The coincidence of the double content of paramyosin found in this paper (compared to, e.g., *Lethocerus* filaments[2, 11]) and six subfilaments in the core of bee filaments instead of three (as found in solid myosin filaments of the fleshfly or *Lethocerus*)[9], suggests that the additional paramyosin might be located in the center of the core of the bee filaments, giving rise to the inner set of subfilaments. It would be interesting to know whether there is space left for core proteins other than paramyosin.

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Comparison of lysine and tryptophan catabolizing enzymes in rat and bovine tissues

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Summary. Earlier studies indicate that α-aminoadipate aminotransferase (AadAT) and kynurenine aminotransferase (KAT) activities from rat tissues are associated with a single protein. However, our recent studies indicate that AadAT activity from bovine liver and kidney is not associated with KAT activity. To test whether the lysine and tryptophan catabolism in bovine tissues differ from that in rat tissues, we compared the activities of enzymes involved in lysine and tryptophan pathways in rat and bovine tissues. The activities of lysine catabolizing enzymes such as AadAT, lysine α-ketoglutarate reductase and saccharopine dehydrogenase in the bovine tissues were significantly lower than those found in rat tissues. The activities of tryptophan catabolizing enzymes such as KAT and kynurenine hydroxylase in the bovine tissues were negligible as compared to those in rat tissues. The results suggest that lysine is degraded via the saccharopine pathway in the livers and kidneys of both species but the metabolism of tryptophan in bovine tissues may be different from that in rat tissues.
Key words. Lysine metabolism; tryptophan metabolism; bovine liver; bovine kidney; rat liver, rat liver, rat kidney.

Lysine and tryptophan are the two essential amino acids whose catabolic pathways appear to be interrelated because, they share a common intermediate, α-ketoadipic acid. α-Aminoadipate aminotransferase (AadAT, EC 2.6.1.39) and kynurenine aminotransferase (KAT, EC 2.6.1.7) are the enzymes of lysine and tryptophan catabolic pathways, respectively. Earlier studies in rat tissues indicated that AadAT and KAT are properties of a single protein[2, 3]. However, recently we found that AadAT from bovine kidney was not associated with KAT activity[4]. Another report suggested that in human tissues, the transamination of kynurenine may be similar to that in bovine tissues but different from that in rat tissues[5]. To test whether the lysine and tryptophan catabolism in bovine tissues differ from that in rat tissues, we compared the enzyme activities of the lysine and tryptophan catabolic pathways in kidneys and livers of rat and bovine.

Materials and methods

Chemicals. L-Lysine, L-tryptophan, L-saccharopine, α-ketoglutarate, α-ketoadipate, L-kynurenine sulfate, pyridoxal-5′-phosphate, NADPH and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals used were of analytical grade.

Animals. Five adult male albino Sprague-Dawley rats, weighing 250–300 g were purchased from Charles River Laboratories (Wilmington, ME). Bovine livers and kidneys from five different animals were obtained from a local slaughterhouse where the tissues were removed immediately after sacrificing the animal.

Enzyme assays. Rats were sacrificed by decapitation and their livers and kidneys were immediately removed. Rat and bovine livers or kidneys were homogenized (10 % w/v) in 10 mM Tris-HCl, pH 7.0. The homogenates were centrifuged at 600 × g for 10 min and the resulting super-

natants were used for all enzyme assays except tryptophan pyrrolase. For assaying tryptophan pyrrolase activity, liver and kidneys were homogenized (10 % w/v) in 0.02 M potassium phosphate, pH 7.0 containing 0.14 M KCl. The homogenate was centrifuged at 6,000 × g for 10 min in a refrigerated centrifuge and the supernatant was used for the assay.

