Pseudoisozymes of Hepatic Tyrosine Aminotransferase*

(Received for publication, August 8, 1973)

CAROLYN J. SPENCER AND THOMAS D. GELEHRTER

From the Departments of Human Genetics, Medicine and Pediatrics, Yale University School of Medicine, New Haven, Connecticut 06510

SUMMARY

Dexamethasone, insulin, and serum induce an additive increase in the activity of tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) in hepatoma cells in tissue culture. By the criteria of immunotitration, heat stability, and electrophoresis on polyacrylamide gels, we have shown previously that all three effectors increase the cellular concentration of the same protein (GELEHRTER, T. D., EMMANUEL, J. R., SPENCER, C. J. (1972) J. Biol. Chem. 247, 6197-6203). During the course of these studies however, we observed an apparent isozyme of tyrosine aminotransferase whose activity is not enhanced by these three inducers.

This minor, more cathodal form is also a heat-stable, cytoplasmic enzyme which differs from the major anodal form in net charge but not molecular weight. Unlike the anodal form, however, this enzyme can utilize oxalacetate in place of α-ketoglutarate in the transamination of tyrosine, and can transaminate aspartate as well as tyrosine. A similar, heat-stable, cathodal form with some biochemical properties has also been found in cytoplasmic extracts of adult and fetal rat liver.

We have used immunologic criteria to characterize further the apparent heterogeneity of hepatic tyrosine aminotransferase. Incubation of extracts of hepatoma cells, and adult, and fetal rat liver with antisera to purified rat liver soluble tyrosine aminotransferase inactivates only the anodal enzyme; whereas incubation with antisera to purified pig heart soluble aspartate aminotransferase (L-aspartate:Z-oxo-glutarate aminotransferase, EC 2.6.1.1) inactivates only the cathodal form. Thus, the latter activity presumably represents a cytosolic enzyme, but unlike H-1, is capable of utilizing oxalacetate in place of α-ketoglutarate in the transamination of tyrosine, and can actively transaminate aspartate.

These two properties differentiate H-3 from the soluble tyrosine aminotransferase described in rat liver, since purified rat liver tyrosine aminotransferase manifests an absolute requirement for α-ketoglutarate and does not transaminate aspartate (8). Aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1), on the other hand, can utilize oxalacetate, but there is disagreement in the literature as to whether this enzyme can (9) or cannot (10) transaminate tyrosine. Therefore, although H-3 might represent aspartate aminotransferase, it might also represent a previously undescribed tyrosine aminotransferase isozyme, or possibly the expressed tyrosine aminotransferase (H-1) in HTC cells, but differs in net charge. Like the latter, H-3 appears to be a heat-stable cytoplasmic enzyme, but unlike H-1, is capable of utilizing oxalacetate in place of α-ketoglutarate in the transamination of tyrosine, and can actively transaminate aspartate.

During the course of these studies, however, we observed apparent heterogeneity of tyrosine aminotransferase activity in HTC cells which was not previously recognized. We demonstrated electrophoretically a second enzyme capable of transamaining tyrosine, which is not affected by dexamethasone, insulin, or serum. Preliminary experiments suggested that this enzyme, designated H-3 on the basis of its electrophoretic mobility, has the same molecular weight as the major cytosolic tyrosine aminotransferase (H-1) in HTC cells, but differs in net charge. Like the latter, H-3 appears to be a heat-stable cytoplasmic enzyme, but unlike H-1, is capable of utilizing oxalacetate in place of α-ketoglutarate in the transamination of tyrosine, and can actively transaminate aspartate.

These properties differentiate H-3 from the soluble tyrosine aminotransferase described in rat liver, since purified rat liver tyrosine aminotransferase manifests an absolute requirement for α-ketoglutarate and does not transaminate aspartate (8). Aspartate aminotransferase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1), on the other hand, can utilize oxalacetate, but there is disagreement in the literature as to whether this enzyme can (9) or cannot (10) transaminate tyrosine. Therefore, although H-3 might represent aspartate aminotransferase, it might also represent a previously undescribed tyrosine aminotransferase isozyme, or possibly the expression in the neoplastic hepatoma cell of a fetal transaminase not expressed in adult liver (11).

