Depression of Tryptophan Pyrrolase Induction in Regenerating Rat Liver.* (31464)

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Many oncologists have examined both human and animal tumors in an attempt to determine differences in their enzyme complement or biosynthetic pathways which might prove causally related to the neoplastic state. Examination of the "minimum deviation" hepatomas(1), malignant tumors which have an enzyme complement quite similar to that of normal liver, has suggested that malignancy may be associated with abnormalities in enzymatic regulatory mechanisms(2). One such mechanism is the induction by cortisone of increased tryptophan pyrrolase activity(3). This hepatic enzyme can normally be induced to levels of activity approximately 5 times that of baseline, 4 to 5 hours after cortisone administration(4). This induction is due to an increased rate of synthesis of enzyme protein. The induction can be blocked by actinomycin D(5), and this is taken as evidence that enzyme induction by cortisone depends on synthesis of new messenger RNA and is DNA-directed. Several of these hepatomas, although they demonstrated tryptophan pyrrolase activity, failed to show any rise in enzyme activity after cortisone administration and the rest showed only slight increases when compared with the response of normal liver tissue(6). Other regulatory mechanisms were similarly depressed or absent(7). Therefore, the hypothesis has been presented that one critical factor in neoplasia may be a loss of responsiveness to controlling regulatory processes.(7).

There is no reason to assume, however, that these changes observed in hepatomas are necessarily related to their neoplastic character. Rather, the differences observed between hepatoma and normal liver tissue might be the result of comparing a dividing population of cells with the mitotically inactive adult liver. Therefore, it seemed appropriate to study tryptophan pyrrolase induction by cortisone in rats subjected to 70% partial hepatectomy, a procedure which initiates considerable mitotic activity in the remaining liver(8) and can serve as a model for enzyme induction in non-neoplastic dividing hepatic cells. Previous experiments have indicated that there is indeed a depression of tryptophan pyrrolase induction by cortisone 48 hours after 70% hepatectomy(9,10). However, a large portion of the mitotic activity which follows partial hepatectomy has taken place by 48 hours(11), and we therefore chose to investigate the induction of tryptophan pyrrolase activity in the early hours following operation during the preparation of the hepatocytes for mitosis.

Material and methods. Adrenalectomized male Sprague-Dawley rats (Charles River Farms), weighing approximately 250 g, were used throughout the experiments in order to eliminate endogenous cortisone secretion. Seventy percent hepatectomy (or a sham operation) was performed 5-7 days after adrenalectomy between 8-11 a.m.(12). At various times following the operation, hydrocortisone phosphate (5 mg/100 g body weight) was administered intraperitoneally. Four hours after steroid administration, the animals were sacrificed and the right posterior lobe of the liver was removed for enzyme assay. Tryptophan pyrrolase activity was determined by the method of Knox(4) using added hematin(9). Five to 12 animals were studied at each time period and standard errors of the means were calculated.

A second group of animals identically prepared by operation and steroid administration was treated with intraperitoneal colchicine (1 mg/g body weight) at 26 hours and sacrificed at 32 hours. Three sections of liver were

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TABLE I. Mitotic Indices in Regenerating Rat Liver.

Procedure	Mitotic index ± S.E.
Hep(12)	4.73 ± 1.10
Hep adrex(12)	5.03 ± 1.00
Sham adrex(9)	0.28 ± 0.10
Hep adrex + cortisone	
6 hr(6)	5.2 ± 1.4
21 " (4)	4.9 ± 1.8

Figures in parentheses = No. of animals.

prepared from each rat by standard histologic techniques. All nuclei were counted for an average count of 12,000 nuclei per animal, and the mitotic index was then calculated.

Results. The data of Table I indicate that adrenalectomy did not appreciably change the mitotic response to 70% partial hepatectomy and that sham operation did not cause a significant degree of mitotic activity. Also, administration of high doses of cortisone did not appreciably alter the mitotic response in adrenalectomized, partially hepatectomized rats, whether the hormone was given soon (6 hours) or late (21 hours) after the procedure.

Fig. 1 summarizes enzyme activity obtained when hydrocortisone was administered at the times indicated following operation. The sham operated animals demonstrated a lessened response to hydrocortisone induction in the early post-operative period, reaching a normal value by 12 hours. The hepatectomized rats displayed a similar but more profound depression of response which improved with time following operation also to 12 hours. However, at 18 hours, the degree of enzyme induction was again depressed and this depression remained through 24 hours. At 48 hours the value observed for partially hepatectomized rats was 15% less than controls.

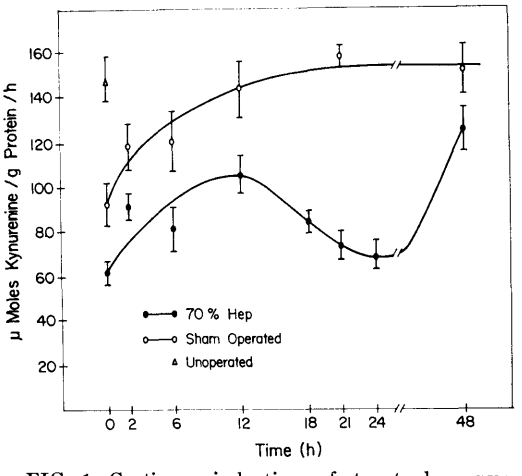

FIG. 1. Cortisone induction of tryptophan pyrrolase in regenerating rat liver. Brackets indicate standard errors of the means.

