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Developmental Biology of Antarctic Birds in Regard to the Organic Phosphate Compounds of Erythrocytes

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Abstract : A survey on the content of phosphorylated metabolic intermediates of erythrocytes from penguins and skuas of different stages of development was carried out. Embryos (25th and 30th day of incubation), chicks (2, 4, 14, 28-days old) and adult adelie penguins (*Pygoscellis adeliae*), and 2-days old and adult skuas (*Chataracta mackormicki*) were the birds studied. Penguin eggs and chicks were obtained at the rookery of the Arctowski Polish Antarctic Station, and adult and chicken skuas, nearby the Brazilian Antarctic Station Commander Ferraz.

Key words : penguins, skuas, antarctic birds, erythrocytes, organic phosphate compounds, biological development, developmental biology

Introduction

In an earlier report by Rapoport and Guest (1941), it has been recognized that the erythrocytres of most mammals, after birth, contain high concentrations of 2, 3-bisphosphoglycerate (2, 3-DPG), while the erythrocytes of birds possess instead an inositol polyphosphate, originally thought to be myoinositol hexaphosphate (phytic acid). Studying also the blood of reptiles, amphibians and fish, Rapoport and Guest (1941) came to the general conclusion that phosphoglycerate was not found in the bloods of species which normally have nucleated erythrocytes.

After the pioneering work of Rapoport and Guest (1941), research on the comparative biochemistry of phosphorylated metabolic intermediates of red blood cells from many animals and their physiological role have been the subject of several papers. A general account of the patterns of phosphate compounds in red blood cells of man and animals was given by Bartlett (1970). He stated that the spectrum of phosphates in the red blood cells has certain distinguished features as compared to other tissues, but there are larger species differences. Bartlett (1976) also studied the phosphate compounds in red cells of reptiles, amphibians and fish ; and Bartlett and Borghese (1976) assayed the content of phosphate compounds in red cells of the chicken and duck embryo hatchling.

The relationships between the major phosphorylated metabolic intermediates and oxygen affinity of whole blood in chick embryos and chicks and several galliformes were studied by Isaacks et al. (1976a and b).

Although it should be expected that Antarctic birds possess a similar pattern of erythrocytic

organic phosphate metabolic intermediates, as it was found in avians in general, this research was undertaken with the aim to have a deeper insight on the biochemical and physiological behavior of those important members of the Antarctic environment.

Rosa et al. (1989) carried out a comparative study on blood glucose partition and glucose metabolism of penguin erythrocytes and somatic tissues. It was found that almost the whole blood glucose is compartmentalized at the plasma site, the red blood cells being ineffective in regard to glucose metabolism. Levels of enzymes of the carbohydrate metabolism were estimated in the erythrocytes of both gentoo and chinstrap penguins as well as in the somatic tissues of the gentoo. On the other hand, one of us (Rodrigues, 1987) carried out an extensive study on the hexokinase activity of chicken erythrocytes and on the cell content an the physiological behavior of inositol pentaphosphate (IP5). He found out that IP5 could account for the defective metabolism of the avian erythrocyte due to the property of this compound to chelate magnesium and thus to alter the intracellular homeostasis in regard to the distribution of this important enzyme cofactor.

Thus, it is the main objective of the present paper to show the data obtained by the assay of posphorylated metabolic intermediates in the red cell of penguins and skuas in different stages of development and to correlate the data found with the general features of the metabolic properties of their respective erythrocytes.

Material and Methods

For the experiments reported in the present paper, embryos, chickens and adult adelie penguins and chickens and adult skuas were used.

Adult penguins were captured among the ones wondering on the beach in front of the Brazilian Station. The birds were kept at a special place and set free immediately upon the collection of blood. Adult skuas and one 2-days old chicken were also captured in the proximities of the Brazilian Station. The adult skuas were set free upon the collection of blood. Embryos and chickens of adelie penguins were from the rookery nearby the Arctowski Polish Station. They were obtained during the hatching seasons of 1988 and 1989.

In order to collect blood from penguin embryos, previously marked eggs were removed from the nests and carefully transported to the laboratory. Eggs on the 25th and 30th day of incubation were placed in a Petri's dish and opened at the blunt pole. The embryos were removed and the blood collected by means of a Pasteur's pipette and transferred to a test tube containing EDTA. After pooling the collected samples, the blood was spun down 10 min at 1500 rpm and the sedimented erythrocytes washed three times with cold saline. A total of 14 embryos were used for the experiment, 9 on the 25th and 5 on the 30th day of incubation. Four adelie chicks, 2, 4, 14 and 28-days old respectively, were sacrificed by decapitation, the blood collected in heparin and the tissues and organs used for enzymological and metabolic experiments. From the adult penguins, venous blood samples were drawn from the cubital vein of the flipper by means of "vacutainer" syringes containing heparin. The collected blood was spun down 10 min at 1500 rpm and the sedimented red blood cells washed three times with cold saline and used for organic phosphate analysis.

