NEOPLASTIC TRANSFORMATION-LINKED ALTERATIONS IN ADENYLOSUCCINATE SYNTHETASE ACTIVITY

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SUMMARY: Adenylosuccinate synthetase has been measured in rats in normal, differentiating, and regenerating liver, transplantable hepatomas of different growth rates, kidney cortex, and a transplantable kidney tumor. The activity was increased 1.6 to 3.7-fold in all the tumors. The activity showed no correlation with the degree of histological or biochemical differentiation of the tumors, nor with their growth rate. Adenylosuccinate synthetase activity in regenerating liver was unchanged and in neonatal liver it was much lower than in adult liver. It is concluded that the ubiquitous increase in the tumors of liver and kidney was linked with the neoplastic transformation.

Previous studies in this and other laboratories have demonstrated profound changes in the balance of purine biosynthesis and catabolism of malignant tissues. Human and rat hepatomas and rat kidney tumors all show significant increases, relative to appropriate control tissues, in the activity of glutamine PRPP amidotransferase, EC 2.4.2.14, which is the first enzyme in the de novo purine biosynthetic pathway (1,2). Several tumors, including rat hepatomas, are reported to have decreases in the activity of xanthine oxidase, EC 1.2.3.2, which is regarded as the rate-limiting enzyme of purine catabolism (3,4). These observations directed our attention to the metabolic disposition of IMP, since this nucleotide occupies a central position in purine metabolism (Fig. 1). It seemed probable that the enzymes utilizing IMP might act as key control points for the pathways of purine interconversion. Recent studies (5) showed that IMP dehydrogenase, EC 1.2.1.14, the first enzyme of the guanine nucleotide branch, was increased in rat hepatomas and kidney tumors, and that the increases correlated with the tumor growth rates. The IMP dehydrogenase activity of liver

was also increased following partial hepatectomy. These observations made it important to study the behavior of adenylosuccinate synthetase, EC 6.3.4.4, under conditions of normal and malignant cell proliferation, since this is the first enzyme of the adenine nucleotide branch, and competes with IMP dehydrogenase for IMP. The enzyme catalyzes the reaction: IMP + GTP + aspartate = GDP + P_i + adenylosuccinate.

Adenylosuccinate synthetase was studied in great detail in rabbit muscle (6) It has also been examined in rat liver (7) and Novikoff hepatoma (7,8). However, comparative measurements of the enzyme in tumors and in the homologous normal tissues have not been published. This paper presents studies of the enzyme in rat hepatomas and kidney tumor, compared to the normal liver and kidney cortex, respectively. Levels of the enzyme in two non-malignant proliferating hepatic cell populations - regenerating liver and differentiating neonatal liver - are also reported.

MATERIALS AND METHODS.

Substrates and cofactors were purchased from Sigma Chem. Co., St. Louis, Mo. 8-[^{3}H]-IMP was obtained from Amersham/Searle, Arlington Heights, Ill., at a specific activity of 5.5 Ci/mmol. Phosphoethyleneimine-cellulose thin layer plates were from Macherey-Nagel, Düren, Germany.

Animals and tumors: Inbred male animals were kept in separate cages with water and Purina chow available ad libitum. Hepatomas 3924A and 3683 were carried in ACI/N rats; all other tumors in Buffalo strain rats. The tumors were implanted subcutaneously, and harvested at a diameter of about 1.5 cm. Rats were killed between 9 a.m. and 10 a.m. in all experiments. Animals were stunned, decapitated, and bled; tumors were removed, and freed from necrotic, hemorrhagic, and non-tumor material. Normal rats of the same sex, strain and age were killed at the same time for control tissues. The properties of the tumors used are described in ref. 9.

Regenerating liver: ACI/N rats of 200 g body weight were used. Partial hepatectomy was carried out under ether anesthesia by the standard method (10). Sham-operated animals were used as controls.

Studies on differentiating liver: When using rats under 7 days old, 3 or more samples were prepared, each containing pooled livers of 4-6 animals. Beyond this age individual livers were used. The developmental changes occurring in livers of neonatal rats are discussed in ref. 11. Cellularity was assessed by counting of orcein-stained nuclei (12).

