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Hypoglycemia in *Columba livia* Due to the Infection of Apicomplexan Parasites

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ABSTRACT: The impact of two apicomplexan parasites, *Haemoproteus* and *Plasmodium* species in naturally infected *Columba livia* was evaluated in order to examine the effects on the host. *Haemoproteus* occurred at a higher prevalence (55.63%) in *Columba livia* as compared to *Plasmodium* (6.76%). Blood sugar level was found to be 273.27 ± 1.13 mg/dl and 269.25 ± 1.18 mg/dl in uninfected male and female C. livia respectively. In lone infection of *Haemoproteus* the values of glucose decreased by 16.37% in male and 18.17% in female pigeons. The values decreased by 21.27% and 21.99% in male and female pigeons respectively under concurrent infections of *Haemoproteus* and *Plasmodium* as compared to uninfected *C. livia* indicating that hypoglycemia is a frequent outcome of apicomplexan infection and that the manifestation is greater under concurrent infectivity.

Key words: Glucose, *Haemoproteus*, *Plasmodium*, concomitant

Introduction

Intracellular parasitism involves series of subtle interrelationships between parasite and its host cell (Trager, 1974). Of special interest is the nature of biosynthetic lesions in the parasite, and the parasite's corresponding dependence on its host for factors essential to its metabolism. Although the impact of blood parasite infection on passerine birds is potentially great, little is known of their biochemical effects. Biochemical analysis is widely used to diagnose and characterize diseases in most species of animals. It is less utilized in avian species perhaps due to the relatively low economic value of individual animals. However, biochemical analysis is a useful research or diagnostic implement, especially with diseases having a poorly understood pathogenesis. Clinical chemistry is a very useful tool in veterinary medicine for helping in the development of a diagnosis and prognosis of avian diseases (Lewandowski *et al.*, 1986) as also for the interpretation of the normal reference values for each species.

Glucose is an essential metabolic fuel for the growth and reproduction of erythrocytic stage of malaria (Mc Kee, 1951; Honigberg, 1967). Indeed, one of the most striking characteristic is its high rate of glucose consumption. Glucose is an important form of energy currency that can be transported between a number of different tissues of the body through the bloodstream. Evidence was presented in 1901 and confirmed by Bell (1957) that the blood sugar of birds is in the form of D-glucose, as in mammals. A measure of its importance in birds may be gauged from the high blood glucose concentrations in a wild range of birds which are generally higher than in mammals and, in contrast with mammals, the steady-state levels are much less susceptible to change starvation.

At present, the information concerning blood chemistry in birds is very limited. Most of the present knowledge of avian physiology is concerned with domestic species, while physiological trends associated with free-living individuals have been only partially studied (Kren *et al.*, 1972; Chilgren and deGraw, 1977; deGraw *et al.*, 1979 Gee *et al.*, 1981; Leonard, 1982; Puerta *et al.*, 1990; Alonso *et al.*, 1991 and Peinado *et al.*, 1992). Studies were under taken to observe the glucose values under lone (*Haemoproteus*) and dual (*Haemoproteus* and *Plasmodium*) apicomplexan infectivity and the values compared with the controls in order to observe changes in glucose metabolism, if any.

Materials and Methods

One milliliter of Blood was collected directly from the brachial vein of each *Columba livia* Gmelin (n=128) which were collected from different sources of Bareilly including bird market, college campus, hostel's garden, and old buildings and kept in cages. For this study, the pigeons were divided into six groups: Group AM (Uninfected male pigeons), Group AF (Uninfected female pigeons), Group BM (*Haemoproteu* infected male pigeons), Group BF (*Haemoproteus* infected female pigeons), Group CM (*Haemoproteus* and *Plasmodium* infected male pigeons) and Group CF (*Haemoproteus* and *Plasmodium* infected female pigeons) and maintained in the laboratory at 38-40^{0}C, fed on grains (30 gms per day) and provided water (1 litre per 20 pigeons). A drop of blood placed on a clean microscopic slide and the remaining blood was transferred to a clean glass vial containing EDTA (Ethylene Diamine Tetra Acetic Acid) as an anticoagulant for biochemical studies. Blood smears were prepared to detect the parasites, according to Gupta (1986) and stained in Giemsa's with phosphate buffer (pH 7.4) in the ratio of 1:7 for 3 hours. The slides were washed, dried and examined for blood parasites at 1000x. Parasite species were identified using morphologic characteristics (Garnham, 1966).

The values of glucose were recorded by collecting plasma in small tubes by centrifugation of the blood at 12000 rpm for 5 minutes according to Oser (1965) by autoanalyser and with the help of OGENT Glucose kit. The values are expressed in mg/100 ml of blood. The analyses were completed within one day of collection.

