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The Dedifferentiated Pattern of Enzymes in Livers of Tumor-Bearing Rats¹

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Annemarie Herzfeld and Olga Greengard

2776400
Department of Biological Chemistry, Harvard Medical School, and the Cancer
Research Institute, New England Deaconess Hospital, Boston, Mass. 02215

Running Title: Host liver enzymes

SUMMARY

In livers of adult rats, 7 days after the subcutaneous implantation of neoplasm, there is a significant decrease in the level of ornithine aminotransferase and glucokinase and an increase in that of hexokinase. The concentrations of some 10 additional enzymes also change in the course of the ensuing week, while the levels of 9 or 10 other enzymes and the liver size remain normal. In suckling rats, an implanted tumor inhibits the developmental formation of 4 liver enzymes (ornithine aminotransferase, glucokinase, glutamine synthetase and malate-NADP dehydrogenase) but does not interfere with the premature rise of ornithine aminotransferase upon an injection of glucocorticoid.

These results together with previous observations demonstrate that enzymes which increase in liver upon tumor transplantation are among those that are high in hepatomas and in fetal liver; those which decrease are low or absent in hepatomas or fetal liver and tend to emerge only during the late suckling period of normal differentiation. Thus, the enzymic composition of host liver deviates from normal adult liver in the same direction as do well-differentiated hepatomas and immature liver but to a much lesser extent.

INTRODUCTION

The livers of tumor-bearing animals are known to exhibit a variety of biochemical abnormalities including quantitative changes in the activity of several enzymes (10, 31, 35). Any one of these changes may also occur under other pathological conditions, such as abnormal nutritional or hormonal states and tissue necrosis, and thus none have been proved to be specific for the presence of neoplastic growth in the organism. However, the direction in which the overall enzymic composition changes may be uniquely related to this condition. To test this possibility, we measured the level of 20 randomly selected enzymes in adult rat liver 4-23 days after tumor transplantation, before the onset of major deterioration in the health of the animals. The effect of tumor-bearing on the normal and induced developmental formation of liver enzymes in suckling rats was also explored. The results suggest that the enzymic composition of host livers tends to acquire features of "undifferentiation".

METHODS

Host livers were from NEDH rats carrying Walker 256 carcinoma or from Fischer rats carrying mammary carcinoma DMBA 5A. Control livers were from rats of the same strain, age and body weight as the host livers. Morris hepatomas 7800 and 7777, used in some of the experiments, were grown in Buffalo rats. The volume doubling time of the tumors, determined as described previously (20), were 0.86, 1.07, 5.0 and 1.5 days for Walker 256, 5A, 7800 and 7777, respectively. Fetal livers were obtained on the 18th day of gestation.

Total body X-irradiation of 10-day-old rats of 250 kv was applied at 70 cm distance and at a rate of 69 r per min. The rats were returned to their mothers (and untreated littermates) for nursing; in the next 10 days, they showed a normal gain in body weight.

Enzyme activities (see Table 2 for E.C. numbers) in μ moles per min per g tissue wet weight, were determined in extracts of freshly excised liver by previously established methods (ornithine aminotransferase (14), glucokinase and hexokinase (16), cathepsin (1), alanine aminotransferase (29), glutamine synthetase (21), malate-NADP dehydrogenase (7), tryptophan oxygenase (26), glucose-6-phosphate dehydrogenase (30), aspartate transcarbamylase (15), phosphoserine phosphatase (19), glutamate dehydrogenase (33), cytochrome c reductase and glucose-6-phosphatase (5), phosphorylase (36), aldolase (3), arginase (9), aspartate aminotransferase (13) and ATP-citrate lyase (7)). The activities of the first 8 of these enzymes in the standard tissue, adult male rat liver, are given in Tables 1 and 3; others are to be found in the above references in parentheses. For phosphorylase and glutamate dehydrogenase, the activities were 5.2 ± 0.7 and 64 ± 9 units per g liver of adult, male rats. Protein was determined by the method of Lowry *et al.* (23).

