Theor. Appl. Genet. 55, 113-117 (1979)

THEORETICAL AND APPLIED GENETICS

Differences in Red Cell Shape of Two Inbred Chicken Lines

K. Hála and J. Plachý
Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

H. Knížetová and B. Kníže
Department of General Zoology, Faculty of Science, Charles University, Prague (Czechoslovakia)

A. Stratil
Institute of Animal Physiology and Genetics, Czechoslovak Academy of Sciences, Liběchov (Czechoslovakia)

J. Derka
University School of Veterinary Medicine, Brno (Czechoslovakia)

Summary. Comparison of two inbred chicken lines ($F_x >$ 99.9%) revealed significant differences in shape of the red blood cells (RBC). The length-width index was lower for both sexes in IC-line (1.46) when compared to CB-line chickens (1.88). Phenotypic expression of this character in F_1 hybrids and both backcross groups corresponded to the common manifestations of the metric parameters. The index in F_1 hybrid chickens deviated from intermediate values with the dominant tendency to oval RBC. An analysis of the segregating first backcross generation chickens did not show any association between RBC shape and the genotype in the blood group systems B, C, I, and D and the IgG allotypes. The differences in RBC shape were probably not associated with the survival of RBC in the blood circulation.

Key words: Red cell shape – Inbred chicken lines

Introduction

The results of the evaluation of variations and differences in the haematological characters within individual species are discordant and depend largely on the origin and physiological state of the animals compared (Tanaka and Rosenberg 1954; Lucas and Jamroz 1961).

The comparison of the inbred lines in which a certain phenotypic uniformity of the characteristics corresponds to genetic homogeneity may disclose some more distinct differences between the lines C and W, which had been brother × sister mated for over 20 generations, Jaffe (1960) found a reduction in the number of red blood cells (RBC) in the C line. In addition, he observed differences in resistance of the RBC from both these lines to osmotic pressure. Karakoz et al. (1977) investigated the haematological characteristics of 4 inbred chicken lines and confirmed that erythrocyte concentration, haematocrit values and haemoglobin content (mg %) were lower in C-lines chickens compared to the W- and I-line chickens.

In this study, we have compared the size and shape of RBC in IC- and CB-line chickens, F_1 hybrids and in both groups of backcrosses, that is, IC × F_1 and CB × F_1. The possible relationship between RBC shape and the genotype in some blood group systems and the IgG allotypes has been investigated.

Material and Methods

The inbred chicken lines IC and CB were described in earlier papers (Hála et al. 1966; Hašek et al. 1966). All chickens were reared under standard conditions in houses at the farm of the Institute of Molecular Genetics, Czechoslovak Academy of Sciences, at Koleč. Five adult cocks and 5 adult hens from each of the inbred lines and from the F_1 hybrid groups were used in the experiments. The groups of the first backcross (B_1) generation chickens included 38 birds.

The size of the RBC was defined as lengths and widths measured with an ocular micrometer at a magnification of 60 × 15. The shape of the RBC was expressed in terms of a length-width index. The two characters were estimated on dry unstained smears of at least 60 cells from each chicken.

The relationship between RBC shape and the blood group systems B, C, I, and D (system designations conform to the internationally accepted nomenclature, allele designations will be those currently used in our laboratory) was investigated in segregating

Table 1. Size (μ) and shape (length-width index) of red blood cells in adult cocks and hens of the inbred lines IC, CB, and their F_1 and B_1 crosses

		IC			CB			IC × CB			IC × (IC × CB)F_1			CB × (IC × CB)F_1		
		Length	Width	Index	Length	Width	Index	Length	Width	Index	Length	Width	Index	Length	Width	Index
♂♂	Mean	10.82	7.47	1.43	12.34	6.63	1.87	11.94	6.91	1.73	11.63	7.13	1.63	11.89	6.92	1.72
	Standard deviation	0.082	0.037	0.025	0.287	0.141	0.035	0.234	0.212	0.050	0.165	0.141	0.041	0.126	0.160	0.046
	Standard error	0.037	0.017	0.011	0.128	0.063	0.016	0.105	0.095	0.022	0.052	0.044	0.013	0.040	0.051	0.015
	Coefficient of variance	0.7	0.5	1.7	2.3	2.1	1.9	2.0	3.1	2.9	1.4	2.0	2.5	1.1	2.3	2.7
♀♀	Mean	10.05	6.91	1.47	11.60	6.15	1.89	11.15	6.32	1.77	11.34	6.84	1.66	11.79	6.75	1.75
	Standard deviation	0.102	0.071	0.035	0.239	0.150	0.052	0.121	0.122	0.016	0.381	0.185	0.076	0.286	0.178	0.060
	Standard error	0.046	0.032	0.016	0.107	0.067	0.023	0.054	0.055	0.007	0.144	0.070	0.029	0.090	0.056	0.019
	Coefficient of variance	1.0	1.0	2.4	2.1	2.4	2.8	1.1	1.9	0.9	3.4	2.7	4.6	2.4	2.6	3.4

chickens of both B_1 generations. Antisera were prepared by the standard procedure (Hála 1977).

