Relationship Between Cathepsin Activity, Sulfhydryl Levels, and Mitotic Activity in Regenerating Liver.* (27145)

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Fluctuations in cell sulfhydryl (SH) levels associated with cell division have been described in a wide variety of species: sea urchin eggs, lily anther, and *Escherichia coli* (1). In addition, Sylven and his co-workers (2), found cyclic fluctuations in cathepsin activity in yeast cultures dividing synchronously. The question raised in these experiments was: do a mature tissue and a mammalian tissue exhibit similar fluctuations in SH levels and cathepsin activity during cell division? Since synchronous division is a prerequisite, regenerating liver was selected because, about 30 hours after partial hepatectomy, an explosive and relatively synchronous division of parenchymal cells occurs.

Materials and methods. The experimental plan was as follows: partial hepatectomy was performed on 150-200 g male rats, and 12, 24, 36, 48, and 96 hours thereafter, groups of 8 rats were sacrificed by decapitation, and a liver homogenate prepared. Aliquots of the homogenate were analyzed for cathepsin activity according to the technic of Anson(3). Nonprotein sulfhydryl (NPSH) and protein sulfhydryl (PSH) were determined according to the amperometric technic of Benesch and his co-workers(4). Only freely reacting SH moities in non-denatured proteins are determined by the method of Benesch. The mitotic index of parenchymal cells only was determined from microscopic sections of liver. Surgery and sacrifice were performed at the same time of day to avoid the diurnal variations in SH level and mitotic activity (5,6). Sham surgical controls were performed.

Results. The results are given in Fig. 1. A peak of mitosis occurred at 36 hours. The NPSH level did not significantly change for 24 hours, then a marked and abrupt increase occurred coincident with the mitotic peak, followed by a rapid rate of decrease for the succeeding 12 hours, then a slower rate of decrease for at least another 48 hours; the levels remained above those of the 0 hour control. PSH levels remained unchanged for 12 hours, then decreased continuously for 48 hours, and remained at this level for the succeeding 48 hours. Cathepsin activity was unchanged 12 hours after hepatectomy; a minimum occurred at 24 hours with return to control level at 36 hours; 48 hours after hepatectomy activity began to increase.

Discussion. These data derived from regenerating liver follow the general observation that cell division is associated with high SH levels. In addition, SH levels are apparently cyclic as in the case of the sea urchin egg or the lily (*Lilium longflorum*) anther. Liver cells proceed to divide asynchronously after the initial outburst of mitosis; thus SH levels would not show maxima and minima after the first cycle. We have not determined the nature of the SH compounds undergoing cyclic change. In lily anthers the compound presumably is glutathione; in sea urchin eggs, a high molecular weight polypeptide. In sea urchin egg and lily anthers, the SH increase precedes mitosis, followed by a fall sometime after nuclear division; whereas in regenerating liver peak SH levels coincide with peak mitotic activity and continue in a sustained manner after the initial mitosis subsides. Other investigators(7,8) have found increased SH levels in regenerating liver though coincidence with mitosis was not demonstrated. The technics used would have tended to minimize fluctuations.

Neither the cause nor the function of the increased SH level is known. Functionally speaking, the never less than normal levels probably preclude any major influence on the SH dependent enzymes. Moreover, since the greatest rates of RNA, DNA, and protein synthesis occur before 24 hours, a relationship here is unlikely. Since the PSH level falls and NPSH rises, a change in PSH-NPSH re-

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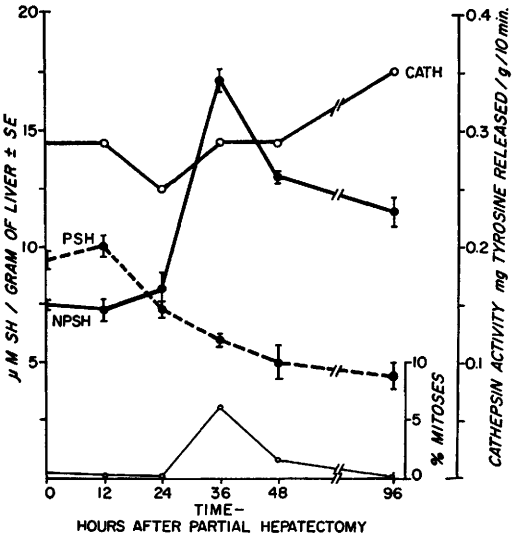

FIG. 1. Fluctuations in non-protein sulfhydryl (NPSH), protein sulfhydryl (PSH) levels, cathepsin (Cath) activity and mitotic activity in regenerating liver. The S.E. for cathepsin activity was too small to be displayed on a graph of this scale.

lationships seems to be a likely area for further investigation. Change in protein structure, (not synthesis) including the cell wall (9), might well be the basic phenomenon, with fluctuating SH levels a tangible result of this change.

Cathepsin activity seems to follow a pattern exhibited by many enzymes in regenerating liver; an initial decrease followed by an increase(10,11). On the basis of these findings no case for a unique relationship between cathepsin activity and cell division in regenerating liver can be made.

Summary. Cell division in regenerating liver is associated with a high sulfhydryl level showing a maximum at peak mitotic activity.

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Translocation of Bacteriophage Across the Intestinal Wall of the Rat.* (27146)

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Particulate matter and large molecules introduced into the lumen of the intestine appear in the blood stream and other body fluids. The particle size varies from that of bacteria(1,2) to that of botulinum toxin(3, 4) and native proteins in food(5).

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Keller and Engley(6) showed that bacteriophage for *Bacillus megaterium* appears in blood within minutes after being administered *per os,* to mice. These workers left open the question as to the primary mode of entry of this phage into the blood. In the work presented here phage is shown to enter primarily into the intestinal lymph, and only sporadically and in small numbers directly into portal blood.

Methods and materials. Rats (Long-Evans