Hemoglobin Concentration in Normal Diploid and Intersex Triploid Chickens: Genetic Inactivation or Canalization?

Abstract. *Of 19 adult intersex chickens examined, 17 were triploid animals with ZZW sex chromosomes. Triploid erythrocytes were about 1.5 times the size of diploid erythrocytes, and were present in amounts that were about two-thirds the number of diploid erythrocytes. Both diploid and triploid animals had similar hematocrits and hemoglobin concentrations. Triploidy is reflected in a relative increase in RNA, as well as in DNA, per erythrocyte. This, in addition to the trisomic mode of inheritance observed in several genetically distinct loci determining isoantigens, indicates that all three sets of chromosomes in the triploid animals are genetically active. Apparently, there is a homeostatic mechanism maintaining the concentration of hemoglobin for the species in spite of triploidy and its effect on erythrocyte size.*

An intersex individual appears in approximately 0.05 percent of the females in commercial egg-producing flocks of hybrid chickens (*1*). Karyotype analysis of 19 of these intersex adults revealed that two were mosaic for male and female complements (2A-ZZ/2A-ZW; or 3A-ZZZ/2A-ZW), while the rest were homogeneous triploid animals with *ZZW* sex chromosomes. Thus, about 90 percent of these intersex adults were triploid animals (3A-ZZW). Triploid erythrocytes (RBC's) are about 1.5 times the size of diploid RBC's, and the concentration of hemoglobin in the blood was expected to show a corresponding difference. However, both triploid and diploid animals exhibited very similar hemoglobin concentrations. Such similarity in hemoglobin concentration can only be achieved if there is a mechanism that maintains a constant amount despite the obvious difference in RBC size between diploid and triploid animals.

Three groups of adult hybrid chickens (five males, five females, and five triploid intersexes), all about 8 months of age, were compared with regard to their RBC volume, number of RBC's per cubic millimeter of circulating blood, hematocrit, DNA content per cell, RNA content per cell, and hemoglobin concentration (Table 1).

Standard procedures were used in the determinations of RBC number and volume; the data for both were taken from ten blood samples collected from each individual. Hemoglobin was determined by the cyanemethemoglobin method (*2*), and total DNA and RNA were determined by the Schmidt-Thannhauser technique (*3*).

The mean RBC volume for triploid intersex animals is 146 μm^3, which differs significantly from the 104 μm^3 found in the diploid animals; there is no significant difference between the predicted value for the RBC volume in triploid animals ($1.5 \times 104 = 156$ μm^3) and the observed (146 μm^3) value. However, the RBC count in the blood of triploid animals (2.364×10^6 per cubic millimeter) is significantly smaller than that in the diploid animals (3.439×10^6 per cubic millmeter). Surprisingly, 3.439 is not significantly different from 1.5 multiples of 2.364. Therefore, RBC size in triploid animals is about 3/2 that of the diploid animals, and this ratio coincides with differences in their ploidy; however, the number of RBC's in triploid animals is only 2/3 of that of the diploids. Thus, the increase in volume in the triploid RBC's is accompanied by a reciprocal decrease in the number of RBC's (Fig. 1). As a result, the packed volume of RBC's and the concentration of hemoglobin in blood are expected to be about equal in both diploid and triploid animals. The available data support this expectation (Table 1 and Fig. 1).

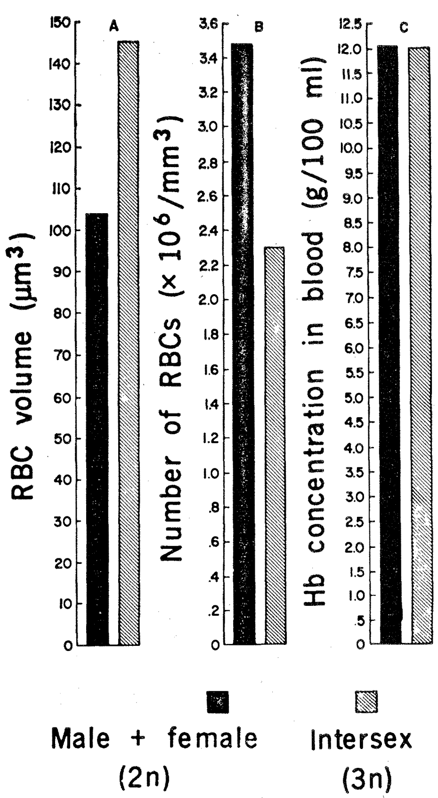

Fig. 1. Differences between mean RBC volume, mean RBC number, and mean hemoglobin concentration of triploid intersex chickens and combined male and female chickens; $2n$, diploid; $3n$, triploid.

A similar relationship exists in macrocytic anemia in man where the number of RBC's is much lower than normal, but the packed red cell volume and hemoglobin concentration are normal (*4*). In man, however, macrocytic anemia is either a megaloblastic one (due to deficiency of vitamin B_{12} or folic acid), or is caused by a variety of diseases. In this case, whole-body triploidy, known to be lethal in man (*5*), is definitely not involved. The megaloblastic macrocytic anemias result in certain disorders of hemopoiesis, including megaloblastic changes in bone marrow, but they usually respond to the administration of vitamin B_{12} and folic acid, which restore normal RBC size and number (*4*). It is unlikely that nutritional therapy would change the RBC number and size of healthy triploid chickens into that of normal diploid ones.

