tient's extract nonvolatile radioactive material was formed only during the first few minutes, in contrast to product formation with control enzyme which was proportional to time. Sephadex treatment of the patient's extract largely prevented synthesis of the nonvolatile radioactive material, whereas control enzyme activity was little affected by such treatment.
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L-Asparaginase: Inhibition of Early Mitosis in Regenerating Rat Liver

Abstract. L-*Asparaginase in agouti serum and in extracts from* Escherichia coli *inhibits the early wave of mitosis occurring in rat liver approximately 30 hours after hepatectomy, but even with continued treatment of the animal the later wave at 50 hours is not inhibited. This result differs from the permanent inhibition of growth which asparaginase causes in various tumors.*

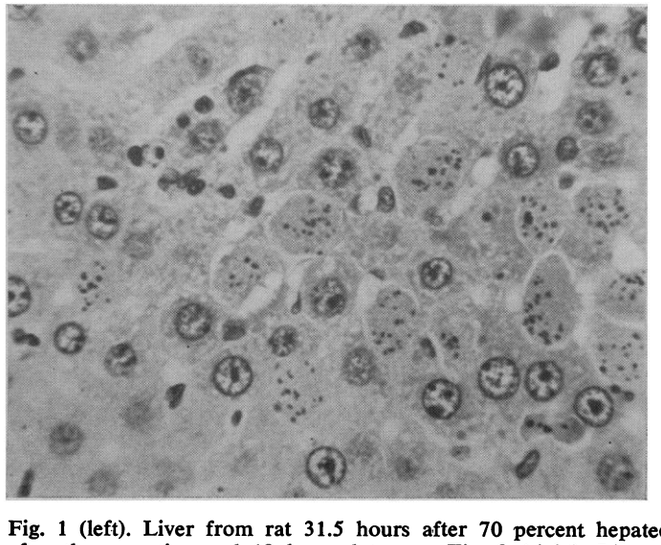
L-Asparaginases of high substrate avidity strongly inhibit the growth of a number of animal tumors of both lymphoid and nonlymphoid origin (*1, 2*). Such enzymes are found in the serums of the guinea pig, agouti, and closely related species, but not in those of other animals (*2, 3*). They are also found in bacteria (*4*). The enzymes produce little general toxicity in treated animals. In earlier studies, animals given asparaginase for prolonged periods maintained normal weights and activity, and no abnormality was found in nonneoplastic tissues upon their inspection with the naked eye or histological examination (*5, 6*). But asparaginase can affect nonneoplastic tissue, as we now show. This enzyme inhibits the early mitosis of the regenerating rat liver to an extent only observed before with toxic amounts of antimetabolites (*7*).

After removal of 70 percent of the liver in the normal rat, an extremely rapid process of growth is initiated, which results in the almost complete restoration of the liver mass within 1 week. The sequence of events by which this takes place has been the subject of considerable study. Following stages of RNA, protein, and DNA synthesis, a wave of mitosis occurs between 25 and 36 hours after hepatectomy, and a later, smaller wave takes place between 47 and 53 hours (*8*). We have examined the effect of asparaginase on liver mitosis using enzyme from two sources: agouti serum, obtained by cardiac puncture of mature animals (*2*), and *Escherichia coli* extract, purified by salt fractionation and chromatography essentially by the method of Mashburn and Wriston (*4*).

Hepatectomy or sham operation was performed on male Sprague-Dawley rats (250 ± 25 g) as described previously (*9*). Immediately afterwards, asparaginase preparations or control solutions were injected intravenously. Further injections of asparaginase or other solutions were given at 12-hour intervals. Rats were killed 31.5 or 52.5 hours after the operation, in each case 6 hours after the intraperitoneal injection of colchicine (1.0 mg/kg) given to arrest mitoses. Livers and other organs were fixed in 10 percent formalin, sectioned at 5 μ, and stained with hematoxylin and eosin. The number of mitoses in 12,000 nuclei were counted in each liver; a notable uniformity was found between counts from sections of different lobes of the organ.

