—研究ノート—
Sceintific Note

Ureogenesis in Antarctic Birds — Blood Levels of Nitrogen Compounds and Liver and Kidney Arginase in Penguins*

Edson RODRIGUES[1], Rubens ROSA[2] and Metry BACILA[3]**

南極鳥類血液中の尿素形成: ペンギン血液中の窒素化合物と肝臓・腎臓中アルギナーゼの量

Edson RODRIGUES[1], Rubens ROSA[2] and Metry BACILA[3]**

要旨: アデリーペンギン属の, ゼンツーペンギン *Pygoscelis papua*, アゴヒゲペンギン *Pygoscelis antarctica*, アデリーペンギン *Pygoscelis adeliae* の肝臓・腎臓中のアルギナーゼ量と動力学についての研究を行った. ゼンツーペンギンでは 24 時間絶食個体に比べて, 給餌個体の血中尿素量は高かった. アゴヒゲペンギンの場合, 給餌個体の血中尿素量は, 絶食個体の 1.5 倍であった. そして, 給餌したゼンツーペンギンの血中尿素濃度は, 絶食個体の 3.5 倍であった. また, アゴヒゲペンギン給餌個体の尿酸値は絶食個体の 2 倍, ゼンツーペンギンは 4.8 倍であった. 肝臓中のアルギナーゼ活性は, アゴヒゲペンギン成体でタンパク質 1 mg 当たり 561 mU, ゼンツーペンギン成体では 208 mU であった. 肝臓ホモジナイズから得たアルギナーゼの L-アルギニンに対するミカエリス定数 Km は pH 9.5 で 16.0±2.0 mM であった. 一般に鳥類のアルギナーゼは, 100-200 mM の高い Km 値を示す. 高タンパク餌料, 高血中尿素量は, 肝臓アルギナーゼの量とアルギナーゼの基質に対する高い親和性の結果である.

***Abstract*:** A study was carried out on the levels and the kinetics of liver and kidney arginase from *Pygoscelis* penguins, the gentoo *Pygoscelis papua*, the chinstrap, *Pygoscelis antarctica*, and the Adelie, *Pygoscelis adeliae*. Higher values of blood urea were found in the gentoo penguins in the native state when compared with specimens maintained in the fasting state for 24 hours. In the chinstrap penguin *Pygoscelis antarctica* the average value for blood urea was 1.5 times higher in the native state than in the fasting condition. In the native gentoo

[1] Faculdade de Ciências Farmacêuticas, Universidade São Francisco, Avenida São Francisco de Assis, 218, 12900-000, Bragança Paulista, SP, Brasil.
[2] Brazilian Antarctic Program. Laboratório de Piscicultura, Departamento de Zootecnia, Universidade Federal do Paraná, Curitiba, PR, Brasil.
[3] Laboratório de Piscicultura, Universidade Federal do Paraná, Rua dos Funcionários, 1540, CEP 80. 035-050, Curitiba, PR, Brasil.
* With a grant-in-aid from Brazilian Antarctic Program (Brazilian Antarctic Program, Laboratório de Piscicultura, Universidade Federal do Paraná, Curitiba, PR, Brasil)-PROANTAR-CNPq.
** To whom all correspondence should be sent: Prof. Metry BACILA.

penguin *P. papua* the relative increase in the blood urea concentration is as high as 3.5 times in regard to the levels found in the fasting state. In regard to the blood levels of uric acid, the difference between the native state and the fasting state is 2.0 times for *P. antarctica* and 4.8 times for *P. papua*. Specific activities of arginase assayed in penguin liver were 561 mU/mg protein and 208 mU/mg protein for adult *P. antarctica* and *P. papua* respectively. Kinetic studies with arginase from penguin liver homogenates showed Km values for L-arginine of 16.0±2.0 mM at pH 9.5. Arginase from birds possesses in general high Km values (between 100-200 mM). It seems then that the high protein diet and the high levels of blood urea of penguins are a consequence of the levels of hepatic arginase and the high affinity of this enzyme toward its substrate.

