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DETECTION OF EXTRAHEPATIC CANCER BY ALTERATIONS IN HEPATIC FUNCTIONS

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Abstract—Signs of the biochemical undifferentiation of the liver of rats carrying various subcutaneous neoplasms include progressive decreases in cytochrome *c* reductase which were paralleled by increases in the duration of hexobarbital hypnosis. The sleeping time, proportional to the size of the mammary carcinomas or fibrosarcomas, reverted to normal within 10 days after the complete resection of these tumors, but not if regrowth occurred. Decreases in the histologically normal liver of hepatic ornithine aminotransferase, glucokinase, alanine aminotransferase, and cytochrome *c* reductase, and increases in hexokinase, were also reversible, and reflected sensitively upon the success or incompleteness of tumor eradication. Enzymic and consequent functional alterations in the uninvolved liver in response to the systemic action of neoplasms thus offer new approaches to the detection and prognosis of cancer.

The general deterioration of the organism of cancer subjects is not longer attributed to the "parasitic" nature of neoplasms, but to alterations in the metabolism of anatomically distant tissues in response to as yet unidentified substances that tumors release to the circulation [1]. The liver is a sensitive target; in rats with extrahepatic tumors, alterations in the concentration of numerous hepatic enzymes have long been known to occur. Only a few of these, i.e. the elevation of enzymes exquisitely sensitive to glucocorticoids, represent a non-specific stress response [2]. When searching for a common denominator behind abnormal concentrations of the remaining apparently unrelated enzymes, we demonstrated that they denote a shift in overall enzymic composition toward that of immature liver [3]. This includes enzymic changes shown also to occur 24–48 hr after the injection of tumor extracts [4–6] or upon the perfusion of isolated, normal liver with the blood of tumor-bearing animals [7]. The extent of a given enzymic response may vary with the type of neoplasm, but decreases in the concentration of "adult" enzymes (those emerging at late stages of normal ontogeny), as well as increases in those that were at high levels in the fetus, are common to a variety of cancers (e.g. carcinomas of the mammary and submaxillary glands, sarcoma, hepatoma or lymphoma) [8, 9].

Insofar as changes in host tissues begin long before the onset of cachexia or any alteration in the nutritional state, they might be looked upon not only as causes of the eventual metabolic deterioration, but perhaps also as early signals of neoplastic growth in the organism. Indeed, the above-described process of partial undifferentiation in rat liver begins when the subcutaneous tumors are not yet palpable, 4–6 days after transplantation[8, 9]. It thus became of interest whether signs of this process might be detectable indirectly, with the avoidance of invasive procedures. The feasibility of this hinges on whether deviations in the concentrations of hepatic enzymes, assessed by their activities under optimal conditions (fortified with substrates and cofactors) *in vitro*, are associated with proportionate alterations in the functions of the enzymes *in vivo*. This is one question we began to investigate. The other, pertinent not to the detection of cancer but to assessing the outcome of its therapy, is: to what extent do enzymic or functional changes in the uninvolved liver revert to normal upon surgical eradication of the tumors?

MATERIALS AND METHODS

Tumors (approximately 10^6 cells) were implanted subcutaneously into one of the flanks of adult male albino rats. Mammary carcinoma 5A was grown in CDF Fisher rats; fibrosarcoma, radiation-induced monocytic lymphoma and chemically induced osteosarcoma in KX rats; and Morris hepatoma and renal carcinoma K3 in Buffalo rats. The methods of assay of the five hepatic enzymes were as in previous studies on rats with these tumors [3, 8, 9].

Hexobarbital (150 mg/kg) was injected intraperitoneally. Rats were then placed on their backs; spontaneous standing denoted the end of sleeping time.

RESULTS

Cytochrome *c* reductase (EC 1.6.99.2), a component of the microsomal drug-metabolizing system, emerges at late stages of hepatic ontogeny [10] and was thus one of the enzymes expected to have a decreased concentration in the partially undifferentiated livers of tumor-bearing, adult rats [3]. The present studies confirm this, and show that subcutaneous transplantation of each of six different neoplasms (a chemically induced osteosarcoma, a radiation-induced monocytic lymphoma, Morris hepatoma 7777, renal carcinoma MK1, mammary carcinoma and fibrosarcoma) into three appropriate strains of rats was followed by appreciable diminutions in the cytochrome *c* reductase content of the uninvolved liver. These results are in harmony with the previously noted impaired drug metabolism, and consequent increase in the sensitivity to hypnotic agents, of tumor-