In order to distinguish between these alternatives, and to characterize the basis for the apparent heterogeneity of tyrosine aminotransferase activity in HTC cells, we have utilized the technique of polyacrylamide gel electrophoresis coupled with histochemical staining to analyze the heat stability, amino acid and ketoacid specificity, and antigenic nature of the tyrosine and aspartate aminotransferase activities of cytoplasmic extracts of HTC cells, fetal rat liver, and adult rat liver. We have found an enzyme in both fetal and adult rat liver cytosol identical in

* This investigation was supported by United States Public Health Service Grant GM 17712 from the National Institute of General Medical Sciences.

† The abbreviation used is: HTC, hepatoma tissue culture.
all these respects to H-3, and have demonstrated immunologically that this enzyme appears to be aspartate aminotransferase. Since transaminases may exhibit rather broad substrate specificities (9, 10, 12, 13), and since aspartate aminotransferase is present in hepatocytes in large amounts, our sensitive histochemical assay detected this activity as an “isozyme” of tyrosine aminotransferase. This finding may also explain several other reported examples of what appeared to be multiple molecular forms of tyrosine aminotransferase (5–7, 14).

MATERIALS AND METHODS

Preparation of Cell Extracts—Cell culture, enzyme induction, and preparation of cell extracts in the HTC cells were performed as described previously (4). Livers from adult male Sprague–Dawley rats weighing 200 to 300 g, or fetal rats (1 or 2 days before term) were excised, minced, and washed twice at 4°C in 0.05 M potassium phosphate, pH 6.5, containing 0.2 mM pyridoxal phosphate, 1 mM α-ketoglutarate, and 1 mM EDTA. Homogenates, 25% (v/v), of the adult rat livers and homogenates, 33%, of fetal livers were prepared in a Sorvall Omnimixer, filtered through gauze, and centrifuged for 60 min at 100,000 × g at 4°C. All supernatant fluids were routinely heated at 65°C for 6 min to achieve a 3- to 4-fold purification of tyrosine aminotransferase. After heat treatment the preparations were centrifuged at 45,000 × g for 20 min. These supernatant fluids were used for all enzyme assays and electrophoretic experiments.

Enzyme Assays—Tyrosine aminotransferase was assayed by a modification of the method described by Diamondstone (15). The following stock solutions were prepared. Solution A, 550 mg of α-tyrosine dissolved in 250 ml of 0.125 M dibasic potassium phosphate with the addition of 1.2 ml of 10 N KOH; Solution B, 0.125 M monobasic potassium phosphate; Solution C, 0.5 M α-ketoglutarate in 0.1 M potassium phosphate, pH 7.6; Solution D, 20 mM pyridoxal phosphate; Solution E, bovine serum albumin, 10 mg per ml. The assay mixture was prepared by mixing 5 parts Solution A with 3 parts Solution B, adjusting the pH to 7.6, and finally adding 0.2 parts Solution C, 0.1 part Solution D, and 0.2 parts Solution E. Ten to 100 μl of cell extract were added to 0.8 ml of the assay mix and incubated for 15 min at 37°C. The reaction was stopped by the addition of 0.1 ml of 10 N KOH. Following incubation for an additional 20 min at 37°C, the samples were read at 331 nm against an enzyme blank sample to which KOH had been added prior to addition of the assay mix. One milliunit of tyrosine aminotransferase catalyzes the formation of 1 nmole of p-hydroxyphenylpyruvate per min at 37°C.

Aspartate aminotransferase was assayed by the malate dehydrogenase-linked assay of Sizer and Jenkins (16). One milliunit of aspartate aminotransferase catalyzes the conversion of 1 nmole of oxaloacetate per min at 25°C. Succinate dehydrogenase (17) and glucose 6-phosphate dehydrogenase (18) were assayed by standard methods. Protein was measured by the method of Lowry et al. (19).

