Results. The results are summarized in Table I and Table II.

Table II. Estimation of the molecular weight of proteins in the fat body and midgut of prepupa *G. mellonella* L.

Prepupa of *G. mellonella*			
Molecular weight of proteins			
Midgut	Fat body	Midgut	Fat body
70,000	110,000	520,000	475,000

Zusammenfassung. Mittels dünnschichtiger Gel-Filtration Sephadex G-200 (Superfine) verteilten sich die Hämolymphproteine von Vorpuppen und Puppen der *Galleria mellonella* in 2 Hauptfraktionen. Für die erste Fraktion wurde das Molekulargewicht um 13000, für die zweite Fraktion um 500000 (im Mittel 75% des Gesamteiweisses der Hämolymphe) bestimmt.

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Heterogeneity and Globin Composition of Adult Chicken Hemoglobin

The heterogeneity of hemoglobin (Hb) in an individual of a certain animal species is likely to be a general rule, though the molecular basis and physiological significance of this finding are not yet clearly understood.

We have been interested in the study of hemoglobin composition in amphibia[1], fishes[2], and chickens[3], during both embryonic development and adult life.

The present study reports our findings on the heterogeneity and globin composition of the hemoglobin of adult chickens of both sexes and of various breeds (White Leghorn, Arbor Acres, etc.).

The number and composition of chicken Hb have been the subject of many investigations[4–9]; the discrepancies emerging from different results might be due to the various techniques used, though a strain-dependent heterogeneity cannot be ruled out completely.

Materials and method. Blood samples were drawn by heart- or veni-puncture in heparinized syringes. Red blood cells were then treated as previously reported for human red blood cells[10]. Clear Hb solutions were transformed in the cyano-derivative[11] for prolonged storage and for all the chromatographic and electrophoretic separations. Electrophoresis of Hb was carried out in starch gel at pH 8.6 as previously described[2], or in cellulose acetate at pH 8.5 using the buffer system suggested by HUEHNS[12]. Separations were performed at 5 °C with a current of 400 Vs for 1–2 h. The fractions were stained with Amido Black 10 B, or benzidine, or Ponceau Red (only for cellulose acetate). Chromatography of hemoglobin was carried out by Amberlite CG-50 type II. The original procedure worked out by ALLEN et al.[11] for human hemoglobin has been of great help. A buffer consisting of Na_2HPO_4 (molarity of $Na^+ = 0.092$ + KCN $0.005M$, brought with $5M$ phosphoric acid to pH 7.45) was found to be satisfactory. The Amberlite columns (1×35 cm for analytical, and 2×45 cm for preparative work) were operated at 5 °C, at a flow-rate of 2 ml/h; the elution of Hb types was completed by heating the columns at 28 °C and raising the flow-rate to 3.5–4 ml/h.

Globin chains composition and properties were investigated through starch gel electrophoresis in $6M$ urea, with and without $0.1M$ mercaptoethanol. The following buffer systems were used for preparing the gels: (1) Glycil-glycine ($0.01M$) brought with $5M$ NaOH to pH 9.3; (2) *Tris*-EDTA-borate buffer[12], pH 8.55; (3) *Tris*-EDTA-borate ($0.0187M$ *Tris*, $0.047M$ boric acid, $0.001M$ EDTA), pH 7.3; (4) phosphate buffer (KH_2PO_4 $0.006M$), brought with $1M$ KOH to pH 6.2; (5) acetate buffer (Na acetate $0.005M$), brought with $5M$ acetic acid to pH 5.4; (6) acetate buffer (Na acetate $0.005M$), brought with $5M$ acetic acid to pH 4.7; (7) citric acid-*Tris* (citric acid $0.004M$ + *Tris* $0.009M$), brought with $5M$ KOH to pH 3.5; (8) citric acid-*Tris* (citric acid $0.004M$ + *Tris* $0.009M$), brought with $5M$ KOH to pH 2.8; (9) formic acid buffer, according to MULLER[9], pH 1.8. This buffer was also used without urea. The buffer reservoirs had the same pH and salt composition, but with a buffer concentration about 8 times higher than the corresponding gels; urea and mercaptoethanol were likewise omitted.

Results and discussion. The electrophoresis of chicken hemoglobin, in agreement with other authors[8,9], showed 2 fractions; we have called Hb 1 the minor more anodic component (25–30% of the total Hb), and Hb 2 the major component (70–75% of the total Hb).

