présence des glucocorticostéroïdes. Cet effet a été également observé par SCHWARTZ[16], mais dans des conditions expérimentales différentes, en mesurant l'activité, du système arginine synthétase et dans le foie foetal cultivé in vitro. Cet auteur n'observe aucun effet in vivo après un apport supplémentaire de corticostéroïdes exogènes. Cette observation n'est guère surprenante compte tenu que la quantité de corticostérone endogène normalement présente au cours de cette période est déjà importante[17].

Au contraire ces hormones ne semblent pas indispensables à l'augmentation d'activité de l'argininosuccinase et de l'arginase. Leur rôle ne semble être déterminant qu'après la naissance[18] et à l'âge adulte[11,19]. En ce qui concerne l'arginase, les résultats sont plus surprenants. En effet, bien que l'activité de cette enzyme ne soit pas modifiée par l'absence de corticostéroïdes à partir de 18,5 jours, celle-ci est toutefois augmentée par l'administration au même âge de cortisol exogène. Ces observations impliquent de s'assurer d'une part que la date de mise au repos des surrénales fœtales (18,5 jours) n'est pas trop tardive en mesurant l'activité de cette enzyme chez des fœtus décapités plus tôt (17,5 jours) et d'autre part que la maturité fonctionnelle du foie fœtal pour cette enzyme peut être avancée.

Summary. The activity of three urea-cycle enzymes, argininosuccinate synthetase, argininosuccinase and arginase have been studied in the fœtal and new-born liver of rats. The activity increases with regularity between 17.5 days of pregnancy and birth in control foetuses. The lack of corticosteroid from 18.5 days of pregnancy decreases the activity of argininosuccinate synthetase. After administration of cortisol (hydrocortisone), to these 18.5-day-old foetuses lacking of corticosteroids, both activities of argininosuccinate synthetase and arginase are enhanced.

A. HUSSON, C. GAUTIER et R. VAILLANT[20]

Université de Rouen, Laboratoire d'Endocrinologie U.E.R. des Sciences et des Techniques, Boîte postale 67, F-76130 Mont-Saint-Aignan (France), 5 Mai 1975.

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Sorbitol Dehydrogenase in *Anas platyrhynchos* and *Zenaida auriculata auriculata* During Development

The sorbitol dehydrogenase (E.C.: 1.1.1.14) converts sorbitol to fructose according to the following reaction: Sorbitol + $NAD^+ \xrightarrow{E}$ Fructose + NADH + H^+. BREUSCH[1] was the first who demonstrated the SDH activity in vitro in the cat liver slides. Later, several authors[2-20] studied the enzyme in various tissues of different species and found that it appears in the early stages during the development of chick embryo, varying its activity along the periods studied.

The large distribution of this enzyme, its presence in the germinal epithelium of the individual whose phylogenetic origin is very primitive[10,11], the fact that it appears early during the development, the poor knowledge about its activity, its molecular structure and its physiological role prompted the present study on SDH in several bird species of South American faunes during their development.

Materials and methods. Tissues: Eggs of *Zenaida auriculata auriculata* (golden pidgeon) from Piquillín and Villa Ascasubi (Córdoba, Argentina) and of the *Anas platyrhynchos* ('creole' domestic duck) from local farm were incubated at 37 ± 5°C during 12 and 28 days, respectively. The young golden pidgeons were maintained up to 60 days in captivity; the adult *Zenaida auriculata auriculata* were caught in Córdoba fields and placed in bird-cages. The domestic duck grew in capitivity. The animals were sacrificed by decapitation and the tissues (liver, kidney, testes and ovary) were removed and immediately placed in chilled beakers; all the operations were performed at 4°C.