Discussion. It is probable that the initial depression of tryptophan pyrrolase activity observed in both groups is related to an operative factor such as anesthesia, laparotomy or post-surgical physiological alterations.

The depression of induction observed at 18 hours in partially hepatectomized animals coincides with a rapid rise in DNA synthesis in the regenerating liver(13). If the only factors affecting enzyme induction in partially hepatectomized animals were the effects of operation one would expect that the response curve would parallel that of the controls. Therefore, at 18 to 24 hours a value between 105 and 125 micromoles of kynurenine/hour would be anticipated. The values observed, however, are approximately 40% less than this and it is perhaps corroborative that Grisham(14), using autoradiographic techniques, demonstrated that approximately 40% of the hepatic cells participate in this rapid DNA synthesis. It is possible, therefore, that the depression in inducible enzyme activity seen in the present experiments represents a total suppression of response in those cells preparing to divide, rather than a partial suppression of response in the entire cell population.

It is possible that this lessened response in cells preparing to divide is related to repression of transcriptional and/or translational mechanisms. Garren *et al*(15) have presented evidence for the existence of repressors acting at the translational level which may inhibit the inductive process. Additional evidence for translational inhibition are the findings of Salb and Marcus(16) which demonstrated a decrease in protein synthesis in metaphase HeLa cells, apparently resulting from a protein coating of the ribosomes.

It is possible that, associated with preparation for division, the cell produces repres-

sors which render it refractory to normal inductive stimuli. On the other hand, the duplicating genome itself may be unable to simultaneously participate in other functions.

The data of these experiments indicate that cells preparing to divide are less able to respond to an hormonal stimulus than are nondividing cells. Therefore, the failure or diminution of enzyme induction by steroids in hepatomas is not a specific property of neoplastic cells but may be common to dividing cells.

Summary. The induction of tryptophan pyrrolase by hydrocortisone has been studied in the regenerating rat liver. An initial depression of response, presumably related to the operative procedure, was observed in both sham operated and partially hepatectomized animals. In partially hepatectomized animals, a second depression was observed during that period in which DNA synthesis has been shown to occur. It has been suggested that alterations in enzyme control mechanisms, such as the failure of tryptophan pyrrolase induction by cortisone in minimum deviation hepatomas, may be a fundamental biochemical alteration in tumors. This study shows that a depressed response to hormonal enzyme induction is found in non-neoplastic hepatic cells preparing to divide.

1. Morris, H. P., J. Nat. Cancer Inst., 1955, Suppl. 15, 1535.

2. Potter, V. R., In Cellular Control Mechanisms in Cancer, P. Emmelot, O. Muhlbock, eds., Elsevier. 1964.

3. Pitot, H. C., Morris, H. P., Cancer Res., 1961, v21, 1009.

4. Knox, W. E., Auerbach, V. H., J. Biol. Chem., 1955, v214, 307.

5. Greengard, O., Smith, M. A., Acs, G., ibid., 1963, v238, 1548.

6. Dyer, H. M., Gullino, P., Morris, H. P., Cancer Res., 1964, v24, 97.

7. Pitot; H. C., Cho, Y. S., Prog. Exp. Tumor Res., 1965, v7, 158.

8. Bucher, N. L. R., Intern. Rev. Cytol., 1963, v15, 245.

9. Goldstein, L., Knox, W. E., Ann. N. Y. Acad. Sci., 1963, v111, 233.

10. Greengard, O., Feigelson, P., ibid., 1963, v111, 227.

11. Becker, F. F., Am. J. Path., 1963, v43, 497.

12. Higgins, G. M., Anderson, R. M., Arch. Path., 1931, v12, 186.

13. Tsukada, K., Lieberman, I., J. Biol. Chem., 1964, v239, 1564.

14. Grisham, J. W., Cancer Res., 1962, v22, 842.

15. Garren, L. D., Howell, R. R., Tomkins, G. M., Crocco, R. M., Proc. Nat. Acad. Sci., 1964, v52, 1121.

16. Salb, J. M., Marcus, P. I., ibid., 1965, v54, 1353.

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In vitro Suppression of the Lymphocyte Response to Tuberculin by Live Measles Virus.* (31465)

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It is known that peripheral blood lymphocytes from a tuberculin sensitive individual when exposed to PPD in tissue culture will react by transforming into blastoid cells and by an increased mitotic rate(1). Recent studies have shown that measles-infected tuberculin positive children not only develop a depressed tuberculin skin reaction(2) but also manifest a diminished *in vitro* lymphocyte response to tuberculin(3). Thus, it appeared that in the infected children the measles virus exerted a direct effect on the ability of the peripheral lymphocyte to respond to PPD. To test this hypothesis, both live measles virus, which will multiply in suspensions of human leukocytes(4), and PPD were added to lymphocyte cultures obtained from children

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