Enzyme assays: The procedure for working-up adenylosuccinate synthetase is summarized in Fig. 2. The standard assay mixture contained IMP (0.4 µmole), GTP (0.22 µmole), $MgCl_2$ (16.5 µmole) and aspartate (2.5 µmole) plus enzyme and N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate buffer, 0.05 M, pH 7.2, to a final volume of 1.3 ml. The mixture was equilibrated to 37°, reaction was started by addition of enzyme, and activity was monitored at 280 nm in a Gilford 2400-S spectrophotometer. Rates were corrected for the slight optical density change

which occurred in absence of aspartate.

Chromatographic assay: 8-[^{3}H]-IMP was added to the standard system at 0.5 μCi/ml. The reaction was stopped by perchloric acid, and after removal of the precipitated protein, and neutralization of the supernate with KOH, a mixed carrier solution containing AMP, IMP, XMP, GMP, ADP and adenylosuccinate (all at 10 mM) was added. Aliquots of the mixture were applied to a phosphoethyleneimine-cellulose thin-layer plate and the chromatogram was developed for 2.5 hr with 0.5 M sodium formate, pH 3.4. Carrier spots gave the following R_f values: AMP, 0.74; IMP, 0.51; XMP, 0.38; GMP, 0.26; ADP, 0.12; adenylosuccinate, 0.06; nucleosides and purine bases migrated at the solvent front, and ATP, GDP and GTP remained at the origin. The portions of the plate containing the various compounds were cut out and counted in a scintillation counter.

RESULTS AND DISCUSSION:

Preparations of adenylosuccinate synthetase partially purified (Fig. 2) and assayed optically gave rates which were linear with enzyme amount up to 0.005 I.U./ml, and for up to 15 min. The identity of the reaction was confirmed by the chromatographic assay. A time-, aspartate-, GTP- and enzyme-dependent conversion of IMP radioactivity into adenylosuccinate was catalyzed. No radioactivity was recovered in other nucleotides.

Liver enzyme prepared as described in Fig. 2 was used for preliminary kinetic studies. Apparent Km values were 14 μM for GTP, 32 μM for IMP and 201 μM for aspartate. Enzyme purified from hepatoma 3924A gave very similar values. A recent study in Novikoff hepatoma (8) showed a higher Km for aspartate, making this amino acid one of the rate-limiting factors in purine bio-

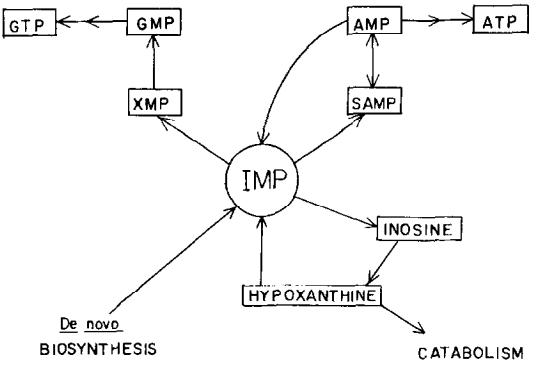

Fig. 1. Schematic metabolic map of some reactions of purine interconversion, showing the strategic role of IMP.
Abbreviation: SAMP, adenylosuccinate.

