An Inherited Glutathione Deficiency and a Concomitant Reduction in Potassium Concentration in Sheep Red Cells

SMITH and OSBURN[1] found that in a flock of 104 sheep the reduced glutathione (GSH) levels in the red cells of 3 individuals were less than 20% of the mean of the rest. They suggested that this deficiency might be genetically controlled. This paper presents the results of a study of the blood GSH levels in 6 different breeds of sheep and confirms the existence of low GSH concentrations in the red cells of some individuals. Evidence is presented that the deficiency is inherited and also that sheep homozygous for the gene responsible have lower than normal red cell potassium concentrations.

Blood samples were collected into heparin by jugular venepuncture from a total of 427 sheep. They were always tested within 24 h of collection and in most cases within 6 h. Whole-blood GSH was measured (in a Unicam SP500 spectrophotometer) using the DTNB (5, 5[1]-dithiobis-(2-nitro benzoic acid)) method[2]. The concentration of GSH per 100 ml packed red cells was calculated from the whole-blood haematocrit values.

Table I shows the GSH concentrations in the 6 different breeds of sheep. In the Finnish Landrace breed, a distinctly bi-modal distribution of GSH values was found. Those sheep with GSH values below 55 mg/100 ml red cells were classified as GSH low type, and those above this value as GSH high type. 25% of the Finnish Landrace sheep were of GSH low type. One individual Soay and 2 Merino cross sheep were also GSH low type, otherwise the sheep of the other breeds were GSH high types. The mean GSH value for high type Finnish Landrace sheep was higher than that in the other breeds.

Table II shows the breeding data for the Finnish Landrace sheep. The results are in accord with the hypothesis that the GSH levels are controlled by a single pair of autosomal alleles, the gene for high GSH (GSH^H) being dominant to that for low (GSH^h). Repeated measurements from the same individuals showed that, in healthy adult sheep, the GSH values (whether high or low) did not change appreciably over the course of 12 months.

When the red cells of the Finnish Landrace sheep were typed for potassium (LK or HK type[3]) it was found that GSH^l type sheep had significantly lower mean red cell potassium values than did GSH^H type sheep (Table III). The same GSH/potassium relationship was apparent whether the potassium concentrations were related to packed cell volumes or to the haemoglobin concentration of the sample. Preliminary studies also indicate that the red cell sodium levels are lower than normal in the GSH^l type sheep and this raises the possibility that the high potassium GSH^l type sheep are the same as the $A1$ potassium type sheep previously described[4]. This is being investigated.

A deficiency of red cell GSH has been found in man[5,6] and this also is inherited as an autosomal recessive disorder[5]. However, unlike the situation in man, this defect

Table I. Reduced glutathione (GSH) in the red cells of different breeds of sheep

Breed	High type No. of sheep	Mean GSH ± S.D. (S.E.) mg per 100 ml red cells	Low type No. of sheep	Mean GSH ± S.D. (S.E.) mg per 100 ml red cells
Finnish Landrace	109	96.65 ± 21.87 (2.09)	31	31.07 ± 9.47 (1.70)
Clun Forest	129	89.85 ± 12.54 (1.10)	0	–
Soay	100	85.82 ± 10.68 (1.06)	1	48.17
Merino Cross	30	89.25 ± 14.95 (2.72)	2	19.46
Welsh Mountain	18	83.82 ± 16.07 (3.78)	0	–
Shetland	7	89.01 ± 15.19 (5.74)	0	–

Table II. Inheritance data for reduced glutathione (GSH) types in Finnish Landrace sheep

GSH type of parents		No. and type of offspring		Totals
Ram	Ewe	High	Low	
High(7)	High(16)	45	8	53
Low(4)	High(11)	16	2	18
High(4)	Low(9)	9	14	23
Low(2)	Low(9)	0	18	18
Presumed genotype				
Hh	*Hh*	25	8	33
Hh	*hh*	9	14	23
hh	*Hh*	1	2	3

Figures in parenthesis are the numbers of different individuals involved in the matings. The *Hh* genotypes were presumed on the basis that a GSH high type sheep was heterozygous if it had either a GSH low type parent or offspring.

