Acute Glycogenolysis: A Major Stimulus of Autophagocytic Activity in Rat Hepatocytes[1] (36634)

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Autophagocytic activity of great intensity is achieved within the residual hepatocytes of rat livers following 70% hepatectomy (1). Within the first 3 hr after operation numerous membrane-bound cytoplasmic sequestra containing a variety of organelles are seen within the hepatocytes. Eventual degradation of the autophagosome's contents occurs following acquisition of lysosomal hydrolases (1, 2). The stimulus to such atuophogocytic activity is unknown. An intense activation also occurs under the influence of the hormone glucagon or following administration of the glucoside phlorizin, also without identification of the stimulus (3, 4). Since the phenomenon of autophagocytosis may have widespread significance in the physiology of cells, the identification of the stimulus to its activity in the regenerating hepatocytes would be of general interest.

Materials and Methods. Albino, male rats (Carworth Farms, CFE) weighing approximately 200 g were used throughout the experiments. The details of the operative procedure have been published previously (5). Seventy % hepatectomy entailed the removal of the left and median lobes. Sham operations were comparable in all details save for the removal of the liver. All operations were performed at 8 AM under light ether anesthesia with free air flow and none lasted more than 2 min. Unless otherwise specified, the rats were fed Purina Rat Chow *ad libitum* throughout the experiments. Food deprivation consisted of removing all food at 8 AM of the day prior to operation and was continued until sacrifice. Water was freely available.

Glucose (Fisher Chemical, reagent grade) was diluted with 0.45% NaCl and was administered as a divided dose in several subcutaneous sites. The standard dose administered at the time of operation was 100 mg/100 g body weight. A second dose of glucose of 50 mg/100 g body weight was administered at 3 hr.

The rats were sacrificed by exsanguination and thin slices of liver were fixed immediately in 100% ethyl alcohol at 2° for 24 hr, sectioned at 3 μm and stained. Periodic acid Schiff (PAS) stains gave the most consistent glycogen staining. PAS-positive, diastase-sensitive materials will be hereafter referred to as glycogen (6). Sections from each lobe of each rat were stained for glycogen and code numbered. One observer (without knowledge of the treatment regimen) graded these on multiple occasions for glycogen content and distribution. Repeated estimates were always consistent with the original determination. For the purpose of determining lobular locations of glycogen, the lobules were divided into thirds as previously described (7). The histologic estimates of glycogen content were invariably consistent with those derived by electron microscopic examination.

Blocks of tissue 1 mm in thickness were fixed at 2° in 0.01 *M* phosphate-buffered 4% glutaraldehyde at pH 7.2 and then further fixed in unbuffered 1% aqueous osmium tetroxide. The specimens were dehydrated by passage through a series of graded alcohols and embedded in Epon 812. Sections were cut on an LKB Ultratome; thick sections were used for examination of the tissues and

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lobular-orientation as previously described (7, 8). Modifications of stains for histochemical analysis of acid–phosphatase activity under light and electron microscopic examination were performed as previously described (1, 8).

A number of grids from each liver specimen were studied and the glycogen content of hepatocytes from various areas of the lobule was estimated. A similar grading of autophagocytosis was based upon the number, size-distribution and content of the sequestra, using a randomized Chalkley-type analysis.

To determine the effects of the various treatments upon mitotic response, groups of six rats (each regimen) were treated with colchicine (1 mg/kg body weight, intraperitoneally) 26 hr after operation and were sacrificed at 32 hr. Three Feulgen-stained sections from each rat were used for total nuclear counts. Approximately 15,000 hepatocyte nuclei were counted and the mitotic index determined.

Results. Morphologic. Seventy % hepatectomy of fed rats resulted in a sequence of histochemical and electron microscopic alterations identical to those which we have previously reported (1, 8). Within 3 hr after operation, stainable glycogen was completely eliminated from the hepatocytes of the portal half of the lobule (peripheral hepatocytes) in every rat examined. Identifiable glycogen had also disappeared from every peripheral hepatocyte when examined by electron microscopy. During this same period but lagging slightly, numerous double membrane-bound cytoplasmic sequestra were identified in these same cells. These structures increased in number rapidly until at 3–6 hr as many as 5–15 could be identified in a single hepatocyte. They often contained degenerating mitochondria and less frequently, membranes or glycogen aggregates.

The size and acid–phosphatase content of these structures increased progressively. By 6 hr every peripheral hepatocyte examined by light microscopy demonstrated numerous acid–phosphatase positive bodies, 1–15 μm in diameter, diffusely spread through their cytoplasm.

