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GLYCATED HEMOGLOBIN IN MUTE SWAN (*CYGNUS OLOR*) AND ROOK (*CORVUS FRUGILEGUS*)

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Abstract—1. Percentages of glycated Hb were determined in 10 young and 10 adult mute swan (*Cygnus olor*), and 4 rook (*Corvus frugilegus*) using affinity microchromatography method.

2. The levels of glycated Hb and blood glucose in young (1.45% per 129 mg/ml) and adult (1.43% per 121 mg/ml) swans were the same.

3. The level of glycated Hb in rook (1.82%) does not differ significantly from that in swan (1.44%) but the level of blood glucose (236 vs 125 mg/ml) differs significantly ($P < 0.001$).

4. The level of glycated Hb probably not only simply reflects dependence on blood glucose level, erythrocyte life span and permeability of erythrocyte membrane, but also on food regime.

INTRODUCTION

The modification of hemoglobins (Hb) by non-enzymatic glycosylation (glycation) involves primarily the formation of a Schiff-base linkage between the oxo-form of carbohydrate, usually glucose, and either the amino group of the amino-terminal amino acid or the epsilon amino group of a lysine residue(s). The extent of hemoglobin glycation is dependent on mean glucose levels (Harding, 1985). Therefore, measurement of glycated Hb is used for the diagnosis and screening of diabetes mellitus, when the normal human maintains a glycated hemoglobin (GHb) level of approximately 6% (4.3–7.7%), while the untreated diabetic can have GHb levels of 10% or greater. Monnier *et al.* (1991) occupied themselves with the question of the proposed role of Maillard reaction in aging process. They pointed out high glycemia levels of birds.

Levels of GHb have been reported in animals such as mice (Koenig and Cerami, 1975), monkeys (Solway *et al.*, 1979; Alperin *et al.*, 1979), sharks (Alayash *et al.*, 1991), white-tailed deer (Jenks *et al.*, 1991), dog (Hasegawa *et al.*, 1991), camel (Al-Ali *et al.*, 1990), ruminants (Richter, 1986), some domestic animals including birds (Rendell *et al.*, 1985) and also invertebrates (McDonald and Kitto, 1989).

Mute swan (*Cygnus olor*) is resident in Czechoslovakia (i.e. in Bohemia). There they are also here plenty of migrants from neighbouring countries (Poland, Austria and mainly Germany) and also some individuals from Baltic countries. In this study we work only on ones from our country (identified by rings). In nature swans eat mainly aquatic and swampy plants, as well as some small crustaceans and insects. In winter swans are fed plentifully by people (bread, pastry). The maximum age was discovered to be 19 years (Hudec *et al.*, 1972).

The rook (*Corvus frugilegus*) is not resident in Czechoslovakia. Rooks bred in Czechoslovakia winter mainly in France (i.e. west to southwest direction) and populations wintering in our country are bred in the east to northeast direction (i.e. Poland and mostly the former Soviet Union; the greatest distance was discovered at the foot of the Ural mountains). Rooks eat vegetables (e.g. corn) and animal food (e.g. insects). Composition of feed is changing by annual seasons. Maximum age was discovered to be 20 years (Hudec *et al.*, 1983).

MATERIALS AND METHODS

Blood samples (*ca* 0.1 ml) were collected from live animals into EDTA-treated tubes. All birds were caught in the morning (to prevent influence from human feeding) and if they had not previously been ringed they were labeled by rings from Bird-ringing Centre (Narodni Museum, Praha). All samples were taken during the period 29 January to 18 March 1992. In the case of swans the specimens were divided into two groups—young (i.e. *ca* 9 months old) and adult (i.e. *ca* age ≥ 1 year and 9 months).

Levels of GHb were determined as a percentage of total hemoglobin using Pierce Glyco-Gel columns (Pierce, Rockford, IL) according to procedures recommended by the manufacturer. Two human samples were used to validate obtained results. Glucose concentration in plasma was determined using the glucose oxidase–peroxidase method, Lachema (Brno, Czechoslovakia).