1. Introduction

In spite of being considered uricotelic in regard to nitrogen metabolism, birds display blood urea levels that change as a function of feeding habits and of the species: domestic fowl, 2.64 mg/d*l* (BELL, 1957) and birds of prey, 36 mg/d*l* (FERRER *et al.*, 1987). In Antarctic birds, ROSA *et al.* (1993) found still higher values for blood serum urea in the skua *Chataracta maccormicki* (54.8 mg/d*l*) and in penguins, *Pygoscelis antarctica* (13.8 mg/d*l*) and *Pygoscelis papua* (6.3 mg/d*l*). However, there are still many doubts in regard to the origin and the fate of the urea nitrogen in birds. LEMOND (1959) reported urea formation by liver slices from Leghorn chickens. KARASAWA and MAEDA (1995) demonstrated *in situ* degradation and absorption of [^{15}N] urea in chicken coeca. Furthermore, blood levels of urea and uric acid are important data for the clinical diagnosis of bird ailments (LUMEIJ, 1987) and as parameters for the fasting conditions experienced by birds in connection with breeding, molting and migration. ROBIN *et al.* (1987) carried out studies on uric acid and urea in relation to the protein catabolism in long-term fasting geese. In the emperor penguin, ROBIN *et al.* (1988) carried out studies of protein and lipid utilization. They found that the rate of body mass loss and plasma uric acid and urea concentration closely reflected the changes in protein utilization, according to particular physiological conditions to which the bird is subjected.

The main aim of the present research (RODRIGUES, 1996b) is to study the correlation between the feeding=native or fasting=state and the levels of blood non-protein nitrogen compounds=urea, uric acid and creatinine=in penguins. A study was also carried out on the levels and the kinetics of liver and kidney arginase from the gentoo penguin *P. papua* and the chinstrap, *P. antarctica*.

2. Materials and Methods

All the management of the birds used in the different steps of the present experiments was carried out upon authorization and according to the rules governing Antarctic research.

Samples of 5 m*l* blood were obtained from adult *Pygoscelis* penguins, the chinstrap *P. antarctica*=25 in the native state of feeding and 6 in fasting state=; the gentoo *P. papua*=6 in the native state of feeding and 6 in fasting state=; and the Adelie *P. adeliae*=

6 in the native state. They were captured between January and February 1994 and December and February 1995 in the vicinities of the Brazilian Antarctic Station, King George Island, South Shetlands. They were wandering, grazing and looked healthy and well fed. The blood was drawn from the flipper cubital vein by means of a vacuum tubing system without anticoagulant. Another group of birds was maintained fasting for 24 hours before the blood sampling. The birds were handled very gently and set free when the sampling was finished.

Care of the blood samples followed normal laboratory procedures. To obtain serum, coagulated blood, after being defibrillated by means of a glass rod, was spun down for 10 min at 700 *g*. The serum samples were used for urea, uric acid and creatinine assays.

Liver and kidneys were obtained from one adult gentoo, two adult chinstraps and one 5-day old and one 10-day old chick chinstraps, and used for arginase studies. After the birds' decapitation, liver and kidneys were rapidly removed, rinsed with a 4°C chilled saline solution and immediately homogenized in a Potter-Elvehjem glass homogenizer. Homogenization was carried out for both liver and kidneys in the proportion of 1 g of tissue for 3 m*l* of 10 mM TRIS-HCl, pH 8.6 buffer, containing 100 mM KCl and 50 mM $MnCl_2$. The homogenates were spun down for 30 min at 12000 *g* and the supernatants used for the assay and kinetic studies of arginase.

