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.

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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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Formation of radioactive gallic acid in *Rhus* and *Acer* leaves from labelled compounds

Compounds fed	Metabolic period (h)	Leaf age	Radioactivity (dpm) of 0.6 ml of the MeOH-HCl leaf-extract of		Specific activity (dpm/μMol) of gallic acid		Distribution[a] of radioactivity in gallic acid (%)	
			Rhus	*Acer*	*Rhus*	*Acer*	*Rhus*	*Acer*
Phenylalanine-U-^{14}C	7	Juvenile	21865	—	414	—	0.16	—
	23	Juvenile	51599	—	417	—	0.23	—
	7	Mature	10914	16848	217	417	0.24	0.31
	23	Mature	10789	18977	322	705	0.42	0.46
Shikimic acid-U-^{14}C	7	Juvenile	91475	—	206	—	0.04	—
	23	Juvenile	93666	—	556	—	0.11	—
	7	Mature	26468	25787	898	380	0.53	0.22
	23	Mature	16513	22213	2649	853	2.46	0.51

[a] The radioactivity (dpm) of 0.6 ml of the MeOH-HCl extract (total vol., 10 ml) was regarded as 100%.

a definite period, each sample was rinsed in water, blotted and weighed. The cut-up leaves were dropped into boiling 0.5% methanol-HCl, heated for a few minutes and then cooled with tap water. The extract was decanted and the residue thoroughly extracted with the same solvent until it became colourless. The combined extract was accurately made up to 10 ml and 0.6 ml aliquots were concentrated to a small volume, spotted on paper and a two-dimensional chromatogram prepared by developing first in *n*-BuOH – HOAc – H_2O (6:1:2) and then 6% acetic acid. The chromatograms were air-dried and examined in UV-light (256 nm) before and after exposure to ammonia vapour when gallic acid and other compounds were detected. The identification of gallic acid has been mentioned in a previous report[5]. Then the chromatograms were covered X-ray film (Sakura, non-screen type) and exposed for 36 days. The autoradiograms obtained were compared with the spots observed above, and the spot containing gallic acid was cut from the chromatogram and put in a vial containing 10 ml of scintillator, which consisted of the mixture of toluene — 2, 5-diphenyloxazole (DPO) – 1, 4-*bis*(2-methylstyryl)-benzene — triton X-100 (3 l:10 g: 1.5 g: 1.5 l). The radioactivity of gallic acid was measured in a Packard liquidscintillation spectrometer Model 3385. A blank area of the chromatogram, equivalent to the gallic acid spot, was used as control. The gallic acid in the eluate from the chromatograms was measured spectrophotometrically at 274 nm and quantitatively determined by comparison with the standard curve of the authentic specimen.

When phenylalanine-^{14}C or shikimic acid-^{14}C was infiltrated into leaves, the incorporation rate of ^{14}C of both precursors into gallic acid was found to be characteristic of the species *Rhus* or *Acer* and the age of their leaves. As shown in the Table, the mature leaves of *Acer buergeranum* utilized phenylalanine and shikimic acid as good precursors for producing gallic acid at the about same rate. Unfortunately, gallic acid in the juvenile *Acer* leaves was insufficiently recovered for the tracer analysis. On the other hand, in the juvenile leaves of *Rhus succedanea*, the incorporation rate (as %) of phenylalanine-^{14}C into gallic acid was about 2 or 4 times that of shikimic acid-^{14}C. In contrast, phenylalanine was effective by only about $^1/_2$ to $^1/_6$ as much as shikimic acid for the synthesis of gallic acid in the mature *Rhus* leaves. Thus the formation of gallic acid in the *Acer* leaves seems to proceed both from direct dehydrogenation of shikimic acid and β-oxidation of the phenylpropanoid at the mature stage. On the other hand, the biosynthesis of gallic acid in the *Rhus* leaves might proceed preferentially by β-oxidation of the phenylpropanoid derived from phenylalanine at the juvenile stage, and in preference by dehydrogenation of shikimic acid at the mature stage, respectively.

Summary. The incorporation of ^{14}C-labelled phenylalanine and shikimic acid into gallic acid by the leaves of *Rhus succedanea* and *Acer buergeranum* suggested that two alternative pathways could exist for the biosynthesis of gallic acid, and that the preferential route for the acid formation seems to be influenced by leaf age and species of the plants.

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Increased Collagen and Glycoprotein Contents of the Denervated Cremaster Muscle of the Bonnet Monkey, *Macaca radiata*

Transection of genitofemoral nerve results in an increase in alkali-soluble collagen and in a progressive atrophy of the monkey cremaster muscle[1]. However, the pool size of neutral salt- and acid-soluble and insoluble[2] collagens of the denervated muscle are not known. We have examined whether this increase in collagen, due to denervation atrophy, was reflected uniformly in all types of collagens.

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