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Isoproterenol-Induced Myocardial Infarction in the Gerbil (*Meriones unguiculatus*)[1] (37958)

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The potent beta-adrenergic stimulating agent isoproterenol will induce myocardial necrosis in rats (1). The severity of the myocardium infarcted may be regulated on a dose:body weight basis. However, the age and body weight and the strain of the rat employed, particularly the Sprague–Dawley rat, will alter the dose:body weight response (2–4). We have been producing massive myocardial infarction in the isoproterenol-resistant Sprague–Dawley rat by using relatively large doses of this catecholamine (2–4). This "syndrome" of massive myocardial infarction is accompanied by congestive heart failure, hyperglycemia, hyperlipidemia, dynamic changes in serum enzymes, blood urea nitrogen, and marked changes in adrenocortical steroids (2–4). The dynamic oscillations in these pathophysiologic parameters reflect the various phases of acute myocardial infarction, i.e., increasing during acute ischemia and infarction and subsidence during the repair phase (2–4). Adrenocortical steroidogenesis also appears to be related to the necrosis and repair phases in that excessive mineralocorticoid production attended by hydrothorax and anuria appears to be related to the acute necrosis phase concomitant with a decreased glucocorticoid production and falling blood pressure, shock, and death (2–4).

The gerbil (*Meriones unguiculatus*), unlike the rat but like man, produces the adrenal steroid 17-hydroxy-corticosterone (cortisol, Cmpd. F) predominantly (5–7). Because of our interest in the role of the adrenal cortex during the duress of acute myocardial infarction, we investigated the pathophysiologic response pattern of the gerbil, a predominantly Cmpd. F producing animal, and its response to myocardial infarct-inducing doses of isoproterenol, comparing this response pattern of the gerbil with that of the Sprague–Dawley rat which produces corticosterone (Cmpd. B) predominantly.

Methods. Adult, male gerbils, 12 months old (Tumblebrook Farms, New York, NY), were fed a commercial diet (Agway Inc., Syracuse, NY) which has a low fat content. In a series of preliminary experiments, the gerbil was found to be much more sensitive to the infarct-inducing effects of isoproterenol compared to the Sprague–Dawley rat, i.e., 25–50 mg of isoproterenol/100 body wt will produce massive myocardial infarction in the Sprague–Dawley rat with 50–60% survival, whereas only 1 mg/100 g body wt was sufficient to induce grossly visible myocardial infarction and 60% survival in the gerbil. Accordingly, 200 gerbils were given two sc injections of isoproterenol, each injection spaced 24 hr apart, at a dose level of 1 mg/100 g body wt. Groups of animals were sacrificed 4, 6, 12, and 24 hr after the first injection and at the same intervals after the second injection, as well

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as 48 hr later (Day 3) and a final group on the seventh day after the second injection. A minimum of 12 animals were used for each determination. A group of 45 gerbils, given two sc injections of distilled water (diluent for isoproterenol), served as controls.

The animals were sacrificed by decapitation to avoid the stress of anesthesia. (Because of the small size of the gerbil, the blood of three gerbils had to be pooled in order to provide sufficient serum to complete the spectrum of multiple determinations described below). Blood was collected from the severed neck vessels and centrifuged (refrigerated) and the following parameters measured using the automated methods prescribed for the Autoanalyzer (Technicon): creatine phosphokinase (CPK), triglycerides, free fatty acids, total cholesterol, glucose, and blood urea nitrogen (BUN). The serum cortisol or 17-hydroxy-corticosterone levels were determined by the Van der Vies method (8) as modified by Smith and Muehlbaecher (9).

The heart of each animal was carefully examined for any gross evidence of pathology. The final body weight, heart, kidney, adrenal and thymus glands, and testes were carefully trimmed of extraneous tissue and weighed. The heart and other pertinent organs from each animal were fixed in 10% buffered neutral formalin (Lillie) for histopathological analyses.