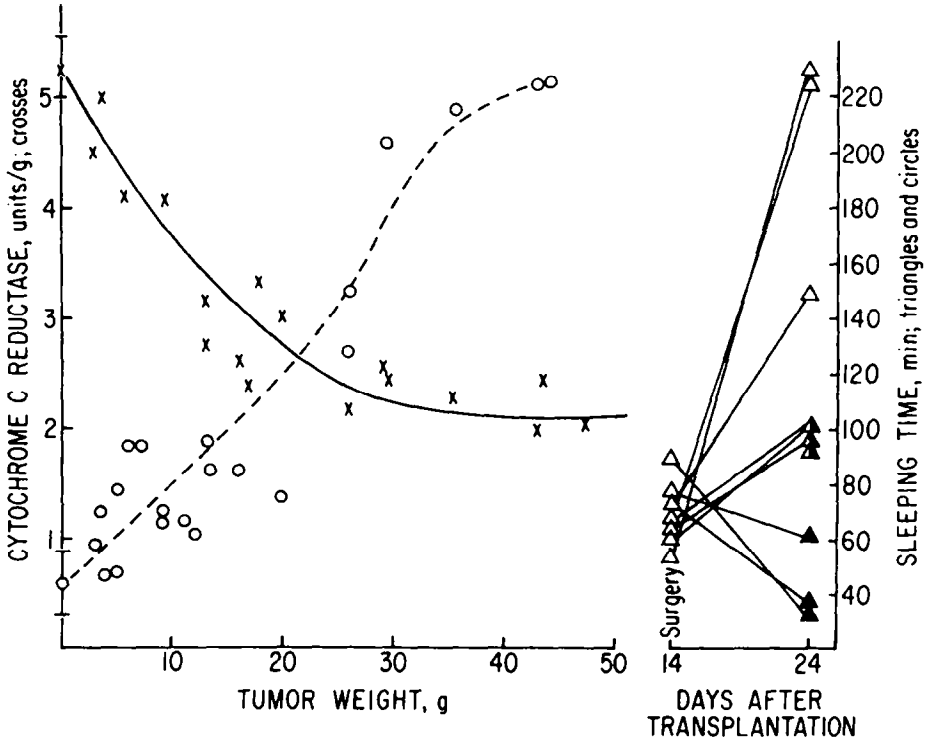

Fig. 1. Changes of hepatic cytochrome *c* reductase content and hexobarbital sleeping time with fibrosarcoma growth. Tumors (approximately 10^6 cells) were implanted subcutaneously into one of the flanks of adult male KX rats. Body weight, and the protein content, weight and histology of the liver, remained normal for 40 days. Left side: points refer to individual rats without tumors (abscissa = 0) or 4–30 days after transplantation. Cytochrome *c* reductase activity (μmoles/min/g of liver, crosses) and hexobarbital sleeping time (open circles, right ordinate) are plotted against tumor weight. Right side: each line connects the sleeping time of one rat measured twice: a few hours before surgery (when tumors were 5–9 g) and 10 days later. Autopsy examination detected no tumor (▲) or 0.2 to 4.5 g regrowth (▲) of the apparently totally resected tumors. In the three sham-operated animals (△) at 30 days, the tumors weighed 30–50 g. At the end of the experiments, the body weight of the rats was 278–310 g.

bearing rats [11, 12]. It remained to test for the quantitative relationship of such sensitivity to the extent of loss of hepatic cytochrome *c* reductase.

The inverse correlation between the activity of the hepatic cytochrome *c* reductase and the hypnotic effect of hexobarbital in fibrosarcoma-bearing rats is depicted on the left side of Fig. 1. Changes in these two parameters with increasing tumor weight began with an approximately linear drop and rise respectively. They became less steep when the growth rate, log tumor volume days after transplantation, was no longer linear [13]. Both curves closely resembled those obtained in studies on twenty-six rats of the CDF strain carrying another non-metastic tumor, mammary carcinoma 5A. Observations on rats implanted with lymphoma or hepatoma revealed a similar relationship between sleeping time and hepatic enzyme activity, but whether the rate of change in both parameters with tumor size (or time after transplantation) varies significantly with the type of neoplasm remains to be established.