IgG allotypes were detected by double diffusion in agar (Derka 1971) using alloantisera produced in inbred chickens.

The haemoglobin patterns and their migration were studied by starch gel electrophoresis using the Tris-EDTA-boric acid buffer, pH 8.9 (Gahne et al. 1960; Stratil and Valenta 1976).

The survival time of RBC in the circulation was estimated by the elimination of chromium (^{51}Cr)-labelled erythrocytes (Hartmanová and Hála 1976).

The significance of the differences was determined by the Student t-test and the intra- and interline variations by the analysis of variance.

Results

The lengths, widths and length-width indexes of RBC are shown in Table 1. RBC were shorter ($P < 0.01$) and wider in IC-line compared to CB-line chickens and the values were significantly higher ($P < 0.01$) for males versus females. Because the RBC shape was not markedly influenced by sex, the values for males and females could be evaluated simultaneously. The results clearly showed that IC-line chickens had round RBC with a length-width index of 1.46, whereas oval RBC (index 1.88) were characteristic for CB-line birds. This difference was highly significant ($P < 0.01$). The difference between the lines was seen even when the native preparations were evaluated.

The results of crosses suggest that the genetic determination of RBC shape is probably not a simple one. The length-width index of (IC × CB)F_1 hybrids was approximately intermediate (1.75) with the predominant shape of RBD tending to an oval form. In view of the high degree of homozygosity of the IC and CB lines it could be supposed that the distribution of individual values in these lines and their F_1 hybrids essentially represented a nongenetic variability of RBC shape. The discrete phenotypic categories were not carried in the segregating backcross populations. In the first back-cross generation chickens of the IC × F_1 combination, a shift of the values in the direction of the IC line and a slight increase in variability was seen. The differences in RBC shape between individual groups were significant ($P < 0.01$) except for the differences between F_1 hybrids and the backcross populations to the CB line. The expected variations between the groups were considerably higher than the within-group variations (Table 2).

The analysis of variance in the B_1 progeny segregating in the major histocompatibility B system (Table 3) as well as in the C, I, and D blood group systems did not reveal any association between the respective genotype and the RBC shape. Nor was any relationship found between the IgG allotypes and the length-width index of RBC (Table 4). Just as Jaffe (1960) observed no difference in electrophoretic mobility of haemoglobins between the C and

W lines, we also found that the IC and CB lines had the same electrophoretic patterns.

The survival time of RBC in the blood circulation was essentially the same in both lines examined, thus suggesting that it was not associated with the differences in RBC shape. We tried to influence the shape of RBC in CB-line females by repeated blood collection. The CB- and IC-line hens were bled six times within 14 days. A total of 100 ml of blood was collected which corresponded to approximately 10% of their body weight. Repeated collections had no influence on the shape of RBC. The length-width was 1.87 for CB-line chickens.

Table 2. Analysis of variance of red blood cell shape in groups of chickens examined (IC, CB, F_1 and 2 B_1 generations)

Variance	SS	df	MS	F
Intergroup	0.9866	4	0.2466	104.7[a]
Intragroup	0.1461	62	0.0024	
Total	1.1327	66		

SS = sum of squares; df = degrees of freedom; MS = mean square
[a] Execceds the high-significance level (0.01)

Discussion

Inbred lines provide a suitable material for studies on genetically determined differences. Differences in some properties of RBC have been shown in females of the C and W lines which have been maintained by brother × sister mating from 1932 and 1944, respectively (Jaffe 1960; Karakoz et al. 1977). In the present experiments, there was a significant difference in the length-width of RBC between the CB and IC lines. Oval RBC with a mean index of 1.88 were typical for CB-line chickens, whereas round RBC with an index of 1.46 predominated in IC-line birds. The length-width index of RBC was found to be a relatively stable character of an inbred line. This has been observed for 3 generations.

The indexes in (IC × CB)F_1 hybrids deviated somewhat from intermediate values with the tendency to an oval form of RBC. No distinct segregation for the discrete phenotypic categories occurred in backcrosses. Only in B_1 generation chickens of the IC × (IC × CB) was there a shift towards the lower values of the index. A slight increase in the variability of individual values was also noted in the two B_1 groups.

