Erythropoietic Response in Chickens with Inherited Muscular Dystrophy.* (31343)

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Studies on the genetically dystrophic chicken led us to determine whether the erythropoietic response of dystrophic chickens is normal. Reticulocyte numbers were determined in birds of different ages. The reticulocyte response to blood loss and to reduced oxygen pressure was measured in dystrophic chickens from a strain of New Hampshires with inherited muscular dystrophy(1) and in normal birds.

Methods. Experiment 1. Blood samples were taken from dystrophic chickens varying in age from 9 days to 18 months. The blood was stained with brilliant cresyl blue and smears of the stained preparation counterstained with Wright's stain. The procedure is outlined elsewhere(2). The slides were examined microscopically and the number of reticulocytes (cells showing any reticular material) was determined on the basis of a counted thousand red blood cells.

Experiment 2. Four normal and 4 dystrophic New Hampshire male birds approximately 5 months old were used in the experiment. The birds were taken from floor pens and transferred to cages. Reticulocyte counts were made at the time of transfer. After the birds had been caged for 2 days, 50 ml of blood were drawn from each bird by cardiac puncture. Reticulocyte counts were made again at this time and at intervals thereafter until 23 days had elapsed after blood withdrawal. Total blood cell counts were also determined periodically.

Experiment 3. After reticulocyte counts had returned to normal in the above experiment, the birds were used in a further experiment to study the reticulocyte response to hypoxia. The birds were placed in a cage unit entirely encased in transparent plastic. The air within the plastic "tent" was gradually replaced with a mixture of 12% oxygen and 88% nitrogen. The birds were maintained in this atmosphere with continuous ventilation for 23 hours. During this time the birds had free access to feed and water.

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TABLE I. Reticulocyte Counts in Chickens of a Dystrophic Strain of New Hampshires.

Age of birds	Sex	No. of birds examined	Avg reticulocyte count, %	Range
9 days	Unknown	2	19.6	18.4–20.8
2 mo	"	3	15.7	11.9–21.4
3 "	♂	2	11.1	10.9–11.3
3 "	♀	2	11.9	10.7–13.2
5– 6 "	♀	5	4.6	3.1– 6.0
8–10 "	♂	6	5.2	4.5– 5.9
Ca. 18 mo	♂	25	4.3	2.1–11.9
Idem	♀	8	3.5	2.7– 4.6

Reticulocyte and total red cell counts were made on the day following restoration of normal atmosphere and at intervals thereafter until 25 days had elapsed.

Results and discussion. The reticulocyte counts in dystrophic chickens of different ages are given in Table 1. The counts show a similar decline with increasing age to maturity to that reported for normal chickens (2). The values found in the mature birds of the dystrophic strain of New Hampshires were lower than those observed in mature birds of any of the breeds which had been studied previously. It should be noted, however, that mature birds of a normal strain of New Hampshires had reticulocyte counts ranging from 5.4 to 14.1 with an average count of 7.9%. This average value is also lower than that found for birds of the other breeds tested. The low reticulocyte count in the dystrophic New Hampshire birds may accordingly be a strain variation of a normal breed characteristic and be physiologically unrelated to the condition of muscular dystrophy.

Reticulocytosis is a consistent response of normal animals to severe loss of blood and to reduced atmospheric pressure. There seems, however, to be little known with regard to the reticulocyte response of the chicken in

TABLE II. Reticulocytosis in Normal and Dystrophic Chickens Following Blood Loss.

Normal bird No.	1		2		3		4	
	Retic, %	RBC, millions/mm³	Retic, %	RBC, millions/mm³	Retic, %	RBC, millions/mm³	Retic, %	RBC, millions/mm³
Original count	6.7		7.0		8.5		7.0	
Caged for 2 days	8.9		9.7		8.9		9.2	
Days after blood loss:								
1	7.9		10.4		6.5		10.0	
2	12.5		14.5		9.8		7.2	
4	25.0		21.6		15.4		16.0	
5		2.39		2.67		2.45		2.39
9	22.9	2.98	25.0	2.89	23.9	2.95	19.2	2.87
16	6.9	3.22	9.4	3.27	7.4	3.37	11.6	3.00
23	7.0	3.37	10.0	3.25	7.6	3.50	9.3	3.32
Dystrophic bird No.	5		6		7		8	
Original count	11.9		5.0		6.8		4.6	
Caged for 2 days	11.0		6.2		7.9		5.8	
Days after blood loss:								
1	22.9		14.5		10.0		14.1	
2	27.5		22.5		23.1		26.3	
4	30.6		26.5		13.4		35.9	
5		2.78		2.77		2.65		2.57
9	25.8	2.85	24.1	2.88	24.3	2.85	31.8	2.65
16	11.5	2.88	10.3	2.89	9.0	3.27	11.3	2.95
23	8.0	3.59	5.8	2.93	6.5	3.57	4.4	3.12

