rate outward current mechanisms in starfish oocytes showed that total potassium conductance also changed from a rapidly inactivating form to a slowly inactivating or noninactivating form during egg maturation (*8*). The interpretation in that study was that potassium inactivation disappears at maturation and that the transient outward current is modified to become the delayed rectifier. In the *Drosophila* DLM, however, there appear to be two distinct currents with different kinetics of activation as well as inactivation. In voltage clamp experiments in mature fibers, shifting the holding potential to more positive values than −60 mV not only eliminates the peaking component of outward current, but also reveals the much slower activation time of the delayed rectifier.

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7. Experiments were performed at temperatures between 10° and 14°C. The preparation was cooled with a Cambion Peltier plate. To expose the muscles to saline, we removed a small portion of the dorsal pupal case and opened the scutellum with microdissection needles. To prevent infusion of the saline throughout the pupal case, we sealed off the exposed area with petroleum jelly. To respirate the preparations, we opened the ventral part of the pupal case and exposed it to an airstream. The resting potentials of early (72-hour) muscle fibers averaged −48 mV with or without respiration. In mature (96-hour) pupae, resting potentials of muscle fibers averaged −60 mV and required respiration to maintain polarization at that level. Resting potentials are commonly more negative than potassium reversal potentials in insect muscle fibers. This is probably because membrane potentials are maintained at a more negative level by active metabolic processes [H. Huddart and D. W. Wood, *Comp. Biochem. Physiol.* **18**, 681(1966); M. B. Rheuben, *J. Physiol. (London)* **225**, 529 (1972)]. The composition of the saline used in these experiments was NaCl, 128 m*M*; KCl, 4.7 m*M*; $CaCl_2$, 1.8 m*M*; and phosphate, 1.0 m*M*; *p*H 6.9. In experiments with 96-hour pupae, calcium was removed from the saline to block the inward current, and 1.4 m*M* [ethylenebis(oxyethylenenitrilo)]tetraacetic acid was added. Magnesium chloride (14 m*M*) was added for membrane stability. The DLM fibers are nearly isopotential cylinders, measuring approximately 0.1 by 0.8 mm. Approximate passive membrane properties: R_m = 2 kilohm/cm^2; C_m = 9 μF/cm^2. Stocks of *Drosophila melanogaster* used were of the wild-type Canton-S strain.
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Carbamoyl Phosphate Synthetase (Glutamine-Hydrolyzing): Increased Activity in Cancer Cells

Abstract. *The specific activity of carbamoyl phosphate synthetase (glutamine-hydrolyzing), the first and rate-limiting enzyme of de novo uridine 5′-triphosphate biosynthesis, was increased in 13 transplantable hepatomas, particularly in the rapidly growing tumors (5.7- to 9.5-fold), and the rise was correlated with tumor growth rates. Thus, synthetase activity was linked with both hepatic neoplastic transformation and progression. Synthetase specific activity was also elevated in a transplantable sarcoma (18-fold) and a kidney adenocarcinoma (5-fold). The increased activity should enhance the capacity of the pathway and should confer selective advantages to cancer cells.*

Neoplastic cells express a program that in pyrimidine metabolism requires increased activities of the key enzymes of biosynthesis and decreased activities of those of degradation (*1–3*). We now report that the specific activity of carbamoyl phosphate synthetase (glutamine-hydrolyzing), E.C. 6.3.5.5 (CPS II), the first and rate-limiting enzyme of de novo biosynthesis of uridine 5′-triphosphate (UTP) (*4, 5*), was increased in 13 rat hepatomas, and the rise was positively correlated with the increase in the growth rates of the tumors. These observations were made in experiments designed to resolve an apparent contradiction in that the concentration of cytidine 5′-triphosphate (CTP) in the rapidly growing hepatoma 3924A was increased fivefold over that in normal liver, but the UTP pool was unchanged (*6*). Since UTP is a feedback inhibitor of CPS II (*5*), the lack of increase in UTP concentration in the hepatoma might be advantageous to the cancer cells by not blocking the activity of CPS II. A selective metabolic advantage might also be conferred on cancer cells if there was an increase in CPS II activity. We tested this hypothesis by investigating the behavior of the activity of CPS II in chemically induced, transplantable solid hepatomas carried in inbred rats and in the normal, differentiating, and regenerating liver.