Polyacrylamide Gel Electrophoresis—Sample preparation and electrophoresis were carried out as described previously (4), except that α-ketoglutarate was omitted from the cathode chamber, resulting in a more rapid electrophoretic separation. Electrophoresis was routinely performed at 3 mA per gel for 90 min at room temperature, using 0.5 × 7.5 cm, 6% acrylamide running gels. Further experimental details are contained in the legends to the figures. Histochemical staining of tyrosine aminotransferase was performed as reported previously (4). Aspartate aminotransferase was assayed histochemically by a modification of the method of DeLorenzo and Ruddle (20), using Fast Violet B Salt (6-benzamido-4-methoxy-m-toluidine, diazonium chloride; diazotate-6-benzamido-4-methoxy-m-toluidine chloride), obtained from Sigma.

Inactivation of Aminotransferase Activities by Specific Antisera—Antiserum prepared in sheep to highly purified soluble rat liver tyrosine aminotransferase was provided by Dr. David W. Martin, Jr. (4, 21), and antiserum prepared in rabbits to purified soluble pig heart aspartate aminotransferase was kindly given to us by Dr. Marino Martinez-Carrion (9). Enzyme preparations were mixed with the antibody and incubated at 37°C for 2 hours. The samples were then kept at 4°C overnight and centrifuged at 17,000 × g for 20 min at 4°C. Supernatants were assayed for enzymatic activity and aliquots applied to acrylamide gels.

RESULTS

Heterogeneity of Tyrosine Aminotransferase Activity in HTC Cells—The apparent heterogeneity of tyrosine aminotransferase activity in HTC cells is shown in Fig. 1 (top). In this experiment, an extract of deaminohexose-induced HTC cells was subjected to electrophoresis, followed by histochemical staining at 60°C for tyrosine aminotransferase activity. After 10 min, a single band of activity is seen near the anode. After 20 min of staining, this band has become more prominent and a more cathodal band, designated H-3 is now apparent. With increasing time of staining, both bands become more intense. A band of intermediate mobility, H-2, is occasionally seen, and can be appreciated in this figure. The staining of the intermediate band is quite variable, and we have shown previously that this band represents an enzymatically active oligomeric form of H-3 (4).

The increase in histochemically assayable tyrosine aminotransferase activity with time of staining, expressed as the area under the peaks as measured by planimetry, is shown in Fig. 1 (bottom). Histochemically assayable tyrosine aminotransferase activity in H-1 increases more or less linearly for approximately 20 min, after which it appears to reach a plateau. Stainable activity in H-3, on the other hand, does not usually appear until approximately 20 min, and then increases linearly with time. The interval during which histochemical activity is linear with time depends on the amount of enzyme applied to the gel. Thus, if one stains the gels for a brief interval, the density of the major band (H-1) is proportional to the total amount of tyrosine aminotransferase applied to the gel, providing a reasonably accurate quantitative estimate of enzyme activity.

If the gels are stained for a longer period such as 60 to 120 min, the assay becomes more sensitive in that smaller amounts of tyrosine aminotransferase activity can be detected, but quantitation is lost. Under these conditions, the proportion of total transaminase activity attributable to the minor form will be overestimated. In the experiments described below, the longer staining periods have usually been employed in order to define the qualitative characteristics of the minor form of tyrosine aminotransferase activity.
ferase Activities in HTC Cells—Since cytosol tyrosine and aspartate aminotransferases reportedly differ in their amino acid and ketoacid specificities (8–10), we have examined these properties of H-1 and H-3 from HTC cell extracts. Fig. 2 compares the histochemical assay of tyrosine aminotransferase using oxalacetate in place of α-ketoglutarate as the ketoacid. Both H-1 and H-3 activities are demonstrable in the presence of α-ketoglutarate; however, only H-3 is able to transaminate tyrosine using oxalacetate as the amino group acceptor, confirming that H-1 requires α-ketoglutarate. In the absence of either ketoacid there is no histochemical tyrosine aminotransferase activity.

Since H-3 is capable of transamminating aspartate we have studied the effect of L-aspartate on the transamination of tyrosine. In the presence of aspartate, the transamination of tyrosine by H-1 is not inhibited and in fact may actually be enhanced somewhat, in contrast, the transamination of tyrosine by H-3 is markedly inhibited by aspartate in the staining mixture. Fig. 3 demonstrates the inhibition of staining with increasing concentrations of aspartate. At 8 mM L-aspartate, a concentration half that of the substrate monoiodotyrosine, tyrosine aminotransferase activity in H-3 is inhibited by approximately 50%.

