CYTOCHEMICAL LOCALIZATION OF SORBITOL DEHYDROGENASE IN KIDNEY AND LIVER FROM *Myiopsitta m. monachus* (BODDAERT, 1783), DURING EMBRYONIC DEVELOPMENT

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SUMMARY

A histochemical study to determine the localization of sorbitol dehydrogenase (SDH) in kidney and liver from embryionic, young and adult *Myiopsitta m. monachus* was performed. The enzyme activity increased with age in both organs. In the kidney, the enzyme appeared at the proximal convoluted tubules, and increased in the basal cytoplasm of the tubular cells. In the liver the localization was diffuse in the lobule but more intense in the cytoplasm of hepatocytes, especially in the perinuclear areas. These studies indicate that the cytochemical enzyme localization differs in this species, which is more evolutioned than *Gallus gallus*, and would be related to ontogenetic and phylogenetic differentiation.

Key words: sorbitoldehydro genase - cytochemistry - Kidney - liver Myiopsitta M. Monachus - embriology.

INTRODUCTION

The enzyme SDH (L-iditol: NAD oxidoreductase; E.C.1.1.1.14.) was studied by biochemical methods in several adult tissues such as liver and kidney [5,8], testes [1], placenta [6], etc., from different species.

In a previous study [4], it was postulated that this enzyme would be involved in metabolic processes or membrane transport in some organs of the chick embryo.

Besides, the enzyme presents different molecular forms in South American avian tissues before and after hatching [9].

The cytochemical localization of this enzyme in one of this species-*Myiopsitta m. monachus*, was performed to determine the phylogenetic and ontogenetic relation in this specimen, which is more evolutioned than *Gallus gallus*.

MATERIAL AND METHODS

Eighteen days-old *Myiopsitta m. monachus* embryos, young (three months after hatching) and adult forms (over one year of age) were trapped in the areas of Piquillin and Villa Ascasubi, Córdoba, Argentina.

Kidney and liver were dissected out from each animal at the three stages.

For histochemical studies the material was frozen in a bath of 2-methyl butane chilled with liquid air. Sections of 10 µm were cut in a Lapshaw criostat at −25ºC. The activity of SDH was demonstrated by the method described by Johnson [7]. Sections were fixed for 45 min at 0ºC in a solution containing 10% formaldehyde, 50% ethanol and Tris base (600 mM) adjusted at pH 7.0 with acetic acid. Tissues were af-

terwards treated for 5 min with acetone at 0ºC to remove lipids and rinsed three times in a cold Tris base (300 mM) - HCL buffer, pH 8.8, for 5 min to remove soluble factors [7]. The abbreviations used are: Tris, tris (hydroxymethyl) aminomethane; NBT, 2,2-di-p-nitrophenyl-5,5' diphenyl-3,3' (3,3' -dimethoxy-4,4-biphenylene) ditetrazolium chloride; NAD, nicotine adenine dinucleotide; PMS; phenazine methosulfate.

Sections were incubated in a medium containing: Tris base (300 mM)-HCL buffer pH8.8, NBT (0.3 mM); sorbitol (30 mM); NAD (1 mM) and PMS (15nM) during 30 - 60 min at 37°C and then were removed and treated with 10% formaldehyde-50% ethanol in equal parts, washed in distilled water and mounted in glycerogel. Control sections were incubated in substrate-free medium and heat- inactivated sections (in boiled water for 10 min), were incubated in the complete medium. Tissues fixed in Bouin's liquid were dehydrated and embedded in paraffin and serial sections were stained with Harris' hematoxylin and eosin to obtain other morphological controls.

RESULTS

In the embryonic kidney, a low enzymatic activity appeared in the cytoplasm at the level of the proximal convoluted tubules as a diffuse substance or as fine granulations; nuclei were not stained. A slight reaction appeared in Bowman's capsule (Fig. 1).

After hatching, the activity increased, being more marked in the basal cytoplasmic areas or the convoluted tubules. The Bowman's capsule presented a mild reaction (Fig. 2). Although this localizations was maintained in the adult kidney, the enzyme actitvity rose to a high level (Fig. 3).

The embryonic liver showed a light and diffuse localization of formazan in the cytoplasm of hepatocytes. Connective tissue was not reactive.

The enzyme activity increased in the postnatal and adult liver. The reaction was strong in the cytoplasm of adult hepatocytes surrounding the nucleus. All the lobules' areas presented an homogeneous staining (Fig. 4).