Table 3. Analysis of variance of red blood cell shape in B_1 generation chickens segregating in the B system

Group	Variance	SS	df	MS	F
♂ IC × ♀ (IC × CB) F_1	Between segregants B^{13}/B^{13} and B^1/B^{13}	0.0020	1	0.0020	0.61
	Within segregants	0.0488	15	0.0033	
	Total	0.0508	16		
♂ CB × ♀ (IC × CB) F_1	Between segregants B^1/B^1 and B^1/B^{13}	0.0035	1	0.0035	1.12
	Within segregants	0.0595	19	0.0031	
	Total	0.0630	20		

See Table 2 for explanations

Table 4. Indexes of red blood cell shape in B_1 crosses in relation to the genotype of some blood group loci and the IgG allotype

Mating		Blood group locus									
		B		C		I		D		Allotype	
		B^{13}/B^{13}	B^1/B^{13}	C^{13}/C^{13}	C^1/C^{13}	I^{13}/I^{13}	I^1/I^{13}	D^{13}/D^{13}	D^1/D^{13}	IC/IC	IC/CB
IC × (CB × IC) F_1	Mean	1.62	1.66	1.64	1.65	1.65	1.64	1.60	1.66	1.64	1.65
	n	6	11	10	7	11	6	4	13	10	7
		B^1/B^1	B^1/B^{13}	C^1/C^1	C^1/C^{13}	I^1/I^1	I^1/I^{13}	D^1/D^1	D^1/D^{13}	CB/CB	CB/IC
CB × (CB × IC) F_1	Mean	1.75	1.72	1.73	1.75	1.75	1.71	NT		1.73	1.74
	n	14	7	12	9	13	8			12	8

n = number of birds in group, NT = Not tested. The genotype could not be determined because only antiserum against Dl antigen was available

The phenotypic expression of RBC shape in the original parental lines and hybrid groups essentially corresponded to the manifestations of the common metric characters. An analogous problem of the analysis of size and shape of the head and other parts of sperms in inbred lines of rabbits and mice had been described in the literature. Beatty (1971) reported high coefficients of heritability from 0.56-0.97 for some parameters. In our experiments we found the expected high variations in RBC shape between the groups (MS = 0.2466), whereas the within-group variations were very low (MS = 0.0024) owing to the high degree of inbreeding of the original parental lines ($F_x > 99.9\%$). Although the variations in the RBC length-width index within individual chickens were not included in the analysis, it could be concluded that this intra-individual variability was high. The intra-individual variation coefficients were 6.7-9.4, whereas the coefficients for individual chickens within a group were on average substantially lower (v = 2.6). The intra-individual variations in the index illustrate how much RBC shape is affected by the non-genetic factors.

The intra-individual variations in RBC shape may be due to a simultaneous presence of cells of various developmental stages in the blood circulation. The differentiation of individual RBC types during embryogenesis and in the beginning of postembryonic life has been described in detail (Alexander and Schjeide 1953; Lucas and Jamroz 1961; Lemež 1964, 1977; Barrett and Scheinberg 1972; and others). According to Bruns and Ingram (1973), oval cells with oval nuclei are the predominant mature definitive RBC (90.4%) in adult chickens. In addition, mature round cells with round nuclei (9%), mature oval cells with round nuclei (0.2%), and immature erythrocytes (0.2%) are present. More than 95% of mature oval cells have been reported in adult ducks (Attardi et al. 1970).

Our present study was mainly cytometric in nature, the evaluation was done on dry, unstained smears, and therefore the frequency of residual RBC types could not be estimated. Nevertheless, it could be expected that the majority of the cells were of the definitive type. The survival time of RBC in the blood circulation of both starting parental lines was approximately the same. The length-width index did not change after repeated blood collections, and this character could therefore not be taken as a typical one for the early maturation stages of RBC.

According to Barrett and Scheinberg (1972), RBC of definitive shape appear after the last mitotic division, that is, during postmitotic maturation. The shape of RBC, as of other cell types, has been thoroughly studied in relation to the role of the microtubules or microfilaments (Barrett and Dawson 1974). Many investigators believe that spectrin, a membrane protein, is an effective regulator or modulator of the shape of the erythrocyte (Birchmeier and Singer 1977; Shohet and Greenquist 1977). The present results suggest that the pleiotropic or other genetic effects of the blood group antigens examined are not reflected in the changes of RBC shape. The differences in the length-width index of RBC are not associated with the differences in IgG allotypes, nor are they caused by differences in the electrophoretic patterns of haemoglobins which are identical in both lines. In agreement with Gilbert (1965) we found that RBC were larger in male chickens than in female chickens but the length-width index was practically the same for both sexes.