Methods for the maintenance of normal and tumor-bearing animals, for killing the animals and for excising liver and tumors, and studies on differentiating and regenerating liver have been reported (*1–3*). The growth rates of the hepatoma lines ranged from 2 to 52 weeks, measured as the time required for the tumors, which had been transplanted subcutaneously, to attain a diameter of 1.5 cm. Rat liver contains a mitochondrial enzyme, the ammonia- and *N*-acetyl-L-glutamate–dependent carbamoyl phosphate synthetase, E.C. 6.3.4.16 (CPS I), which provides carbamoyl phosphate for urea synthesis, as well as CPS II, a cytosolic enzyme which is solely responsible for pyrimidine biosynthesis (*5*). The activity of liver CPS I is 1000 times greater than that of CPS II. In the preparation of homogenates, the mitochondrial CPS I might be liberated, and it could interfere with accurate measurement of CPS II activity in crude extracts. By modifying a purification procedure for the rat liver enzyme (*7*), we developed a simple and reproducible technique for preparing an enzyme fraction for the CPS II assay; this method was applied to normal livers and hepatomas (legend to Fig. 1). That the partially purified CPS II obtained was not contaminated with CPS I was verified by absence of activation with acetylglutamate plus ammonium chloride. To calculate the CPS II activity of the soluble supernatant, we used the recovery of the activity of aspartate carbamoyltransferase, E.C. 2.1.3.2, in the same samples in which CPS II activity was measured, since aspartate carbamoyltransferase exists as a multienzyme complex with CPS II (*7, 8*). The recovery of CPS II activity was in good agreement with that observed for the highly purified enzyme added at the step of tissue homogenization (*9*). Typically, CPS II was purified about 11- and 33-fold from the 105,000*g* supernatant fractions of hepatoma 3924A and the control liver, respectively. Specific activities for both purified enzymes were 809 and 302 nmole/hour per milligram of protein, and

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thus the specific activities of CPS II calculated for the crude extracts were 73.3 and 9.2 nmole/hour per milligram of protein. In hepatoma 3924A, therefore, CPS II activity was 8.0 times the control liver value (Fig. 1).

The kinetic properties of purified CPS II from hepatoma 3924A and liver were similar to those of hepatoma 3924A CPS II in a crude preparation. The *p*H optimum was 7.4. The apparent values of the Michaelis constant (K_m), as determined by a standard method (double-reciprocal plot), for L-glutamine, ammonium chloride, magnesium adenosine triphosphate (ATP) (in the presence of excess Mg^{2+} at 5 m*M*), and bicarbonate (in the presence of 50 m*M* KCl) were 0.017, 17, 2.4, and 2.1 m*M*, respectively. Free Mg^{2+} at 0.24 m*M* was required to achieve half-maximal activation of the enzyme. In the absence of excess Mg^{2+} (equal amounts of $MgCl_2$ and ATP), the saturation kinetics for MgATP showed a sigmoidal curve, and the activity at 50 percent substrate saturation ($S_{0.5}$) was 23 m*M*. Enzyme activity was proportionate with the amount of enzyme added and with the incubation time (at least three times that of the standard assay).

The specific activities of CPS II in 13 transplantable hepatomas as a percentage of the values for normal control rats are shown in Fig. 1. In liver, the enzyme activity ranged from 8.0 to 10.7 nmole/hour per milligram of protein, the lowest activities among the enzymes in the de novo and salvage synthesis of UTP (*3, 10*). The CPS II activity was significantly increased in all the hepatomas examined ($P < .05$): in the slowest growing hepatoma 9618A, the increase was 1.5-fold; in the tumors with medium growth rates, 2.4- to 4.8-fold; and in the rapidly growing tumors, 5.7- to 9.5-fold. The enzyme activities were correlated positively with the tumor growth rate, with a Spearman's rank correlation coefficient (r_s) of .940, significant at the 1 percent level. In five transplantable mammary carcinomas, CPS II activity was also proportional to the growth rates (*11*).