The numerous mitoses of the early wave are obvious in a control rat killed 31.5 hours after hepatectomy (Fig. 1). The scattered distribution of the chromosomes is the result of treatment with colchicine. By contrast, in a similar rat treated with 2.0 ml of agouti serum [810 units of asparaginase assayed according to Broome (*10*)] at the time of hepatectomy and 12 hours later, no evidence of mitotic activity is visible. Nonetheless, the hepatic cells do not appear abnormal so far as can be observed with light microscopy. The nonregenerating liver contains few mitoses (Table 1); 31.5 hours after hepatectomy the mitotic index has risen to 5.4 percent. At this time in hepatectomized animals given asparaginase either in agouti serum or in the preparation from *E. coli* almost no mitoses can be counted. The total quantities of asparaginase given are not large compared to those that inhibit the growth of rat tumors (*5, 11*).

Normal rabbit serum and a crude preparation of *E. coli* glutaminase, both of which lack asparaginase activity, fail to inhibit mitosis. In other experiments



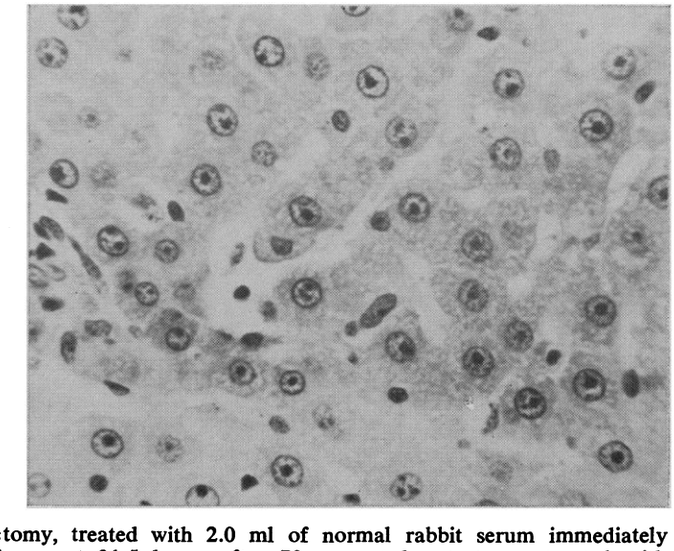


Fig. 1 (left). Liver from rat 31.5 hours after 70 percent hepatectomy, treated with 2.0 ml of normal rabbit serum immediately after the operation and 12 hours later. Fig. 2 (right). Liver from rat 31.5 hours after 70 percent hepatectomy, treated with agouti serum as described in the text (× 450).

Table 1. Effect of asparaginase on mitosis of liver cells from rats killed 31.5 hours after hepatectomy. Numbers in parenthesis denote numbers of livers examined. Mitotic index is the proportion of hepatocytes in mitosis per 100 cells. Hepatectomy was performed on animals under ether anesthesia, and 70 percent of the organ was removed. In animals subjected to sham operation the abdomen was opened, and the liver was manipulated as it would be in hepatectomy, but none of it was excised. All treated animals received intravenous injections immediately after hepatectomy and 12 hours later. The substances used and the amounts at each injection were: normal rabbit serum, 2 ml; agouti serum, 2 ml containing 745 to 840 units of asparaginase; *E. coli* glutaminase preparation, 1 ml of solution in Ringer solution containing 5.0 mg and 162 units of glutaminase; *E. coli* asparaginase, 1 ml of solution in Ringer solution containing 1.0 mg and 431 units of asparaginase.