Supernatant fractions obtained by the centrifugation of homogenates at $106,000 \times g$ for 30 min were electrophoresed on starch gel at pH 8.6 in EBT buffer (0.02 M EDTA, 0.5 M boric acid and 0.9 M Tris, diluted 1:20 for dissolving the starch and 1:7 for the electrode compartments) at 280 V, 25 mA for 14 hr at 4°. Hexokinase activity was detected *in situ* as described previously (17) except that β -mercaptoethanol was omitted from the mixture and glucose concentrations of 0.5 mM were used so that glucokinase remained undetected.

RESULTS

Adult rats. Table 1 summarizes some of the observations made on adult tumor-bearing rats. Values in the last 11 rows are expressed relative to those obtained for control rats; thus, numbers greater and smaller than 1.0 reflect increases and decreases, respectively. It may be seen that up to 14 days after transplantation there was no significant alteration in body weight and in the weight or protein content of the liver. There may have been a small decrease in dry weight. However, significant increases or decreases of enzyme levels occurred.

The decrease in the ornithine aminotransferase level was the earliest major change observed in the liver: it was seen clearly 4 days after the transplantation of Walker 256 (see Table 1) when the tumors are usually not yet palpable. Continued tumor growth caused relatively little further change in the level of this enzyme: there was no correlation between liver ornithine aminotransferase activity and tumor size (ranging from 7-55 g). The rise in hexokinase and decrease in glucokinase were also detectable within 7 days after transplantation, while alanine aminotransferase and malate-NADP dehydrogenase required 14 days to exhibit a significant decrease in activity. The level of glutamine synthetase may have decreased before the 14th day when the first measurement was done. These effects were not specific for the particular tumor or rat strain since in livers of Fischer rats carrying DMBA 5A tumors the enzyme changes were similar (see Table 1). (The rise in cathepsin has only been studied in liver of rats carrying 5A tumors.) The implantation of non-neoplastic tissues (i.e., mince of embryonic liver and kidney) was without effect on the enzymes studied so that effects are unlikely to be due to transplantation of tissue per se.

Hexokinase is known to exist in liver in more than one molecular form (17). To establish whether the increase in hexokinase activity in host livers, as shown in Table 1, can be attributed specifically to one of its isozymes, extracts of hepatic tissues were subjected to electrophoresis. Fig. 1 shows that the amount of isozyme III was much higher in host liver than in normal adult liver. This isozyme also predominated in hepatoma 7800 but was almost undetectable in fetal liver which contains isozyme I (present in all hepatic tissues) and also IA (absent from other hepatic tissues).

The first column of Table 2 presents the complete lists of observations made on host livers. The list includes the enzymes already shown in Table 1 as well as those which, in the same experiments, underwent small or no changes in level as a result of tumor transplantation; previous results with 3 enzymes (catalase, phosphofructokinase and serine dehydratase) not assayed in the present studies, are also included. The standard deviations (see Table 1) were such that small differences between control and host liver are probably not significant. Values above 1.25 or below 0.85 in the first column of Table 2 can be considered to reflect real changes in the level of the enzymes in host liver. Some of the present observations, such as the decreases of glutamine synthetase (34) and arginase (10), and the rise in glucose-6-phosphate dehydrogenase (35) and cathepsin (2) in host livers are in qualitative agreement with previous reports. We confirmed the rise of tryptophan oxygenase and tyrosine aminotransferase (8) in host liver but neither this enzyme nor tyrosine aminotransferase are listed in Table 2 because their augmentation after tumor transplantation, as in many other stressful states, is attributable to enhanced adrenocortical secretion (8, see also Table 3 and last paragraph of Results).

All of the enzymes which were determined in host liver have been also studied in fetal liver and hepatomas. The results obtained for all tissues (given in the 2nd, 3rd and 4th columns of Table 2) were normalized, taking the values in normal adult liver as 1.0. Thus, Table 2 contains in a suitable form the data necessary for comparing the quantitative pattern of enzymes in host liver with the patterns of other hepatic tissues. The classification of enzymes in this list and the conclusion based on the inter-tissue comparisons will be given in the Discussion: in general, immature liver and hepatomas share some of their enzymic characteristics with host liver.