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

Results. Although 40% of the gerbils died after two injections of isoproterenol (1 mg/100 g body wt) during the acute myocardial ischemia phase (Days 1 and 2), there were only a few streaks of grossly visible necrosis in the left ventricle on Day 3, when confluent myocardial necrosis reaches a zenith and is readily apparent in the rat (2–3). Unlike the rat, the gerbil manifested very little evidence of congestive heart failure, i.e., hydrothorax, which is a consistent finding in the Sprague–Dawley rat when subjected to a massive myocardial infarct. Like the rat, these gerbils immediately became prostrate and showed extreme tachycardia within minutes after the first injection of isoproterenol. Six to twenty-four hours later, however, they showed progressive evidence of recovery despite anuria. Following the second injection of isoproterenol, the gerbils again manifested tachycardia, anuria, and prostration. Most of the animals succumbed after the second injection (Day 2). By Day 3, myocardial necrosis (by gross and histopathologic examination) reached a maximum and then rapidly became resolved during Days 4–8.

In addition to the dynamic changes in the gross appearances of the hearts of these animals, their livers showed a marked but evanescent fatty metamorphosis which became evident within hours after the first injection, becoming progressively intense and maximal by Day 3, but then began to fade, becoming almost completely free of lipid by Day 8. Periadrenal and mesenteric adipose tissue rapidly disappeared (Days 1 and 2). Gravimetric analysis of the daily, temporal changes in organ and body weights demonstrated a statistically significant ($P < 0.001$) and progressive increase in adrenal glandular weight (Day 0 = 21.5 ± 3 mg vs Day 3 = 23 ± 4 mg vs Day 8 =29 ± 3 mg) concomitant with progressively severe (and statistically significant ($P < 0.001$)) thymus gland involution (Day 0 = 105 ± 2 mg vs Day 3 = 38 ± 6 mg vs Day 8 = 35 ± 5 mg). Hearts, kidneys, and body weight were greatly increased in size and weight ($P < 0.001$), reaching a peak on Day 3 when myocardial necrosis was most pronounced, and then all showing reduction in size, virtually to normal, by Day 8 when myocardial repair was established, e.g., heart weights: Day 0 = 237 ± 4 mg vs Day 3 = 338 ± 18 vs Day 8 = 252 ± 9 mg.

Chemical analysis of serum. Creatine phosphokinase (CPK): Serum CPK levels began to rise within minutes after the first injection of isoproterenol (Fig. 1). CPK levels continued to rise markedly ($P < 0.001$), reaching a maximum 12 hr after the first injection, and then began to recede

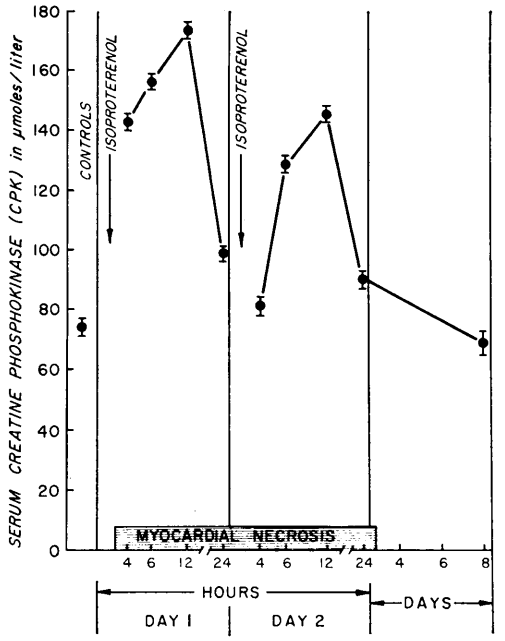

FIG. 1. Changes in serum CPK levels (mean ± standard error) of adult, male gerbils at various time intervals after two sc injections of isoproterenol spaced 24 hr apart. Myocardial necrosis is an on-going event and becomes maximal between the second and third day. Myocardial repair is rapid and occurs between the fourth and eighth day.