Human diploid-triploid mosaics have hemopoietic systems with diploid cells (*6*). Triploid cells exhibit severe mitotic disturbances in vitro, suggesting that a hemopoietic system with triploid cells cannot function normally and, in competition with diploid cells, the triploid line is gradually eliminated (*7*). However, there is no evidence that triploid cells are at a selective disadvantage in either the cultures from a mosaic human fetus (*8*), or in the hemopoietic systems of other mammalian and avian diploid-triploid mosaics (*9*). Furthermore, all homogeneous triploid chickens as well as the only two known cases of pure triploid fetuses that developed to term in man (*10*) had hemopoietic systems with triploid cells. Ohno *et al.* (*11*), who identified the first adult triploid chicken, demonstrated the possibility of a viable hemopoietic system in this chicken by counting the number of triploid bone marrow cells. These observations provide evidence that a triploid system may function normally.

The fact that both triploid and diploid chickens exhibit very similar hemoglobin concentrations suggests the possibility of either genetic inactivation of one set of chromosomes in the triploid cells, or the presence of a homeostatic system which maintains a metabolically desirable hemoglobin concentration in spite of the difference in RBC size.

Data on chemical extractions indicate that the triploid cells have 1.4 times the DNA content (in each RBC) of that in the diploid cells (Table 1). The same ratio of 1.4/1.0 was also observed in the cytophotometric determination of relative DNA content per nucleus (*12*). This ratio is fairly close to the expected ratio of 1.5, and the difference may be due to slight errors in the determination procedures. Triploid erythrocytes contain about twice the amount of RNA as do the diploid ones (Table 1). Examination of the genetic segregation for loci determining isoantigens show that three isoantigens, each determined by a separate isoallele, may be present on the erythrocytes of a single triploid intersex individual. This has been demonstrated for several genetically distinct loci that determine isoantigens (*13*). Such results indicate that all three sets of chromosomes in these triploid animals are equally active genetically. Furthermore, in a given tissue, the loci that are genetically active in protein synthesis are seemingly the same in both diploid and triploid cells. These two groups produce identical patterns of protein bands in polyacrylamide gel electrophoresis, suggesting that triploidy does not suppress certain genes that are normally active, nor does it activate other genes that are normally inactive in the diploid cells.

The lack of evidence for inactivation of one set of chromosomes in triploid intersex animals leaves developmental homeostasis as a possible mechanism to account for the similarity in hemoglobin concentration in diploid and triploid chickens. Developmental homeostasis, which Waddington (*14*) prefers to call canalization, leads to a standard phenotype in spite of genetic or environmental changes. This homeostasis does not imply a stationary state (*14*) but rather a dynamic stability that is brought about by changes in some lesser processes that result in the stability of a vital process, such as the constancy of blood composition in vertebrates (*15*). Therefore, in a vital trait as hemoglobin content of blood, an amount essential for normal metabolic activity has most likely been already achieved and stabilized in the diploid animals as a result of long-term natural selection. This concentration is also maintained in the triploid animals by a reduction in their RBC number to compensate for the increase in RBC size resulting from triploidy. In this case, the processes that control RBC number seem to change in order to maintain a constant concentration of hemoglobin for the species. Such a reduction in RBC number is probably mediated by erythropoietin, which induces erythropoiesis and controls hemoglobin synthesis (*16*). In higher plants, the most immediate and universal effect of polyploidy is an increase in cell size. This does not always increase the size of the plant as a whole, or even its individual organs, since a common effect of polyploidy is a reduction in the number of cell divisions which take place during development (*17*). A similar process may take place in triploid chickens, as all of them encountered so far possess a general body size that is intermediate between normal males and females (*1*). These triploid animals have a packed RBC volume that is similar to that in normal animals (Table 1), and, most likely, they possess comparable total blood volume and total RBC mass. The total mass of living material produced during development is apparently fixed by the genetic constitution of the species, and diploidy seems to be the most optimum degree of ploidy for gene expression in higher organisms.

Table 1. Differences between adult triploid intersex chickens and normal adult diploid chickens of both sexes (five triploid, five male, and five female chickens); $3n$, triploid; $2n$, diploid.

Parameter (mean)	$3n$	$2n$	$3n/2n$
RBC volume (μm^3)	146	104	3/2*
RBC count ($\times 10^6/mm^3$)	2.364	3.439	2/3*
RBC packed volume (hematocrit in %)	34.4	35.8	1.0†
Hemoglobin content (g/100 ml)	12.10	12.13	1.0†
DNA/cell ($\times 10^{-12}$ g)	1.65	1.20	1.4
RNA/cell ($\times 10^{-12}$ g)	0.29	0.14	2.1

* Based on the results of the statistical analysis and testing by the nonparametric Mann-Whitney test. † Based on the results of statistical analysis by the Student *t*-test for determining the difference between two means.

Changes in ploidy result in changes in gene dosage and, therefore, must have a definite effect on nucleocytoplasmic interactions. Detailed examination of nucleocytoplasmic relations in polyploid animals of several amphibian species reveals a normal course of morphogenesis during the development of triploid, tetraploid, and pentaploid embryos; thus, whole sets of genes may be multiplied in these amphibians up to the pentaploid level without disturbing the harmonious activity of the whole genotype as long as all genes are present in the same dosage (*18*).

Our study represents the only known case of a normally functioning homeostasic hemopoietic system in adult triploid higher vertebrates. Such homeostasis may be characteristic in other cases of naturally occurring polyploidy in lower vertebrates, such as those in fishes and amphibians.

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References and Notes

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