Suckling rats. Several enzymes (Tables 1 and 2) which decrease in amount in host livers appear in normal liver at rather late stages of development. To test whether the presence of a tumor in the organism might interfere with the normal process of enzymic differentiation, tumors were implanted into 10-14 day old rats. The levels of some liver enzymes, known to emerge around the third postnatal week were subsequently determined. In suckling rats transplanted Walker tumor grew at a rate similar to that in adult rats; the body weights of these rats remained normal but in the younger age group there was a 20% increase in liver weight. (Table 3). At the age of 20 days, livers of control rats attained almost adult levels of ornithine aminotransferase while those of their tumor-bearing littermates contained very little (see also Chart 1). Liver glucokinase, which is absent up to the age of 15 days (16), reached almost half the adult level in 20-day-old control rats while in the experimental littermates it remained too low to be accurately measurable (Table 3). Two other enzymes in the cluster which rise in the late suckling period (4) were prevented from reaching their adult level at the normal time, i.e., at the age of 20

(glutamine synthetase) or 30 days (malate-NADP dehydrogenase), respectively (Table 3). Hexokinase, unlike the other enzymes in Table 3, is very active in fetal liver and decreases to adult values around the time of birth (16); in 20-day-old host livers, hexokinase rose (Table 3) to levels normally seen in fetal liver.

In rats, both tumor growth (8) and irradiation (32) , cause pituitary stimulation, resulting in the secretion of glucocorticoids. Response to stress becomes pronounced in rats on about the 12th postnatal day (22); since glucocorticoids are essential for the emergence of tryptophan oxygenase (6) and ornithine aminotransferase (12) around this time, stress may enhance the developmental formation of these enzymes. We subjected 10-day-old rats to 300 r whole body irradiation; 8 days later tryptophan oxygenase was at twice the adult level (2.1 ± 0.3 units/g; adult = 1.0 units/g) and ornithine aminotransferase attained 0.6 ± 0.06 units/g. In the nonirradiated litter mates the level of tryptophan oxygenase was only 0.4 ± 0.05 units/g. and that of ornithine aminotransferase 0.25 ± 0.08 units/g. Thus, irradiation, despite imposing a serious debility (survival time was less than 3 weeks), enhanced the developmental formation of both tryptophan oxygenase and ornithine aminotransferase. Stress associated with tumor bearing also enhanced the formation of tryptophan oxygenase (Table 3). However, in the same host livers, the level of ornithine aminotransferase was lower than in the controls (Chart 1); tumor bearing interfered with as yet unidentified factors involved in the developmental formation of ornithine aminotransferase. This interference is apparently overcome by injected hydrocortisone which, as seen in Chart 1, evoked the formation of

ornithine aminotransferase as effectively in tumor bearing as in normal suckling rats. It should be noted that an injection of hydrocortisone does not raise the level of liver ornithine aminotransferase in normal adult rats and did not restore the loss seen in tumor-bearing adult rats.

DISCUSSION

Adaptation to the presence of a tumor in the organism and to consequent alterations in nutritional and endocrine states may result in a multiplicity of apparently unrelated enzymic abnormalities in the liver. Some of these abnormalities are clearly not unique to the condition of tumor bearing. The increase in host liver of the level of enzymes exquisitely sensitive to glucocorticoids (tryptophan oxygenase and tyrosine aminotransferase (8), omitted from Table 2) must be considered as a non-specific phenomenon, resulting from "stress". However, the remarkable decrease in ornithine aminotransferase in adult liver as early as 4 days after transplantation, and the changes in the levels of additional enzymes within the next 10 days may be more specifically related to the condition of tumor bearing. In suckling rats some of the effects of a transplanted tumor on the liver were more striking than in adult rats and may extend to many enzymes in addition to the ones investigated. Thus, tissues of the developing animal which are still in the process of differentiation may provide sensitive systems for studying the mechanisms underlying the systemic effects of the tumors. We have no indication about the nature of substances which might mediate these effects but one of the consequences of the process, the particular quantitative pattern of enzymes seen in host liver, may be interpretable by comparison with that exhibited by other normal and pathological variants of hepatic tissue.