The specific activity of CPS II in the liver of 6-day-old rats was 2.2 times that of normal adult rats, and in 24-hour regenerating liver was 1.5 times that of the liver of sham-operated control rats. Thus, the CPS II activities in differentiating and regenerating livers are less than those in the hepatomas of medium growth rates, and the marked elevation of CPS II activity in rapidly growing hepatomas is characteristic of neoplastic proliferation. Specific activity of CPS II was also increased in a renal tumor (MK-3) (5.0-fold) and in a sarcoma (18.1-fold) in comparison with values of the respective normal tissues.

The de novo pathway of pyrimidine synthesis is a major source of uridine nucleotides (*12*). Pyrazofurin, a metabolite of which yields a competitive inhibitor of orotidine 5′-phosphate (OMP) decarboxylase, reduced the pools of UTP, CTP, deoxythymidine 5′-triphosphate (dTTP), and deoxycytidine 5′-triphosphate (dCTP) in rat hepatoma cells (*2, 13*). In hepatomas, therefore, the de novo pathway might also provide a major part of the uridine nucleotides synthesized. The increased activity of CPS II, the rate-limiting step of de novo UTP biosynthesis, may well be critical for the pyrimidine supply in cancer cells. In a display of an integrated program in hepatomas, there are also increases in activities of other enzymes of de novo UTP synthesis (aspartate carbamoyltransferase, dihydro-orotase, orotate phosphoribosyltransferase, and OMP decarboxylase) (*2, 10*) and in those of the salvage enzymes, uridine kinase, uridine phosphorylase, and uracil phosphoribosyltransferase (*2, 3*). By contrast, the activity of dihydrouracil dehydrogenase, the rate-limiting catabolic enzyme for pyrimidines, was decreased (*14*). This integrated eyzyme imbalance provides a heightened capacity for synthesis and a decreased capacity for catabolism of the precursors for pyrimidine production. These alterations should confer selective reproductive advantages on cancer cells.

In this hepatoma model, alterations linked to transformation and progression have been demonstrated for activities of key enzymes for glucose catabolism (hexokinase, phosphofructokinase, pyruvate kinase) (*1*), guanosine 5′-triphosphate synthesis (inosine 5′-phosphate dehydrogenase) (*15*), and pyrimidine and DNA production (CTP synthetase, thymidine kinase, ribonucleotide reductase, DNA polymerase) (*3, 16–18*). Increased CPS II activity in cancer cells is in line with these observations and supports the molecular correlation concept of neoplasia (*1*).