For the determination of the organic phosphate content in the erythrocytes, the packed red cells were deproteinized with $HClO_4$ in the proportion of 1 ml of packed cells to 2 ml of the perchloric acid solution. This step was followed by 20 min centrifugation at 5,000 rpm, the supernatant being collected and its pH adjusted to 3.0 with a 6N solution of Na_2CO_3, allowed to rest in an ice cold bath and then spun down 20 min at 5,000 rpm. The supernatant was collected and used for the assay of the phos-

phorylated metabolic intermediates by ion-exchange chromatography (Bartlett, 1968) with Dowex AG 1X8 formate columns, using a linear ammonium formate : formic acid (40 : 60, pH 3.45) gradient, and quantitated according to the method proposed by Isaacks et al. (1979). Inorganic phosphate and organic phosphorus were assayed with malachite green according to the method of Hess and Derr (1974).

Results

Table 1 shows the relative values of the different phosphorylated metabolic intermediates and inorganic phosphate found in the erythrocytes of embryos (25th and 30th day of incubation) of 2, 4, 14 and 28-days old chicks, and of adult adelie penguins.

Two typical profiles of the ion-exchange chromatography separation of the phosphorylated metabolic intermediates of erythrocytes from Antarctic birds are shown in Figure 1 and Figure 2. Thus, Figure 1 shows the profile corresponding to the erythrocytes from adelie penguins in the 30th day of incubation, in which a very neat peak of 2,3-DPG is present as well as a small and less meaningful peak of IP5. On the other hand, Figure 2 shows a similar experiment carried out with erythrocytes from an adult skua, showing a very neat peak of IP5.

A comparison between the relative concentrations of phosphorylated metabolic intermediates from a 2-days old skua and adult ones is shown in Figure 3. It can be observed that 2,3-DPG is already absent in the erythrocyte from the younger bird.

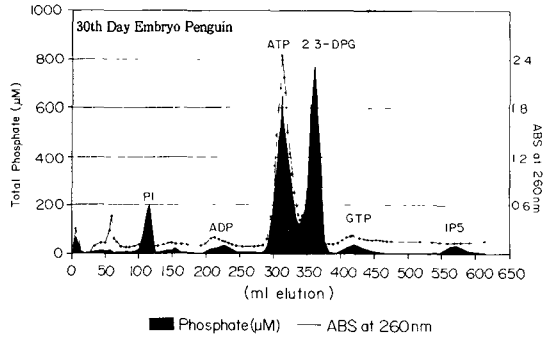

Fig. 1. Profile of the ion-exchange chromatography of phosphorylated metabolic intermediates found in erythrocytes from 30th day embryos of adelie penguins *Pygoscellis adeliae.*

Table 1. *Relative percentual values of phosphorylated metabolic intermediates in erythrocytes from Pygoscellis adeliae* penguins of different stages of development.*

Compound	Embryos (days of incubation)		Chicks (days old)				Adult
	25th	30th	2	4	14	28	
AMP	1.7	0.3	6.3	2.3	1.3	0.6	2.8
Pi	7.3	6.6	20.9	10.5	7.0	23.1	8.8
IMP	—	1.6	8.3	1.3	1.4	3.2	4.6
ADP	6.2	3.5	14.6	14.4	12.3	9.2	8.0
ATP	63.3	39.8	24.7	64.4	65.7	50.1	31.0
2, 3-DPG	9.9	38.4	2.3	—	—	—	—
GTP	3.8	3.9	2.0	0.8	2.8	2.3	6.3
IP5	—	2.8	9.7	3.5	6.1	7.8	37.3
ANOTHERS	6.8	3.0	4.0	2.8	3.4	3.6	1.2

* Relative values in percent total phosphates.
Abbreviations : AMP, Adenosine monophosphate; ADP, Adenosine diphosphate; ATP, Adenosine triphosphate 2,3-DPG, 2,3-bisphosphoglycerate; GTP, guanosine triphosphate; IMP, inosine monophosphate; Pi, inorganic phosphate.