In 1947, having found low levels of catalase, arginase, D-aminoacid-oxidase and riboflavin in hepatomas as well as in host livers, Greenstein (10) suggested that "What apparently happens is a generalized and systemic 'cancerousness' of the organs of the tumor-bearing animals, so that the liver, for example, of such an animal takes on certain neoplastic features. These features may be of relatively small degree compared with those of the hepatoma, but they are, nevertheless, in the direction of the hepatoma." Twenty years later, prompted by the behavior of an equally small number of enzymes studied, Suda et al. (31) suggested that the biochemistry of host livers is essentially "immature". There is no contradiction between the two suggestions. Assessment of large numbers of enzymes proved recently that hepatomas and fetal liver have similar quantitative patterns of enzymes (18); isozymic profiles of enzymes which exist in more than one molecular form reveal also qualitative similarities between fetal liver and hepatomas (3, 18). Thus, insofar as host livers may be "cancerous", they may also be immature. But quantification of large numbers of enzymes, in tissues including fetal liver and hepatomas, is required to prove such a tendency. Before discussing these quantitative comparisons, the less well documented qualitative alterations in isozyme pattern need be mentioned. The L or "adult" form of pyruvate kinase, absent from fetal and neoplastic liver (3), was reported to decrease preferentially in host livers (31). Variants of hexokinase also reflect the similarity of host liver to hepatoma or to immature liver. Isozyme III, though absent from fetal liver, is the prominent form of hexokinase in both host liver and hepatomas (Fig. 1). As seen in Table 2, An increase in hexokinase to glucokinase ratio is clearly shared by fetal, host and neoplastic liver. Normal

adult liver contains much more glucokinase than hexokinase; it is noteworthy that the decrease in glucokinase during starvation or lack of insulin is not coupled to an increase in hexokinase (25), while in fetal, host and neoplastic livers the low glucokinase levels are always associated with hexokinase levels higher than in normal adult liver. In Table 2 we summarized in a normalized manner enzymes in host liver juxtaposed with much previously accumulated and some newly acquired information about fetal liver and hepatomas.

To facilitate the comparison between the quantitative pattern of enzymes in host liver and fetal liver, the enzymes are divided into two main groups. Knox (18) distinguished between "fetal-type" enzymes, already significant in the fetal tissue, and "adult-type" enzymes which appear later in development. In Table 2, under "Fetal Liver", 0.39 L.U. is the lowest level for enzymes classified as "fetal type" and 0.22 is the highest level for those classified as "adult type". The first two columns of Table 2 reveal (comparisons with hepatomas will be summarized later) that all the enzymes which increase in host liver (i.e., > 1 L.U.) are among "fetal-type" enzymes. This phenomenon is probably not restricted to the enzymes so far studied: the reported increases in DNA synthesis (28) and mitotic rate (24) in host livers suggest that several enzymes involved in the synthesis of macromolecules, and highly active in normal fetal liver, may increase in host livers. It may also be seen from the first two columns of Table 2 that all the enzymes which decrease in host liver (i.e., < 1 L.U.) are "adult-type" enzymes.

It is possible to subdivide the "adult-type" enzymes according to the age at which they emerge in the course of normal differentiation (4).

The 5 enzymes in group a belong to the late fetal cluster; they increase rapidly during the last 3 or 4 days of gestation. The next 3 enzymes (b) show biphasic developmental patterns; they appear during late fetal life but undergo a second upsurge in the neonate (glucose-6-phosphatase) or in the third postnatal week (catalase and arginase). The last 6 (c) emerge only after birth: one with the neonatal cluster (serine dehydratase), the others with the late suckling cluster (4). It may be seen that none of the 5 enzymes of group a has decreased significantly in host liver while 2 of group b and all of group c did. It thus appears that the enzymes which decrease in host liver are not randomly distributed among the "adult-type" enzymes: they are all among those that develop late. This suggests that we might be dealing with an almost orderly reversal of the normal process of differentiation. In partial summary of Table 2, the "fetal-type" enzymes that are high in fetal liver tend also to be elevated in host liver. The "adult-type" enzymes that are low in fetal liver also tend to be low in host liver. This latter tendency is more marked for those enzymes developing late. The pattern of enzymes in the host liver thus changes from that of normal liver toward the pattern in immature (early postnatal) liver.