Neither sham operation nor ether anesthesia for as long as 5 min resulted in a significant loss of glycogen or alteration of the lysosomal composition of these cells.

Food deprivation of rats for 24 hr eliminated almost all stainable glycogen from their peripheral hepatocytes, as judged by light and electron microscopic examination. When such food-deprived, glycogen depleted rats underwent 70% hepatectomy they displayed no increase in autophagocytic activity nor any significant alteration in acid–phosphatase staining pattern.

It was possible to prevent glycogen loss from regenerating hepatocytes by supplying the rats with a large quantity of exogenous glucose (9, 10). The administration of 1 mg of glucose per g body weight at the time of operation and a half dose administered at 3 hr completely prevented the expected loss of glycogen from the hepatocytes. When such glucose treated rats were examined at 3 and 6 hr, they displayed no increase in autophagocytic activity nor any significant alteration in acid–phosphatase staining pattern.

Mitotic Activity. Neither food deprivation for 24 hr prior to 70% hepatectomy nor glucose administration altered the expected mitotic response significantly. The mitotic response of fed, 70% hepatectomized rats was 6.1 $\pm$ 2.4% (SE). Rats which were deprived of food demonstrated a mitotic response of 5.7 $\pm$ 1.6%, and those to which glucose was administered, 3.8 $\pm$ 1.8%. These differences were not significantly different when examined by the student's t test.

Discussion. A series of characteristic alterations of the hepatocyte's vacuolar system occurs in response to challenges as varied as cell injury, physiologic demand or pharmacologic alteration (1, 12). Sequestration of cytoplasmic matrix and organelles by endoplasmic reticulum occurs rapidly. Subsequently, there is a fusion of these sequestra with hydrolase-containing lysosomes which results in the degradation of the autophagosomes' content. Although the formation of the sequestered body and its fusion with lysosomes to form autophagosomes has been studied in the greatest detail, no evidence has been offered as to the nature of the stimulus of these events.

One alteration which occurs after many of these challenges is the rapid depletion of the

hepatocyte's glycogen. In previous reports of the events following 70% hepatectomy of fed rats and confirmed in the present experiments, acute glycogenolysis invariably preceded visible activation of the vacuolar system. This massive glycogenolysis is apparently a response to an acute increase in energy demand per hepatocyte, possibly resulting from the increased requirements of these cells themselves in addition to extrahepatic demand. The present experiments suggest that the sequestration of cytoplasm and lysosomal fusion of hepatocytes which occurs within the rats subsequent to several physiologic and pharmacologic challenges, represent a response to this acute glycogenolysis.

When glycogen was absent from the peripheral hepatocytes at the time of 70% hepatectomy no visible alteration of the vacuolar system occurred. Similarly, the vacuolar system remained inactive if sufficient exogenous glucose was supplied to prevent breakdown of hepatocyte glycogen stress subsequent to operation.

The mechanism by which massive glycogenolysis results in cytoplasmic sequestration and lysosomal response is uncertain. Membrane formation about damaged cell organelles has been reported and a large aggregate of partially degraded glycogen may simulate cytoplasmic damage. It is also possible that a high, local concentration of oligosaccharides may alter the osmotic milieu, directly activating membranous engulfment (13). However, glycogenolysis represents a process intimately related to energy production involving many substances which are vital to other cell functions. It remains possible that sequestration and lysosomal activity result from an alteration in such substances.

In conclusion, it is suggested that stimuli of hepatocyte autophagocytosis and of its vacuolar system, such as hypoxia, endotoxin and others, may act totally or in part via acute glycogenolysis. It is obvious that in many instances direct injury to cell parts may induce the sequestration, lysosomal sequence without glycogen intervention (14). We have been able to produce such changes by the use of phlorizin in fasted rats. However, the sequence of events herein described may be representative of a general physiologic pathway in which the acute breakdown of storage materials results in intervention of the vacuolar system with subsequent acceleration of the breakdown of the stores. A sequence of this type has been demonstrated for the breakdown of lipids of brown fat cells during cold fasting (15) and for the prostate following castration (16).

Summary. Following 70% hepatectomy of fed rats intense autophagocytic activity is evident in the residual hepatocytes. Prior to visible alteration of the vacuolar system, massive glycogenolysis occurs. Elimination of hepatocyte glycogen by food deprivation prior to 70% hepatectomy or prevention of postoperative glycogenolysis by administration of exogenous glucose both prevent the expected autophagocytic activity. It was concluded that acute, massive glycogenolysis is one stimulus to hepatocyte autophagocytic activity.

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