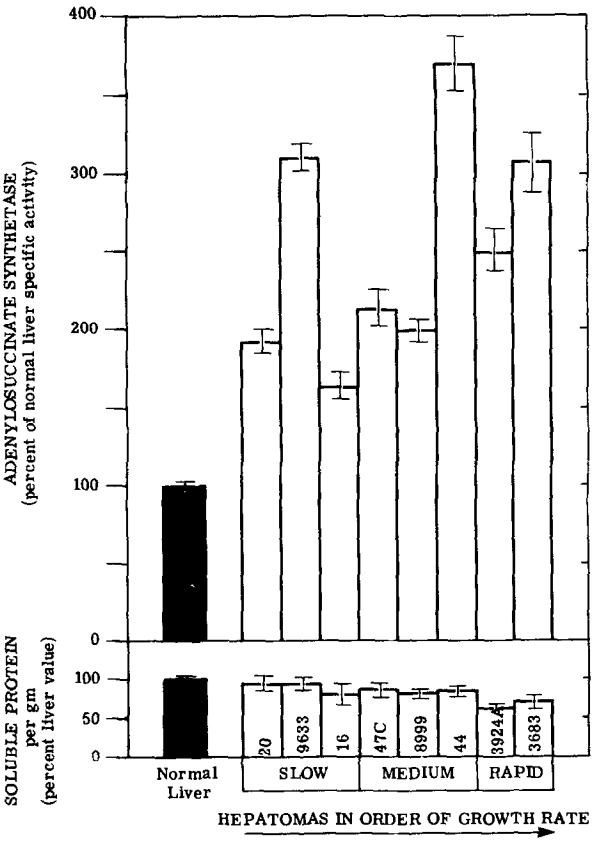

Fig. 2. Adenylosuccinate synthetase in rat hepatomas. 25% (w/v) homogenates were prepared in 0.02 M tris chloride, pH 7.4 containing 1 mM EDTA. 100,000 g supernates were added to 10 vol. acetone at -60°. 200 mg of the dried acetone powder were suspended in 8 ml of 0.05 M tris chloride, pH 7.4, containing 1 mM dithioerythritol (tris/DTE). Insoluble material was removed by centrifugation, and the supernate was treated with 1.25 ml calcium phosphate gel. After a further centrifugation (50,000 g, 10 min) the supernatant solution was fractionated with ammonium sulfate. The portion precipitating between 45% and 70% saturation was redissolved in 2 ml of tris/DTE and dialyzed for 3 hr against 1 l. of the same buffer. The resulting material was the only fraction containing measurable adenylosuccinate synthetase, and it was free from interfering activities. The data in the upper histogram show enzyme activities (plotted as percent of control liver values) representing mean ± S.E. of 5 animals in each group. The lower histogram shows protein content of the 100,000 g supernates, as measured by the Biuret method.

synthesis; the present results with hepatoma 3924A indicate that this behavior is not a general property of rapidly growing hepatomas.

The mean activity in the normal liver samples was 34.9 nmole substrate

converted/hr/mg soluble protein, with a coefficient of variation of 18%. The soluble (i.e. 100,000 g supernate) protein content was 88 mg/g wet weight of liver. Activities found in the hepatomas are summarized in Fig. 2. All the hepatomas showed increases in the specific activity of this enzyme, ranging from 1.6- to 3.7-fold. Even the slowest growing hepatomas, with intervals of a year or more between successive transplants, showed this increase, and there was no correlation of activity with the tumor growth rate.

Adenylosuccinate synthetase was purified in a similar manner from rat kidney cortex. The mean activity was 20.2 nmole/hr/mg protein (protein: 61.7 mg/g wet weight). Mean activity in three MK3 transplantable kidney tumors was 63.6 nmole/hr/mg protein (range: 59.6 - 67.4; protein content: 58.5 mg/g wet weight), equivalent to 315% of normal kidney activity.

To examine the behavior of adenylosuccinate synthetase in a non-malignant hyperplastic situation, we measured the activity of this enzyme in regenerating rat liver. Samples were taken 12, 18, 24, 48, 72 and 96 hr following partial hepatectomy. At each time point, 5 or 6 partially-hepatectomized rats and 5 or 6 sham-operated rats were killed. Adenylosuccinate synthetase was partially purified (Fig. 2) and assayed as described in the Methods section. Activity in all sham-operated groups was between 92% and 107% of normal. The activity in all partially hepatectomized groups was within 9% of normal, except the 24 hr point, which was 113% of normal; this small elevation was not statistically significant at the 5% level. We conclude that proliferation of hepatic cells does not, in itself, involve an increase in adenylosuccinate synthetase activity.