Tissue homogenates were made in 3 or 4 volumes of ice cold 0.15 *M* NaCl containing 0.003 *M* $NaHCO_3$ using a TenBroeck glass homogenizer. The supernatant obtained by centrifuging the homogenate at 27,000 *g* for 30 min in

Table I. SDH specific activity during the development of *Zenaida auriculata auriculata*

Tissue	Embryo	Stage	
		Young	Adult
Liver	2.15 ± 0.04 (4)	4.75 ± 0.02 (6)	10.70 ± 0.15 (6)
Kidney	3.00 ± 0.06 (4)	5.70 ± 0.68 (6)	10.20 ± 0.26 (6)
Testes	0.51 ± 0.05 (4)	1.63 ± 0.14 (6)	3.75 ± 0.19 (6)
Ovary	0.40 ± 0.08 (4)	0.99 ± 0.04 (6)	1.29 ± 0.02 (6)

Specific activity in 10^{-3} nmoles NADH formed per min per mg protein ± SEM. Figures in brackets indicate number of determinations.

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a Sorvall refrigerated centrifuge, rotor SS-34, at 2 °C was removed and centrifuged again as above. The remaining supernatant was dialyzed against 0.15 *M* NaCl plus 0.003 *M* $NaHCO_3$ for 24 h. and used for enzymatic assay.

Protein determination: the Biuret method[21] was used. Bovine globulin (Nutritional Biochemical Corp.) dissolved in water was used as standard. Enzyme assay: Sorbitol dehydrogenase was estimated by the reduction of NAD observing spectrophotometrically the increase in absorption at 340 nm during the production of fructose from sorbitol. Two cuvettes were used for each determination. The experimental cuvette contained 0.5 ml of 0.5 *M Tris*-HCl buffer pH 8.2; 0.1 ml NAD (10 mg per ml); 0.3 ml of 1 *M* sorbitol; 0.2 ml of tissue extract and 1.9 ml of water. The substrate concentration was varied for the kinetic studies. In the blank cuvette, the substrate was omitted. The change in optical density against time was determined usually at 1 min intervals over a 20 min period. The reaction was carried out at 24 °C. The specific activity was expressed as 10^{-3} nmoles of NADH formed per mg of protein, per min. The electrophoretic procedure was performed according to MURRAY et al.[14]. The *t*-test was used to test the significance of activity. Only values at level $p < 0.05$ were considered significant. The standard deviation was calculated from 6 determinations in each experiment.

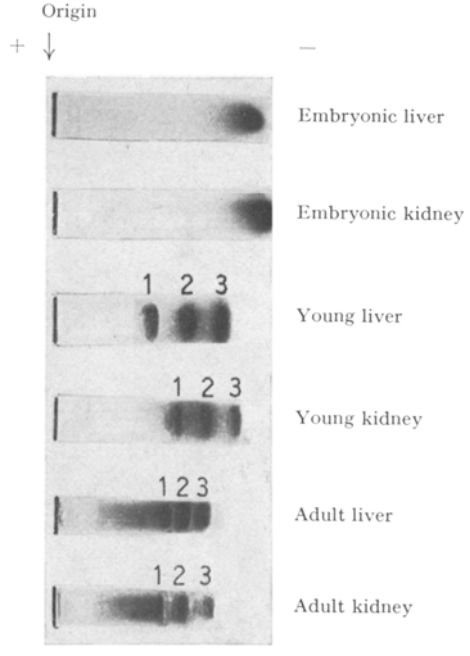

Fig. 1. Electrophoretic patterns of SDH in liver and kidney from *Zenaida auriculata auriculata* of different ages. All of the homogenates were electrophoresed simultaneously in the same starch block.

