

Letter to the Editor

## Lack of Proportionality between Rate of Cell Division and Induction of Tumors in Carcinogen-exposed Regenerating Livers<sup>1</sup>

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### ABSTRACT

Although a single pulse of dimethylnitrosamine administered during the regenerative response of liver subsequent to 70% hepatectomy resulted in more primary hepatocellular carcinomas in treated livers than in controls, the response was not proportionate to the level of cell division. Further, the use of 55-g male Sprague-Dawley rats that displayed an extremely active regenerative response did not significantly shorten the lag period before the appearance of tumors. Of additional interest was the finding that the post-S period might be even more susceptible to dimethylnitrosamine than the S phase was. These results support the suggestion that many aspects of the interaction between carcinogens and dividing cells and the requirement for subsequent events for the development remain unclear.

A major goal of many recent experiments in chemical carcinogenesis has been to simplify the regimens involved in identifying obligatory components in the sequence of alterations which result in cancer (8, 10, 13). The enhanced susceptibility of the dividing cell and, in particular, the hepatocytes of regenerating liver has been frequently utilized in this effort. Numerous publications report the appearance of PHC<sup>2</sup> as a result of exposure of the dividing hepatocyte to a pulse of carcinogen which was ineffective in the control animals (3, 4, 6, 7, 12, 14). Further, even when failing to result in PHC, this combination may prepare (initiate) the liver so that a secondary stimulus, such as phenobarbital, makes manifest the potential for hepatocarcinogenesis (9, 10, 13). Two operational problems of the carcinogen-regenerating liver regimen are the long lag period before the appearance of the PHC and, generally, a low yield of cancer (4). An additional point which requires resolution has been that, although it is generally assumed that the period of DNA synthesis is the most sensitive phase of the cell cycle, conflicting data exist (3, 5, 11). In an attempt to decrease the lag time, to increase tumor yield, and to answer the question of the relative sensitivity of different phases of cell division, 55-g rats were used as the source of regenerating liver. Previous results from many experiments in this laboratory and other laboratories have demonstrated several potential advantages of rats of this age when compared to the (young) adult.

The number of residual hepatocytes demonstrating DNA synthesis during the first wave of cell division subsequent to operation in these rats is at least twice that of older animals,

and the postmitotic period is more clearly defined (1, 2). It has also been shown that the susceptibility of animals of this age is greater than that of an adult animal when exposed to chronic doses of standard carcinogens such as *N*-2-acetylaminofluorene (8), a finding in keeping with the demonstration that they have an intense metabolic capability for activating *N*-2-acetylaminofluorene in the Ames assay.<sup>3</sup>

To determine the effectiveness of this approach, male CFE Sprague-Dawley rats obtained from Charles River Farms underwent 70% hepatectomy at 55 or 175 g body weight. The operations were carried out between 8 and 10 a.m., and the course of DNA synthesis of the first responsive wave of cell division was determined by tritiated thymidine incorporation and DNA analysis. This analysis, which was confirmatory of many prior experiments, demonstrated that peak DNA synthesis was between the 20th and 22nd hr for the younger rats, and between the 24th and 26th hr for the older. The specific activity of the former was approximately 2.5 times greater than that of the latter at peak synthesis. The chemical agents were administered i.p. (Table 1). In each instance, sham-operated control animals received twice the dose of the carcinogen in an attempt to compensate for the effect of smaller liver mass in partially hepatectomized rats. The rats were observed clinically by palpation on a monthly basis and by laparotomies when tumor was suspected. In addition, monthly determinations of circulating  $\alpha$ -fetoprotein were generously performed by Dr. S. Sell, as another means of determining the presence of tumors. The experiment was terminated at 2 years of age by the sacrifice of all surviving animals.