Literature

Alexander, G.V.; Schjeide, O.A.: Studies of the New Hampshire chicken embryo. 1. The red blood cell. Anat. Rec. **115**, 383-384 (1953)

Attardi, G.; Parnas, H.; Attardi, B.: Pattern of RNA synthesis in duck erythrocytes in relationship to the stage of cell differentiation. Exp. Cell Res. **62**, 11-31 (1970)

Barrett, L.A.; Scheinberg, S.L.: The development of avian red cell shape. J. Exp. Zool. **182**, 1-13 (1972)

Barrett, L.A.; Dawson, R.B.: Avian erythrocyte development: Microtubules and the formation of the disk shape. Dev. Biol. **36**, 72-81 (1974)

Beatty, R.A.: The genetics of size and shape of spermatozoan organelles. In: Proc. Int. Symp. Genetics of the Spermatozon, Edinburgh 1971 (eds. Beatty, R.A.; Gluecksohn-Waelsch, S.), pp. 97-115. Edinburgh-New York: pub. 1972

Birchmeier, W.; Singer, S.J.: Muscle G actin is an inhibitor of ATP-induced erythrocyte ghost shape changes and endocytosis. Biochem. Biophys. Res. Comm. 77, 1354-1360 (1977)

Bruns, G.A.P.; Ingram, V.M.: The erythroid cells and hemoglobins of the chick embryo. Phil. Trans. Roy. Soc. London **266**, 225-305 (1973)

Derka, J.: Allotypes of inbred lines of chickens. In: Proc. Seventh Int. Symp. on Laboratory Animals, Hrubá Skála 1971 (eds. Karakoz, I.; Houška, F.), pp. 107-111. Prague: Czechoslovak Scientific-Technical Society, Agriculture Section 1971

Gahne, B.; Rendel, J.; Venge, O.: Inheritance of beta-globulins in serum and milk from cattle. Nature **186**, 907-908 (1960)

Gilbert, A.B.: Sex differences in the erythrocyte of the adult domestic fowl. Res. Vet. Sci. **6**, 114-116 (1965)

Hála, K.: The major histocompatibility system of the chicken. In: The major histocompatibility system in man and animals (ed. Götze, D.), pp. 291-312. Berlin-Heidelberg-New York: Springer 1977

Hála, K.; Hašek, M.; Hložánek, K.; Hort, J.; Knížetová, F.; Mervartová, H.: Syngeneic lines of chickens. 2. Inbreeding and selection within the M, W and I lines and crosses between the C, M and W lines. Folia biol. (Praha) **12**, 407-421 (1966)

Hartmanová, J.; Hála, K.: Development of immunity against antigens of the A and B blood groups during postembryogenesis in chickens. Folia biol. (Praha) **22**, 264-272 (1976)

Hašek, M.; Knížetová, F.; Mervartová, H.: Syngeneic lines of chickens. I. Inbreeding and selection by means of skin grafts and tests for erythrocyte antigens in C line chickens. Folia biol. (Praha) **12**, 335-353 (1966)

Jaffe, P.: Differences in numbers of erythrocytes between inbred lines of chickens. Nature **186**, 978-989 (1960)

Karakoz, I.; Miglová, M.; Blaszczyk, B.: Haematology of inbred chicken lines. Folia biol. (Praha) **23**, 419-420 (1977)

Lemež, L.: The blood of chick embryos. Quantitative enbryology at a cellular level. Adv. Morphogenesis 3, 197-245 (1964)

Lemež, L.: Quantitative Studie der fünf Typen der definitiven Erythrozyten (EII) beim Hühnerembryo. Verh. Anat. Ges. 71, 235-238 (1977)

Lucas, A.M.; Jamroz, C.: Atlas of avian hematology. Washington: Agric. Monograph 1961

Shohet, S.B.; Greenquist, A.C.: Possible roles for membrane protein phosphorylation in the control of erythrocyte shape. Blood Cells 3, 115-133 (1977)

Stratil, A.; Valenta, M.: Ontogenetic changes in the haemoglobins of geese, ducks, chickens and turkeys. Comp. Biochem. Physiol. 55B, 145-149 (1976)

Tanaka, T.; Rosenberg, M.M.: Relationship between hemoglobin levels in chickens and certain characters of economic importance. Poultry Sci. 33, 821-827 (1954)

Received December 1, 1978
Communicated by H. Abplanalp

Dr. K. Hála
Institute of Molecular Genetics
Czechoslovak Academy of Sciences
Prague (Czechoslovakia)