Dot blot hybridization using cytoplasmic extracts is inappropriate for determination of mRNA levels in regenerating liver

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Because of its convenience, dot blot hybridization of cytoplasmic extracts (DBH) (1) is widely used for measurement of mRNA levels in tissues. However, purification of RNA followed by Northern analysis (NA) (2) is likely to produce more reliable estimates of mRNA levels since any contaminants are removed.

Levels of the mRNA for 20 proteins in rat liver after partial hepatectomy or induction of inflammation were determined by DBH and by NA (cDNA probes as described in ref. 3). Typical examples are shown in Fig. 1. During the inflammatory response, mRNA levels measured by NA were very similar to the values determined (3) by DBH for each mRNA at all time points, within the range expected for biological variation (Fig. 1). All mRNA levels determined in regenerating liver by DBH differed substantially from those obtained by NA. For most proteins during liver regeneration, mRNA levels measured by DBH were consistently higher than levels measured by NA (Fig. 1). RNA

degradation was minimal in all samples and could not explain the difference.

The mRNA levels determined in regenerating liver by DBH also differed substantially among proteins (Fig. 1) indicating that material present in cytoplasmic extracts from regenerating livers, but not in extracts from the livers of rats suffering from acute inflammation, interacts with cDNA in a sequence-related manner.

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Figure 1. Levels of mRNA in the livers of rats at various times after induction of an acute inflammation by subcutaneous turpentine injection or after partial hepatectomy. Values determined by NA, $\square$ — $\square$, for pooled samples from 4–8 rats per time point are compared with levels determined by DBH, $\blacksquare$ — $\blacksquare$, mean $\pm$ 1 standard error for 5–8 rats per time point. Levels of mRNA are expressed as a ratio to the level in healthy animals. ADH, alcohol dehydrogenase; Apo AIV, apolipoprotein AIV.

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