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Some Factors Which Decrease Arterial Saturation in Bird Malaria-Ducks Infected with *P. lophurae*.*†

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The failure of oxygen transport during the terminal stage of *P. lophurae* infection in the duck has been suggested by Rigdon and Rostorfer[1] to be the cause of death. The anemic anoxia is so severe that the oxygen capacity of the blood is only 18 to 19% of the normal. Accompanying this low oxygen capacity there is a marked decrease in percentage of saturation of the blood *in vitro*. Wong[2] was able to demonstrate a decrease in arterial saturation *in vivo* during malarial paroxysms in neurosyphilitic patients undergoing malarial fever therapy. Arterial saturations as low as 70% were noted during the peak of the fever. In discussing the cause of this failure of arterial saturation Wong states, "the presence of some factor which was inhibiting full oxygenation of the blood hemoglobin might be proposed as an explanation of this phenomenon."

This "factor" which Wong suggests might conceivably be either chemical in nature and peculiar to malaria, or it might be a combined physiological action such as the combined effect of temperature increase, acidosis or alkalosis. Furthermore, the mechanics of the circulation and respiration such as the speed of circulation and fall of alveolar partial pressure of oxygen might be contributory factors. In other words this same phenomenon might be observed generally in severe febrile conditions. On the other hand, if some factor prevents oxygen uptake by the hemoglobin in *P. vivax* infection in the human, one might expect to find a similar factor in the blood in other types of malaria, for example, *P. lophurae* infections in the duck. Accordingly, blood from *P. lophurae* infected ducks was investigated *in vitro* with the aim of demonstrating the presence of some factor inhibiting oxygenation of the blood. The relationship of oxygen saturation to pH, pK, CO_2 combining power, and temperature in malarial-infected duck blood is reported and discussed in this paper.

Methods and Materials. In this study *P. lophurae* obtained from highly parasitized donor birds was used as the inoculum to infect young white Pekin ducks from 15 to 20 days old. The parasitemia was followed by counting the number of parasitized cells in 500 red cells from smears stained with a combination of Giemsa's and Wright's stains. Standard methods were used in making the total red cell counts. Young erythrocytes were differentiated from adult red cells by the darker staining of the cytoplasm and rounder nucleus. Blood samples for analytical purposes were obtained by heart puncture from 2 or 3 birds in the same stage of infection. These samples were pooled. Coagulation was prevented by using 1 ml of a 1% solution of the sodium salt of heparin for each 9 ml of blood. All cell counts and gas analyses were obtained from these pooled samples. Equilibration and gas analyses were carried out in the manner described by Rostorfer and Rigdon.[3] Normal and malarial duck blood was equilibrated at 38°C and 42°C and the oxygen dissociation curves at these temperatures were calculated by the use of the formula $= Y = 100\,kX^n/1 + kX^n$.

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† Research paper 585 Journal Series, University of Arkansas.

1 Rigdon, R. H., and Rostorfer, H. H., *Nat. J. Mal. Soc.*, in press.

2 Wong, Yan Tim, *Science*, 1945, **102**, 278.

3 Rostorfer, H. H., and Rigdon, R. H., *Am. J. Physiol.*, 1946, **146**, 222.

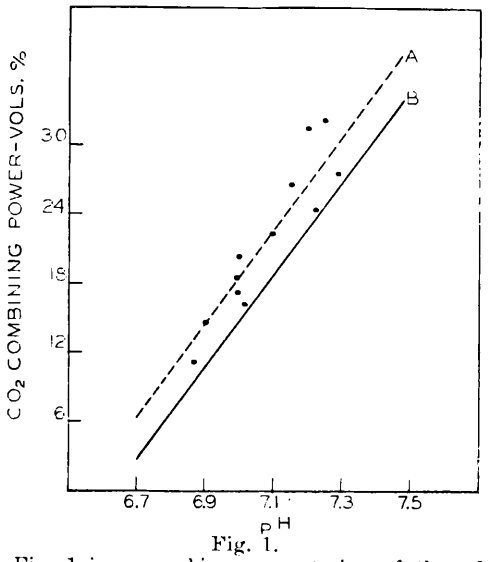

Fig. 1.

Fig. 1 is a graphic representation of the relationship of CO_2 combining power and pH of (A) normal duck blood and (B) blood of malarial-infected ducks during different stages of the infection. The normal relationship (line B) is calculated by the use of the normal pK of 6.20. The pK for line A is the average pK for malarial blood, namely 6.05.