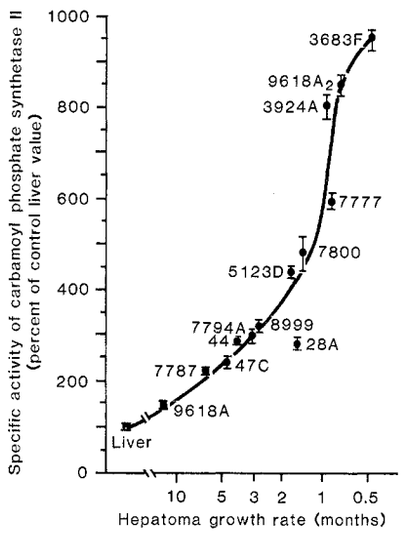

Fig. 1. Progression-linked increase in carbamoyl phosphate synthetase II activity in rat hepatomas of different growth rates. Growth rate was measured in months required for tumors to reach a diameter of 1.5 cm. Tumors are arranged according to their growth rates, 9618A being the slowest and 3683F the fastest growing neoplasm. Each number denotes a transplantable solid hepatoma line. Data are means ± standard errors for four or more rats in each group. Specific activities were plotted as percentage of corresponding normal liver values. Enzyme activities of all hepatomas were significantly higher than those of the normal liver ($P < .05$). Tissue homogenates (20 percent) were prepared at 0°C in a medium (*p*H 7.0) containing 30 percent (weight to volume) glycerol, 10 m*M* $MgCl_2$, 1.0 m*M* sodium EDTA, 50 m*M* potassium phosphate, 4.0 m*M* L-glutamine, and 1.0 m*M* dithiothreitol (DTT). The CPS II was partially purified by a modification (up to the first hydroxylapatite treatment) of the method of Mori *et al.* (*7*). Then the enzyme fraction was desalted in a column of previously packed Sephadex G-25M (PD-10) (Pharmacia) that had been equilibrated with 2.0 m*M* potassium phosphate (*p*H 7.0) containing 30 percent (by volume) dimethyl sulfoxide (DMSO), 5.0 percent (weight to volume) glycerol, and 1.0 m*M* DTT. The reaction mixture (0.3 ml, *p*H 7.5) for the assay of CPS II contained potassium [^{14}C]bicarbonate (1600 to 1800 cpm/nmole), 16.7 m*M*; L-glutamine, 1.0 m*M*; ATP, 10 m*M*; $MgCl_2$, 15 m*M*; potassium 4-(2-hydroxyethyl)-1-piperazineethanesulfonate (Hepes), 50 m*M*; DMSO, 7.5 percent (by volume); glycerol, 2.5 percent (weight to volume); DTT, 1.0 m*M*; L-ornithine, 5.0 m*M*; ornithine carbamoyltransferase from *Streptococcus faecalis* (Sigma), 3.0 units (micromoles per minute); and the enzyme preparation (*19*). The mixture was incubated at 37°C for 15 minutes, and the reaction was stopped by the addition of 3.0*N* formic acid (0.1 ml). After the mixture was dried on solid KOH in a vacuum desiccator, the residual material was dissolved in 0.5 ml of distilled water and mixed with 10 ml of Aquasol-2 (New England Nuclear). The radioactivity incorporated into L-citrulline was determined in a Packard Tri-Carb liquid scintillation spectrometer. The activity of CPS II in the soluble supernatant was calculated by the recovery of aspartate carbamoyltransferase activity (*20*). Estimates of the recovery of CPS II activity are given in text. Protein concentrations were determined as described in (*21*).

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Electroreception in Lampreys: Evidence That the Earliest Vertebrates Were Electroreceptive

Abstract. *Evoked potential and unit responses from the lamprey brain to weak electric fields demonstrate that lampreys have an electrosensory system as sensitive as those of other electroreceptive fishes. Electrosensory responses were recorded in the dorsal medulla, the midbrain torus semicircularis, and the optic tectum. Similarities in the structure of the anterior lateral line nerves and medullary organization between lampreys and several primitive jawed fishes indicate that the electroreceptive systems are homologous in these taxa. Thus electroreception was probably present in the earliest vertebrates ancestral to both agnathans and gnathostomes.*

Marked similarities between lampreys and cartilaginous fishes in the morphology of the lateral line nerves and the specific projections of these nerves in the brain suggest that lampreys, like sharks, skates, and rays, may be electroreceptive (*1*). The octavolateralis area of the lamprey medulla, like that in cartilaginous fishes, is divided into dorsal, medial, and ventral nuclei (*2, 3*) (Fig. 1). The anterior lateral line nerve in both lampreys and cartilaginous fishes is divided into dorsal and ventral roots, which enter the dorsal and medial octavolateralis nuclei, respectively. However, the posterior lateral line nerve has a single root and enters only the medial nucleus. The ventral octavolateralis nucleus does not receive lateral line input, but is the main target of the entering eighth nerve fibers (*4–6*).