RESULTS AND DISCUSSION

Results of the glycation assays are shown in Table 1. This table shows that there is no significant difference between young and adult swans' GHb level as well as their plasma glucose level. There is also no significant difference between mute swan and rook in GHb levels, but there is a significant difference ($P < 0.001$) between glucose levels in these species. Correlation between date of sample collection and levels of GHb and glucose is shown in Fig. 1. It seems that GHb level depends on earlier glucose level than actual, and that these levels significantly alter. It may reflect changes in composition of food, consequently the proportion between feeding by people and natural feeding, and also the differences

Table 1. Serum glucose levels and glycated hemoglobin (GHb) content in erythrocytes of mute swan and rook

	N	Glucose (mg/dl)	%GHb
Mute swan (*Cygnus olor*)			
Young	10	129 ± 21	1.45 ± 0.42
Adult	10	121 ± 15	1.43 ± 0.38
All	20	125 ± 18	1.44 ± 0.39
Rook (*Corvus frugilegus*)	4	236 ± 31	1.82 ± 0.56

in the quality of this natural food. It is possible that there is no simple straight relationship between GHb and glucose. Jenks *et al.* (1991) in their study on white-tailed deer found out serum glucose and GHb levels of captive deer 173.0 ± 6.2 mg/dl and 2.5 ± 0.1%, resp. and in wild deer 119.9 ± 8.4 mg/dl, resp. 3.7 ± 0.4%. In this study the seasons varied in which blood samples were collected. Seasonal differences were also reported for camel: 4.39 ± 0.69% GHb in winter and 5.5 ± 0.38% GHb in summer (Al-Ali *et al.*, 1990; Alayash and Wilson, 1987).

Glycation of animal hemoglobins has been shown to occur in a similar fashion to those of humans. In humans, the major sites of *in vivo* glycation were found to be (in order of prevalence) β-valine-1, β-lysine-66, α-lysine-61, β-lysine-17. The minor sites were α-valine-1, α-lysine-40, β-lysine-8, and β-lysine-144 (Shapiro *et al.*, 1980). In bird hemoglobins all these sites were found (e.g. hemoglobin sequences of mute swan). In adult birds there are two kinds of hemoglobins—HbA and HbD, which have the same β-chains and different α-chains. Presence of HbD in swan is only low, *ca* 5–10% (Oberthr *et al.*, 1982; Kleinschmidt and Sgouros, 1987).

It is proposed from the results of glycated hemoglobins on animals, that the value of GHb (except for blood glucose concentration) is also dependent on erythrocyte life span and so can be used as a valid index for the survival of erythrocytes. Rendell *et al.* (1985) also measured GHb in some species of domestic birds. Their results were substantially lower than ours—chicken 0.54 ± 0.11%, duck 0.47 ± 0.05% and turkey 0.95 ± 0.06% GHb. These low results can be explained by low permeability of erythrocyte membrane. We think that these results may be also influenced by food regimes. This opinion supports the finding of Gallaher and Schaubert (1990) that the level of GHb in diabetic rat is reduced by feeding with diet containing guar gum fiber, but is not influenced by any other fiber diet (e.g. cellulose, sugar beet, etc.). Our results reflect the wild living birds feeding in winter and from these results we proposed influence of food (and from this probably derived influence of seasons) on glycation of hemoglobin.

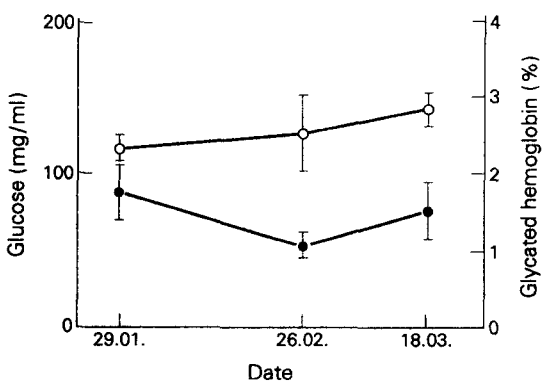

Fig. 1. Values of glycated hemoglobin (solid circles) and serum glucose concentration (open circles) in blood samples collected on 29th January ($N = 6$), 26th February ($N = 4$) and 18th March ($N = 5$) from mute swans.

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