AMINO ACID CONCENTRATING ABILITY OF SLOWLY GROWING AUTOCHTHONOUS HEPATOMAS AND HOST LIVERS

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SUMMARY:

To examine whether an increase in the intracellular concentration of amino acids always accompanies the development of a neoplasm, slowly growing autochthonous hepatomas (originating in the place where found) were induced by feeding 0.02% acetylaminofluorene followed by 0.05% phenobarbital to Buffalo strain rats. It was found that the resulting hepatomas (123 from 43 animals) concentrated less than one half the amount of non-metabolizable alpha-aminoisobutyric acid (AIB) than did the respective host and control livers when AIB was injected 24 hrs before sacrifice. While an increase in the ability to concentrate amino acids may be necessary for rapid growth, it is not a concomitant of neoplastic transformation. The narrow range of AIB concentrations found among these 123 autochthonous tumors compared to a wide spectrum of AIB concentrations found in several transplantable Morris hepatoma lines suggests that these tumors are at one of the earliest stages of their progression.

INTRODUCTION

Malignancy-associated alterations in the transport of nutrients across the cell membrane have been investigated by several workers and Holley (1) has expressed the view that "the primary cause of tumor growth is the increased concentration of critical nutrients inside the cell". In general, transformed cells show an increased uptake of sugars compared to normal cells (2-10). Although there are now conflicting reports regarding the increase in amino acid transport of tumor cells compared to normal cells (11-15), Johnston and Scholefield (16) suggested in 1965 that "tumors, in general, are able to concentrate amino acids in vivo and to compete favorably with most other tissues for whatever amino acids are available". Similarly,

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Christensen and Henderson (17) had proposed in 1952 that the rapid growth of neoplastic cells was due to their greater ability to accumulate free amino acids.

The AIB concentrating ability of several transplantable Morris hepatomas, *in vivo*, was investigated in this laboratory (18-20) and a wide range in the concentrating ability of these hepatoma lines was found both above and below that of normal liver. The present results suggest that an increase in the ability to concentrate amino acids may be a concomitant of rapid growth but not of neoplastic transformation *per se*.

MATERIALS AND METHODS

In the studies reported here amino acid concentrating ability was determined in 123 autochthonous rat hepatomas and the adjacent portions of 43 host livers by examining the equilibrium concentration, relative to blood, of 1-[^{14}C] AIB injected intraperitoneally (1 μCi in 0.125 μmoles per 100 gm body weight) 24 hours before sacrifice, according to the techniques previously employed (18-20). Normal livers from 14 rats raised on non-carcinogenic diets were used as additional controls.

The procedure used in the development of autochthonous tumors was modified from the protocol described by Peraino *et al.* (21-24). Briefly 41 males and 41 females of the Buffalo strain of rats, when 30 days old, were fed a 30% casein diet containing 0.02% 2-acetylaminofluorene (AAF) as an "initiator". After 30 days of feeding the AAF diet, the AAF in the diet was replaced by 0.05% phenobarbital (PB) as a "promoter".

During the entire course of experiments the rat room was lighted between 20:30 and 8:30 hrs and kept dark between 8:30 and 20:30 hrs. Up to 245 days on PB diet the animals were fed *ad libitum*, then for 3 days food was provided only during the first 8 hrs of the dark period. Starting on 248th day of PB diet food was available only for the first 2 hrs of the dark period. The 32 males and 11 females that had survived were killed after 259, 288, or 295 days on the phenobarbital-containing diet, with some additional manipulations including single injections of N-nitrosodimethylamine (DMN, 5.5 or 11.0 mg/kg body weight) 23 or 30 days before the end of the experiment. Many females had been killed earlier because of large mammary tumors. The animals were killed by decapitation between 14:00 and 16:00 hrs (5 1/2 to 7 1/2 hrs after the start of dark period). The easily dissected nodules that resulted are considered to be comparable to the nodules that have been described by Farber (25) as hyperplastic and by a committee of pathologists as "neoplastic" (26). The uniformity of the AIB ratios seen in these nodules may indicate that the nodules may be the earliest stages in the development of the hepatocellular carcinomas (27).