Serum urea concentration was assayed by the urease method according to BERGMEYER (1985) and by the diacetilmonoxime method of WYBENGA *et al.* (1971). Uric acid was assayed by the method of CARAWAY (1955), and creatinine by the method of SLOT (1965). Liver and kidney arginase activities were assayed after a preactivation step of 30 min at 37°C (FUENTES *et al.*, 1994), at this same temperature. The reaction medium contained 20 mM TRIS-HCl pH 9.5, 1 mM $MgCl_2$ and 100 mM arginine. The urea formed was assayed according to the method of GEYER and DABICH (1971). One unit of arginase was defined as the amount of enzyme that produces 1 μM of urea per minute at 37°C. Protein contents of the preparations were assayed by the method of LOWRY *et al.* (1951).

3. Results

Levels of blood urea and uric acid in native and fasting Pygoscelis penguins. Blood levels of urea and uric acid assayed in native and fasting penguins gave the results shown in Fig. 1. In the birds' fasting state there is a decrease in the concentration of both N excreta compounds as compared to the levels found in the native state. When chinstrap and gentoo penguins were subjected to a fasting period of 24 hours, a very significant fall in the values of blood urea and uric acid occurred. In gentoos, blood urea concentration was 3.5 times higher in native birds than in fasting ones ($P<0.001$). In regard to uric acid, the difference was as high as 4.5 times ($P<0.001$).

Concentration of blood urea and uric acid in chinstrap penguins Pygoscelis antarctica. Figure 2 shows the results of a study of the distribution of urea and uric acid in a population of native and fasting chinstrap penguins *Pygoscelis antarctica*.

Distribution of blood urea and uric acid in papua penguins Pygoscelis papua. Figure 3 shows the results of a study of the distribution of urea and uric acid in a population of native and fasting gentoo penguins *Pygoscelis papua*.

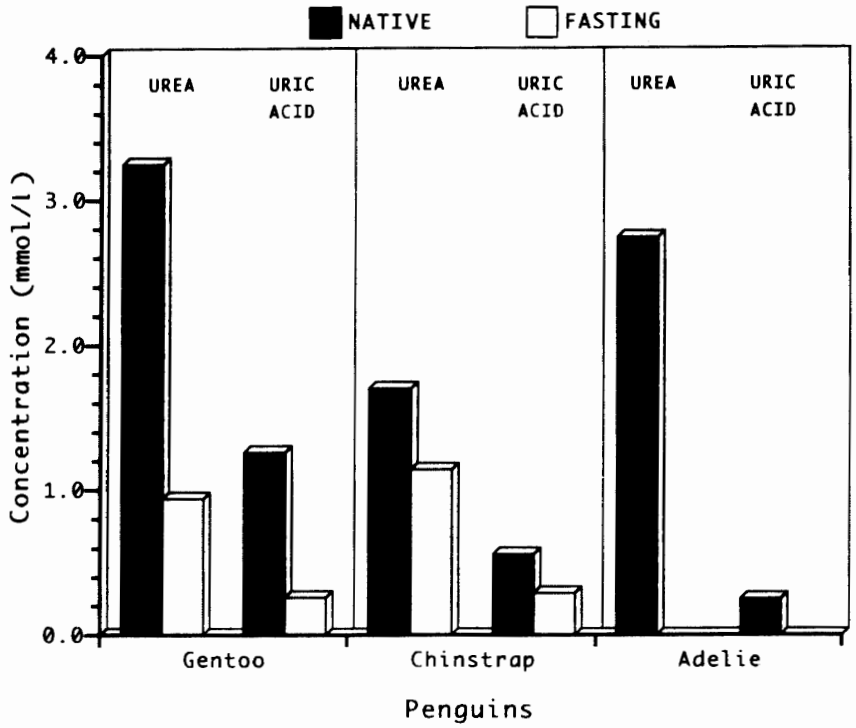

Fig. 1. Blood concentration in mmol/l of urea and uric acid in native and fasting Pygoscelis penguins. For Adélie penguins, values are shown only for the birds in the native state.