The percentage of PHC induced by dimethylnitrosamine in the 175-g rat was at the lower level of those reported in other studies (4). However, this finding confirms previous results in our own laboratory, where the induction of PHC in adult rats by this procedure has been inconsistent and invariably limited. We cannot explain the variance between our results and those in other laboratories, save for the differences that may exist in maintenance, diet, strain, etc., or perhaps the criteria for the diagnosis of PHC. The use of a semisynthetic diet in our experiments may be particularly pertinent. These explanations may also account for the lack of PHC with 7,12-dimethylbenz( $\alpha$ )anthracene.

However, it is also striking that the use of the young rat, with its greatly increased amplitude and synchrony of DNA synthesis, did not commensurately increase the yield of carcinoma. Further, the lag time was not significantly shorter than that seen in the older animal. A similar and striking failure to demonstrate an increase of tumor induction proportionate to

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<sup>2</sup> The abbreviation used is: PHC, primary hepatocellular carcinomas.  
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<sup>3</sup> D. L. Stout and F. F. Becker, unpublished data.

Table 1  
Effect of partial hepatectomy on induction of PHC by chemical carcinogens

Carcinogen	Wt of animals <sup>a</sup> (g)	Time of treatment (hr)	Dose <sup>b</sup> (mg/kg)	Effective no. of survivors	PHC <sup>c</sup>	% <sup>d</sup>
Dimethylnitrosamine	200	No PH <sup>e</sup>	20	24	0	9.0
		24	10	22	2	
	55	No PH	18	19	0	11.0
		6	9	12	0	
		20	9	18	2	
7,12-Dimethylbenz(α)anthracene	55	No PH	40	14	0	17.0
		6	20	9	0	
		20	20	16	0	
		30	20	14	0	
N-Hydroxyacetylaminofluorene	55	No PH	80	16	0	
		6	40	7	0	
		20	40	16	0	
		30	40	19	0	

<sup>a</sup> Weights are approximate, as obtained at the time of operation. All survivors were killed at 2 years of age. Twenty-five rats were used for each group.

<sup>b</sup> The dose of carcinogen used was determined as the 10 to 20% lethal dose, at 6 hr, after partial hepatectomy, the time of maximum sensitivity. All carcinogens were administered i.p. Dimethylnitrosamine was dissolved in 0.9% NaCl solution; 7,12-dimethylbenz(α)anthracene and N-hydroxyacetylaminofluorene were in emulsion (7).

<sup>c</sup> All of the PHC appeared between 14 and 19 months after treatment and were characterized by histological examination. There were well differentiated, and each demonstrated clearly defined invasion. Of 7, 3 were productive of α-fetoprotein.

<sup>d</sup> Those rats which survived at least 1 year after treatment and subsequently demonstrated PHC.

<sup>e</sup> PH, partial hepatectomy.

the rate of cell division or DNA synthesis was reported by Pound and Lawson (11). These results suggest that the administration of carcinogen during cell division is not in itself sufficient to produce the "momentum" to result in a high yield of tumors. It emphasizes the requirement for additional alterations to further the carcinogenic sequence, as has been reported by a number of authors (8, 9, 12). Thus, the combination of carcinogen and dividing cell may represent an initiating complex which is adequate to complete the evolutionary sequence in only a limited manner modified by stochastic variation. This suggestion has been made recently by others (9, 13).

Despite the need for metabolism of the carcinogen, which makes uncertain the period during which the active derivative might be present, previous reports have created the impression that the heightened sensitivity of regenerating liver is due mainly to DNA synthesis. However, other studies have suggested that the period of maximal sensitivity is not yet defined

(3, 5, 11). Although the total number of tumors of this current study is too small for statistical analysis, an intriguing finding was the greater productivity of PHC by rats challenged in the post-S period of the cell cycle. Current experiments aimed at analyzing the sensitivity of this period are under way.

In addition to several interesting findings, these experiments should serve as a caution to investigators who utilize the combination of regenerating liver and carcinogen as a "carcinogenic regimen" for subsequent examination of biological or biochemical agents. At the least, an appropriate "monitor" of such studies in each laboratory must be the demonstration that PHC are actually induced by the combination utilized.

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