Boord and his colleagues (*4, 7*) have suggested that the dorsal root of the anterior lateral line nerve in sharks is composed solely of electroreceptive fibers innervating head ampullary organs, whereas the ventral root is composed of mechanoreceptive fibers innervating head neuromast organs. This anatomical hypothesis was recently confirmed by electrophysiological recordings from single units in the dorsal and ventral roots of the anterior lateral line nerve of the thornback ray (*8*). Additional physiological evidence revealed that the dorsal octavolateralis nucleus in these rays is the primary medullary target of the entering electroreceptive fibers. Thus, the presence of a dorsal octavolateralis nucleus in the medulla of fishes should indicate the presence of electroreception, and the existence of this nucleus in lampreys suggests that these fishes are electroreceptive.

To test this hypothesis, we have studied an anadromous lamprey (*Lampetra tridentata* Richardson) that spawns in rivers from Alaska to southern California, but spends most of its adult life in the Pacific Ocean. Adult animals (60 to 70 cm in total length, 0.5 to 0.7 kg) were captured in the course of their upstream spawning migration in the Columbia River and transported to the laboratory, where they were held and tested in spring water (resistivity, 9.0×10^3 ohm-cm).

Electrophysiological responses to sensory stimuli were recorded after briefly anesthetizing animals by immersing them in tricaine methanesulfonate (0.01 percent) and surgically exposing the dorsal surface of the brain. Animals were then paralyzed by an intramuscular injection of tubocurarine chloride (4 mg per kilogram of body weight) and posi-

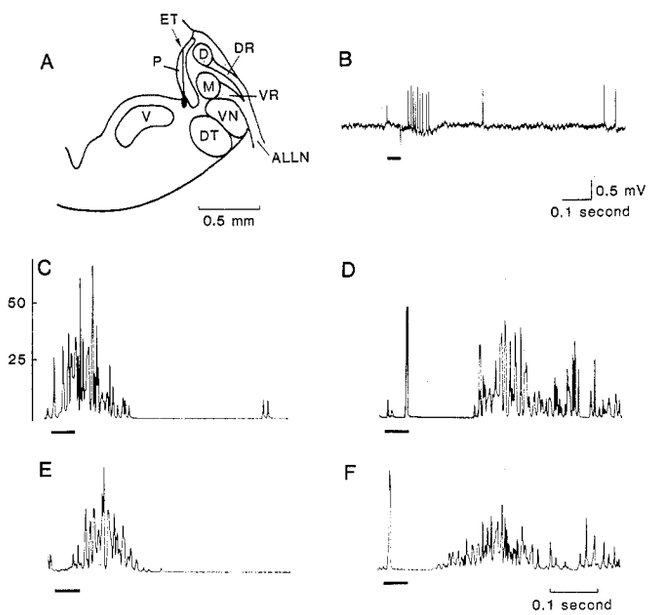

Fig. 1. Single unit responses to weak electric field stimuli. (A) Transverse section through the medulla oblongata at the level of entry of the anterior lateral line nerve. The cell in (B to F) was recorded at the surface of the indicated electrode track in the periventricular layer of cells, the processes of which form the neuropil of the dorsal octavolateralis nucleus. (B) Evoked potential and superimposed single unit response to an electric field stimulus presented as a 50 msec d-c pulse indicated by the line beneath the record. The 50 μV/cm field was oriented parallel to the longitudinal axis of the fish, with the caudal end positive relative to rostral. (C to F) Poststimulus-time histograms of responses (20 in each case) of the unit in (B) to four different orientations and polarities of the stimulus field. (C) Transverse to the longitudinal body axis, left side positive. (D) Transverse, right positive. (E) Parallel to the longitudinal axis, caudal end positive. (F) Parallel, rostral positive. Abbreviations: *ALLN*, anterior lateral line nerve; *D*, neuropil of dorsal octavolateralis nucleus; *DR*, dorsal root of anterior lateral line nerve; *DT*, descending trigeminal tract and nucleus; *ET*, electrode track; *M*, neuropil of medial octavolateralis nucleus; *P*, plate of cells whose processes form the neuropils of the octavolateralis nuclei; *VN*, ventral octavolateralis nucleus; *VR*, ventral root of anterior lateral line nerve; *V*, trigeminal motor nucleus.

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