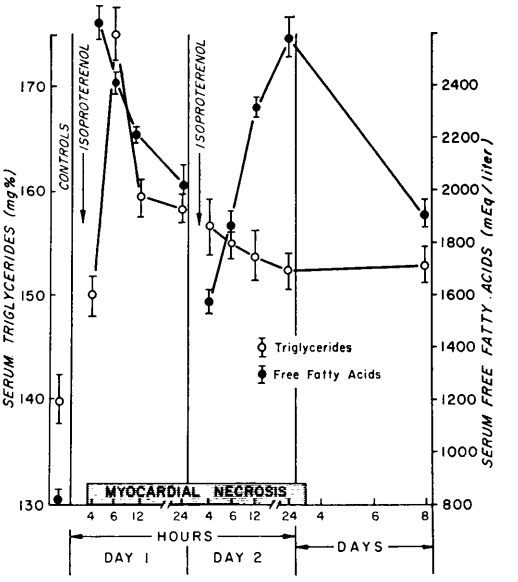

FIG. 2. Changes in serum triglycerides and free fatty acids.

coincident with the apparent recovery of the animals from the cardiac-stimulating effects of isoproterenol. Following the second injection of isoproterenol, circulating CPK levels were again increased until maximum myocardial necrosis was attained (Fig. 1). As the myocardium began to undergo repair, the serum CPK levels fell and were restored, once again, to normal levels.

Triglycerides, free fatty acids, and cholesterol: On Days 1 and 2, when peripheral adipose tissues were undergoing active dissolution and the liver displayed extensive fatty metamorphosis, serum triglycerides and free fatty acids became greatly elevated ($P < 0.001$). Although the serum triglyceride levels were promptly and greatly increased on Day 1, there was little or no response to the second injection of isoproterenol on Day 2 (Fig. 2). Free fatty acids showed very marked increases after each of the two injections of isoproterenol, but remained at a distinctly above-normal level during the remaining course of the experiment (Days 3–8) (Fig. 2). Serum cholesterol changes followed a similar pattern of dynamic increase after each injection of isoproterenol and, like the free fatty acids, remained at above-normal levels during Days 3–8 (Fig. 3).

Glucose and BUN: Prompt and marked hyperglycemia developed during the ischemia and necrosis phase (Days 1 and 2) but remained elevated, as did cholesterol and free fatty acids, during the myocardial repair phase (Fig. 3). BUN levels did not change remarkably.

Cmpd. F: The circulating Cmpd. F level was unusually elevated during the early stages of myocardial ischemia (Day 1) (Fig. 4). However, no further increase in Cmpd. F production was elicited on Day 2, when cardiac necrosis was actively becoming established. Instead, Cmpd. F levels fell precipitously and steadily and remained at considerably below-normal levels throughout the myocardial repair phase (Days 3–8).

Histopathology. Instead of the confluent,

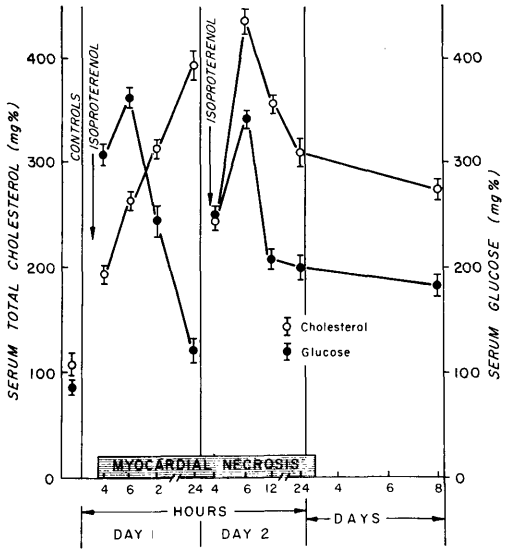

FIG. 3. Changes in serum total cholesterol and glucose.