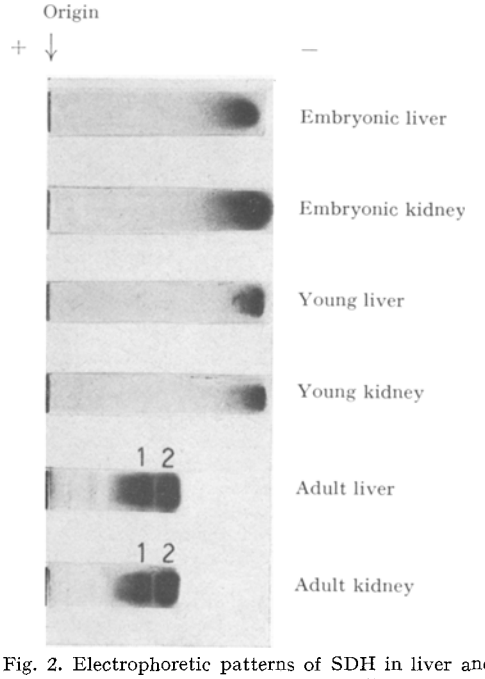

Fig. 2. Electrophoretic patterns of SDH in liver and kidney from *Anas platyrhynchos* of different ages. All of the homogenates were electrophoresed simultaneously in the same starch block.

Results. The studies performed in liver, kidney, testes and ovary of golden pidgeon during development showed that the specific activity is low in liver at embryonic stages; it increases in the young state and rises to its maximal value in the adulthood. The difference between the two last stages was significant (Table I). During *Anas platyrhynchos* development the SDH activity is lower in the embryonic step, and increases progressively during young and adult period (Table II).

In view of these results, it was interesting to study by means of electrophoresis the possibility that the multiple molecular forms of this enzyme change according to the specific activity during development.

The electrophoretic studies were performed in kidney and liver of each species. *Zenaida auriculata auriculata* shows 1 band in the embryonic stage. In the youngstage and in adult tissues, 3 bands were detected with a high enzymatic activity (Figure 1); in *Anas platyrhynchos*,

Table II. SDH specific activity during the development of *Anas platyrhynchos*

Tissue	Embryo	Stage	
		Young	Adult
Liver	7.01 ± 0.05 (4)	10.80 ± 1.50 (6)	20.50 ± 0.05 (6)
Kidney	4.61 ± 0.07 (4)	10.40 ± 0.08 (6)	14.60 ± 1.24 (6)
Testes	0.60 ± 0.05 (4)	1.56 ± 0.10 (6)	3.98 ± 0.71 (6)
Ovary	0.53 ± 0.07 (4)	0.91 ± 0.09 (6)	2.50 ± 0.02 (6)

Specific activity in 10^{-3} nmoles NADH formed per min per mg protein ± SEM. Figures in brackets indicate number of determinations.

Table III. Michaelis constants of SDH from *Zenaida auriculata auriculata* and *Anas platyrhynchos* during development

Tissue	*Zenaida auriculata auriculata*		*Anas platyrhynchos*	
	Young stage (m*M*)	Adult stage (m*M*)	Young stage (m*M*)	Adult stage (m*M*)
Liver	3.1	0.7	1.1	0.4
Kidney	8.0	0.4	3.3	0.3

The standard assay conditions were used; the concentration of sorbitol was varied from 0 to 5 m*M*. The K_m was calculated from a Lineweaver-Burk plot.

the enzyme showed 1 band in liver and kidney of embryonic and young tissues. The zymogram changed in the adult in which liver and kidney showed 2 bands (Figure 2).

The kinetic studies were performed with different concentrations of substrate in order to obtain data on the catalytic properties of this enzyme in the liver and kidney of *Anas platyrhynchos* and *Zenaida auriculata auriculata* during their development. The K_m values were determined by plotting the data according to Lineweaver and Burk. As shown in Table III, a decrease of K_m in the adult period of both tissues in the species is observed.

