while the second is observable only with GTP (figure 2). In parenchyma tissue subjected to the same electrophoresis and staining conditions, a sole band of enzyme activity could be seen either with ATP or GTP (not shown), and it can be proposed that this ATPase is similar to the microvessel one. Protein staining (fast green) shows homogeneous protein bands at the sites of the enzymatic activities.

The kinetic studies indicate that in microvessels GTPase activity is distinct from ATPase. If ATPase is the nonspecific enzyme (i.e. uses either GTP or ATP as substrate) differences are to be attributed to the substrate-specific enzyme (or enzyme-like activity), GTPase.

On the other hand, in parenchyma, ATPase 'works' with either substrate, but is probably less active with GTP. This ATPase is perhaps similar to the capillary ATPase. Raison d'être of the separate GTPase in brain microvessels is unclear. One can speculate that 2 phosphohydrolase activities have to be separated in this brain compartment for the sake of the normal blood-brain functioning. Namely, ATPase is essential for the maintenance of the large potassium gradient between plasma and brain tissue[15], which occurs at the brain capillary surface[16]. Therefore, ATPase is to be strictly regulated according to its role in potassium transport. But GTP is very potent inhibitor of hexokinase (EC 2.7.1.1)[17], and there are indications that this enzyme participates in glucose transport from blood to brain[7,8]. It is obvious that hexokinase must be operative and regulated according to the energy demands of the brain tissue as a whole, and all 'locally-born' inhibitors such as GTP have to be removed as quickly as possible, but without interference with other systems (i.e. the ATPase-related transport system).

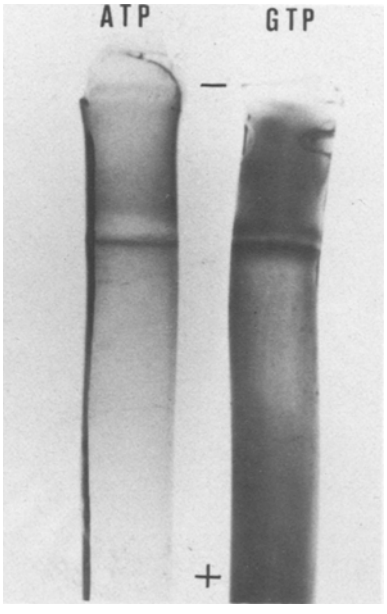

Fig. 2. Representative disc-acrylamide gel electrophoresis of isolated rat brain microvessel specimens. Respective substrates are indicated on the picture.

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Evidence for the existence of two carbonic anhydrase isozymes in rabbit ciliary body

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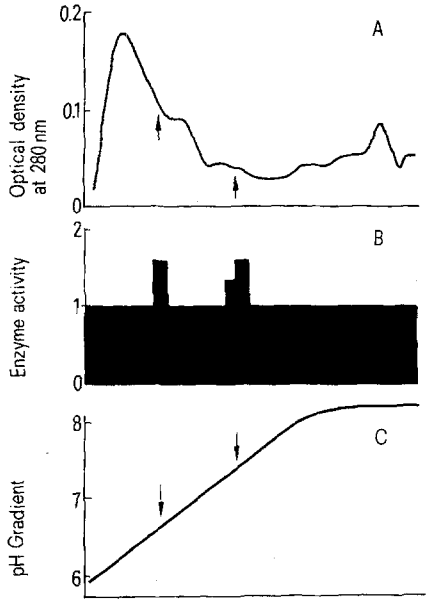
Summary. Two carbonic anhydrase isozymes were found in the rabbit ciliary body. Their isoelectric points were determined to be pH 6.6 and pH 7.4, respectively. The total activity of the higher isoelectric point isozyme was higher than that of the lower isoelectric point isozyme.

Carbonic anhydrase (E.C. 4.2.1.1) is believed to play an important role in aqueous humor formation in the ciliary body[1]. Although 2 isozymes of the enzyme (CA I and CA II) have now been isolated from many mammalian tissues[2], no one has ever succeeded in isolating the isozyme(s) from the rabbit ciliary body. Using the gel electrofocussing technique[3], the authors have isolated 2 carbonic anhydrase isozymes from the rabbit ciliary body.