Results

Results from haemato-parasitological examination of thin blood smears revealed apicomplexans of two genera, *Haemoproteus* and *Plasmodium.* Out of 266 pigeons sampled, 148 pigeons were positive for *Haemoproteus* at a prevalence of 55.63%. Only 18 pigeons (2.67%) had a mixed infection with *Haemoproteus* and *Plasmodium* and 130 pigeons (48.87%) had *Haemoproteus* infection alone and no pigeons was positive for *Plasmodium* alone. The infection rates of *Haemoproteus* were also reassessed according to the sex of the host. In case of *Plasmodium,* only five females and 13 males were infected with this parasite. The values of glucose in uninfected *C. livia* were 273.27 ± 1.13 mg/dl and 269.25 ± 1.18 mg/dl in AM (n=10) and AF (n=10) groups respectively and there was a slight difference between the values of uninfected male and female pigeons. A comparison of these values with infected groups (Group B and Group C), indicated that, the values of glucose declined sharply. In Group BM (n=50), the values were 228.52 ± 1.77 mg/dl and in Group BF (n=40), 220.30 ± 1.94 mg/dl. The values further decreased to 215.14 ± 1.53 mg/dl and 210.00 ± 1.67 mg/dl in Group CM (n=13) and Group CF (n=5) which were parasitized by concomitant infection of *Haemoproteus* and *Plasmodium*. The mean values of Group A was 271.26 ± 1.15 mg/dl as compared to Group B (224.55 ± 1.85 mg/dl) and Group C (221.57 ± 1.60 mg/dl) pigeons (Table 1).

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Table-1:- Glucose values in *Columba livia* due to the infection of erythrocytic parasites

Groups		Glucose (mg/dl)
Group A (Uninfected)	Group AM (n=10)	273.27 ±1.13
	Group AF (n=10)	269.25 ±1.18
Average		271.26 ± 1.15
Group B (Infected with lone infection*)	Group BM (n=50)	228.52 ±1.77
	Group BF (n=40)	220.30 ± 1.94
Average		224.91 ± 1.85
Group C (Concomitant Infection*)	Group CM (n=13)	215.14 ±1.53
	Group CF (n=5)	210.00 ±1.67
Average		212.57 ±1.60

***** *Haemoproteus*
** *Haemopoteus+Plasmodium*

Discussion

During their intra-erythrocytic growth phase, the malarial parasites have ready access to blood glucose. It has been reported that in the monkey malaria, *Plasmodium knowlesi,* the parasitized erythrocyte consumes up to 25 times more glucose than a normal cell (Mc Kee *et al*. 1946) while in the bird malaria , *P. gallinacium* and *P.relictum*, the infected red cell utilized 5 to 10 times more glucose than the uninfected cells (Khabir and Manwell, 1955; Manwell and Feigelson, 1949 and Warren and Manwell, 1954). In the present study, the value of glucose lowered by about 16 to 22% in the infected groups of *C. livia.*

The uptake of carbohydrates has been investigated in only a small number of parasites including haemoflagellate Protozoa (*Trypanosoma lewisi, T. gambiense*), species of Digenia (*Fasciola* and *Schistosoma*) and species of Cestoda (*Hymenolepis*). *In vitro*, glucose is actively transported the across the membrane of parasite. Absorption of glucose is competitively inhibited by some sugar (D-allose, α methyl glucose, 6-deoxyglucose, 3-O- methylglucose and 1-deoxyglucose) but not by others (fractose, L-fucose, 1-5 anhydro D-mannitol). The uptake system is stereospecific, temperature dependent, with a Q_{10} (for the temperature range 15 - 40 ^{0}C) of 2.4, and is inhibited by metabolic poisons (p-mercuribenzoate, iodacetate and 2,4 – dinitrophenol). The parasite can accumulate glucose if incubated in 5 mM glucose for 60 minutes, an internal concentration of 25 mM is attained. It has been proposed that galactose enters parasite via the glucose transport system.

Intra-erythrocytic stages of *Plasmodium* lack carbohydrate reserves and consequently their primary source of energy is glucose from the blood streams. Although the pO_2 in the blood is high, *P. falciparum* does not oxidize glucose completely to CO_2 and H_2O. It has been shown that *in vitro,* infected human red cells (10^9) consume 122 ± 34 nmol glucose per 24h compared to 4.6 ± 1.5 nmol glucose per 24h for uninfected cells. Glucose consumption was lower in ring forms than other stages and the schizont stages were shown to produce most of the lactate (Jensen *et al.,* 1983; Sherman, 1991).

It was originally thought that the hexose monophosphate shunt was in operation in the malarial parasite, because the first enzyme of the shunt, G6PD (glucose-6-phosphate dehydrogenase) was lacking. However, this enzyme has since been identified in *P. falciparum, P. knowlesi* and *P. berghei* (Sherman, 1991). Although the parasite G6PD has a higher affinity for glucose -6- phosphate than does the host enzyme, the contribution to the ribose pool of the parasite appears to be minor. It is of special interest to note that in certain parts of the world, a deficiency in G6PD in human erythrocyte which is an X-linked abnormality occurrs more commonly in high malaria regions, suggesting that such a deficiency provides some selective advantage for human survival. This speculation is also supported by the results from *in vitro* experiments in which growth of plasmodia was retarded in G6PD – deficient cells (Sherman, 1991).