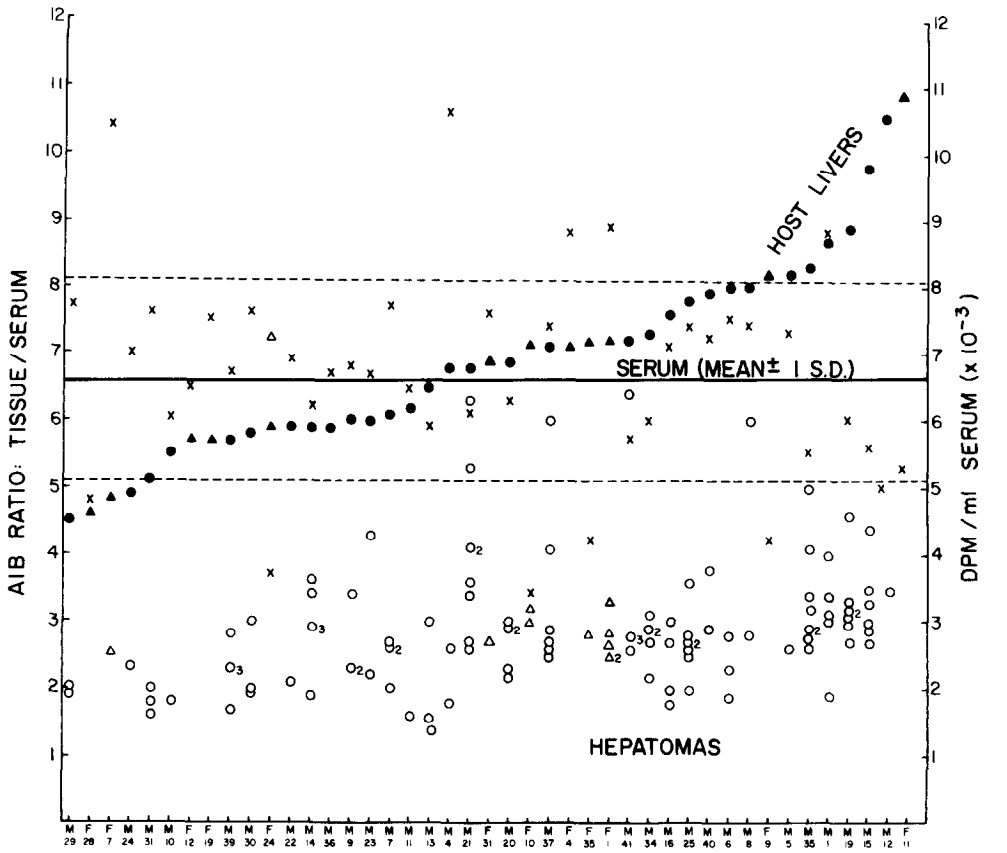

Figure 1.

Comparative AIB concentrating ability of the livers and hepatomas from male and female rats. The AIB concentrating ability is expressed as AIB ratio [(DPM/g liver or hepatoma)/(DPM/ml serum)]. Symbols used are as follows: ● liver male rats, ▲ liver female rats, O hepatoma male rats, △ hepatoma female rats. The letters M or F on the abscissa identify the sex of the animal. Rat numbers are also shown. The AIB ratios for the host livers from 31 male and 12 female rats are arbitrarily arranged in ascending order and the AIB ratios for the hepatomas are shown under or above the corresponding host livers. For livers where no corresponding hepatomas are shown, no macroscopic hepatomas were found. Letters on the lower right corner of a O or △ show the number of hepatomas, from the same liver, that had the same AIB ratio. The mean ± SEM of the AIB ratios for host livers from male, female and males and females combined were 6.97 ± 0.26, 6.77 ± 0.48, 6.91 ± 0.23 respectively. The difference between the means for males and females is statistically non-significant. The corresponding values for the AIB ratios for the hepatomas from males, females and the two together were 2.92 ± 0.09, 3.20 ± 0.41 and 2.95 ± 0.09 respectively. Again the difference between the means for the males and females is non-significant. The difference between the means of the AIB ratios of livers and hepatomas is significant at $p < 0.001$, for both male and female rats.

The number of DPM/ml serum X 10^{-3}, for each rat is shown by X. The mean ± 1SD for serum radioactivity are shown by solid and dotted lines, respectively. Ninety-five percent of the serum radioactivity values lie within mean ± 2 SD.

RESULTS AND DISCUSSION

The AIB concentrating ability of 43 host livers and 123 macroscopically individual hepatomas is shown in Fig. 1. This figure shows that most of the hepatomas concentrated AIB to less than one half the extent seen in their corresponding host livers. Our results on AIB ratios for host livers and normal (non-tumor bearing) livers are comparable to those of Scott et al. (20) for the livers of rats bearing transplantable Morris hepatomas.

Different hepatomas from the same liver, in general, showed very similar AIB concentrating ability. Nonetheless at least two-fold differences in the equilibrium concentration of AIB could be observed in some cases (Figure 1). The same general similarity in the ability to concentrate AIB which was found among hepatomas within a single liver was also found among hepatomas from different animals. The sex of the animal did not seem to affect the AIB concentrating ability of either host livers or hepatomas, since the AIB ratio for the livers and hepatomas from both sexes was found within the range of AIB distribution for all the animals.