through and through necrosis observed in Sprague–Dawley rats when they develop massive, isoproterenol-induced myocardial necrosis (2–4), these gerbils manifested grossly visible but limited, longitudinal streaks confined entirely to the myocardium proper without any evidence of ischemic necrosis in the endo- or epicardium. These distinct but limited streaks, confined to the apex and left ventricle, were most prominent on Day 3, becoming progressively indistinct during each successive day of repair (Days 4–8). Little or no myocardial fibrosis could be found microscopically by Day 8. However, scattered foci of degenerating muscle fibers covered with granular, deeply basophilic material were encountered (Fig. 5). Fibroblasts and white blood cells were absent. This granular, basophilic material stained, very positively, for calcium and mucopolysaccharide. Microscopically, the fatty livers displayed intense lipid infiltration without any specific pattern in a generalized parenchymatous distribution (Fig. 6). The gerbil has abundant and hyperplastic pancreatic islet tissue with intensely staining beta cells (Fig. 7). During the hyperglycemia of both the acute ischemia, necrosis, and repair phases, the islets of these gerbils manifested intense depletion of beta cell granularity and an unusual peri-insular infiltration of large, globoid adipocytes (Fig. 8).

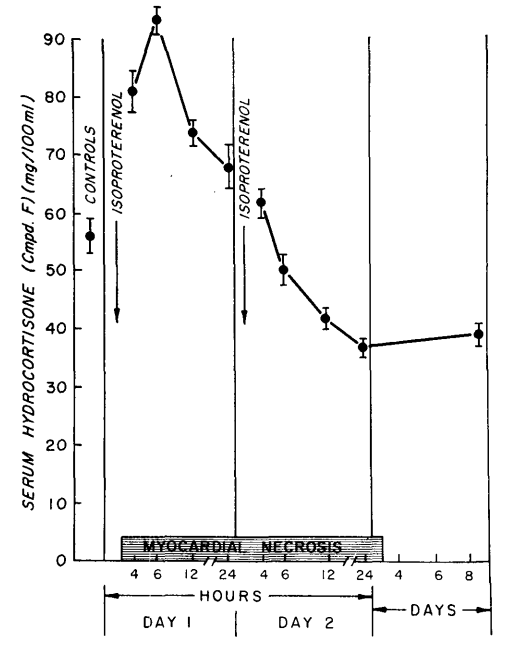

FIG. 4. Changes in serum hydrocortisone.

Discussion. These findings demonstrate that although the rat and the gerbil vary in their sensitivity to an isoproterenol-induced myocardial infarction, their overall pathophysiologic response pattern is quite similar. It is of interest that although the rat produces Cmpd. B and the gerbil Cmpd. F, they each manifest very marked increase in Cmpd. B or F production during the initial duress of acute myocardial ischemia (Day 1) followed by a definite period of subnormal Cmpd. B or F steroidogenesis during the necrosis and repair phases (Days 2–8). Our investigations of massive myocardial infarction in the Sprague–Dawley rat indicate that under these circumstances, adrenocortical steroidogenic pathways favor the production of mineralocorticoids, e.g., aldosterone (3) almost to the exclusion of glucocorticoids. This excessive production of aldosterone is related to the anuria and congestive heart failure, the decreased glucocorticoid production (Day 2) is related