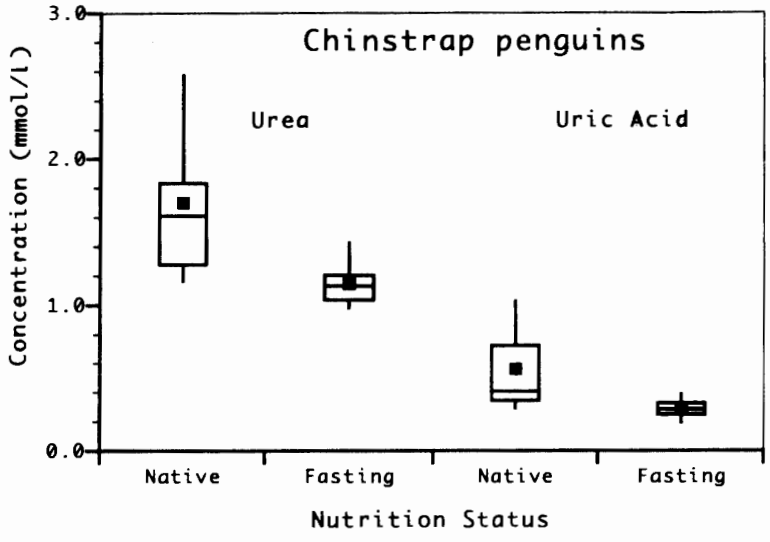

Fig. 2. Plot of the distribution of blood urea and uric acid in a population of native and fasting Pygoscelis antarctica. Values shown are averages of 25 assays in birds in the native state and 6 in the fasting state.

Blood creatinine in native and fasting Pygoscelis penguins. Figure 4 shows the results of an assay of blood levels of creatinine in a population of native and fasting chinstrap and gentoo penguins. A significant difference in the blood values of creatinine

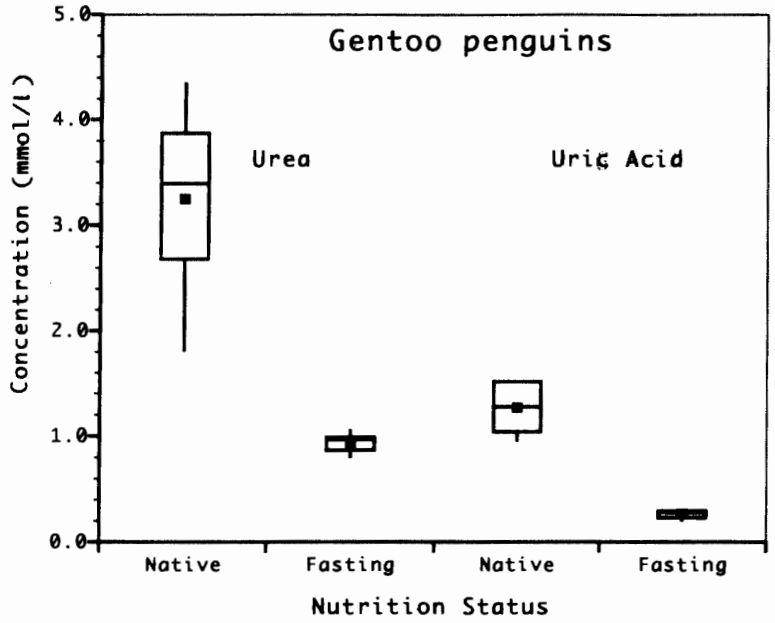

Fig. 3. Same as Fig. 2 but for fasting Pygoscelis papua and 6 assays.

