these the presence of many heterochromatic bodies in interphase nuclei made sex chromatin diagnosis impossible. This same difficulty was encountered in some rat tissues that contained numerous chromocentres[14].

The current concept that sex chromatin represents the inactive X in female interphase nuclei does not explain the presence of a sex chromatin body in any male cells. It is known to occur at a low but variable frequency in apparently normal human males[12].

OHNO et al.[4] interpret the chromatin mass in the male Virginian opossum as representing the Y chromosome. The size difference between male and female chromatin masses is attributed to the length of the X and Y chromosomes, the X being twice as long as the Y.

In *P. nasuta* there is the same size relation between the X and Y chromosomes[7], but not, as mentioned earlier between the male and female sex chromatin masses. If one accepts the interpretation that the male sex chromatin in the marsupials studied so far is the Y chromosome, then in *P. nasuta* the extent of heterochromatization of the X and Y chromosomes in interphase nuclei is equivalent.

In conclusion since both male and female corneal epithelial cells of *P. nasuta* contain sex chromatin, this tissue cannot be used for nuclear sexing in this animal. In addition due to its sex chromosome anomaly any general conclusions regarding the sex chromatin situation in other Australian marsupials cannot be inferred from this study[15].

Résumé. La chromatine sexuelle des cellules de l'epithelium corneal de mâles et femelles adultes du Marsupial australien *Parmeles nasuta* Geoffroy a été étudiée. Les cellules des mâles comme celles des femelles manifestent l'existence de la chromatine sexuelle dans le noyau interphasique. Il n'y a pas de différence de taille entre chromatine sexuelle mâle et femelle.

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A γ-Globin-Chain Mutation in the Hemoglobin 1 (Hb1) of the Domestic Chicken

In a previous work[1] we have shown, in agreement with other authors[2,3], that chicken hemoglobin (Hb) is not homogeneous, but is composed of 2 fractions, easily detectable by electrophoretic or chromatographic techniques. We have called Hb1 the minor fraction, and Hb2 the major one. The 2 hemoglobins seemed to have one globin chain in common, α, and 2 different counterpart globin chains, β, and γ: thus the molecular 'formula' of Hb1 is $\alpha_2\gamma_2$, and that of Hb2 is $\alpha_2\beta_2$.

We have recently described the occasional finding of a 'mutant' Hb in the domestic chicken (breed Arbor Acres and Vantress)[4]. By routine starch gel analysis, a certain number of chickens were found to have 3 Hb fractions instead of 2. Crossing experiment revealed that this pattern corresponds to a heterozygous condition; in fact we could obtain the segregation of 2 homozygous groups of chickens, one group with the 'normal' Hb pattern, and the other with the 'mutant' Hb fraction. The present study was undertaken for the purpose of identifying the mutation responsible for the appearance of the new hemoglobin type.

Materials and methods. The heterozygous cocks and hens were isolated and their eggs collected and incubated. The homozygous 'normal' and 'mutant' chickens were identified by analysing the Hb pattern of the hatched chickens[6]. The analysis was carried out by starch gel electrophoresis at pH 8.6, as previously reported[1].

The purification of the Hb types present in 'normal' and in 'mutant' chickens, was achieved by chromatography on Amberlite CG-50 columns[1]. The globin chain types present in the total lysate, and in purified Hb fractions, were studied by starch gel electrophoresis in 6 M urea at pH 3.6 as previously reported[1]. A new buffer system was devised for a better characterization of the 2 γ globin chains. Its composition was the following: 0.2 M succinic acid brought with solid *Tris* to pH 5.4, diluted 50 times for the gels and 6 times for the buffer reservoirs.

Results and discussion. The electrophoretic pattern of the hemoglobins under investigation is presented in Figure 1. The migration of Hb2 is the same in the 3 conditions considered, while the electrophoretic mobility of the Hb1 fractions differ in 'normal' and in 'mutant' homozygotes. In the 'heterozygous' condition, both Hb1 types are present; in this last case, the relative distribution of the Hb fractions, as determined by analysis of the starch gel with a Chromoscan, is Hb1 'mutant' = 25%, Hb1 'normal' = 25%, Hb2 = 50%.

The hemoglobins from 'normal' and 'mutant' chickens have been purified on Amberlite CG-50 columns. As previously reported[1], this resin allows the separation of Hb1 from Hb2; moreover Hb1 can be separated into 2 fractions, which we have called Hb1 and Hb1a: despite their different chromatographic behaviour, the 2 fractions have been shown to have the same electrophoretic mobility

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[6] The crossing experiments, using heterozygous cocks and hens, gave a total of 314 hatched chickens, distributed as follows: Homozygous 'normal' = 100, heterozygous = 155, and homozygous 'mutant' = 59. No explanation can be given up to now, for the unexpectedly low number of the homozygous 'mutant' chickens, as the post-hatching development of the 'mutants' seems to be normal.

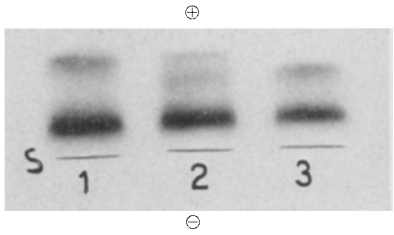

Fig. 1. Starch gel electrophoresis in *Tris*-Borate system at pH 8.6[5], of the hemoglobins under investigation. From left to right: 1, hemoglobins from 'mutant' chickens; 2, hemoglobins from 'heterozygous' chickens; 3, hemoglobins from 'normal' chickens. Staining by benzidine.