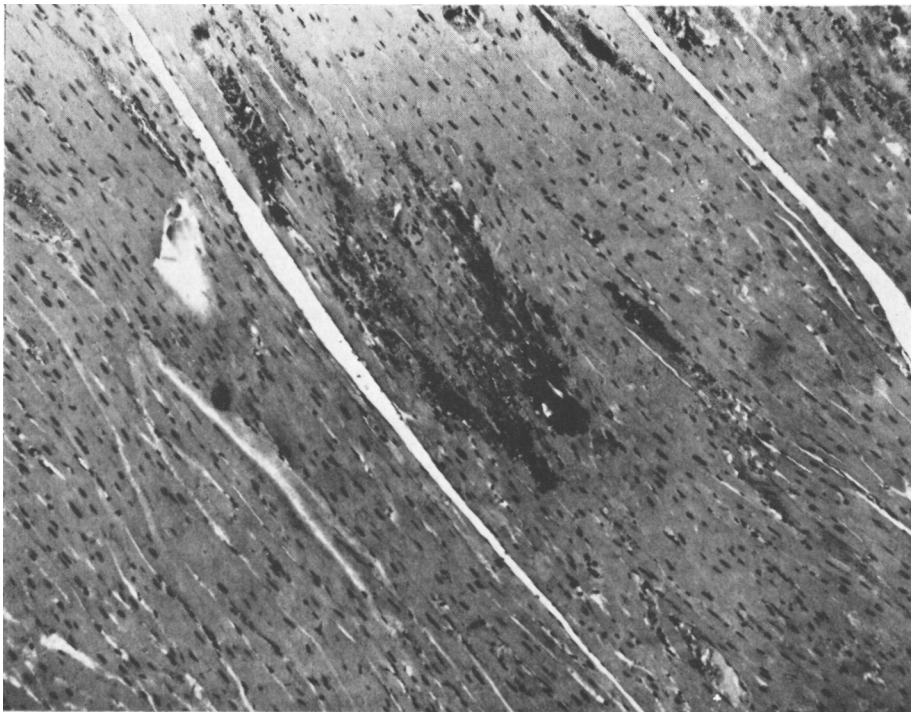

FIG. 5. Foci of persistent myocardial damage in an adult, male gerbil 8 days after the induction of myocardial necrosis with isoproterenol. The injured myocardial foci are covered with a strongly basophilic, granular material (deep black in photo) which appears to be an admixture of calcium and mucopolysaccharide. There are no white blood cells or fibroblasts. H & E, ×50.

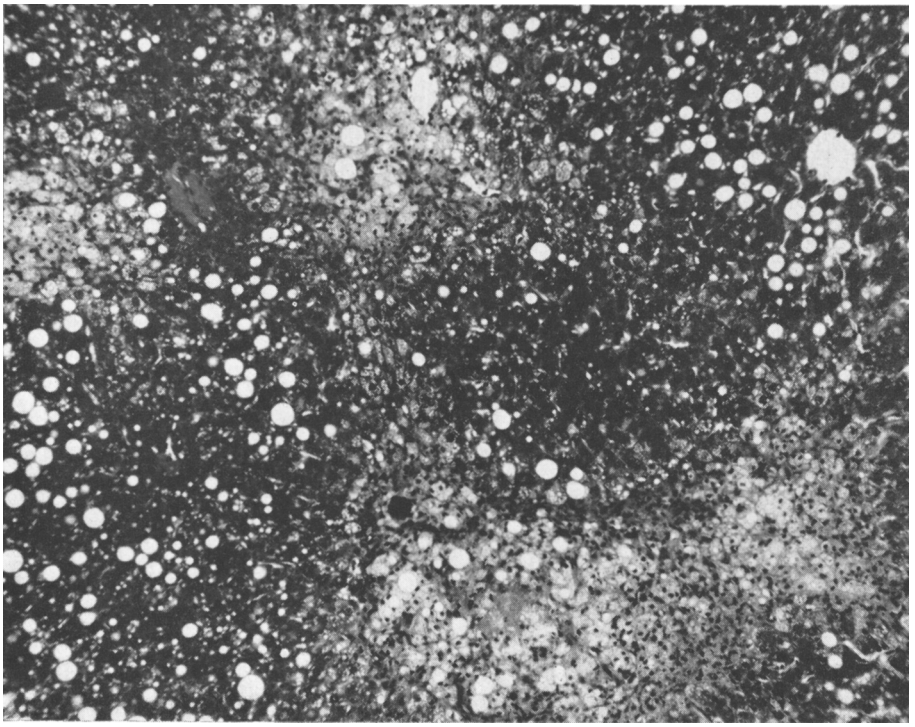

FIG. 6. Severe hepatic steatosis which develops in the gerbil during the first two days of acute cardiac ischemia and beginning necrosis. The lipid occurs as single, isogenous or coalescent globules. There is no distinct lobular pattern of distribution. H & E, ×150.