The tendency in host liver for the decrease of enzymes which develop late can also be established by a criterion independent of the present classification. Of the 16 enzymes with fetal levels less than 0.5, 8 are significantly decreased (0.85 or less) in host liver and none are increased. Of the 6 enzymes with fetal values above 0.5, 5 are significantly increased (> 1.25) in host liver and none are decreased. Random assortment of the enzymes in fetal and host livers in this manner is extremely unlikely ($\chi^2 = 17.61$, 2 d.f., $P < 0.001$).

It has been shown that the quantitative pattern of enzymes in hepatomas resembles that of fetal liver and that fast-growing hepatomas exhibit this resemblance to a greater extent than do slow-growing hepatomas (18). Since host liver, as discussed earlier, acquires some features of immaturity, it would also show a resemblance to hepatomas, particularly to slow-growing ones. As seen in Table 2 the enzyme levels in host liver tend to deviate from 1.0 (i.e., from the normal adult liver) in the same direction as they do in hepatomas; the magnitude of the deviations (i.e., the difference from 1.0) and the number of enzymes which show an altered level is greater in hepatomas than in host liver. On a scale of divergence from normal adult liver, slow-growing hepatomas would fall between host liver and fast-growing hepatomas, though nearer to the latter. Well-differentiated, minimal deviation hepatomas are being extensively studied in the hope of approaching the essence of neoplasia. The ideal material, a "least deviated" or minimum deviation hepatoma postulated by Potter (27), would probably grow at too slow a rate to reveal its existence to the observer. The enzymic abnormalities detected in host liver, qualitatively similar but much less extensive than those in the slowest-growing hepatoma, may include the sought for signs of minimum deviation associated with neoplastic transformation.

ACKNOWLEDGMENTS

We are grateful to Dr. W. E. Knox for enlightening discussions, to Dr. F. Farron for carrying out the electrophoreses, to Dr. J. Li and Mr. Norbert Lempert for the determinations of cathepsins and aldolase. Thanks are due to Dr. S. C. Jamdar for the study of irradiated rats.

FOOTNOTE

¹This study was supported by U. S. Public Health Service grants. CA-08676 and CA-05060, both from the National Cancer Institute, HD-04532, from the National Institute of Child Health and Human Development and RR-05591, from General Research Support Branch, Division of Research Resources; and by the U. S. Atomic Energy Commission Contract AT(11-1)-3085 with the New England Deaconess Hospital (100-3085-5).

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Table 1

The effect of transplanted tumors on livers of adult rats

Rats were all 49-52 days old. All values (mean $\pm$ SD) except those for tumor weight are relative (host/control of the same age and strain). The absolute values (mean $\pm$ SD) of enzyme activities (units/g) in control livers are in parentheses. For the experiment done 7 days after implantation, 13 experimental and 10 control rats were used; for the others, 7 of each.