Lysine α-ketoglutarate reductase (EC 1.5.1.8) and saccharopine dehydrogenase[6] (EC 1.5.1.9), AadAT[7], tryptophan pyrrolase[8] (EC 1.13.11.11), KAT[9], L-kynurenine hydrolase[10] (EC 3.7.1.3), L-kynurenine hydroxylase[11] (EC 1.14.1.2) and aminoadipic semialdehyde glutamate reductase[12] (EC 1.5.1.10) activities were assayed as described[6-12]. All enzyme activities are expressed as nmoles of product formed/min/g fresh tissues except for aminoadipic semialdehyde glutamate reductase which is expressed as the units/g where 1 unit is the amount of enzyme that causes a change in absorbance of 0.001/min at 25 °C[12]. The protein concentrations were determined as described by Lowry et al.[13] using bovine serum albumin as standard. Student's t-test was used to calculate the statistical difference. p-Values < 0.05 were considered statistically significant.

Results and discussion

The bovine liver contained appreciable levels of enzyme activities involved in the lysine catabolism. However, all the activities were significantly lower than those in rat liver. In rat liver, the activity of the first enzyme of the lysine catabolic pathway, lysine α-ketoglutarate dehydrogenase, was about 5 times higher than that in bovine liver (table 1). The second enzyme, saccharopine dehydrogenase was also significantly higher in rat liver than in bovine liver. Another important enzyme of the lysine catabolic pathway, AadAT, was also significantly higher in rat liver than in the bovine liver. Nevertheless, all the enzymes of the lysine catabolic pathway were present in the bovine liver indicating that in the bovine liver, lysine is catabolized via the saccharopine pathway.

Table 1. Comparison of the enzyme activities of lysine and tryptophan catabolism in bovine and rat liver

Enzyme	Bovine	Rat
Lysine catabolism		
Lysine α-ketoglutarate reductase	93 ± 7	424 ± 17*
Saccharopine dehydrogenase	234 ± 15	412 ± 46*
α-Aminoadipate aminotransferase	662 ± 27	1662 ± 158*
Aminoadipic semialdehyde glutamate reductase	7.4 ± 0.5	11 ± 2
Tryptophan catabolism		
Tryptophan pyrrolase	3.8 ± 0.2	16 ± 2*
Kynurenine aminotransferase	< DL	168 ± 22*
Kynurenine hydroxylase	25 ± 1.5	37 ± 5
Kynurenine hydrolase	22 ± 1.5	20 ± 3.8
Protein (mg/g)	146 ± 13	143 ± 29

Results (nmol/min/g tissue) are means ± SEM (n = 5). An asterisk indicates statistically significant difference ($p < 0.05$) as compared to bovine liver. < DL indicates that the activities were below the detection limit of the assay.

Table 2. Comparison of the enzyme activities of lysine and tryptophan catabolism in bovine and rat kidney

Enzyme	Bovine	Rat
Lysine catabolism		
Lysine α-ketoglutarate reductase	140 ± 8	439 ± 72*
Saccharopine dehydrogenase	294 ± 34	24 ± 2*
α-Aminoadipate aminotransferase	2236 ± 27	4701 ± 225*
Aminoadipic semialdehyde glutamate reductase	25 ± 1.5	7.1 ± 1.5*
Tryptophan catabolism		
Tryptophan pyrrolase	< DL	< DL
Kynurenine aminotransferase	< DL	991 ± 38*
Kynurenine hydroxylase	< DL	24.1 ± 1.6*
Kynurenine hydrolase	22.5 ± 1.5	27 ± 2
Protein (mg/g)	164 ± 10	115 ± 11

Results (nmol/min/g tissue) are means ± SEM (n = 5). An asterisk indicates statistically significant difference ($p < 0.05$) as compared to bovine kidney. < DL indicates that the activities were below detection limit of the assay.

Tryptophan pyrrolase, the first enzyme of the tryptophan catabolic pathway, converts tryptophan into N-formylkynurenine. The activity of this enzyme was significantly lower in bovine liver as compared to that in the rat liver. The activities of kynurenine hydrolase and the kynurenine hydroxylase in the livers of two species were comparable. While rat liver contained appreciable amounts of KAT activity, we could not detect any KAT activity in bovine liver (table 1). This is an interesting observation because earlier reports have indicated that AadAT and KAT activites in rat liver and kidney reside on the same protein[2,3]. Our results suggest that in bovine liver, these two activities may not be associated with the same protein.