Materials and methods. Erythrocyte-free ciliary body was obtained as follows: An albino rabbit was anesthetized with sodium pentobarbital and 5000 units of heparin sodium was i.v. injected. After the heparinization the rabbit was perfused (via the carotid artery) with 5 l of physiological saline (the 1st l of saline containing 10000 units of heparin sodium, the 2nd l containing 5000 units of heparin sodium and the last 3 l containing no heparin sodium). The absence of red cells in the perfusate was checked by the determination of iron by atomic absorption photometry (Model 170-50 Atomic Absorption Spectrophotometer, Hitachi, Japan). After the perfusion the eyeball was enucleated and the ciliary body was excised under a microscope. 100 mg of the ciliary body was homogenized with 100 µl of 0.6 M sucrose solution. After centrifugation the homogenate was subjected to gel electrofocussing. The gel electrofocussing was



Carbonic anhydrase activities after gel electrofocussing. *A* After the electrofocussing the gel was scanned at 280 nm using an ISCO Gel Scanner, Model 659, attached to Model UA-5 Absorbance Monitor (Instrumentation Specialities Company, USA). *B* The gel was sliced into sections of 3 mm and they were immersed in 4.0 ml of 0.025 M Tris-H_2SO_4 buffer, pH 8.2. After shaking the enzyme activity was determined. Carrier ampholytes did not affect the enzyme activity in the concentration used in the present experiment. The enzyme activity was defined as the reciprocal of the ratio of the hydration rate of CO_2 to the nonenzymatic hydration rate of CO_2. The hydration rate was determined with Model F-5ss pH Meter (Horiba, Japan). *C* The 3rd gel was also sliced into sections of 3 mm and immersed in 1.0 ml of distilled water and the pH was determined with a Model HM-5A pH Meter (Toa Electronics, Japan).

performed according to the method described by Wrigley[3]. 3 columns were run simultaneously; on to one the ciliary body homogenate was loaded, on to another red cell hemolysate was loaded, and on to the 3rd sucrose solution was loaded. The 3rd column was used to check the pH gradient. Enzyme activity was determined according to Carter et al.[4].

Results and discussion. It has been a question for a long time whether the ciliary body contains CA I, CA II or both, since Wistrand and Rao[5] failed to produce a convincing result in their immunological study. The present experimental observation appears in the figure. 2 carbonic anhydrase activities were found in the pH gradient acrylamide gel. Their isoelectric point was determined to be pH 6.6 and pH 7.4, respectively. (The values for human erythrocyte CA I and CA II were reported to be pH 6.57 and pH 7.36[6]). Both activities were completely inhibited by 5×10^{-5} M acetazolamide. The same result was also obtained in rabbit red cell hemolysate. Consequently, the authors conclude that the ciliary body contains both isozymes. The total activity of the higher isoelectric point isozyme was higher than that of the lower isoelectric point isozyme.

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Enzymic 'imprinting' as the result of early postnatal administration of enzyme inducers to animals

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Summary. Early postnatal treatment of rats or mice with different enzyme inducers (cortisol, galactose, steroid which induces mixed function oxidases) results in long-lasting changes in the activity of corresponding inducible enzymes.

A number of hormones, nutrients and xenobiotics act as inducers of adaptive enzymes which mediate the effects of hormones, or metabolize nutrients and xenobiotics[1-9]. Induction is provided by DNA-dependent synthesis of RNA molecules which control, in turn, the synthesis of the respective enzymes. Thus, glucocorticoids stimulate the synthesis of the key gluconeogenic enzymes in rat liver[1]; gastrin (pentagastrin) induces histidine decarboxylase in rat gastric mucosa[2,3]; administration of galactose leads to induction of rat liver enzymes converting galactose into glucose[4]; amino acids are responsible for the induction of enzymes of the urea cycle[5]; certain xenobiotics induce microsomal enzymes in liver, ensuring the elimination of these foreign compounds[6]. A series of experimental papers on this subject has been published and recent reviews have been concerned with the patterns of enzyme induction in animals[6-9].

It has been shown earlier in this laboratory that long-term treatment of adult rats with cortisol[10], insulin[11], pentagastrin[12] or galactose[13], after an induction of adaptive enzymes, produces a stable refractoriness to the administered inducers. In young animals, unresponsiveness to an inducer develops more readily and is retained longer than in adults. It was suggested that the administration of an inducer in the early postnatal period would have more pronounced and continuous after effects.

To verify this suggestion, rats and mice were treated with inducers in the early postnatal period, and the activities of the inducible enzymes were assayed when they were adults.

Materials and methods. Cortisol in doses of 50 g in 0.1 ml of saline per animal was injected daily i.p. to Wistar rats from days 1 to 16 after birth. Rats of the same strain were injected i.p. with galactose (1 mg in 0.1 ml of saline per g b.wt daily) from days 1 to 14 of postnatal life. Neonatal mice of the hypercholesterolemic SWR/y strain were treated with 16 α-isothiocyanopregnenalone-3-acetate (IPA), a potent inducer of microsomal mixed function oxidases[14]. Each animal received orally 0.2 mg of IPA from days 2 to 8