Data presented in Fig. 1 show the differences and similarities discussed above, but the similarities in AIB ratio among the livers and among the hepatomas or the differences in the AIB concentrating ability of hepatomas vs. livers can be more explicit if the distribution of the AIB ratios of all the livers is compared to that of all the hepatomas. Such a comparison between hepatomas and livers is shown in Fig. 2. This figure shows the distribution of 43 livers and 123 hepatomas over a range of AIB ratios from a minimum of 1.40 to a maximum of 10.90. The difference between the means of AIB ratios for hepatomas and host livers is highly significant (Fig. 2).

It should be noted that no distinction is made as to the AIB ratios for animals that received high, low or no DMN late in the experiment, because the AIB ratios of DMN-treated livers or hepatomas did not differ significantly from those of the non-treated animals. Actually DMN was injected to see if

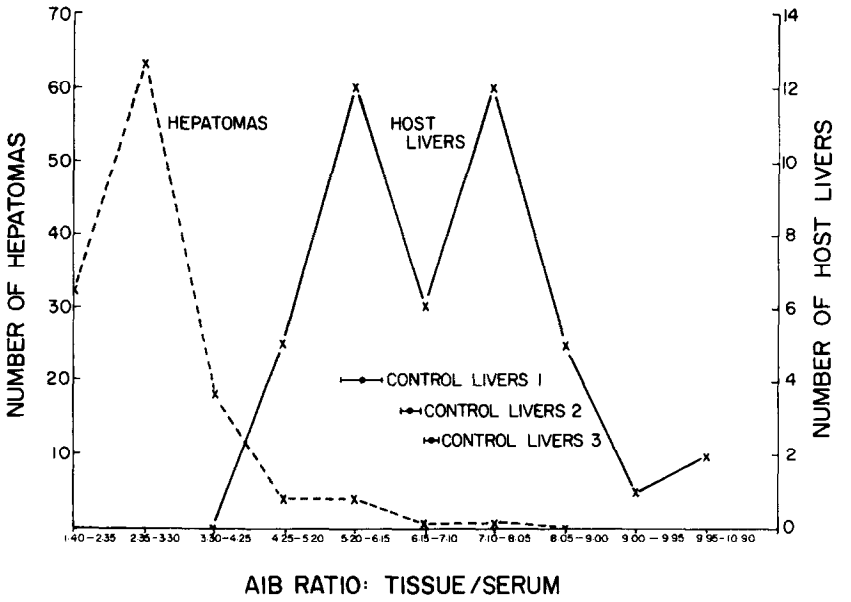

Figure 2.

The distribution of the AIB ratios of 123 hepatomas and 43 host livers over their range, from 1.40 to 10.90. The entire range of 9.50 is divided into ten equal groups. The dotted and solid lines represent hepatomas and host livers respectively. Control livers 1 represents the mean ± SEM of the AIB ratio for 3 Sprague-Dawley rats raised on a 30% protein, non-carcinogenic diet and killed at the start of dark period. Control livers 2 are the same as control livers 1, except these 3 animals were killed 5 hrs after the start of the dark period. Control livers 3 represent the mean + SEM of AIB ratios for 8 Sprague-Dawley rats raised on 60% protein, non-carcinogenic diet and killed at the start of the dark period.

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it would convert any individual slowly growing hepatoma cells to fast growing hepatoma clones. Such conversions as may have occurred would not be expected to alter the entire population of a slowly growing nodule or to result in large masses of fast growing cells in the time allowed. Results on this subject based on autoradiographic and other parameters will be published separately.

In view of the poor AIB concentrating ability of the slowly growing autochthonous hepatomas (present report) and those of slowly growing transplantable Morris hepatomas (20) compared to the normal livers, we conclude that an increase in the ability to concentrate amino acids does not always accompany the neoplastic transformation.

The findings on the 123 autochthonous hepatomas summarized in Fig. 2 have significance relative to the concept of clonal evolution (28) and to the minimal deviation concept (29). It is implicit in the minimal deviation hypothesis that the closer the hepatomas conform to the hypothetical minimum deviation, the more alike they will appear (27,29). The relatively low and narrow range of AIB ratios seen in the hepatomas (Fig. 2) and the fact that most of the various lines of transplantable Morris hepatomas show much higher values that are characteristic of each line (18-20) suggest that the hepatomas seen in the present study are examples of the earliest stages of progression along the branched pathway that leads to the vast diversity seen in AIB ratios among the transplantable hepatomas. Even within the uniformity with respect to AIB ratios, these hepatomas show considerable diversity with respect to some enzyme activities (not shown). Thus the definition of the critical phenotypic alteration in transformation remains undefined. That progression can indeed occur in the transplantable lines is illustrated in the case of the lines derived from the original No. 5123 (28). The autochthonous hepatomas in the present study approach the minimal deviation type (slowly growing, highly differentiated) and hence they resemble each other with respect to AIB transport rather than exhibiting the diversity seen in the entire spectrum of Morris hepatomas. Autochthonous hepatomas produced by minimal dosages of initiators as in the present report should be a valuable experimental tool in testing claims as to the critical changes that produce "transformation".

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