Discussion. The present paper shows that SDH activity increased with development. This activity in liver and kidney is higher in adult animals than in young ones. A similar phenomenon was found for other dehydrogenases[22], such as lactic, malic and glycerol 1-P dehydrogenase[23] which reveal a 10-fold increase in activity from the fetal period to adulthood in rat liver. Enzymatic activity in liver and kidney of various mammalian species is higher than in birds[2,14]. The specific activity of bird testes is lower than that found in guinea-pig, bull, mouse, rat and monkey testes. It is of interest that adult bird testes show higher enzymatic activity than that of toad, starfish and rabbit[24]. The results indicate a species specificity among birds. The rise in activity developed by the tissues studied in adult birds in relation with the young stage was accompanied by a decrease of the K_m values.

The change in the Michaelis constant during development may reflect the change in the multiple molecular forms observed by electrophoresis. When this method was applied to SDH in the liver and kidney of both species, changes already shown for various enzymes during development, were observed in the zymogram[25]. It is interesting to notice the specific development in both species.

Op't Hof et al.[26] found 5 SDH bands in pig liver and suggested a tetrameric structure. It is likely that this structure is typical for SDH in various species. Although fewer bands were found in our study, the literature offers many examples[27] in which the number of subunits does not fit the number of bands found experimentally.

Our observations show that, similar to the mammalian enzyme[14], SDH of birds exhibit multiple molecular forms.

Summary. Sorbitol dehydrogenase (E.C.N.1.1.1.14) was studied in liver, kidney and gonads of *Zenaida auriculata auriculata* (golden pidgeon) and of *Anas platyrhynchos* (creole domestic duck) from South American faunes. The specific activity of SDH increased from embryonic to adult stage and is higher in the *Anas platyrhynchos* tissues. The electrophoretic studies performed in liver and kidney of both species during development showed variations in the number and intensity of the bands in accordance with the age and the species.

Sofia P. de Fabro, Adriana L. Goldemberg and Noemi B. de Speroni[28]

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Incorporation Rate of Shikimic Acid-^{14}C and Phenylalanine-^{14}C into Gallic Acid in *Rhus* and *Acer* Leaves

There have been numerous studies of the biosynthesis of gallic acid and at least three pathways have been proposed for its biosynthesis in higher plants. Zenk[1] has formulated a conventional pathway from L-phenylalanine to 3,4,5-trihydroxycinnamic acid followed by β-oxidation to gallic acid. However, the trihydroxylated intermediate has never been found in the plant kingdom, and the study of Kato et al.[2] with a homogenate of *Pelargonium* leaves has shown that gallic acid-^{14}C is formed from protocatechuic acid-^{14}C, and the latter acid might be formed by β-oxidation of caffeic acid[3], although direct conversion of protocatechuic acid-^{14}C to gallic acid could not be detected in *Rhus typhina*. On the other hand, Conn and Swain[4] suggested that a third route to gallic acid, the direct dehydrogenation of 3-dehydroshikimic acid, existed in plants such as *Geranium pyrenaicum*. It is possible that several pathways may exist for the biosynthesis of any metabolite. The present work deals, therefore, with this problem by the use of shikimic acid-^{14}C and L-phenylalanine-^{14}C to indicate the dominant pathway for the biosynthesis of gallic acid in *Rhus* and *Acer* leaves.

L-Phenylalanine-U-^{14}C (422 mCi/m*M*) and shikimic acid-U-^{14}C (1.86 mCi/m*M*) were obtained from Dai-ichi Chemical Co. Ltd., Tokyo, Japan and New England Nuclear, Corp. USA, respectively.

Four leaves of juvenile stage (collected 26 April) and 1 leaf of mature stage (collected 26 June) were selected. The petiole was removed from each leaf with a razor blade. The leaf blades (0.23 g of the juvenile and 0.24 g of the mature, respectively) were immersed in a solution (0.1 ml) of L-phenylalanine-U-^{14}C (1 μCi) or shikimic acid-U-^{14}C (1 μCi) with air temperature about 27 °C for 7 h (continuous illumination) to 23 h (9 h light and then 14 h darkness) using light of 8,000 lux. The solution was taken up in 1 h and then followed with H_2O. After feeding for

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