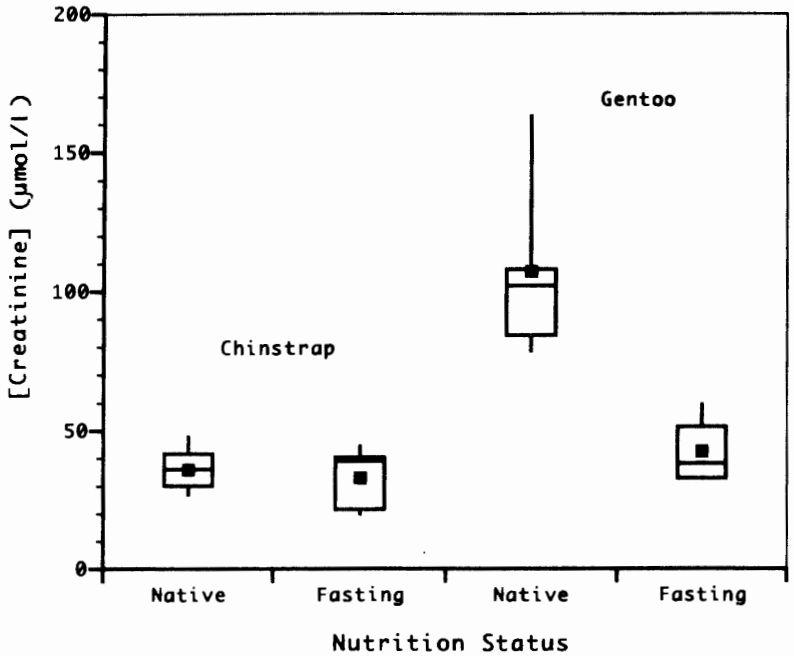

Fig. 4. Levels of blood creatinine in native and fasting chinstrap and gentoo penguins. Averages are taken over 25 assays for native and 6 for fasting chinstraps and 6 for native and fasting gentoos.

was found in native and fasting gentoo penguins but not in chinstraps.

Levels of liver arginase in adult gentoo and in 5- and 10-day old and adult chinstrap penguins. Figure 5 shows a higher content of liver arginase in the adult gentoo when in native condition compared with the adult chinstrap. There is also evidence that

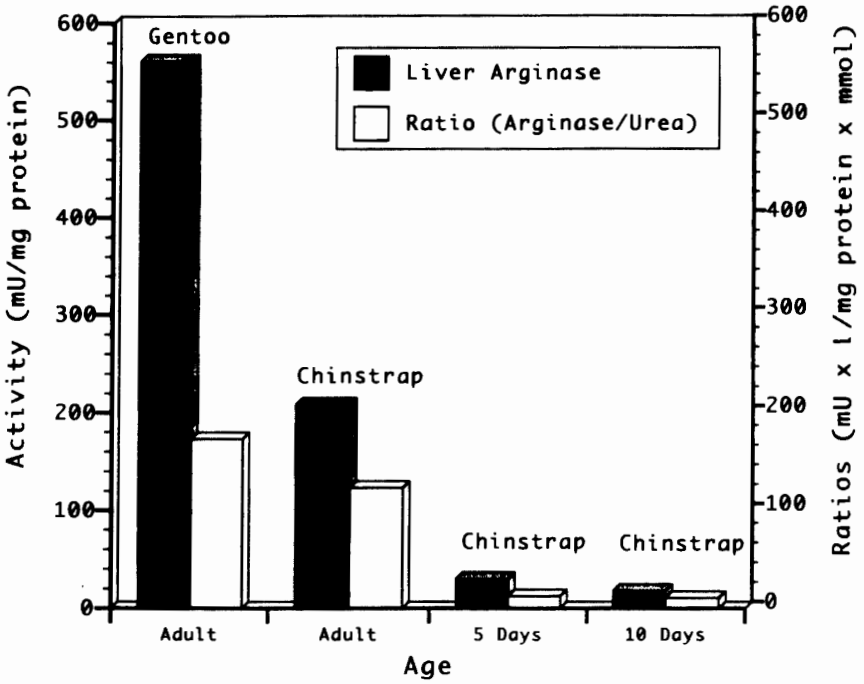

Fig. 5. Levels of liver arginase in adult gentoos and in 5- and 10-day old and adult chinstrap penguins and ratios between liver arginase and blood urea in the same group of birds.