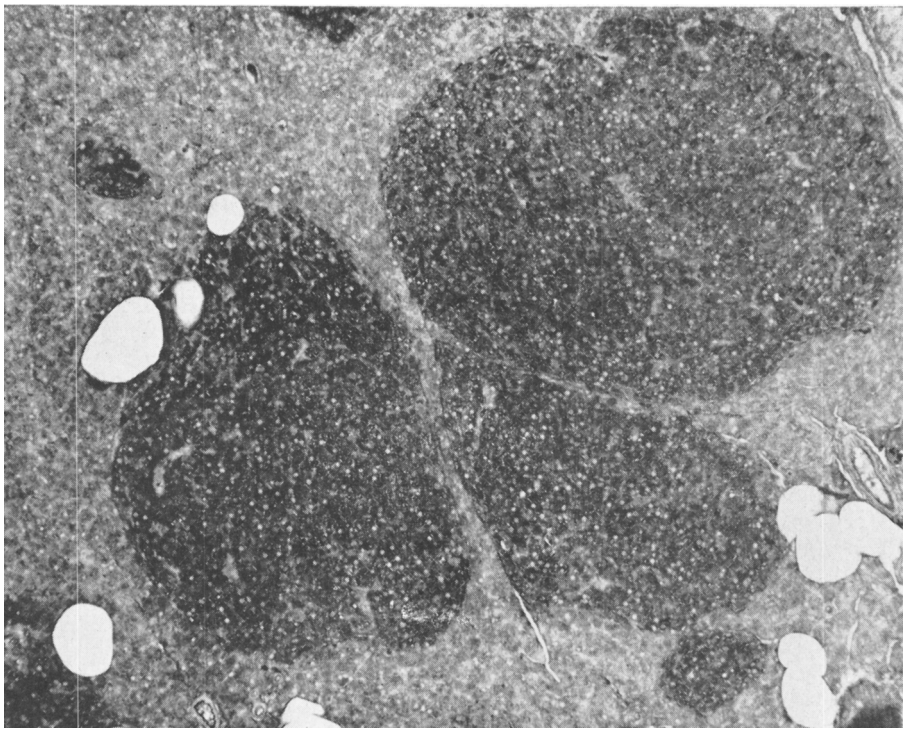

FIG. 7. Pancreatic islet tissue of a control, male gerbil demonstrating the florid islet tissue of the gerbil with deeply staining beta cells. Ald. fuchsin, ×150.