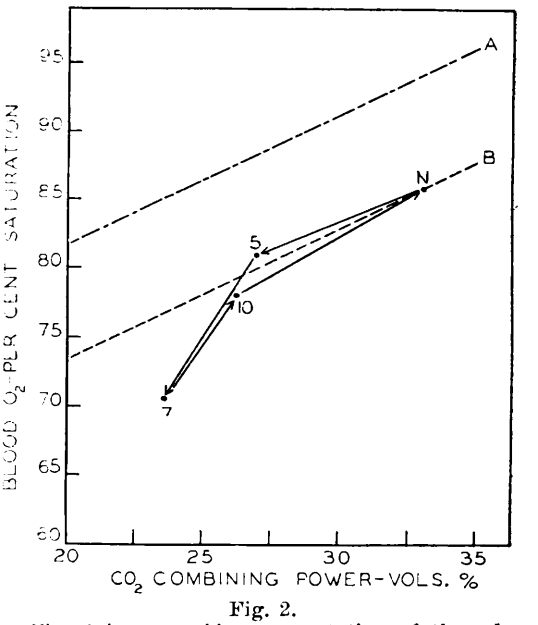

Fig. 2.

Fig. 2 is a graphic representation of the relationship of the percentage of oxygen saturation at an O_2 tension of 83 mm Hg with the CO_2 combining power for (N) normal duck blood, (5) fifth day after inoculation, (7) seventh day after inoculation, and (10) tenth day after inoculation. Line A is the rate of fall in saturation with increasing CO_2 tension in the equilibration gases. Line B is the theoretical rate of decreasing saturation with decreasing CO_2 combining power.

The constant k and the exponent n were calculated from the data obtained by equilibrating samples at 52 and 83 mm Hg of oxygen tension. The pH was determined on samples equilibrated at 31 mm Hg of CO_2 tension by the use of the glass electrode. The pK was calculated by the use of the formula $pH = pK + \log BHCO_3/\text{Free } CO_2$.

Results. A severe anemia develops during the course of *P. lophurae* infection in the duck. In the terminal stage of the infection the blood may have only 15% of normal oxygen capacity. As the anemia progresses there is a decrease in pH which is definitely correlated with the degree of anemia, at least during the terminal stages of the disease. pH values of heparinized blood taken during the 5th and 6th day after inoculation have been recorded as low as 6.89. A fall in pH from the normal of 7.40 to 7.25 was noted as early as the third day.

The CO_2 combining power of the whole blood out of normal young ducks, which was heparinized as described and equilibrated with 31 mm Hg of CO_2 tension, was found to be between 33 and 34 volumes %. There is a gradual fall in CO_2 combining power during the course of the malarial infection. Values as low as 14 volumes % have been observed. This shift of CO_2 combining power is accompanied by a corresponding fall in pH. The relationship of CO_2 combining power and pH is shown in Fig. 1.

The pK of normal blood from young ducks is approximately 6.2. However, during the course of malarial infection the pK falls to values as low as 6.05. In Fig. 1, line B represents the relationship between CO_2 combining power, pH and pK for the blood of normal young ducks. Line A is the average relationship of these 3 values during the course of infection. It can be seen in Fig. 1 that the pH is lower at the same level of CO_2 combining power in the malarial blood in comparison to the normal blood.

The decrease in blood oxygen saturation which accompanies the decrease in CO_2 combining power is shown in Fig. 2. Line A is

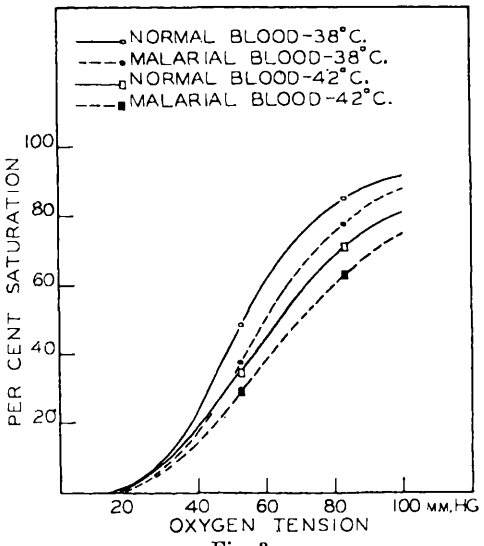

Fig. 3.

Fig. 3 is the graphic representation of the effect of increasing temperature on normal and malarial duck blood.

the normal fall in oxygen saturation which results from increasing the tension of CO_2 in the equilibrating gas. Line B is the normal fall in oxygen saturation with decreasing CO_2 combining power of the blood. According to Adair[4] and Barcroft[5] there is a linear relationship between O_2 uptake with CO_2 combining power and pH which holds until the lower levels of pH are reached when the percentage of O_2 saturation fails to continue to fall with further decrease in pH. It will be noted that malarial blood bears out this relationship until the 7th day of the infection. At this time there is a deviation from the linear relationship. This deviation is in the opposite direction from that which normally occurs at lower pH values. However, if the birds recover from the infection the normal linear relationship is restored by the 10th day.

The one discernible difference in the malarial blood between the 5th and 7th day of infection lies in the great increase in young red cells by the 7th day. At this time and later, if the bird survives, the young cells may reach numbers of between 200 and 400 per 500 red cells. These cells have been shown by Rostorfer and Rigdon[6] to have hemoglobin which is apparently more susceptible to the CO_2 effect, to carry less hemoglobin per cell, and to be less effective as O_2 carriers. By the 10th day after inoculation most of the young red cells have disappeared from the blood stream, and by this time the original linear relation between CO_2 combining power and O_2 saturation has been restored.