In 1971, Rosso *et al.* [12] reported that ablation of Walker carcinoma decreased the duration of pentobarbital hypnosis of the experimental rats. The present observations on two strains of rats with three different neoplasms were in accord with this, and also showed corresponding increases in the level of hepatic cytochrome *c* reductase. The results of typical experiments on rats bearing fibrosarcoma or mammary carcinoma are illustrated on the right side of Fig. 1 and the last part of Table 1 respectively. Ten days after surgery, the duration of hexobarbital sleeping time was much higher in all sham-operated animals than in those subjected to tumor resection. In the majority of cases, tumor resection decreased the values to normal; in others, it arrested or slowed down the progressive increases in sleeping time. The reason for this variation became apparent upon autopsy. In some cases our inexpert surgery failed to eradicate the neoplastic cells completely. The sleeping time of these rats did not revert to normal, whereas it did in rats with no detectable regrowth (see Fig. 1 and Table 1). Lymphoma implantation had a similar effect on sleeping time, on cytochrome *c* reductase or on other hepatic enzymes (see also Ref. 8), and its complete eradication also resulted in the normalization of sleeping time. However, rats in which this normalization failed to occur exhibited secondary lesions in the lung rather than regrowth of the lymphoma at the original site. None of the other tumors studied have a tendency for metastasis, and microscopic examination could not detect neoplastic growth or other histological abnormalities in the liver of rats carrying the transplanted mammary carcinoma or fibrosarcoma.

The decrease in cytochrome *c* reductase was also

Table 1. Effect of mammary carcinoma and its resection on hepatic enzymes and hexobarbital hypnosis*

	Days after transplantation:				
		20	30	30 Surgery:	30
	Control		Sham operated on day 20	Resected on day 20: Regrowth	Resected on day 20: No regrowth
Ornithine aminotransferase	3.07 ± 0.51	1.4 ± 0.3	1.09 ± 0.3	1.8 ± 0.5	3.0 ± 0.3
Alanine aminotransferase	59.7 ± 10.0	38.7 ± 6.0	31.8 ± 13.0	37.9 ± 7.2	50.7 ± 7.0
Glucokinase	1.89 ± 0.43	0.6 ± 0.4	0.02 ± 0.01	1.06 ± 0.5	1.8 ± 0.4
Hexokinase	0.24 ± 0.04	0.4 ± 0.05	0.51 ± 0.14	0.38 ± 0.14	0.33 ± 0.05
Cytochrome *c* reductase	5.0 ± 0.8	3.3 ± 0.2	2.1 ± 0.8	3.2 ± 0.6	4.2 ± 0.9
Sleeping time (min)	45 →	70 →	200		
	33 →	85 →	255		
	34 →	62 →	267		
	40 →	118 →		86	
	42 →	79 →		70	
	50 →	96 →		115	
	49 →	96 →			60
	53 →	70 →			53
	46 →	73 →			44
	45 →	88 →			45

* Enzyme activities (μmoles/min/g of liver), were determined in untreated, control rats (column 1) and in four groups of experimental rats without (columns 2 and 3) and after (columns 4 and 5) resection of the transplanted mammary carcinoma. The values are means ± S.D. of results on eight to ten (columns 1 and 2) or on three to five rats (columns 3, 4 and 5). Statistically significant ($P < 0.01$) were the differences for each enzyme in columns 1 and 2, for ornithine aminotransferase, glucokinase and hexokinase in column 3 compared to column 5, and for ornithine aminotransferase in column 4 compared to column 5. Hexobarbital sleeping time was determined in ten rats three times (arrows connect values for each individual): at the time of transplantation (control), 20 days later (just before surgery) and 30 days later (i.e. 10 days after surgery, last two columns). The mean (± S.D.) sleeping times in columns 1, 2, 3, 4 and 5 were: 44 ± 6.2, 84 ± 16, 241 ± 29, 90 ± 15 and 50 ± 6.5 respectively. Sham operation itself had no effect on the enzymes, sleeping time or tumor growth. For some additional details see the legend of Fig. 1.

reversed by tumor resection. However, since this initial study avoided the inconvenience of taking sequential liver biopsy samples, the values (means of results on different individuals) do not reflect the results of surgery as conclusively as did the sleeping time tests where each animal served as its own control.

