DECREASED IN VIVO DISAPPEARANCE OF LABELLED LIVER PROTEIN AFTER PARTIAL HEPATECTOMY

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SUMMARY

Two groups of six mice each were injected intraperitoneally with L-arginine (guanido-^{14}C); 36 hours later 2/3 of the liver was removed from the animals in one group. The removed tissue served as control for both groups. Three days after the operation the regenerating livers still contained 104% of the total protein radioactivity whereas the normal livers had lost 50% of it.

Attempts to explain the net protein gain during liver regeneration have included studies on the rate of protein synthesis (1,2,3) and the proportion of exported proteins (4). I present in this communication an experiment indicating a dramatic reduction in the rate of protein degradation following partial hepatectomy.

PROCEDURE

Male, 6 weeks old CD-1 mice were purchased from Charles River Laboratories. They were kept for two weeks before and during the experiment with Purina rat chow and water ad libitum. Two groups of six mice each were used. Each animal was injected intraperitoneally with 6 μcuries per 100 g of body weight of L-arginine (guanido-^{14}C) (Schwarz Bioresearch, 46 μcuries per μmole). Thirty six hours after the injection the median and left lateral lobes of the liver were surgically removed (5) in one of the groups. The removed tissue was used as control for both groups. In ten additional animals this portion comprised 66.7±0.6 per cent (range 63-70) of the total liver weight. Three days after the partial hepatectomy (or 4.5 days after

the injection) all animals were sacrificed. The protein radioactivity and protein content of the livers, including the tissue removed by surgery, were determined as follows. Each liver was homogenized with two volumes of water. For the determination of protein radioactivity, duplicate samples (0.2 ml) of the homogenate were precipitated with 3 ml of 5% trichloroacetic acid, heated for 15 min. at 90°C and centrifuged. The precipitate was washed successively with 3 ml of 5% trichloroacetic acid, alcohol-ether-chloroform (2:2:1) and acetone. The final pellet was allowed to dry, ground with a glass rod to a fine powder, dissolved in 1 ml of hyamine (Packard Instrument Co.), mixed with 20 ml of toluene-scintillation fluid (Permaflour, Packard Instrument Co.) and counted in a Packard scintillation counter with 67% efficiency. Protein content was determined in duplicate aliquots of the same homogenate by the procedure of Lowry et.al.(6).

RESULTS

The results are presented in the table. Note that in comparing the normal group with the control values of the operated group the data is presented in cpm per mg of protein. In the regenerating livers allowance has to be made for the dilution of the radioactive protein by growth. Thus, in this case the results are represented by the total protein radioactivity of the regenerating 1/3 of the organ compared with the total protein radioactivity of the 2/3 previously removed from the same animal. Since the portion removed by surgery represents twice as much tissue as the portion left to regenerate, the percent value in each animal is calculated as $\frac{\text{total cpm (regenerating)}}{\text{total cpm (control)}} \times 2 \times 100$. The normal livers show a 50% decrease in the protein radioactivity when compared with the control values, three days earlier. In the same time the regenerating livers show no appreciable decrease in the total protein radioactivity.

DISCUSSION

The use of arginine labelled with ^{14}C in the guanido group is particularly

TABLE 1

days after injection	1.5		4.5	
days after hepatectomy	--		3	
	CONTROL		REGENERATING	
mouse #	c.p.m. / mg protein	total[a] c.p.m.	total[b] c.p.m.	per[c] cent
1	20.8	5350	2810	105
2	20.4	5310	2800	105
3	23.8	5750	3930	137
4	31.6	7070	3280	93
5	21.9	6300	3000	95
6	18.7	5650	2490	89
	22.9±1.9 (100%)			104% ±7
			NORMAL	
mouse #			c.p.m. / mg protein	
7			9.5	
8			13.3	
9			12.6	
10			11.6	
11			8.3	
12			13.3	
			11.4±0.9 (50%)	

Table 1. Liver protein radioactivity 1.5 days (control) and 4.5 days (normal and regenerating) after the intraperitoneal injection of L-arginine (guanido-^{14}C). The control and regenerating values were obtained in the same animals (#1 to 6). (a) total protein radioactivity of the 67% of the liver removed during surgery; (b) total protein radioactivity of the remaining 33%, 3 days later; (c) percent radioactivity calculated as $\frac{(b)}{(a)} \times 2 \times 100$ (further explanations in the text).

suited for *in vivo* degradation experiments of liver protein. The high arginase activity in this organ, resulting in the elimination of the radioactivity as urea, reduces the chances of reincorporation of the labelled amino acid (7). The decrease of the total liver protein radioactivity in

the normal animals to one half in three days is consistent with reports of a half-life of 3.3 days reported for the same label in the rat (8). With such a high turnover rate, its drastic decrease during regeneration should contribute substantially to the liver growth.

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