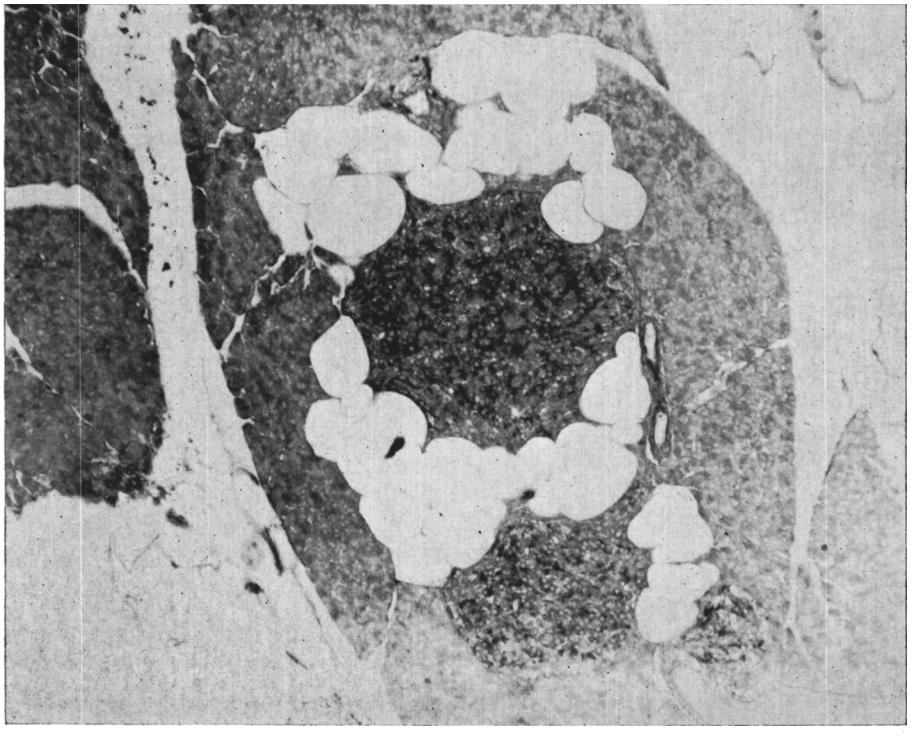

FIG. 8. Islet tissue of a gerbil during the acute stages of myocardial infarction (Day 3). The diameter of the islet tissue is greatly contracted (cf. Fig. 7), the beta cells are degranulated, and the islets are surrounded by large globoid adipocytes. Ald. fuchsin, ×100.

to the shock, prostration, and low blood pressure (in Sprague–Dawley rats) on Day 2.

It is now well-established that serum CPK levels are a useful index of early moycardial ischemia and infarction. The dynamic oscillations in the CPK levels of these gerbils on Days 1 and 2 attest to the myocardial ischemia-inducing effects of the potent beta-adrenergic stimulating agent isoproterenol. The somewhat blunted response in CPK on Day 2 in these gerbils is much more marked in the Sprague–Dawley rats (10). We ascribe this blunted CPK response to the rat's inability to switch from aerobic to anaerobic metabolism and the irreversible myocardial damage caused by the second challenge of isoproterenol (10). The acute dissolution of peripheral adipose tissue sites, the hepatic steatosis, and the islet beta cell degranulation correlate well with the hyperlipidemia and hyperglycemia. In the Long–Evans rat, the fatty liver stores great quantities of triglycerides during the acute phase of cardiac ischemia (11). This could account for the lack of increase in circulating triglycerides in the gerbil on Day 2. However, we have no ready explanation for the dynamic increases in free fatty acids and cholesterol on Days 1 and 2 and their persistence at above-normal levels during Days 3–8. A similar pathophysiologic constellation of serum enzyme elevation, hepatic steatosis, hyperlipidemia, beta cell degranulation, hyperglycemia, and abnormal adrenal steroid production also occurs in gerbils subjected to acute cerebral ischemia (12) and in repeatedly bred gerbils which develop atherosclerosis spontaneously (13). Finally, it is our contention that this characteristic pattern of waxing and waning of these metabolic parameters in the circulation as well as in key organs, e.g., adrenal, thymus, liver, and pancreas, may be a reflection of a pathophysiologic response pattern which coincides with the area and the severity of the myocardium infarcted.

Summary. Adult, male gerbils were subjected to two sc injections of the potent beta-adrenergic stimulating agent isoproterenol. The animals promptly manifested severe tachycardia and became prostrate, and as the cardiac-stimulating effects of the drug began to wane, most of the animals began to recover. However, after the second injection of the drug, 30–40% of the animals succumbed due to shock and myocardial infarction. Myocardial necrosis, consisting of longitudinal streaks limited to the myocardium proper, became maximal on Day 3 and was no longer grossly visible by Day 8. Microscopically, scattered foci of damaged muscle cells covered with a granular admixture of calcium and mucopolysaccharide were still apparent with little or no evidence of fibroblasts or white blood cells. Concomitant with the acute myocardial ischemia, severe hepatic steatosis, islet beta cell degranulation, marked adrenal hypertrophy, and thymus gland involution, these gerbils displayed dynamic changes in circulating CPK, triglycerides, free fatty acids, cholesterol, glucose, and Cmpd. F. The changes in circulating Cmpd. F suggest that after an initial response to the duress of acute cardiac ischemia, the adrenal cortex of the gerbil is incapable of producing additional quantities of Cmpd. F. It is proposed that the pathophysiologic changes observed both in key organs and in the circulation are related to the extent and severity of the myocardium infarcted.

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