The effects of the acidosis and temperature upon malarial blood in relation to the same effects on normal duck blood are shown in Fig. 3. The combined effects of a rise of 4°C in temperature and the acidosis cause the dissociation curves to markedly shift to the right. The malarial blood at 42°C has only approximately 70% of the oxygen combining capacity of normal duck blood at 100 mm Hg of O_2 tension.

In order to demonstrate the effect of any substance or "factor" in the plasma of malarial blood which might affect the cells and decrease oxygen uptake, samples, of 10 ml of heparinized malarial and normal duck blood, were centrifuged and the plasma was drawn off by careful aspiration. The plasma of malarial blood was placed on normal cells and the normal plasma was placed on malarial cells and carefully brought up to the original volumes. These samples were equilibrated with air and with 83 mm Hg O_2 tension and 31 mm Hg CO_2 tension and compared with the data obtained on the original bloods. No difference in O_2 capacities was noted between the original malarial blood and the blood which was composed of malarial cells and normal plasma. Nor was there any demonstrable difference between the O_2 capacities of the original normal blood and that for normal cells and malarial plasma. There was a fall, however, in percentage of saturation in the case of normal cells and malarial plasma equilibrated at 83 mm Hg of O_2 tension and 31 mm Hg of CO_2 tension. There was also a fall in CO_2 combining power in this mixture of malarial plasma and normal cells

[4] Adair, G. S., *J. Biol. Chem.*, 1925, **63**, 529.

[5] Barcroft, J., Camis, M., Mathison, C. G., Roberts, F., and Ryffel, J. H., *Phil. Trans. London*, Series B, 1914-15, ccvi, 69.

[6] Rostorfer, H. H., and Rigdon, R. H., *Am. J. Clin. Path.*, in press.

which seems sufficient to account for the decreased percentage of saturation. There seemed to be no decrease in oxygen uptake by the normal cells in the presence of malarial plasma; nor were the malarial cells benefitted by the normal plasma excepting the increase in saturations resulting from the rise in CO_2 combining power due to the presence of normal plasma.

Discussion. During the course of malarial infection in the duck an anemia develops which is accompanied by a fall in pH, a marked decrease in CO_2 combining power and a decrease in pK. There is also a shift of the oxygen dissociation curve to the right and a decrease in saturation of the blood *in vitro*. This decrease in saturation bears a linear relationship with the pH and the CO_2 combining power with the exceptional case of the blood taken from ducks on the 6th and 7th day of the infection. During this time young red cells make up from 60 to 80% of the total number of cells present. These cells have been demonstrated to have an abnormal behavior with respect of O_2 capacity, acid base binding power and dissociation curve. It is the authors' opinion that the young cells present on the 6th and 7th days after inoculation are responsible for the abnormal decrease in O_2 saturation with the decreased CO_2 combining power which occurs at this time.

When the effect of temperature is superimposed on the effect of acidosis the percentage of saturation is greatly lowered at the higher oxygen tensions of the equilibrating gas. There seems to be no need for the assumption of a "factor," such as suggested by Wong, which prevents the O_2 saturation of blood other than the decreased CO_2 combining power, the fall in pH, the presence of young cells and increased temperature in the case of *P. lophurae* infected duck blood to account for the decrease in oxygen saturation of the blood *in vitro*.

It should be pointed out here that Cullen *et al.*[7] have investigated arterial oxygen saturation in patients whose body temperatures have been raised in the fever box and have observed decreases in arterial saturation down to 70% of the normal value. It should be noted also that the data obtained by Wong are correlated absolutely with the degree of fever. It is quite possible that the decreased arterial saturation in severe febrile condition has a physiological basis. Various investigators are not in agreement as to the mechanisms involved in the cause of decreased O_2 saturations in febrile conditions but the phenomenon does not seem to be peculiar to the fever of malarial infection.

Summary. Data have been presented which indicate that the decreased arterial saturation, *in vitro*, of the blood of malarial-infected ducks may be caused by the state of acidosis and the presence of great numbers of young red cells during the terminal stage of *P. lophurae* infection. The combined effect of increased temperature and acidosis on the *in vitro* oxygen saturation is sufficient to reduce the percentage of saturation to 70%. No evidence was obtained for the existence of a "factor" in malarial duck blood which would prevent the saturation of the hemoglobin of normal red cells obtained from uninfected birds.

[7] Cullen, S. C., Weir, E. F., and Cook, E., *Anesthesiology*, 1942, **3**, 123.

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Effects of Reduced Caloric Intake on Leucocyte Count of the Rat.*

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The purpose of the present communication is to report the effects of reduced caloric intake on the leucocyte count of the rat. It

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