this regard, although it has been stated(3) that the haematopoietic system is more reactive in chickens than in mammals. In Exp. 2 the amount of blood withdrawn represented approximately one-sixth of the total volume. The normal birds weighed an average of 3.1 kg and the dystrophic birds 3.3 kg. The amount of blood withdrawn was not altered in proportion to the body weight because of the differences in the proportions of different tissues between the normal and the dystrophic birds. The reticulocyte and erythrocyte counts determined during the experiment are given in Table II. The increase in reticulocyte counts occurred more rapidly in the dystrophic than in the normal birds. Blood samples taken on the second day after the birds had been bled showed that over 20% of the red blood cells from the dystrophic birds were reticulated. In 2 of the dystrophic birds the level increased to over 30% during the subsequent 2 days. By the 9th day the number of reticulocytes was declining in the dystrophic birds, whereas it was at its highest level in 3 of the 4 normal birds. Thereafter, the reticulocyte count approached the original level by the 16th day in the normal birds and by the 23rd day in the dystrophic birds. No appreciable differences were found in the erythrocyte counts of the birds of the two strains.

In the third experiment subjection of the birds to reduced atmospheric oxygen pressure for a period of 23 hours did not impose so great a stimulus to erythropoiesis as did blood loss in the previous experiment. The reticulocyte and total erythrocyte counts of the birds are shown in Table III. The only bird in which the reticulocyte level increased above 20% was dystrophic bird no. 8. This was the only bird which showed acute discomfort in the artificial atmosphere. It had also demonstrated the greatest reticulocyte response after blood loss. The degree of response of the other dystrophic birds was similar to that of the normal birds except that the maximum reticulocyte count tended to occur earlier in the case of the dystrophic birds. An interesting difference was found between the dystrophic and the normal birds in the total number

TABLE III. Reticulocytosis in Normal and Dystrophic Chickens Following Hypoxia.

Normal bird No.	1		2		3		4	
	Retic, %	RBC, millions/mm³	Retic, %	RBC, millions/mm³	Retic, %	RBC, millions/mm³	Retic, %	RBC, millions/mm³
Days after hypoxia:								
1	9.6	3.25	9.9	3.10	10.6	3.53	12.6	3.11
3	11.7	3.30	10.7	3.45	10.4	3.45	12.0	3.59
5	11.8	3.59	9.2	3.42	11.3	3.42	12.6	3.51
8	12.2	3.20	11.3	3.20	13.8	3.21	16.6	3.39
11	12.9		15.1		9.9		14.0	
18	13.7		10.4		8.5		13.0	
25	8.1	3.62	9.2	3.30	7.2	3.42	8.8	3.62
Dystrophic bird No.	5		6		7		8	
Days after hypoxia:								
1	11.5	3.27	8.5	2.88	13.3	3.53	8.6	3.13
3	14.6	3.56	8.9	3.62	13.1	3.92	11.7	3.40
5	17.5	3.72	10.4	3.99	13.5	4.48	17.4	2.27
8	12.8	3.46	11.7	3.48	7.2	4.11	26.4	3.05
11	14.1		10.2		9.9		16.9	
18	11.9		10.9		8.3		8.4	
25	7.3	3.15	9.6	2.89	6.8	3.16	5.0	3.60

of red blood cells present in the blood subsequent to the experimental treatment. On the 5th day after return of the birds to normal atmosphere the counts in the dystrophic birds (with the exception of no. 8 which showed erratic fluctuations) had markedly increased and by the 25th day had decreased to or below the original level. A similar sequence of changes was not evident in the erythrocyte counts of the normal birds.

