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Oxygen Affinity of Red Cells Hemolysates of Different Hen Breeds

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Summary. Oxygen affinity and number of -SH groups of hemoglobins have rather constant values for hens belonging to Leghorn, Whiterock, Rhode Island Red, Sussex and Cornish breeds, and for hybrids between Rhode Island Red and Leghorn, irrespective of the breed. Number of -SH groups in red cell hemolysates amounts to 8 per mole Hb (7,88 - 8,48), $\log p_{50}$ = 1,04 - 0,94; value of "n" for Leghorns is 1,70, for Whiterocks 2,80.

Introduction

Despite numerous investigations performed within the last decade, both spacial structure and functional properties of avian hemoglobin (Hb) have not been sufficiently elucidated, and in some cases the obtained data are contradictory (Van der Helm and Huisman 1958; Huisman *et al.* 1964; Muller 1961; Matsuda et al. 1964; Schnek *et al.* 1972). Hemoglobins of birds, including poultry, as well as hemoglobin of mammals, have been proved to be heterogeneous. Dunlap *et al.* (1956) revealed two hemoglobin fractions in hen blood, the main fraction constituting about 80 % of the total hemoglobin, and the minor fraction (20 %) of higher electrophoretic mobility (Saha 1964). However, D'Amelio and Salvo (1959), D'Amelio (1966) and Kołątaj (1963) found three fractions in hen hemoglobin. The number of fractions equal to 2, 3 or 5 proved to be dependent on the buffer applied during electrophoretic separation (Schall and Turba 1963).

Moreover, apart from cytoplasmic hemoglobin, nuclear hemoglobin was found in hens (Wierzbicki 1974).

The capability for reversible binding of oxygen by divalent heme iron is a basic biological function of respiratory proteins. Binding of oxygen by molecules of tetrameric hemoglobin is accompanied by the phenomenon of "heme-heme" interaction and oxygenation curves have a sigmoidal shape. Sigmoidal curves are described by the Hill equation, so "p_{50}" and "n" values constitute parameters characterizing quantitatively the oxygen affinity of hemoglobins and are indicators of their physiological features (Antonini and Brunori 1971). Affinities of hemoglobins for oxygen exhibit a rather strict dependence on the amount of free -SH groups in the molecule (Riggs 1969). Taking into account the above data, it seemed interesting to determine the oxygen affinity of hemoglobin in hemolysates of red blood cells of different hen breeds, characterized by changeable features (for example egg production) simultaneously with determination of the number of their free-SH groups.

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Materials and Methods

Blood of 5 hen breeds, Leghorn, Rhode Island Red, Sussex, Whiterock and Cornish, as well as of crossbreeds Rd × Lg and Lg × Rd, was used for investigations. All the birds were reared at the poultry farm of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Michrów, under identical food and case conditions. The blood was taken from weaning vena by syringe with heparine. The red cells were sedimented and washed with 0.9 % NaCl and hemolysed with an equal amount of water. The hemolysate was centrifuged at 10,000 rpm for 20 min. to remove fragments of membrane and nuclei. The purified hemolysate was used to determine oxygen dissociation curves and the number of -SH groups.

Oxygen affinity of the hemolysate was determined spectrophotometrically according to Asakura *et al.* (1964). The number of -SH groups was determined spectrophotometrically with sodium p-chloromercuribenzoic acid at 250 nm, according to Boyer (1954); p-CMB of concentration $1\text{-}2 \times 10^{-3}$ M, in 0,05 M phosphate buffer pH, was used for titrations of 3 ml hemolysate in the same buffer. Hemoglobin concentration in the hemolysate was calculated from absorbance at 542 mm, assuming that $HbO_2 E^{1\%}_{1\,cm} = 8,4$. Spectrophotometric measurements were performed in a Carl Zeiss spectrophotometer, type VSV - 2P.