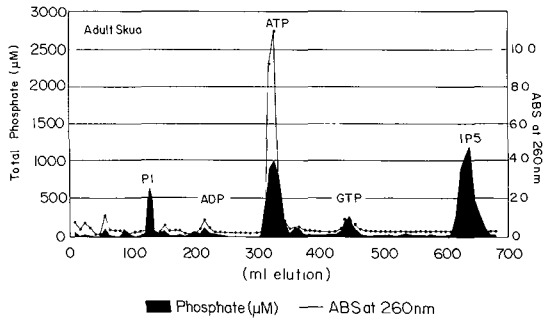

Fig. 2. Profile of the ion-exchange chromatography of phosphorylated metabolic intermediates found in erythrocytes from adult skua *Chataracta mackcormicki*

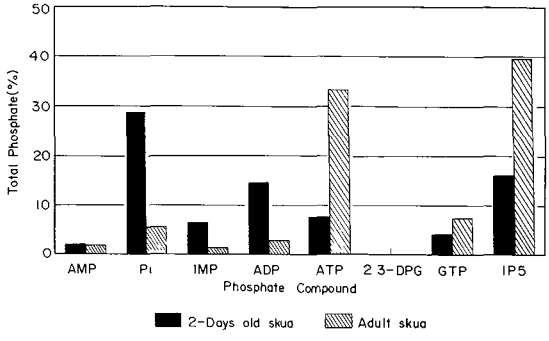

Fig. 3. Comparative amounts of inorganic phosphate and phosphorylated metabolic intermediates in the erythrocytes of 2-days old and adult skuas.

Discussion

Changes in the profiles of phosphorylated metabolic intermediates in the red blood cells of penguins and skuas show some features that deserve comments.

Penguin embryos in the 30th day of incubation—about two to three days before hatching—possess an erythrocyte in which both 2,3-DPG and IP5 are present, the concentration of 2,3-DPG being much higher than that one of the IP5. However, IP5 is completely absent in the erythrocytes of the 25th day embryos. This allows us to place the beginning of the IP5 synthesis in between the 25th and the 30th day of incubation of the egg. The fact that 2,3-DPG is found in lower concentrations in the 25th day embryo than in the 30th day ones, means that the rate of 2,3-DPG synthesis and accumulation is still increasing during this period of the embryonic development of the bird.

One way to interpret the facts regarding the changes in the levels of phosphorylated metabolic intermediates in penguins is shown in Figure 4.

It can be observed that the concentration of 2,3-DPG in the penguin erythrocyte raises from the 25th up to the 30th day of the embryo development, decreasing there upon and becoming almost completely absent in the 2-days old chick. Coincidentally, IP5 concentration begins to raise around the 30th day of the egg incubation, becoming exponential from the 28-days old chick up to the adult penguin. Although adenosine triphosphate (ATP) is the most abundant phosphorylated metabolic intermediate in the penguin red blood cell, it displays a profile of concentration which counteracts the behavior of the other main components, namely 2,3-DPG and IP5, in regard to their metabolism and, of course, their concentration, both depending on the ATP phosphate and potencial energy for their respective synthesis.

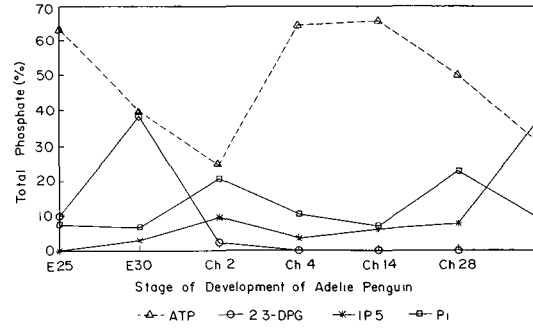

Fig. 4. Comparative profiles of ATP, 2, 3—DPG, IP5 and inorganic phosphate during the biological development of penguins. E25 and E30, embryos in the 25th and 30th day of incubation, ch2, ch4, ch14, ch28, 2, 4, 14, 28—days old chick, A, adult penguin.

In this way, between the phosphorylated metabolic intermediate and the inorganic phosphate of the penguin red cell there is a dynamic equilibrium established by the metabolic activity of the erythrocyte along its life span.

According to Rodrigues (1987), the total concentration of phosphate within the chicken erythrocytes is fairly constant, so that along the biological development of the bird occurs a redistribution of the phosphate between the different kinds of organic molecules which are part of the cell environment.

Rosa et al. (1989) showed that penguin erythrocytes possess a defective glucose metabolism, the red blood cell being inefective in regard to glucose metabolism. This property of the penguin red cell could be explained by the fact that IP5, which accumulates in the red blood cell after the hatching and during the adult life, is a very effective Mg^{2+} chelating agent competing this way for this cofactor with several enzymes. For instance, Williamson (1970) pointed out that the concentration of Mg. Adenosin diphosphate (ADP) in the erythrocyte is probably below the Km for phosphoglycerate kinase and therefore may exert effective control on the enzyme velocity.

The skua follows very closely the general pattern established for penguins, even for the metabolic behavior of the erythrocyte (unpublished data from this Laboratory).

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