Elution of Enzyme Activity from Polyacrylamide Gels—In order to examine further the properties of the two tyrosine transami-
nating activities, the enzymes were electrophoretically eluted from the gel. After locating the bands of activity histochemically in two gels, the corresponding segments of unstained gels were removed, placed in glass tubes, and subjected to electrophoresis under these same conditions for an additional 30 min. The eluted material was collected in a small dialysis bag attached to the anodal end of the gel tube (29). The material eluted from H-1 contained tyrosine aminotransferase activity when assayed biochemically as described under "Materials and Methods." When this material was subjected to re-electrophoresis, a single band of histochemical activity was seen in the position of H-1. In contrast, the material eluted from H-3 contained biochemically assayable aspartate aminotransferase but not tyrosine aminotransferase activity. When this material was subjected to re-electrophoresis a single band of aspartate aminotransferase activity was seen in the expected position of H-3. In addition, however, tyrosine aminotransferase activity was also demonstrated histochemically in the same position. It is unlikely that the elution procedure inactivated tyrosine aminotransferase since histochemically assayable enzyme was demonstrable upon re-electrophoresis. The possibility that an inhibitor of tyrosine aminotransferase was eluted at the same time as the enzyme was eliminated by experiments in which eluates of H-3 were mixed either with cell extracts or with eluates of H-1. Furthermore it is unlikely that the discrepancy between the biochemical and histochemical activity of tyrosine aminotransferase of H-3 is a result of using tyrosine as the substrate for the biochemical assay and the more soluble monooiodotyrosine for the histochemical assay. Both tyrosine and monooiodotyrosine are equally effective in the histochemical assay of tyrosine aminotransferase in both H-1 and H-3, and in the biochemical assay of H-1. We believe that the difference between the biochemical and histochemical activity of H-3 is a function of the duration and temperature of the assays. The histochemical assay is carried out at 60° for 30 to 90 min, while the biochemical assay is routinely performed at 37° for 15 min. Cells stained histochemically at 37° also develop tyrosine aminotransferase activity migrating in the H-3 position; however, this occurs only after staining for about 2 hours. Conversely, when the biochemical assay is performed at 60° for 15 min, low levels of tyrosine aminotransferase activity are demonstrable in eluates of H-3.

Differentiation of Cytoplasmic and Mitochondrial Aminotransferase. It has been reported recently, that in rat liver mitochondria the tyrosine aminotransferase and aspartate aminotransferase activities are the result of the same enzyme (9). It seems very unlikely, however, that H-3 represents this mitochondrial transaminase. The mitochondrial enzyme is quite heat-stable. Furthermore, the two forms of tyrosine aminotransferase activity demonstrated in Fig. 1 are clearly heat-stable. These extracts have been subjected to heating at 65° for 6 min prior to electrophoresis, and the histochemical staining itself is performed at 60°. Furthermore, the cell extracts were prepared under conditions in which mitochondria should be sedimented by centrifugation. In order to confirm this latter point, HTC cells were homogenized in 0.05 m potassium phosphate, pH 7.6, containing 0.2 mM pyridoxal phosphate and 1 mM α-ketoglutarate. Sucrose was added to a final concentration of 0.25 m, and a mitochondrial pellet was collected by centrifugation at 20,000 × g for 20 min at 4°. All of the total cellular succinate dehydrogenase activity was found in the mitochondrial pellet and none in the supernatant; 98 to 99% of the total glucose 6-phosphate dehydrogenase activity was found in the supernatant fraction. When the mitochondrial extract was subjected to electrophoresis and histochemical staining at 37°, a single rather broad band of tyrosine aminotransferase activity was observed near the cathode, not in the position of any of the previously described tyrosine aminotransferase activities. After heating the mitochondrial preparation for 6 min at 65° no histochemical tyrosine aminotransferase activity was demonstrable. Therefore, it appears that H-3, like H-1, represents a heat-stable, soluble transaminase, which, unlike H-1, is capable of transaminating aspartate as well as tyrosine, and of utilizing oxalacetate in place of α-ketoglutarate in the transamination of tyrosine.