Tumor carried	Walker 256			DMBA 5A	
	4	7	14	13	23
Days post implantation	4	7	14	13	23
Tumor weight (g)	<1	10.3 $\pm$ 2.5	21.0 $\pm$ 14	8.40 $\pm$ 3	49.0 $\pm$ 10
Body weight (- tumor)	1.10 $\pm$ 0.06	1.07 $\pm$ 0.09	1.01 $\pm$ 0.05	1.03 $\pm$ 0.26	0.74 $\pm$ 0.14
Liver weight	1.10 $\pm$ 0.01	1.21 $\pm$ 0.08	1.04 $\pm$ 0.02	1.10 $\pm$ 0.12	0.82 $\pm$ 0.08
Liver protein (mg/g)		0.93 $\pm$ 0.05	0.98 $\pm$ 0.09		1.01 $\pm$ 0.03
Liver dry weight (mg/g)		0.91 $\pm$ 0.02	0.87 $\pm$ 0.07		0.87 $\pm$ 0.03
Liver enzyme activities					
Hexokinase (0.21 $\pm$ 0.03)	1.25 $\pm$ 0.03	1.99 $\pm$ 0.07	1.75 $\pm$ 0.06		2.35 $\pm$ 0.40
Cathepsin (2.5 $\pm$ 0.3)				1.40 $\pm$ 0.30	1.7 $\pm$ 0.20
Ornithine aminotransferase (3.1 $\pm$ 0.5)	0.53 $\pm$ 0.06	0.24 $\pm$ 0.05	0.22 $\pm$ 0.10	0.37 $\pm$ 0.04	0.30 $\pm$ 0.05
Glucokinase (1.03 $\pm$ 0.04)	0.98 $\pm$ 0.03	0.64 $\pm$ 0.06	0.66 $\pm$ 0.01		0.31 $\pm$ 0.08
Glutamine synthetase (1.03 $\pm$ 0.04)			0.64 $\pm$ 0.10	0.93 $\pm$ 0.06	0.60 $\pm$ 0.07
Alanine aminotransferase (40 $\pm$ 6)	0.97 $\pm$ 0.05	0.94 $\pm$ 0.01	0.34 $\pm$ 0.07	0.69 $\pm$ 0.07	0.53 $\pm$ 0.10
Malate-NADP dehydrogenase (1.8 $\pm$ 0.18)	1.03 $\pm$ 0.15	0.90 $\pm$ 0.05	0.67 $\pm$ 0.15	0.83 $\pm$ 0.04	0.41 $\pm$ 0.10

Table 2

Comparison of enzyme levels in hepatic tissues

"Liver units" are relative values; those in normal adult male rat liver are taken as 1.0. Values in italics for fetal liver and hepatomas are from a compilation of literature data by Knox (18, Appendix 2,3); those for aldolases, phosphorylase and glutamate dehydrogenase are from the present experiments on fetal livers (18th day of gestation), hepatoma 7777 ("fast") and hepatoma 7800 ("slow"). Values for host liver are from the present studies (mean of 4 or more) 14 days after the transplantation of Walker 256, or in the case of cathepsin, 23 days after the transplantation of 5A (see Table 1) or from previous reports referenced in parentheses. The solid horizontal line divides the enzymes into "fetal" and "adult" types (see text). The broken lines divide the "adult type" enzymes according to whether they emerge shortly before birth (a), exhibit a prenatal and a postnatal upsurge (b) or emerge postnatally (c); this classification is based on previously reviewed data (4) and, in the case of glutamate dehydrogenase, aspartate aminotransferase and aldolase (FLP), on recent studies (3,11,13).

"Liver Units" (adult liver = 1.0 LU/g)

Enzyme	E.C. No.	Host	Fetal	"Fast"	"Slow"
		Liver	Liver	Hepatoma	Hepatoma
Hexokinase	2.7.1.1	1.75	<u>1.11</u>	<u>6.47</u>	<u>1.78</u>
Glucose-6-phosphate dehydrogenase	1.1.1.49	1.35	<u>0.69</u>	<u>1.95</u>	<u>2.50</u>
Phosphofructokinase	2.7.1.11	3.1(3)	<u>2.16</u>	<u>3.46</u>	<u>1.33</u>
Cathepsin	3.4.4.9	1.7	<u>0.61</u>	<u>0.49</u>	--
Phosphoserine phosphatase	3.1.3.3	1.25	<u>2.84</u>	<u>2.80</u>	<u>0.63</u>
ATP citrate lyase	4.1.3.8	0.9	<u>0.44</u>	<u>0.30</u>	--
Pyruvate kinase	2.7.1.40	0.91	<u>0.39</u>	<u>6.67</u>	<u>0.73</u>
Aldolase (FDP)	4.1.2.13	0.91	6.05	28.4	5.25

