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Variation in Reduced Glutathione in Bloods of Inbred Lines of Chickens.* (22169)

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There can be no doubt that the reduced glutathione (GSH) levels of blood and other tissues are influenced by the adrenal, thyroid, pituitary, pancreatic, and parathyroid hormones(1). There is a paucity of data, however, which evaluate the extent that activities of endocrine glands are under hereditary control and, therefore, the extent that variations in genetically controlled activities of endocrine glands regulate chemical activities of the cells.

Proof of genetic control of vital biochemical reactions in living organisms has been demonstrated principally by results of studies conducted on monoploid microorganisms(2,3). In higher animals, such evidence has resulted from studies of physiological abnormalities, primarily in the human being, from studies of pigment development and body coloration, or the presence or absence of a chemical constituent such as atropinase or a β-globulin in the sera(4-8). Williams *et al.*(9) have suggested a quantitative individuality in metabolism of humans and rats on the basis of variation in patterns of excretion of metabolites in urine. Breed differences in plasma or serum alkaline phosphatase in chickens(10), in thyroxine secretion rate of chickens(11,12), and in total body glutathione concentration in 2- to 14-day-old chicks(13) lay a broad basis for the study of biochemical genetics in chickens, but the majority of such studies has not dealt with these biochemical factors as bases for biological individuality.

Genetically controlled individual quantitative differences in biochemical constituents should tend to become fixed by inbreeding and, as a result of this increase in intra-line genetic uniformity, differences between inbred lines become more easily demonstrable. With availability of inbred lines of chickens within a single breed (White Leghorn) in our

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laboratories, studies have been initiated on constancy of and genetic control of the concentration of GSH in mature chickens.

Methods and materials. Blood levels of reduced glutathione have been determined for mature male and female birds of 3 inbred lines, *viz.*, Lines 22, 23, and 24 of White Leghorns, maintained by the Poultry Husbandry Department, A. and M. College of Texas. These birds were hatched during 1952, 1953, and 1954 seasons. All these lines were inbred to about the same extent. The average inbreeding coefficient of birds used in these experiments was 0.53. Whole blood GSH was estimated by the method of Grunert and Phillips(14). The method was modified by increasing the volume of hemolyzing saponin solution to 4 ml for each ml of blood. Using potassium oxalate as anticoagulant, blood samples were collected by venepuncture and were placed immediately into ice bath at about 0°C. Duplicate analyses were made on samples within 8 hours after collection. Hemoglobin values were determined colorimetrically as oxyhemoglobin and red cell volumes were estimated after centrifugation of blood samples for 45 minutes at 1400 x *g* in Wintrobe tubes.

The data were statistically analyzed according to the methods outlined by Snedecor(15) to evaluate the significance of observed differences. Repeatability estimates were calculated as product-moment correlation coefficients between results of successive determinations. Coefficients of variation were calculated as standard deviation divided by the mean and reported as percentages.

Results. Estimates of repeatability. One group of 18 males of Line 22, housed on the range, had a mean GSH level of 48.8 ± 1.8 mg/100 ml blood. Analyses made on the same birds 5 weeks later showed a mean value of 51.8 ± 1.8 mg/100 ml. The correlation or estimates of repeatability between these successive determinations on the same birds was found to be 0.58 and is statistically significant ($p<0.05$). The coefficient of variation for the first set of determinations was 15.8% and 14.4% for the second. In a second group of males from the same line, the mean GSH levels observed from subsequent determinations on 40 birds were 50.9 ± 1.1 and 63.0 ± 1.4 mg/100 ml blood, respectively. Coefficient of variation was 13.6% in the first, and 14.4% in the second determination. Again the estimate of repeatability between these determinations was found to be 0.58, a correlation which was highly significant statistically ($p<0.01$). Just prior to the second determination, the 40 males described above were moved from one set of open-air quarters to another. In view of the fact that no dietary change was made, it might be possible that the increased level of GSH observed in the second determination could be due to environmental changes. Regardless of whether the difference between means was the result of environmental effects or a possible uncontrolled analytical error, the significant correlation between determinations was indicative of an individuality in the level of blood GSH within an inbred line.

TABLE I. Repeatability Estimates of Reduced Glutathione Levels in Blood of Lines 22, 23, 24, and 22 × 24 Females. Ten birds in each series.

Line	Date	Avg glutathione, mg/100 ml	Estimate of repeatability*	Coeff. of variation, %
22	3/17/55	33.4 ± 1.6†		14.82
	26	39.5 ± 2.1	.90	16.63
23	17	21.7 ± 1.0		15.18
	26	25.7 ± 1.1	.78	13.90
24	17	26.9 ± 1.6		18.83
	26	33.0 ± 1.8	.88	16.89
22 × 24	18	29.0 ± 1.1		11.79
	29	34.9 ± 1.9	.79	16.88

* Significant at .01 level of probability.
† Stand. error of the mean.

Subsequent GSH determinations made on 10 mature females from each of inbred White Leghorn Lines 22, 23, and 24 were used to calculate estimates of repeatability of GSH measurement in the inbred female. In addition, successive determinations were made on 10 females produced from a cross of Line 22 males on Line 24 females. All of these birds were individually caged. Repeatability estimates for all these lines were highly significant (Table I) and ranged from 0.78 to 0.90. Again an increase of 18 to 23% in the aver-

TABLE II. Average Blood Levels of Glutathione of Mature Males from Inbred White Leghorn Lines 22, 23, and 24.