Heterogeneity of Tyrosine Aminotransferase Activity in Adult and Fetal Rat Liver. In order to determine whether this heterogeneity of tyrosine aminotransferase activity might represent the expression in the malignant hepatoma cell of a fetal enzyme not expressed in adult rat liver, we have performed similar studies on liver from adult and fetal rats. Cytoplasmic extracts of adult rat liver were found to have two major heat-stable forms of tyrosine aminotransferase activity as shown in Figs. 4, 5, and 6. The more anodal band, designated AL-1, corresponded to the HTC band H-1, and the more cathodal band, designated AL-3, corresponded to H-3 in electrophoretic mobility. In addition there was a minor band, migrating more slowly than AL-3, which did not correspond to any activity seen in the HTC cells. The relative activity of AL-3 to AL-1 appears to be considerably greater than the relative activities of H-3 to H-1.

Tyrosine aminotransferase is present in very low levels in fetal rat liver, the amount rising rapidly at birth (23). In only one out of five preparations of fetal rat liver (each prepared from the pooled livers of a single litter) was tyrosine aminotransferase activity demonstrable by biochemical assay. This preparation was used for the experiments shown in Figs. 4, 5, and 6. Nevertheless, all five cytoplasmic extracts of fetal liver showed a major band of heat-stable tyrosine aminotransferase activity assayed histochemically, which migrated in the same position as H-3 and is designated as FL-3. In the single preparation which showed tyrosine aminotransferase activity biochemically, there was also a band of histochemical activity, designated FL-1, which migrated in the same position as H-1 in HTC cells.

Fig. 4 demonstrates the electrophoretic mobilities of the two forms of tyrosine aminotransferase activity in HTC cells, adult, and fetal liver at different gel concentrations. It can be seen that H-1, AL-1, and FL-1 are identical in their electrophoretic behavior as are H-3, AL-3, and FL-3. Furthermore, the plot of log Rf against gel concentration for the anodal and cathodal bands from each source yields parallel slopes suggesting that they differ in net charge but not in molecular weight (24). Histochemical assay of aspartate aminotransferase in extracts of adult and fetal liver demonstrated a single band of activity migrating in the position of AL-3 and FL-3, respectively. Furthermore, as is the case in HTC cells, this cathodal band was capable of using oxalacetate in place of α-ketoglutarate in the transamination of tyrosine, and the histochemical activity of tyrosine aminotransferase in this band was inhibited by the addition of asparagine to the staining mixture. The presence of this form in both fetal and adult rat liver clearly eliminates the possibility that this form represents the expression by the malignant hepatoma cell of a fetal gene not expressed in adult liver.

Immunologic Inactivation of Cytoplasmic Tyrosine Aminotransferase Activities in HTC Cells, Adult, and Fetal Liver. Because of the controversy in the literature regarding the ability of cytosol asparate aminotransferase to transaminate tyrosine
The first is the problem of pseudoisozymes separated by electrophoretic or other techniques. Biochemical demonstration of multiple forms of enzyme activity by histochemical or biochemical techniques as described under "Materials and Methods." The relative mobility ($R_f$) of the two major bands of tyrosine aminotransferase activity was determined as described by Rodbard and Crambach (24), and plotted on a log scale against gel concentration. (%) in Fig. 5, extracts of HTC cells, adult, and fetal liver have been incubated with antibody to purified rat liver tyrosine aminotransferase. Following incubation the extracts were subjected to electrophoresis and histochemical staining as described under "Materials and Methods." Incubation with anti-tyrosine aminotransferase antiserum in amounts sufficient to inactivate the enzyme activity in each extract resulted in complete disappearance of histochemical activity in the anodal band, whereas staining of the cathodal band was unaffected. In contrast, when these extracts were incubated with antiserum to purified pig heart cytosol aspartate aminotransferase and then subjected to electrophoresis, histochemical tyrosine aminotransferase activity was completely abolished in the cathodal band but was unaffected in the anodal band (Fig. 6). As expected, histochemical aspartate aminotransferase was simultaneously eliminated.