The enzyme activities of lysine and tryptophan catabolic pathways in rat and bovine kidneys are shown in table 2. The activities of lysine α-ketoglutarate reductase and α-aminoadipate aminotransferase were significantly higher in rat kidney than those in bovine kidney. However, aminoadipic semialdehyde glutamate reductase and saccharopine dehydrogenase activities were significantly higher in the bovine kidney than those in rat kidney (table 2).

In bovine kidney, all activities of the tryptophan catabolic pathway (except for kynurenine hydrolase) were absent indicating that in bovine kidney, the tryptophan catabolic pathway may not be functional. The absence of tryptophan-metabolizing enzymes in bovine kidney is not surprising because according to Tobes and Mason[2], AadAT/KAT in kidney may be more directly associated with lysine rather than tryptophan metabolism. We were unable to detect the first enzyme of the tryptophan catabolic pathway, tryptophan pyrrolase in the rat kidney. Earlier studies were unable to separate AadAT and KAT activities from rat kidney[2]. Although AadAT was present in appreciable amounts in bovine kidney, we were unable to detect any KAT activity (table 2).

This is the first report where the enzyme activities of lysine and tryptophan catabolism have been studied in

bovine tissues. The presence of the enzymes involved in lysine catabolism in bovine tissues suggest that lysine is catabolized via saccharopine pathway in bovine liver as well as in kidney. Our results indicate that the main catabolic pathway in the two species is similar. But the absence of KAT activity in bovine liver and kidney suggests that in bovine tissues, AadAT and KAT activities are not the properties of a single protein. Our results indicate that the transamination of kynurenine and hydroxykynurenine to kynurenic acid and xanthurenic acid does not occur in bovine tissues.

Tryptophan and its metabolites may be toxic when tryptophan is taken in higher doses as a sedative. Several cases of eosinophilia-myalgia associated with L-tryptophan have been reported[14]. Tryptophan also plays a role in some forms of cancers in which plasma levels and urinary excretion of tryptophan metabolites is increased[14–16]. This shows the interest for studies on tryptophan metabolism. Earlier studies of Kido[5] suggested that the transamination of kynurenine in the liver of monkey and human may be similar to that in the bovine liver. Further studies on the metabolism of tryptophan in bovine and other mammalian tissues may help to understand the role of tryptophan and its metabolites in various disorders.

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Phase-shift and memorization of the circadian rhythm of transpiration of *Tamarix aphylla*[1]

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Summary. The control of the circadian rhythm of transpiration in *Tamarix aphylla* seems to include two distinct components: an externally induced one, that is initiated by the 'light on' signal, and an endogenous clock, whose memorization of the period length is independent of the instant environmental signal.
Key words. Circadian; rhythm; transpiration; clock.

Endogenous circadian rhythms with periods that range from 21 h to 28 h have been observed and reported for a variety of physiological and biochemical reactions of plants[2,3]. Regular oscillations of transpiration of *Tamarix aphylla* (L.) Karst. (Tamaricaceae) with high values during the days and low values during the nights were observed under field and under controlled environmental conditions[4,5]. Such oscillations have continued even after the plants have been transferred to continuous light, and were preserved under such conditions for at least 8 cycles. The mean period of the oscillations under such conditions was 21.7 h. When plants were moved after some time of training (12 h Light : 12 h Dark) to conditions of continuous darkness, no apparent rhythm of transpiration had developed, except for a minor increase in transpiration that was distinguished during the 'day' time of the first cycle. The question whether the observed circadian rhythm, in continuous light, was indeed under endogenous control, was tackled by the following phase-shifting investigation.

Plants of *Tamarix aphylla* were grown from cuttings under controlled environmental conditions (25 ± 2 °C; ca 70% RH; 12 h L : 12 h D cycles) in a modified 1/2 strength Hoagland's nutrient solution[4]. The plants were placed in 1-l containers with nutrient solution, the containers were sealed with plastic bags and the plants were placed on top of recording balances. The loss of weight, determined every hour, was used as a measure of the transpiration for each plant. At the end of the experiment, the rates of transpiration were calculated per dry