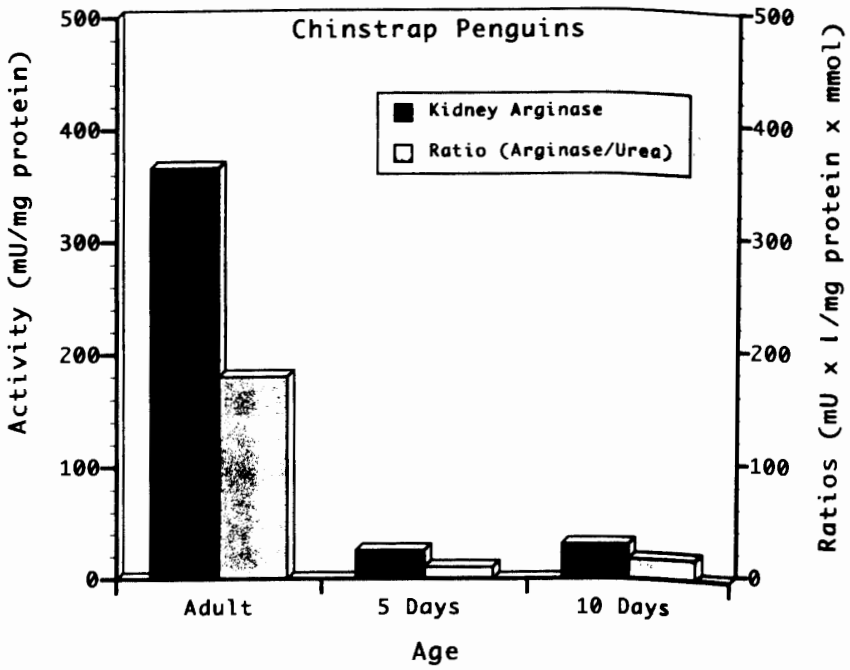

Fig. 6. Levels of kidney arginase in 5- and 10-day old and adult chinstrap penguins and ratios between kidney arginase and blood urea in the same group of birds.

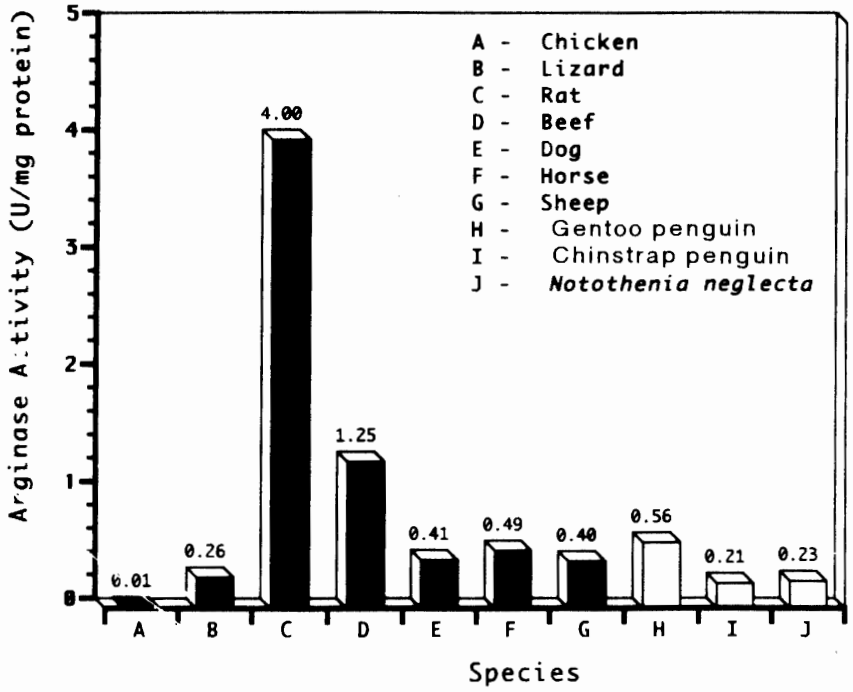

Fig. 7. Comparative levels of specific activities (U/mg protein) between liver arginase from chicken, Gallus gallus domesticus, (0.01 U/mg protein) gentoo (0.56 U/mg protein) and chinstrap (0.21 U/mg protein) penguins. The figure also shows data for fish, reptiles and mammals.

the concentration of liver arginase increases with aging of the bird.