Discussion

Multiple molecular forms of enzymes have been recognized for some time (25, 26). Furthermore, different isozymes may have a heterogeneous distribution between different compartments of the cell and between different tissues, and may be regulated independently by different effectors (25, 26). Ideally, isozymes should be defined by both genetic and biochemical techniques as naturally occurring, noninterconvertible, independent protein species arising from genetically determined differences in primary structure (27). Frequently, however, isozymes are postulated simply on the basis of the histochemical or biochemical demonstration of multiple forms of enzyme activity separated by electrophoretic or other techniques.

Two major classes of error arise from such less rigorous definitions of isozymes. The first is the problem of pseudoisozymes which represent different physicochemical states of the same enzyme arising from modification of a common primary sequence. This situation can arise from in vitro artifacts such as sulfhydryl oxidation during enzyme preparation or storage; an apparent example of this problem are the pseudoisozymes of rabbit skeletal muscle phosphoglucone isomerase reported by Holtmann and co-workers (28). A similar situation could arise from in vivo, post-translational modification of an enzyme by phosphorylation, methylation, carboxylation, addition of carbohydrate groups, aggregation, allosteric modification, etc. This kind of modification could be modulated by hormones, and might account for the apparent isozymes of hepatic tyrosine aminotransferase reported by Iwaski et al. (29) and Holt and Oliver (7). These multiple forms might also represent intermediates in the degradation of the enzyme as suggested by Johnson et al. (30).

It is unlikely that our observations can be explained in this way. When HTC cell extracts are prepared and subjected to electrophoresis in the presence or absence of the reducing agent dithiothreitol, the results are identical. Furthermore, when the material eluted from H-1 and H-3 is subjected to re-electrophoresis we observe histochemical activity of the appropriate kind in a single band with the expected mobility. Finally, the marked differences between H-1 and H-3 in catalytic activity, relative substrate specificity, and antigenic behavior make it unlikely that they could represent interchangeable forms of a single protein.

A second class of pseudoisozymes can arise when two or more electrophoretically distinguishable forms of an enzyme activity are indeed genetically different, but are, in fact, different enzymes with overlapping substrate specificities. We believe that this represents the most likely explanation for our findings. It is also quite possible that this explanation accounts as well for the electrophoretically demonstrated apparent isozymes of tyrosine aminotransferase in mouse liver (5, 6), and in preparations of rat liver purified by affinity chromatography (14).

The anodal transaminase we have demonstrated in extracts of HTC cells (H-1), adult rat liver (AL-1) and in one of five pools of fetal rat liver (FL-1) clearly appears to be tyrosine aminotransferase (EC 2.6.1.5). It is a cytoplasmic, heat-stable enzyme with an absolute requirement for 2-oxoglutarate as ketoacid, and transaminates tyrosine but not aspartate. It accounts for nearly all (>95%) of the soluble tyrosine transaminating activity in HTC cells and is completely inactivated by antiserum to purified rat liver tyrosine aminotransferase. The concentration of this enzyme in HTC cells is increased by incubation of the cells with dexamethasone, insulin, and serum (4). The more cathodal tyrosine aminotransferase activity in HTC cells (H-3), adult rat liver (AL-3), and fetal liver (FL-3) on the other hand, most probably represents aspartate aminotransferase (EC 2.6.1.5). It is also a cytoplasmic, heat-stable enzyme with the same apparent molecular weight as the anodal enzyme described above, but differs in net charge. Unlike the anodal enzyme, it can utilize oxalate in place of 2-oxoglutarate, and can transaminate aspartate as well as tyrosine. It is inactivated by incubation with antiserum prepared against pig heart soluble aspartate aminotransferase but not by antiserum to tyrosine aminotransferase, and its activity in HTC cells is apparently not affected by corticosteroids, insulin, or serum.