Table III. Red cell potassium concentrations in GSH high and GSH low type sheep

GSH type	High potassium type No. tested	K^+ mM/l (mean ± S.E.)	Low potassium type No. tested	K^+ mM/l (mean ± S.E.)
High	31	86.38 ± 1.28	18	31.55 ± 1.24
Low	22	72.09 ± 1.49	14	16.38 ± 0.95

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does not appear to have any obvious deleterious effect on sheep, although experiments in progress indicate that GSH^l type sheep are more prone to kale anaemia than are GSH^H type individuals.

Resumen. Se medió glutation reducido (GSH) en glóbulos rojos de 427 ovejas pertenecientes a 6 razas diferentes. 25% de las ovejas de raza «Finnish Landrace» dieron un contenido de GSH de un tercio del valor obtenido en el resto. Datos familiares indican que esta deficiencia es heredada como un factor autosómico recesivo. En las ovejas que tienen esta deficiencia, la concentración media de potasio en glóbulos rojos fué significativamente menor que la observada en las ovejas con niveles normales de GSH.

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Interspecific Variability of DNA Content in Amphibia[1]

Gene and chromosome duplications are important mechanisms in the evolution of pluricellular organisms. The duplications can be accomplished by several means, such as unequal crossing-over between homologous chromosomes during meiosis, unequal exchange of chromatids during mitosis, redundant duplication of DNA in certain regions of the chromosomes, and polyploidy or polyteny.

Higher vertebrates seem to be remarkably uniform in DNA content. Indeed, the class Mammalia as a whole, the class Aves, and the order Squamata exhibit almost unvarying DNA content values[2–5].

However, among plants and lower animals, several cases of DNA increasing series have been demonstrated. Indeed, the anemone plants of the family Ranunculaceae[6], the grasshoppers of the family Acrididae[7], the teleost fish of the orders Percomorphi and Heterosomata[8], and the frogs of the family Ranidae[9] have stable karyotypes but show increased DNA content values. Proportional increase of DNA was also demonstrated in the polyploid amphibians of the family Ceratophrydidae[10–12]. Attempts to correlate DNA content of vertebrates, especially amphibians, with evolution patterns and ecology have recently been made[13,14].

The present paper deals with DNA content values of 30 species of Salientia, 2 species of Caudata, and 1 species of Gymnophiona.

Material and methods. Smears of air-dried blood were fixed in neutral 50% formol for 1 min, washed in tap and distilled water, hydrolysed in *N* HCl at 60 °C, and stained in Feulgen. 20 blood smears of a *Bufo paracnemis* LUTZ were prepared, air dried and stored. These slides were used as controls for every batch of specimens to be stained. The DNA value of each species was always determined in relation to that of *B. paracnemis*.

The DNA content was determined[15,16] with the histophotometer of VIALLI and PERUGINI[17] (Fratelli Koristka, Italy). The nuclei were enlarged to 1500 diameters, the area of the plug being more or less 20% the optical area of the nucleus. Generally, the mean of 3 plugs through the nuclear area was used but in some cases 4 to 6 measurements were made of material with irregular shaped chromophore masses. For each nucleus, a blank measurement was made and the galvanometer adjusted to the value of 100. Each absorption reading of the plus was given in % of the standard light of the blank. A stabilized lamp with an interference filter of 5400 Å was used.

Through the average determination of absorption for each nucleus, the DNA content was calculated according to the formula $DNA = D^2 (2 - \log In)$. D^2 = optical area of the nucleus; In = value of the absorption, and $2 = \log 100$. For each specimen 25 measurements were obtained and the mean (X), the standard deviation (S), and the standard error of the mean (SE) calculated. The overlapping of segments to 2 SE indicates roughly the significance of the differences between the two mean values at the level of $P \leqq 0.05$[18] (Figure).

In the Table and in the Figure the DNA values were adjusted for the Feulgen baths, and also expressed as % in proportion to mammal DNA measured on lymphocytes. The blood was collected in heparinized capillary tubes and centrifuged. The tubes were sectioned at the leucocytes strate level, smeared on slides, dried, fixed and Feulgen stained. Bird lymphocytes were also measured and compared with erythrocytes in the same smear.

In order to compare amphibian DNA with mammal DNA content, we adjusted mammal lymphocyte DNA values using a ratio between lymphocyte DNA and erythrocyte DNA, determined in the quail.

In the species where more than one specimen was analyzed, the arithmetic mean, the S, and the SE are calculated from the data of all the specimens together.

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