The data of these experiments are interpreted as indicating that the erythropoietic response is not defective in genetically dystrophic chickens despite the low level of reticulocytes normally found in these birds. The greater-than-normal reticulocyte response to blood loss observed in the dystrophic birds is similar to the finding(4) that rats with a decreased rate of erythropoiesis as a result of hypophysectomy, high oxygen pressure, starvation- or transfusion-produced polycythemia, exhibited an exaggerated rate of response to anemic plasma. In the present instance the level of erythropoietin in the dystrophic birds may be assumed to have been low under normal conditions. Both erythropoietin production and the subsequent reticulocytosis were, however, greater than normal in response to appropriate stimulation. The two methods of stimulation employed here induced somewhat different types of response. In the case of blood loss the response was associated with filling a depleted tissue compartment and in the case of hypoxia the response involved increasing the numbers of erythrocytes in order to increase the capacity of the blood for oxygen transport. In both cases, however, the stimulus to the erythropoietic system may be assumed to have resulted from a deficiency in the amount of oxygen supplied to the tissues. The more rapid reticulocyte response following blood loss and the greater elevation in erythrocyte count in response to short-term hypoxia in the dystrophic chickens could be indicative of greater oxygen requirements in these birds, or of greater sensitivity to surges in erythropoietin production. The latter is the more probable explanation since the erythrocyte counts in the dystrophic birds are not higher than normal.

Summary. The reticulocyte counts in birds of a genetically dystrophic strain of New Hampshire chickens are lower than in normal birds of other strains which have been examined. The reticulocyte response to blood loss was more marked in dystrophic than in normal birds. In response to short-term hypoxia the erythrocyte counts were elevated to a greater extent in dystrophic birds than in

normal birds. The results indicate that there is no defect in the erythropoietic response in birds with genetic muscular dystrophy.

1. Asmundson, V. S., Julian, L. M., J. Hered., 1956, v47, 248.
2. Coates, V., March, B. E., Poultry Sci., in press.
3. Lucas, A. M., Jamroz, C., Atlas of Avian Hematology, Agriculture Monograph 25, U.S.D.A., Washington, D. C., 1961, p29.
4. Fried, W., Plzak, L. F., Jacobson, L. O., Goldwasser, E., Proc. Soc. Exp. Biol. and Med., 1957, v94, 237.

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Urinary Protein and Carbohydrate II. Fractionation of Nondialyzable Ammonium Sulfate Precipitable Glycoproteins in Normal Human Urine. (31344)

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Human urine contains a group of carbohydrate-protein complexes (CPC). Their molecular weights vary from 5,000(1) to several million(2). Some of these CPC are dialyzable(3) through cellophane membrane; some are nondialyzable but ultrafiltrable(1,2-6) and others are nondialyzable and not ultrafiltrable(2,7). The Tamm and Horsfall (T & H) mucoid is the only one which has been isolated in pure form and in substantial amount(2,7). Berggard(1) employed dialyzing membranes of various pore sizes to separate the urinary glycoproteins into 3 fractions of different molecular sizes. Using 'pevikon' block electrophoresis, he further demonstrated the multicomponent nature of each of the 3 fractions of the urinary glycoproteins. Recently, he(8) has reported the isolation of a sialic acid-rich glycoprotein fraction from his fraction B (molecular weight between 10,000 and 40,000). For this purpose, he used zone electrophoresis at pH 8.6 to separate the fast-moving albumin fraction from other CPC. The eluate of this portion of the albumin zone was concentrated and subjected to zone electrophoresis at pH 4.5. Under such treatment, the albumin migrated toward the cathode, and the sialic acid-rich fraction and AMP toward the anode. During the process the latter two substances were almost completely separated, one from the other.

By employing ultrafiltration, 'pevikon' zone electrophoresis and gel filtration, Lundblad isolated nonultrafiltrable(9) and ultrafiltrable(10), nondialyzable fucose-rich glycopeptides from normal human urine. Dische *et al*(4) have precipitated the nondialyzable ultrafiltrable glycan of human urine with increasing concentrations of isopropanol and isopropanol-ether, followed by further separation with continuous flow electrophoresis on glass fiber filter paper. At least 40 glycoprotein fractions were thus identified. Hakomori *et al*(11,12) isolated low molecular weight glycoproteins by treating the supernate from 85-90% ETOH precipitation of concentrated urine with lead acetate and Dowex 1-X2 chromatography and gel filtration. Bourrillon *et at*(13) studied the glycoproteins of urine which were soluble in 50% ETOH. Employing the DEAE-cellulose column technic, they were able to separate at least 4 peaks. The early fractions were rich in neutral sugars and the later peaks were rich in sialic acid. The same authors (14) also fractionated the urinary glycoproteins which had been precipitated by the use of 50% ETOH. Forty peaks were separated from the 50% ETOH precipitate fraction. Recently we(15) reported a simple method for fractionation of the nondialyzable urinary CPC. The method was mild and all urinary constituents retained their natural form. The CPC-II fraction in this procedure contains all urinary glycoproteins except