Levels of kidney arginase in 5- and 10-day and adult chinstrap penguins. Figure 6 shows that the level of active arginase in the penguin kidney increases with biological development of the bird. Nevertheless, kidney arginase may not be a limiting factor for the metabolic process of the penguins' urea biosynthesis.

Comparative levels of liver arginase among fish, lizards, penguins, chicken and mammals. Figure 7 shows comparative values of liver arginase activity (U/mg protein) from different animal sources. It is noteworthy that the level of liver arginase of penguins is comparable to those displayed by several mammals including the dog, horse and sheep.

4. Discussion

There is a significant difference between the concentration of blood urea in fasting and native penguins. In the native chinstrap, the concentration of blood urea is about 1.5 times higher than that found in specimens starved for 24 hours. In gentoos, this value is about 3.5 times. Values of uric acid concentration increase about 2.0 and 4.8 times in the blood serum of native chinstrap and gentoo penguins respectively in regard to the values found in specimens starved for 24 hours (Figs. 1–3). Values for creatinine are shown in Fig. 4. It is known that urea and uric acid formation in both ureotelic and uricotelic animals originate from the deamination of gluconeogenic linked aminoacids (CAMPBELL *et al.*, 1987). To carry out the synthesis of 1 mol of urea and 1 mol of uric acid, 2 and 4 mols

of nitrogen are respectively consumed. Accordingly, for comparative purposes, urea and uric acid levels have been calculated in mmol/*l* of nitrogen concentration in the blood of native and fasting penguins.

Values of specific activities for arginase (mU/mg protein) assayed in liver and kidney preparations from adult and chick penguins which were not fasted (Figs. 5 and 6) deserve comment. It is noteworthy that arginase activity rises from 18-30 mU/mg protein in the chick chinstrap liver to 208 mU/mg protein in the adult liver, reaching values as high as 560 mU/mg protein in the adult gentoo (Fig. 5). In the kidney, arginase activity rises from 25-31 in chick preparations to 366 mU/mg in adult preparations. However, no direct correlation between levels of liver arginase and blood urea concentration in the respective birds has been found. It can be seen (Fig. 5) that levels of liver arginase from chick penguins are about 20 times lower than those from the adult penguins but concentration of blood urea is relatively much higher in the chicks' blood (2.43 mmol/*l* in the 5-day old chick and 1.71 mmol/*l* in the 10-day old chick). A similar consideration applies to the levels of kidney arginase activity.

It is instructive to examine the kinetic properties of arginase. For adult gentoo penguin liver arginase, Km values of 16.0±2.0 mM at pH 9.5 and 76.0±18.0 mM at pH 7.4 (RODRIGUES *et al.*, 1996a) have been found. These values correspond to those of arginase of high affinity. They are comparable to the values found in ureotelic animals such as the rat and mouse (arginase Km=20-40 mM), while arginase from lizards, chickens and rattlesnake displays Km values ranging from 100-200 mM (MORA *et al.*, 1965).

A comparative analysis of the specific activity of liver arginase (Fig. 7) shows that penguins and the Antarctic fish *Notothenia neglecta* possess levels of arginase comparable to those of ureotelic mammals such as the dog, horse and sheep. Levels of chick arginase are negligible. Arginase from birds usually possesses high Km values (100-200 mM). It seems then that the high level of blood urea in penguins is not only a consequence of these birds' high protein diet but also of the level of hepatic arginase they possess and the high affinity of this enzyme toward its substrate.

References

BELL, D.J. (1957): Tissue components of the domestic fowl. 2. Blood urea. Biochem. J., **67**, 33-36.