An alternative proliferating hepatic cell population is provided by the neonatal liver. Fig. 3 shows adenylosuccinate synthetase activity in livers of young rats. It should be noted that the mean cell size of baby rat livers is only about 40% of the adult cell size; thus to obtain a meaningful index of rates of gene expression in this situation it is preferable to calculate activity as enzyme units per cell, rather than per g wet weight, or per mg protein. The activity, per cell, in new-born rats was about 13% of the adult value, ris-

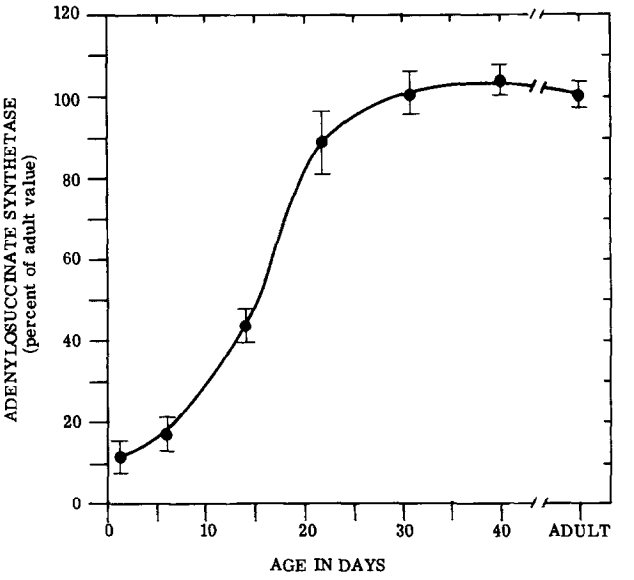

Fig. 3. Adenylosuccinate synthetase in livers of young rats. Activity is expressed as enzyme units per cell, as a percentage of the normal adult value. Vertical lines indicate standard error.

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ing rapidly after a week, and approaching the adult value at about 3 weeks of age.

Adenylosuccinate synthetase clearly has a characteristic pattern in proliferative states; in comparison to normal liver, the activity is increased in hepatic neoplasia, it is unaltered in regeneration and activity is low in differentiating liver. The enzyme activity is not related to tumor proliferative rate since the activity is increased in all the hepatomas. Since the synthetase activity was increased even in the slowest growing, best differentiated tumors the elevation in the hepatomas appears to be linked with the malignant transformation per se. This interpretation is supported by studies with the kidney tumors, which also show increased activity. The behavior of synthetase is in striking contrast to the other enzyme of IMP utilization, IMP dehydrogenase. The latter enzyme is also increased in malignancy, but the increase correlates with tumor growth rate; IMP dehydrogenase was also elevated in non-malignant cell proliferative states, e.g. regenerating liver (5). Adenylosuccinate synthetase, on the other hand, appears to reflect a pattern of reprogramming of gene expression

independent of the rate of cell proliferation, but linked to the neoplastic transformation.

In this Laboratory recently there were discovered four such transformation-linked increases in enzyme activities, and they all relate to an increased capacity in the channeling of precursors to strategic biosynthetic processes. Thus, the elevation in all hepatomas in the activities of glucose-6-phosphate dehydrogenase, EC 1.1.1.49, and transaldolase, EC 2.2.1.2, increases the potential for routing glycolytic intermediates into pentose phosphate biosynthesis (13). The increase in UDP kinase, EC 2.7.4.6, activity should provide a heightened potential for RNA and DNA biosynthesis (14). The rise in amidophosphoribosyltransferase, EC 2.4.2.14, activity should result in an increased potential for de novo IMP biosynthesis (2). In turn, the increased adenylosuccinate synthetase activity should provide an increased potential for the biosynthetic utilization of IMP for the de novo production of adenine nucleotides. These elevations in activities of key enzymes of ribose-5-phosphate and purine biosynthesis, UTP production and adenylosuccinate synthetase activity indicate a reprogramming of gene expression that as an integrated pattern is specific to malignant transformation and should confer selective biological advantages to neoplastic cells.

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