Glutamate dehydrogenase	1.4.1.2	0.95	0.22	0.14	0.23
Aspartate aminotransferase	2.6.1.1	0.98	<u>0.21</u>	<u>0.21</u>	<u>0.54</u>
NADPH cytochrome c oxidoreductase	1.6.99.1	0.88	<u>0.12</u>	<u>0.19</u>	<u>0.39</u>
Phosphorylase	2.4.1.1	0.99	0.10	0.19	0.25
Aldolase (F1P)	4.1.2.7	0.89	0.22	0.11	0.26

Glucose-6-phosphatase	3.1.3.9	1.01	<u>0.09</u>	<u>0.08</u>	<u>0.49</u>
Catalase	1.11.1.6	0.06(1)	<u>0.17</u>	<u>0.09</u>	<u>0.59</u>
Arginase	3.5.3.1	0.85	<u>0.1</u>	<u>0.03</u>	<u>0.33</u>

Serine dehydratase	4.2.1.13	0.64(3)	<u>0.02</u>	<u>0.03</u>	--
Glutamine synthetase	6.3.1.2	0.64	<u>0.14</u>	<u>0.01</u>	<u>0.69</u>
Ornithine aminotransferase	2.6.1.13	0.22	0.05	0.11	0.55
Glucokinase	2.7.1.2	0.66	<u>0.02</u>	<u>0.04</u>	<u>0.22</u>
Alanine aminotransferase	2.6.1.2	0.34	<u>0.2</u>	<u>0.08</u>	--
Malate-NADP dehydrogenase	1.1.1.40	0.67	<u>0.01</u>	<u>1.02</u>	<u>1.93</u>

Table 3

The effect of tumor bearing on enzyme formation in developing livers

Four litters, aged 10 or 14 days, were divided into a control group and one implanted with Walker carcinoma; both groups of each litter were analyzed on the days indicated. Enzyme activities (units/g) for both the control and experimental rats were normalized by dividing them with the activity of the corresponding enzyme in normal adult male liver (tryptophan oxygenase, 3.4 units/g; for others, see Table 1); they are given as means ($\pm$ SD) of measurements on 4-6 rats.

	Control	Host	Control	Host
Days of age at assay	20	20	30	30
Days after implant	--	10	--	16
Body weight (-tumor)(g)	34 $\pm$ 1	34 $\pm$ 5	69 $\pm$ 6	70 $\pm$ 10
Liver weight (g)	1.32 $\pm$ 0.17	1.67 $\pm$ 0.26	3.6 $\pm$ 0.5	3.6 $\pm$ 0.6
Tumor weight (g)		7.5 $\pm$ 4.0		8.6 $\pm$ 0.5
Ornithine aminotransferase	0.52 $\pm$ 0.02	0.19 $\pm$ 0.02		
Glucokinase	0.45 $\pm$ 0.16	<0.10		
Glutamine synthetase	1.0 $\pm$ 0.20	0.60 $\pm$ 0.10		
Malate NADP dehydrogenase			1.10 $\pm$ 0.20	0.64 $\pm$ 0.10
Tryptophan oxygenase	0.50 $\pm$ 0.04	1.0 $\pm$ 0.20		
Hexokinase	1.2 $\pm$ 0.10	2.0 $\pm$ 0.70		

Activity in normal adult = 1.0

CAPTIONS

Fig. 1. Hexokinase isozymes in hepatic tissues. Extracts of fetal liver, hepatoma 7800, adult liver from 1 control and 2 tumor-bearing rats (14 days after the transplantation of Walker 256) were electrophoresed as described under Methods. Isozymes were numbered I, II and III in accordance with Katzen and Schimke (17).

Chart 1. The developmental formation of ornithine aminotransferase in normal and tumor bearing, suckling rats; the effect of hydrocortisone. Full symbols refer to rats without transplants and open symbols to rats implanted with Walker carcinoma at the age of 10 days. In each group some were injected with 2.5 mg per 100 g body weight hydrocortisone acetate 24 hr before assay (triangles). The activities, as in Table 3, are relative to those in normal adult livers.