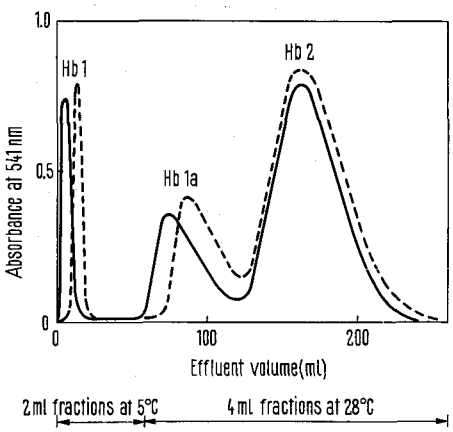

Fig. 2. Elution pattern from Amberlite CG-50 type II columns of 'normal' and 'mutant' hemoglobins. The results obtained in the 2 groups are reported on the same figure for comparison. Cyano Hb was used. The Amberlite column was equilibrated with a buffer of Na_2HPO_4 (molarities: $Na^+ = 0.093$, $KCN = 0.005$) at pH 7.45. The flow-rate was 2 ml/h in the cold, and 4 ml/h at 28 °C. Fractions were collected and analysed at 541 nm. (----), hemoglobins from 'normal' chickens; (——), hemoglobins from 'mutant' chickens.

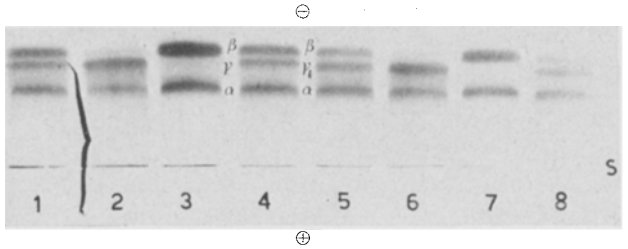

Fig. 3. Starch gel electrophoresis in 6 *M* urea at pH 3.6 of the globin chains present in purified hemoglobin fractions. From left to right: 1, hemoglobins from 'normal' chickens; 2, Hb 1 from 'normal' chickens; 3, Hb 2 from 'normal' chickens; 4, hemoglobins from 'normal' chickens; 5, hemoglobins from 'mutant' chickens; 6, Hb 1 from 'mutant' chickens; 7, Hb 2 from 'mutant' chickens; 8, hemoglobins from 'mutant' chickens. The conditions for the electrophoretic run have been reported previously[1]. Staining by Amido Black 10 B.

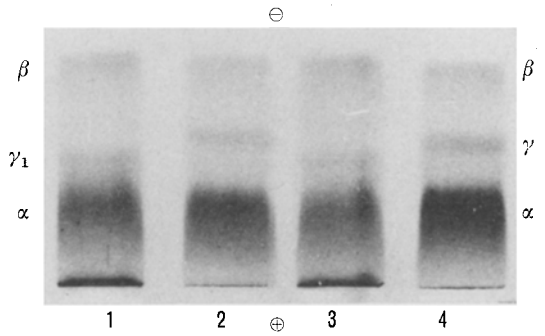

Fig. 4. Starch gel electrophoresis in 6 *M* urea at pH 5.4 of the globin-chains present in the total lysate of 'normal' and 'mutant' chickens. 1 and 3, 'mutant' globins; 2 and 4, 'normal' globins. Succinate-*Tris*-buffer, running time 4 h at 250 V. Staining by Amido Black 10 B.

and the same globin chain composition ($\alpha_2\gamma_2$). In this report Hb1 and Hb1a are both referred to as Hb1. Figure 2 shows the elution profile from Amberlite CG-50, of the Hb types present in 'normal' and in 'mutant' chickens. Hb1, in its 2 fractions, elutes in a slightly different position in 'normal' and in 'mutant' chickens, while Hb2 has the same migration in both conditions. The electrophoretic analysis on starch gel of the purified hemoglobins confirmed that only the Hb1 fraction from 'normal' and 'mutant' chicken displayed a different migration. These results demonstrate that the 'mutant' hemoglobin is Hb1.

In order to determine which one of the globin chains present in Hb1 was affected by the mutation, purified Hb1 from 'normal' and 'mutant' chickens, was submitted to starch gel electrophoresis in 6 *M* urea at pH 3.6[1]. The results obtained are shown in Figure 3. It clearly appears that, while the α- and β-chains have the same electrophoretic behaviour both in 'normal' and 'mutant' chickens, the γ-chain of 'mutant' Hb1 migrates towards the cathode more slowly than the γ-chain from 'normal' Hb1.

This finding receives further support from the data presented in Figure 4, which shows the electrophoretic behaviour of the various globin chains in starch gel-urea at pH 5.4. This pH value, while not satisfactory for a clear migration of the α- and β-chains, is particularly suitable for the differentiation of the 2 γ-chains. Figure 4 shows in fact that the 2 γ-globins from 'normal' and 'mutant' homozygous chickens have a different electrophoretic mobility. It can therefore be concluded that the mutation responsible for the appearance of the new Hb1 type in 'mutant' chickens is likely to be due to an amino acid substitution of the γ-globin chain of Hb1.

Further work is in progress in an attempt to characterize the 'mutant' γ-globin-chain[7].

Riassunto. Attraverso vari incroci è stato possibile isolare un ceppo di polli domestici portatori di una mutazione a carico della emoglobina 1 (Hb1). Mediante tecniche cromatografiche ed elettroforetiche si è potuto accertare che la mutazione riguarda la catena γ globinica della Hb1.

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