In order to ascertain that the reversibility of tumor-induced hepatic changes is a general phenomenon, we also measured some enzymes that are unrelated to drug metabolism, and whose location is not the microsomal but the mitochondrial (ornithine aminotransferase) or soluble (second to fourth in Table 1) fraction. One of these enzymes, hexokinase (EC 2.7.1.1), was shown to increase, while the others (ornithine aminotransferase, EC 2.6.1.13; glucokinase, EC 2.7.1.2; and alanine aminotransferase, EC 2.6.1.2) decreased shortly after tumor transplantation [8, 9]. We found that their levels reverted to normal after complete resection of the mammary carcinoma (cf. columns 1,2 and 5, Table 1) or fibrosarcoma (not shown). Comparison of the mean values of column 4 with 5 indicates that hepatic ornithine aminotransferase and glucokinase may be particularly sensitive indicators of the success of surgery, since values in the absence and presence of regrowth differed from each other considerably.

DISCUSSION

Most currently used biochemical methods for the early diagnosis of cancer are based on the analysis of blood for specific markers, such as abnormal enzymes, glycoproteins and oncofetal antigens. These substances originate from the interior or from the surface of the tumor cells themselves, and become diluted by the large circulatory volume of the body. Therefore, one limitation of the approach is that release of such markers from a very small tumor mass may not involve detectable changes in their blood concentration. Our studies are concerned with a different approach, one based on chemical and functional changes in the uninvolved liver, which represent an amplification of the primary signals, i.e. of the active factors that neoplasms release to the circulation. The observations on cytochrome *c* reductase and hexobarbital hypnosis indicate that relatively small quantitative changes in enzymes in the liver can exert an impact which is significant enough to be detectable by functional tests on the suitably challenged organism *in vivo*.

The decrease in hepatic cytochrome *c* reductase became significant only after the tumors had grown to palpable size. There are several more sensitive indica-

tors, i.e. enzymes whose quantification in the liver distinguishes a normal rat from one which has received a subcutaneous implant of a neoplasms 4–6 days earlier [8]. For such enzymes, too, non-invasive tests (e.g. analysis of body fluids for administered substrates or products) should be feasible. However, we do not know which would respond to neoplastic growth in humans. Potential enzymic indicators of hepatic undifferentiation can be selected from the more than two dozen studied in fetal vs normal adult human liver [14]; however, the identification of the most sensitive and specific ones awaits analysis of an appropriate series of liver biopsy samples.

Since any single enzyme may respond to "non-specific" influences, evidence for the tumor-induced, biochemical immaturity of the liver must rely on the quantifications of several enzymes, from more than one metabolic pathway, and selected so as to include at least one enzyme which is expected to rise rather than decrease in concentration during the process of partial undifferentiation. Some of these deviations from hepatotypic enzyme patterns may also occur in cirrhosis, severe hepatitis or carbon tetrachloride poisoning [15, 16]. However, they can be explained by massive losses in normal liver cells, or by consequent regenerative responses, whereas no such visible signs accompany the biochemical abnormalities in the livers of rats carrying varying neoplasms. Histological normalcy is thus a criterion which distinguishes hepatic abnormalities induced by distant neoplasms from those associated with the above liver diseases. Pertinent in this connection are observations on the increase in serum antipyrine half-life of human subjects: this known sign of liver cirrhosis in man [17] also occurs in patients with hepatic malignancies but is attributable to the defective drug-metabolizing capacity of the uninvolved (tumor-free) parenchyma [18]. Subjects with non-hepatic cancer have unfortunately not been included in the study. The present data on rats with various subcutaneous tumors suggest, however, that this defective drug metabolism is not unique to patients with *hepatic* cancer: it probably reflects upon a more general phenomenon, the biochemical undifferentiation of the liver in response to the systemic action of various neoplasms.

The demonstrated reversibility of the biochemical abnormalities in the livers of rat hosts, the dependence of this process on the type of the resected neoplasm, and correlation with the extent of secondary lesions require further, detailed study. The results indicate, however, that quantification of enzymes in the liver, or of associated functions *in vivo*, can distinguish animals in which tumor resection was apparently successful from those in which obvious regrowth occurred. Such distinction may be a somewhat easier task than the detection of latent neoplasms. Specific diagnosis of the latter, as discussed above, requires a multiplicity of markers. Drug-metabolizing enzymes, for example, are well known to undergo adaptive responses to a variety of physiological states. Change in their amount or function *in vivo* (unless supported by additional signs of hepatic immaturity in histologically normal liver) can certainly not be taken as specific evidence for cancerous growth in the organism, whereas sequential tests under controlled conditions in an individual cancer subject may provide some information about the progress of the disease. At present, however, only studies on the rat are available to indicate that assay of a small, suitable set of enzymes in histologically normal liver could serve to diagnose the presence of neoplastic growth in the organism and also to follow its response to therapeutic intervention.

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