Results and Discussion

Oxygen affinity curves of red blood cell hemolysates of the investigated hen breeds had a typical sigmoidal shape. Numerical data were plotted according to Hill

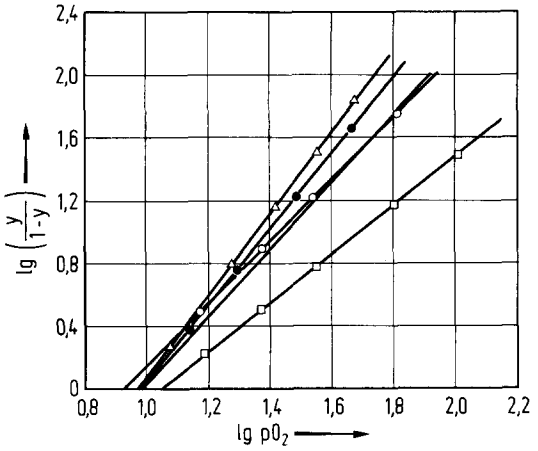

Fig. 1. Oxygen affinity of blood hemolysates ---●--- Cornish, ---□--- Leghorn, ---o--- Sussex, ---------- Rhode Island Red, ---△--- White Rock

(Fig. 1) and from these plots the oxygen affinity value (p_{50}) and the Hill coefficient "n" were determined. These data are summarized in Table 1, where the number of -SH groups per mole protein is also given.

One may infer from the obtained results that the number of -SH groups in red blood cell hemolysates of the investigated hen breeds is constant and amounts to 8 per mole Hb(7,88 - 8,48). No significant differences in oxygen affinity were found. The lowest hemoglobin affinity for oxygen ($\log p_{50}$ = 1,04) was characteristic of Leghorn hens, the highest one of Sussex hens ($\log p_{50}$ = 0,94).

The values of the interaction coefficient "n" showed one more distinct difference, since Leghorn hens were distinguished from other breeds by the highest value of n = 1,70.

Results from former investigations showed that avian blood has a lower oxygen affinity than mammalian blood. It has been established that oxygen affinity of chicken Hb decreases rapidly with age. Oshima *et al.* (1964) and Benesch *et al.* (1968) revealed that the mean factor lowering oxygen affinity of chicken hemoglobin is inositol phosphate (IP) and the concentration of inositol phosphate increases with age. Ochai *et al.* (1972) found that the partial oxygen pressure necessary for 50 % oxygenation of chicken hemoglobin is approximately 20 times lower in purified hemoglobin. Inositol phosphate binds to Hb tetramer in a 1 : 1 molar ratio (Ochai *et al.* 1972; Gondko 1972).

Oxygen affinity of hemoglobin is dependent on the composition and pH of the medium. It is known that phosphate compounds are an important factor in determining this affinity (Gondko 1972).

It seems, from these preliminary studies, that oxygen affinity and number of -SH groups of hemoglobin have quite constant values for hens, irrespective of breed, since no statistically significant differences were found between the investigated breeds. This does not exclude the possibility that such differences exist in oxygen affinity of pure hemoglobin solutions. This question will be the subject of further studies.

Literature

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Table 1. Oxygen affinity and number of -SH groups in hemolysates of different hen breeds

Breed	Number of specimens	$\log p_{50}$ $\bar{x} \pm \bar{s}$	"n" $\bar{x} \pm \bar{s}$	mole-SH / mole Hb $\bar{x} \pm \bar{s}$
Leghorn	19	1,04 ± 0,038	1,70 ± 0,44	8,05 ± 0,38
Rhode Island Red	15	0,98 ± 0,051	2,09 ± 0,43	7,88 ± 0,187
Rd × Lg	5	1,04 ± 0,070	2,11 ± 0,45	8,48 ± 0,30
Lg × Rd	5	1,00 ± 0,037	1,81 ± 0,37	8,27 ± 0,55
Sussex	9	0,94 ± 0,057	2,38 ± 0,40	7,92 ± 0,215
Whiterock	9	1,00 ± 0,070	2,80 ± 0,45	8,03 ± 0,319
Cornish	10	0,98 ± 0,062	2,38 ± 0,31	8,04 ± 0,355

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