BERGMEYER, H.U. (1985): Methods of Enzymatic Analysis. 3rd ed., Vol. 9. Florida, VCH Publ., 449-453.

CAMPBELL, J.W., VORHABEN, J.E. and SMITH, D.D. (1987): Uricotelism: Its nature and origin during the evolution of tetrapod vertebrates. J. Exp. Zool., **243**, 349-363.

CARAWAY, W.T. (1955): Determination of uric acid in serum by a carbonate method. Am. J. Clin. Path., **25**, 840-845.

CHAUDHURI, A.C. (1927): A study of arginase content in the fowl with special reference to sex. Br. J. Exp. Biol., **5**, 97-101

FERRER, M., RODRIGUEZ, T.G., CARRILLO, J.C. and CASTROVIEJO, J. (1987): Hematocrit and blood chemistry values in captive raptors (*Gyps fulvus, Buteo buteo, Milvus migrans, Aquila heliaca*). Comp. Biochem. Physiol., **87A**, 1123-1127.

FUENTES, J.M., CAMPO, M.L. and SOLER, G. (1994): Kinetics of manganese reconstitution and thiol group exposition in dialyzed rat mammary gland arginase. Int. J. Biochem., **26**, 653-659.

GEYER, J.W. and DABICH, D. (1971): Rapid method for the determination of arginase activity in tissue

homogenates. Anal. Biochem., **39**, 412–417.
KARASAWA, Y. and MAEDA, M. (1995): *In situ* degradation and absorption of [^{15}N] urea in chicken cecum. Comp. Biochem. Physiol., **111A**, 223–227.
LEMOND, A. (1959): Urea production in chick liver slices. Can. J. Biochem. Physiol., **37**, 1187–1190.
LOWRY, O.H., ROSEBOROUGH, N.J., FARR, A.C. and RANDAL, R.J. (1951): Protein measurement with the Folin phenol reagent. J. Biol. Chem., **193**, 265–275.
LUMEIJ, J.T. (1987): The diagnostic value of plasma proteins and non-protein nitrogen substances in birds. Vet. Quart., **9**, 262–268.
MORA, J., TARRAB, R., MASTRUSCELLI, J. and SOBERÓN, G. (1965): Characteristics of arginases from ureotelic and non-ureotelic animals. Biochem. J., **96**, 588–594.
ROBIN, J.P., CHEREL, Y., GIRARD, H., GÉLOEN, A. and LE MAHO, Y. (1987): Uric acid and urea in relation to protein catabolism in long-term fasting geese. J. Comp. Physiol., B. **157**, 491–499.
ROBIN, J.P., FRAIN, M., SARDET, C., GROSCOLAS, R. and LE MAHO, Y. (1988): Protein and lipid utilization during long-term fasting emperor penguins. Am. J. Physiol., **254**, R61–68.
RODRIGUES, E., ROSA, R. and BACILA, M. (1996a): Comparative aspects of the nitrogen metabolism in Antarctic birds. Trends Comp. Biochem. Physiol., **2**, 447–463.
RODRIGUES, E., ROSA, R. and BACILA, M. (1996b): Ureogenesis in Antarctic birds. Abstracts of XIX Symposium on Polar Biology, December 5–6, 1996. Tokyo, Natl Inst. Polar Res., 82.
ROSA, C.D., ROSA, R., RODRIGUES, E. and BACILA, M. (1993): Blood constituents and electrophoretic patterns in Antarctic birds: penguins and skuas. Comp. Biochem. Physiol., **104A**, 117–123.
SLOT, C. (1965): Plasma creatinine determination. A new and specific Jaffe reaction method. Scand. J. Clin. Lab. Invest., **17**, 381–387.
WYBENGA, D.RI., DI GIORGIO, J. and PILEGGI, V.J. (1971): Manual and automated methods for urea nitrogen measurement in whole serum. Clin. Chem., **17**, 891–895.

(*Received February 17, 1997; Revised manuscript accepted January 7, 1998*)