a very considerable and rapid increase in hepatic triglycerides, a less marked and less rapid but significant increase in hepatic cholesterol, and only a modest fluctuation in hepatic phospholipid.

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