It has been discovered recently that the tyrosine aminotransferase activity in rat liver mitochondria is identical to the mitochondrial aspartate aminotransferase (9, 10). It has not always
Fig. 5 (top). Inactivation of the anodal tyrosine aminotransferase activity in cytoplasmic extracts of HTC cells, adult and fetal rat liver by antiserum to purified rat liver tyrosine aminotransferase. Heated cytoplasmic extracts of adult and fetal rat liver and dexamethasone-induced HTC cells were prepared and analyzed by electrophoresis on polyacrylamide gels as described under “Materials and Methods.” Histochemical assay was performed at 60° for 60 min in A, and 50 min in B and C. A, HTC cells, ---, no antibody treatment, 10.4 milliunits of tyrosine aminotransferase activity applied to gel; ----, approximately 12 milliunits of enzyme activity were incubated with 30 milliunits of antiserum as described under “Materials and Methods,” and the supernatant containing 0.4 milliunit activity applied to the gel. B, adult rat liver, ---, no antibody treatment, 11.4 milliunits of tyrosine aminotransferase applied to the gel; ----, approximately 15 milliunits of enzyme activity incubated with 37 milliunits of antiserum, and the supernatant containing 1.6 milliunits activity applied to the gel. C, fetal rat liver, ---, no antibody treatment, 2.1 milliunits of tyrosine aminotransferase activity applied to the gel; ----, approximately 5 milliunits of activity incubated with 37 milliunits of antiserum, and the supernatant containing no biochemically assayable activity applied to the gel.

Fig. 6 (bottom). Inactivation of the cathodal tyrosine aminotransferase activity in cytoplasmic extracts of HTC cells, adult and fetal rat liver by antiserum to purified pig heart soluble aspartate aminotransferase. Preparation of cell extracts and incubation with antiserum is described under “Materials and Methods.” Histochemical assay was performed at 60° for 60 min in A, and 50 min in B and C. Note the different absorbance scale in A compared with B and C. A, HTC cells, ---, no antibody treatment, 25.3 milliunits of tyrosine aminotransferase activity and 16.7 milliunits of aspartate aminotransferase activity applied to the gel; ----, approximately 56.1 milliunits of aspartate aminotransferase activity incubated with 17.7 milliunits of antibody, and the supernatant containing 20.5 milliunits of tyrosine aminotransferase and negligible aspartate aminotransferase activity applied to the gel. B, adult rat liver, ---, no antibody treatment, 20.5 milliunits of tyrosine and 198.8 milliunits of aspartate aminotransferase activity applied to the gel; ----, approximately 115.1 milliunits of aspartate aminotransferase activity incubated with 120.8 milliunits of antibody, and the supernatant containing 5.9 milliunits of tyrosine and negligible aspartate aminotransferase activity applied to the gel. C, fetal rat liver, ---, no antibody treatment, 4.3 milliunits of tyrosine and 198.4 milliunits of aspartate aminotransferase activity applied to the gel; ----, approximately 115.9 milliunits of aspartate aminotransferase incubated with 131.2 milliunits of antibody, and the supernatant containing 2 milliunits of tyrosine and no aspartate aminotransferase activity applied to the gel.
been appreciated, however (10), that the cytosol aspartate aminotransferase can also transaminate tyrosine (9) as well as other aromatic amino acids (9, 13). Failure to recognize the latter point has also resulted in the description of a phenylalanine aminotransferase activity in brain (31), which proved to be aspartate aminotransferase (9).

As noted earlier, neoplastic cells may show differences in isozyme patterns from their normal counterparts (11, 32). We have ruled out the possibility that the cathodal form of tyrosine aminotransferase (H-3) in HTC cells might represent the expression by the malignant hepatocyte of a gene expressed in fetal liver but repressed in adult liver, since this enzyme is found in both adult and fetal liver. Furthermore, the observation that there is less aspartate aminotransferase activity in HTC cells than in normal liver is consistent with studies on other hepatomas (33).

In conclusion, we have demonstrated that the apparent heterogeneity of tyrosine aminotransferase activity in HTC cells represents a pseudoisozyme resulting from the rather promiscuous substrate specificity of soluble aspartate aminotransferase. These studies again stress the need for caution in interpreting observations on apparent isozymes based on histochemical and electrophoretic techniques.

REFERENCES
Pseudoisozymes of Hepatic Tyrosine Aminotransferase
Carolyn J. Spencer and Thomas D. Gelehrter


Access the most updated version of this article at http://www.jbc.org/content/249/2/577

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/249/2/577.full.html#ref-list-1