Connective tissue presented a moderate or negative reaction.

DISCUSSION

The cytochemical localization of the enzymes in *Myiopsitta m. monachus* differs from that studied in the chick embryo [4]. In this species, the enzyme was seen at the perinuclear region of the convoluted tubules from kidney at all the ages studied.

In *Myiopsitta m. monachus* the SDH activity that appeared at the basal cytoplasm is probably related to the transport process which occurs at this level. On the contrary, in the liver of this species the localization of the enzyme is perinuclear, and it is associated with glycogen storage. In the chick embryo, hepatic SDH is present in specific areas such as the centrolobular and pericanalicular regions, where the metabolism is conditioned for bile formation.

The increase of enzymatic reaction in both species during development is concomitant with the role that SDH plays on embryonic anaerobic metabolism [2].

Probably, this increment of enzymatic activity is related to the different molecular forms of the SDH detected during development of *Myiopsitta m. monachus* [9].

One molecular form appears in the embryonic and young stages whereas in the adult specimen two bands are found. This event would be related to the differences in the evolution and differentiation processes in both avian species.

RESUMEN

Se realizó un estudio histoquímico para determinar la localización de Sorbitol deshidrogenasa (SDH) en riñón e hígado de *myiopsitta m. monacha* embrionaria, joven y adulta. La acti-

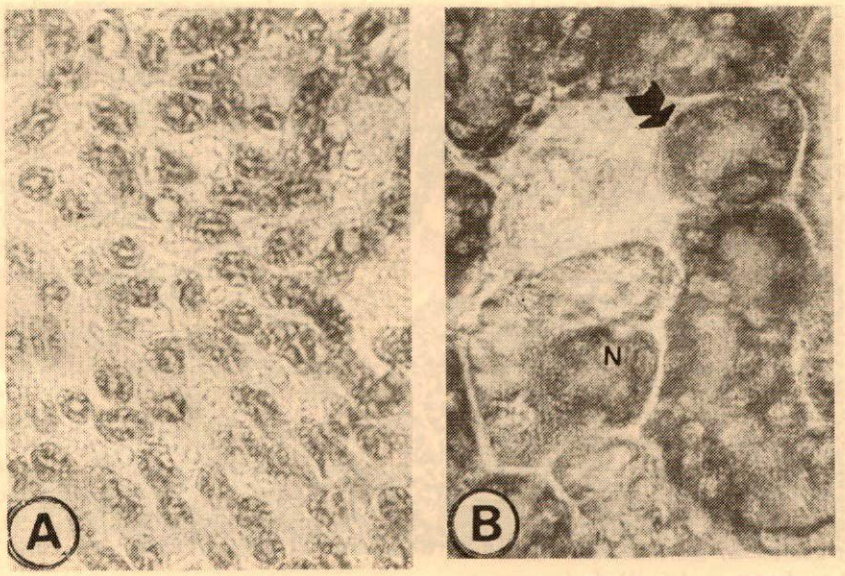

FIGURE 1:
A: Kidney (metanephros) from 18 days-old embryo. Proximal convoluted tubules with slight SDH reaction. X 100.

B: Kidney from young specimen. Proximal convoluted tubules. N: Nucleus. Intense activity of SDH in basal cytoplasm (arrows). x 450.

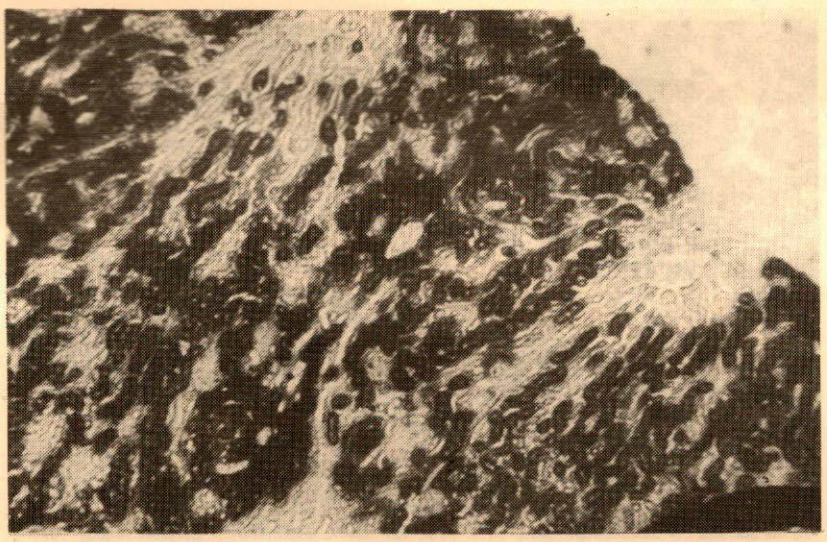

FIGURE 2: Kidney from adult specimen. Strong reaction in proximal convoluted tubule. x 450.