Line	Avg glutathione, mg/100 ml blood				No. of birds
	1952	1953	1954	3 yr avg	
22	51.7 ± 1.1	54.4 ± .9	51.5 ± 2.8	53.3 ± .7	85
23*	44.4 ± 1.6	41.2 ± 1.9	43.3 ± 2.0	43.0 ± 1.1	18
24	56.7 ± 1.1	50.3 ± 1.3	53.8 ± 2.0	52.2 ± 1.1	59

* Highly significantly lower ($p < 0.01$) than other two lines.

age GSH level in the second determination was noted. When the second GSH analyses were made, the environmental temperature was much lower than temperatures existing during first experimental period and possibly may account for the differences. While environmental effects could have affected the level of GSH, the rank order of levels in females as with the results with males, described above, was not appreciably altered.

Genetic Control. Levels of GSH were determined in 162 mature males of the 3 inbred White Leghorn Lines 22, 23, and 24. These birds ranged from about 10 to approximately 30 months of age when the determinations were made. The average levels, expressed in mg of glutathione/100 ml blood, are presented in Table II. These data show that average GSH levels of males from Line 23 were consistently lower over the 3-year period than levels of Lines 22 and 24. The hereditary control of the blood GSH level thus becomes apparent. The data were statistically analyzed for effects of age, inbred line, and dams and sires. Contrary to findings with cattle(16, 17), age was not a significant factor in determining the level of GSH in blood of these birds. The analysis did demonstrate, however, that the lower levels of GSH in birds of Line 23 were highly meaningful statistically ($p<0.01$).

The average GSH levels determined for 18 females from each inbred Line 22, 23, and 24, and 22 females from Line 22 x 24 crosses were 32.0 ± 1.2, 22.5 ± 0.8, 27.3 ± 1.3, and 28.7 ± 0.9 mg/100 ml blood, respectively. The differences between the 3 inbred lines were highly significant statistically. The significant line differences with both males and females provide strong evidence for the genetic control of quantitative differences in blood GSH. It is to be noted also that the levels in females were considerably lower than in males. The glutathione level observed for Line 22 x 24 females fell between the levels of parental lines. Thus, there was no evidence of a heterotic effect on GSH level. Unfortunately, representatives from other crosses between these lines were not available.

Effect of Erythrocyte Concentration. The erythrocyte is the primary source of GSH found in blood, and therefore, variation in number of red cells could account for part of the differences observed in GSH levels. In 52 males from Line 22 with a mean GSH level of 51.2 ± 0.9 mg %, the average hemoglobin content was 12.14 ± 0.23 g/100 ml blood. The correlation of 0.62 between concentrations of these 2 substances was highly significant statistically ($p<0.01$). Yet, in spite of the fact that the erythrocyte is the primary source of GSH in blood, differences in red cell number (indicated by hemoglobin content) accounted for only about 38% of the variation observed in the level of GSH of these males.

GSH levels, hemoglobin content, and red cell volumes were determined for a group of males and females from each inbred line. It is to be noted from the data (Table III) that GSH content of female bloods averaged from 26 to 34% lower than bloods from males of the same lines. However, the hemoglobin content was from 33 to 38% lower and the red cell volume was 32 to 37% lower in females. The major portion of the sex difference in GSH levels within a line was primarily the result of variation in number of erythrocytes as indicated by relative levels of hemoglobin and red cell volume. The decrease in total GSH due to reduction in volume of red cells in the female is partially offset by a 5 to 10% increase in GSH per volume of red cells. The average hemoglobin values and

TABLE III. Average Glutathione Levels, Hemoglobin Values, and Red Cell Volumes of Mature Males and Females from Inbred Lines 22, 23, and 24.

Line	Sex	Glutathione, mg/100 ml	Hemoglobin, g/100 ml	Red cell vol, %	Glutathione-hemoglobin ratio, mg/g	Glutathione conc. in red cells, mg/ml
22	♂	53.5 ± 1.6 (9)*	12.6 ± .5	41.9 ± 1.0	4.25	1.28
	♀	39.5 ± 2.1 (10)	8.4 ± .3	28.5 ± 1.0	4.70	1.39
23	♂	37.9 ± 1.0 (10)	12.3 ± .3	41.1 ± 1.1	3.08	.92
	♀	25.2 ± 1.5 (7)	7.9 ± .4	26.0 ± 1.1	3.19	.97
24	♂	48.8 ± 2.1 (9)	12.7 ± .4	42.3 ± 1.1	3.85	1.15
	♀	34.8 ± 1.1 (8)	7.9 ± .2	27.3 ± .8	4.42	1.27

* The number enclosed in parenthesis indicates the number of birds with all 3 values.

red cell volumes of the 3 inbred lines within sexes are essentially the same. Therefore, the observed line differences in GSH levels are clearly due primarily to different concentrations of GSH within red cells. It may be concluded that while environment and experimental treatment could have some effect, concentration of reduced glutathione within the erythrocyte is conditioned to a large extent by heredity.

Summary. 1. Significant correlations were obtained between successive reduced glutathione (GSH) determinations on the same birds. This was true in both males and females and indicated a high degree of constancy for the GSH level in individual chickens. 2. The blood level of GSH was determined in 162 mature males of 3 age groups from 3 inbred lines of White Leghorns. Age was not a significant factor, but one line had a highly significantly lower GSH level than the other 2 lines. Differences between the GSH levels in females from all 3 inbred lines were highly significant statistically. 3. Females of the 3 inbred lines had much lower GSH levels than the males. However, hemoglobin content and red cell volume of females were also much lower. Since the source of GSH is almost wholly the red cells, the difference in GSH level between males and females within a line could be attributed to relative numbers of red cells present in the sexes. Line differences in males and females appear to result from different concentrations of GSH within the red cell. The data provide evidence that the GSH concentration within the erythrocytes of mature chickens is to a very large extent genetically controlled.

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