Operation	Other treatment	Average mitotic index	Range of mitotic indexes
None	None	0.0	0.1–0.0 (6)
Sham	None	.3	.5– .0 (12)
Hepatectomy	None	5.4	14.7–1.3 (20)
Hepatectomy	Rabbit serum	4.0	12.1–1.5 (4)
Hepatectomy	Agouti serum asparaginase	0.0	0.0–0.0 (8)
Hepatectomy	*E. coli* glutaminase	3.4	7.4–1.3 (4)
Hepatectomy	*E. coli* asparaginase	0.0	0.0–0.0 (8)

Table 2. Effect of asparaginase on mitosis of liver cells from rats killed 52.5 hours after hepatectomy. Experimental methods were similar to those described in Table 1. Treated animals received injections at 12-hour intervals beginning immediately after operation and repeated for the number of times indicated. Preparations injected and quantities used were the same as described in Table 1, except for the substitution of normal horse serum for rabbit serum; neither of these possess asparaginase activity. Numbers in parentheses denote numbers of livers examined.

Operation	Other treatment	Doses (No.)	Average mitotic index	Range of mitotic indexes
Sham	None		0.4	0.5–0.0 (4)
Hepatectomy	None		2.7	6.7–0.7 (12)
Hepatectomy	Agouti serum asparaginase	2	3.7	4.7–2.0 (4)
Hepatectomy	*E. coli* asparaginase	2	3.0	4.1–1.3 (4)
Hepatectomy	Horse serum	4	2.7	7.0–1.1 (4)
Hepatectomy	Agouti serum asparaginase	4	6.4	12.0–3.4 (8)
Hepatectomy	*E. coli* asparaginase	4	7.8	11.0–2.6 (4)

agouti serum was treated with 5-diazo-4-oxo-L-norvaline (DONV), a substance which specifically inactivates asparaginase (*12*). The treated serum does not inhibit mitosis.

The inhibition of mitosis observed in the liver apparently does not occur in other tissues. In sections of ileal epithelium and lymphoid follicles in Peyer's patches obtained from hepatectomized animals, mitoses appear equally numerous in animals treated with asparaginase and in controls.

Although asparaginase almost completely prevents early mitosis after hepatectomy, the inhibition is not permanent. At 52.5 hours (Table 2), hepatectomized but otherwise untreated animals show an average of 2.7 percent of cells in mitosis, a considerably smaller proportion than they have at 31.5 hours. In animals given two injections of asparaginase, enough to fully inhibit mitosis at 31.5 hours, virtually the same number of mitoses are now found as are found in controls. But the number of mitoses in the two groups of animals treated with asparaginase through the whole period after hepatectomy (averages of 7.8 and 6.4 percent, respectively) is notably higher than in any other group. The animals to which we gave asparaginase maintain a high level of enzyme activity in the blood throughout the experiment. Immediately before treatment at 12 hours an average of 31.8 units/ml is found; at 48 hours this has risen to 43.0 units/ml. Thus, the inhibition of mitosis induced by asparaginase is overcome in the regenerating rat liver; indeed, in continuously treated animals, the particularly numerous mitoses seen at 52.5 hours may compensate for the earlier block.

In mice, treatment with asparaginase causes a profound decrease in the concentration of free asparagine in the blood and in both neoplastic and nonneoplastic tissues. The concentration in the liver falls from the normal value of 4.39 ± 1.34 μg/g wet weight to 1.77 ± 0.78 μg/g 18 hours after treatment with doses inhibitory to tumor growth (*13*). It is highly probable that similar changes occur in rats so treated in our experiments. Although adequate for the nondividing cell, the reduced amount of asparagine in the liver after treatment with asparaginase may be critically low for processes essential for cell growth, particularly for the synthesis of large amounts of new protein. In sensitive lymphomas treated with asparaginase or deprived of asparagine protein formation is inhibited (*14, 15*). Asparagine synthesis by these tumor cells is unable to compensate for the decreased amount of exogenous asparagine taken up by the cells under such conditions (*15*). The regenerating liver differs from the lymphomas sensitive to asparaginase in that the inhibitory effects of the enzyme are not permanent; furthermore, after a delay, sufficient asparagine is apparently available for growth. The most likely source of this asparagine is an increased rate of synthesis in the liver cells.

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