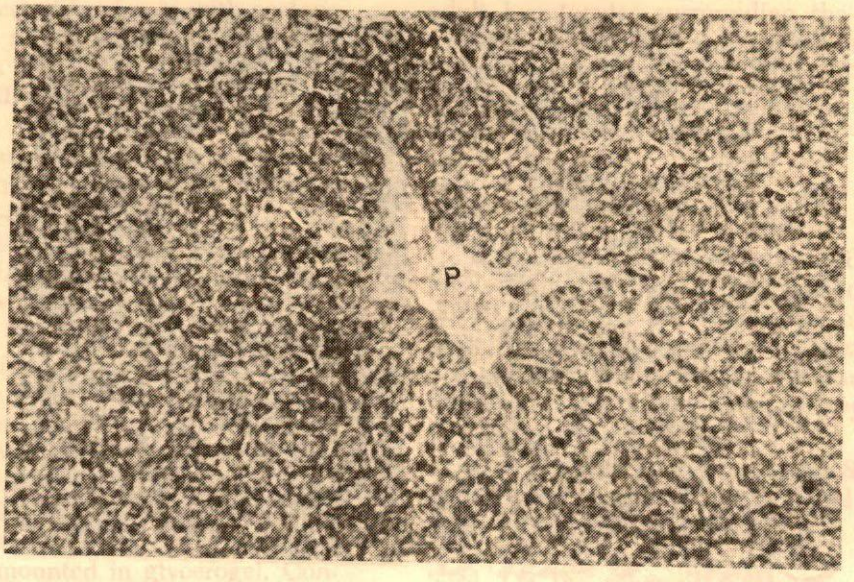

FIGURE 3: Liver from 18 days-old embryo. P: Portal areas. Slight SDH activity in lobules. x 100.

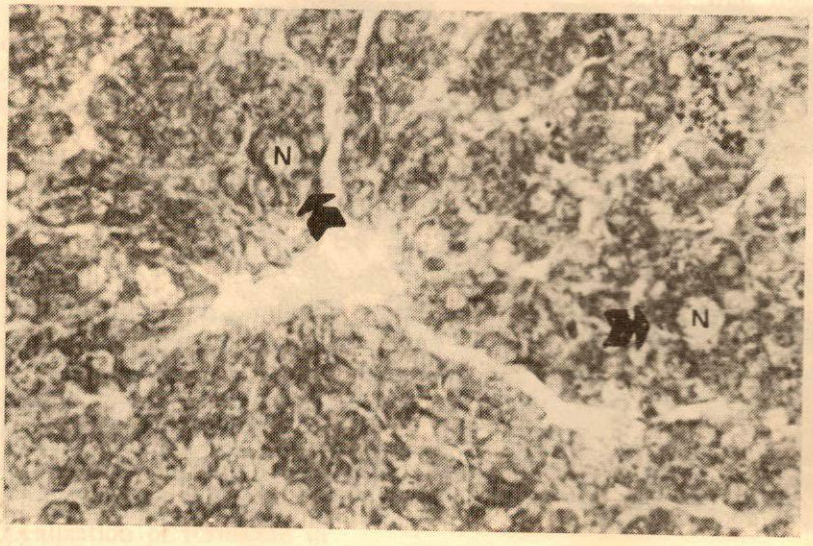

FIGURE 4: Liver from adult specimen. N: Nucleus. Intense SDH activity arround the nuclei (arrows). x 450.

vidad enzimática incrementó con la edad en ambos órganos. En el riñón, la enzima se encontró en los túbulos contorneados proximales, y aumentó en el citoplasma basal de las células tubulares. En el hígado, la localización fue difusa en el lóbulo, pero más intensa en el citoplasma de los hepatocitos, especialmente en las áreas perinucleares. Estos estudios indican que la localización citoquímica de la enzima difiere en esta especie, que es más evolucionada que el *Gallus gallus*, y estaría relacionada con la diferenciación ontogenética y filogenética.

Palabras clave: sorbitol deshidrogenasa - citoquímica - riñón - hígado - Myiopsitta m. monacha - embriología.

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