Malaria plasmodia often produce a marked hypoglycemia in their hosts, at least during the late stages of the infections. They also seem to influence the carbohydrate metabolism of the infected erythrocytes. Fletcher and Maegraith (1962) found increased glucose–6-phosphate dehydrogenase and 6– phosphogluconate dehydrogenase activities in *Plasmodium knowlesi* – infected cells, and Herman *et al.* (1966) observed increased $^{14}CO_2$ production from (1^{-14}C) glucose by *Plasmodium gallinaceum* – infected erythrocytes. Such findings indicate a stimulation of the pentose phosphate pathway of the red cells since the sequence is lacking in the parasites themselves (Von Brand, 1973). It has been suggested that some times, the rising blood sugar during *Plasmodium paroxysms* is because of an excessive breakdown of liver glycogen but on the other hand, during the late stages of infection, plasmodia produce a marked hypoglycemia. Blood sugar levels vary considerably even in healthy birds, in which the mean was found to be 267 ± 9.2 SEm mg per 100 ml plasma. Levels tended to rise in the early stages of the infection, reaching a mean of 305 ± 14.5 mg per 100 ml plasma (based on 10 cases) while parasitemia was 20-49%. At death the mean (determined from 46 birds) had dropped to 155.8 ± 15.5 mg per 100 ml. However there is little reason to think that parasite demand on the host's glycogen reserves acts as a significant factor in causing death, despite the very high parasitemias often reached. (Manwell and Stone, 2007).

Khabir and Manwell (1955) reported that *Plasmodium hexamerium* prefers older erythrocytes as host cells (or such cells may be more hospitable to the parasite). Manwell and Loeffler (1961) found that erythrocytes infected with *Haemoproteus columbae* consumed 100 times more glucose than unparasitized cells, thus considerably lowering host glucose concentration in birds.

How does *Plasmodium* alter the permeability properties of its host cell? *In vivo* studies on carbohydrate transport suggest that three concurrent process are possible to occur using this technique: these are active transport, simple diffusion and a third process known as solvent drug. A problem of any intracellular parasite is to obtain nutrients across two cell membranes, its own and that of the host. *Plasmodium* has overcome this difficulty by inducing, by an unknown mechanism, the host cell to lose (partially) the ability to regulate the passage of molecules across its cell membrane. The host cell becomes freely permeable to many complex molecules which therefore can be taken up with little or no expenditure of energy. This is a considerable advantage to the intracellular parasite.

Current knowledge on the energy metabolism of parasitic protozoa gives some clues as to how they cope with and take advantage of their environment. Blood parasites have an abundant and constant supply of both glucose and oxygen, and some species have adapted to this by evolving a seemingly simple, yet clearly very efficient catabolism of glucose to pyruvate.

Our present knowledge is an interesting example of the exciting biochemical systems that are present in parasitic protozoa. Some groups of parasitic protozoa, such as the sporozoa, are virtually unknown entities in the biochemical sense. Two other possible mechanisms are suggested by Sherman and Tanigoshi (1974). First, the growing parasite releases some substance(s) that promotes the diffusion entry of glucose. Second, increased glucose entry may be due to parasite's induction of anaerobiosis in the host cell thereby increasing the diffusion coefficient of the glucose carrier.

It is logical to ask whether it is proper to speak of the consumption of glucose by malarial parasites at all, since they are within the erythrocytes, and whatever reaches them must come from the interior of the host cell. Perhaps the red cell (especially when it is nucleated,

as in birds) may at least initate the chemical transformations of the well-known Krebs' cycle. Perhaps, parasites (*Plasmodium knowlesi*) could utilize a variety of sugars and also glycerol but no final answer can be given to this question (Fulton, 1939).

Nevertheless, since parasites freed from their host cells have been shown to be able to utilize glucose and some other sugars directly, and since *Plasmodium hexamerium*-infected red cells consume glucose much faster than uninfected mature erythrocytes of apparently similar age, it seems very probable that the plasmodia themselves consume glucose as such. In an extensive review published by McKee (1951) on the carbohydrate metabolism of the malaria parasite, shows the similar pattern.

It has been observed that the glucose consumption of different species of *Plasmodium* varies**:** *Plasmodium gallinaceum*- 3.00 x 10^{-4} mg glucose per hour per 10^{7} square micra of surface area, *Plasmodium relictum*- 4.39 x 10^{-4} mg glucose per hour per 10^{7} square micra of surface area (Warren and Manwell, 1954), and *Plasmodium hexamerium*- 1.42 x 10^{-4} mg glucose per hour per 10^{7} square micra of surface area (Khabir and Manwell, 1955).

The results of this study suggest that glucose consumption rate of *Haemoproteus* and various species of *Plasmodium* is of much the same order resulting in the same trend of depletion in both infected groups of *Columba livia.* It is probably safe to assume that *Haemoproteus* gametocytes and those of *Plasmodium* are very similar in their physiology, both being intracellular parasites of nucleated cells.

It is concluded that the increased entry of glucose in to the malaria-infected cell may be due to an alteration in the simple diffusion component as well as a change the number of carrier-mediated (facilitated diffusion) transport sites.

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