The chromatography on Amberlite columns separated 3 hemoglobin peaks (Figure 1). The first peak is eluted together with some non-heme proteins in the break-through-volume (as shown by the ratio O.D. 541/280), and represents about the 9% of total Hb. The second and the third peak are eluted when the column is heated at 28 °C, and represent respectively the 20 and the 70% of the total Hb. When submitted to starch gel electro-

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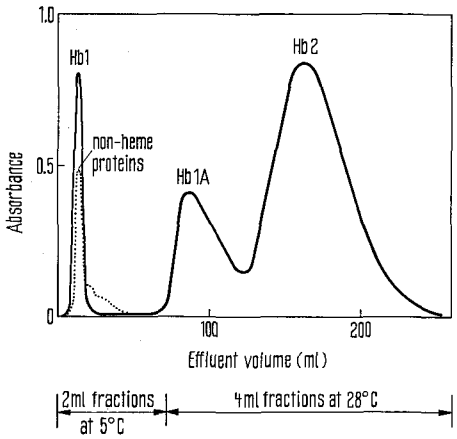

Fig. 1. Column chromatography on Amberlite CG-50, of adult chicken cyano-Hb. Three peaks are present; their identification, after concentration of the corresponding fractions, was achieved by starch gel electrophoresis as reported in the text. Fractions were read with a Gilford model 2000 spectrophotometer. —— absorbance at 541 nm; ----- absorbance at 280 nm.

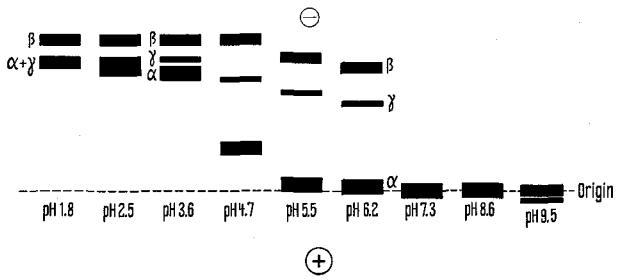

Fig. 2. Electrophoretic behaviour of globin chains at different pH values in starch gel-urea, with and without 0.1 *M* mercaptoethanol. The composition of various buffers is that reported in the text, but almost the same results were obtained with a variety of buffers of different salt composition. Intact hemoglobin, or native globin prepared with the acid-acetone technique[15], were used for the runs; at pH values higher than 4.8–5, only globin was used.

phoresis, the first 2 peaks show the same mobility of the minor fraction Hb 1 of the total lysate, and thus have been called Hb 1 and Hb 1a respectively. The third and largest peak corresponds to the slow-moving Hb 2 fraction of the total lysate and thus is called Hb 2. Hb 1 and Hb 2 are practically pure, while in Hb 1a some Hb 2 is present. Rechromatography can easily overcome this trouble.

Globin chains composition and properties from purified Hb fractions were initially studied with the buffer system at pH 1.8 of MULLER[9] but, at least in our hands, this technique did not allow us to appreciate clearly the differences which could be presumed to exist between the Hb fractions. Thus the behaviour of globin chains was studied in starch gels and urea at different pH values. No effect was observed by the addition of 0.1 *M* mercaptoethanol to the gels; this fact might suggest that the 8 reactive SH groups/mole present on chicken Hb, are not so critical for the maintenance of its structure.

The results presented in Figure 2, show that, under our experimental conditions, the best separations of the globin chains are obtained in the pH range from 3.3–5.5. The pattern obtained in Figure 3 shows that the hemo-

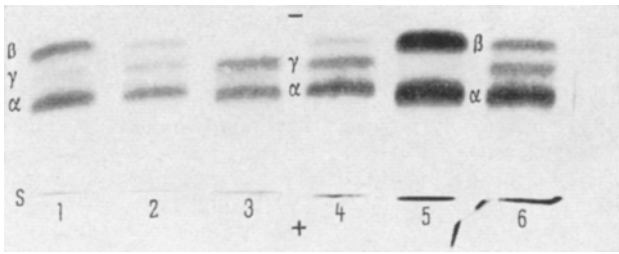

Fig. 3. Electrophoretic pattern in starch gel-urea at pH 3.5, of the globin chains present in chicken hemoglobins. From left to right (1) total globin; (2) total Hb; (3) Hb 1 from Amberlite; (4) Hb 1a from Amberlite; (5) Hb 2 from Amberlite; (6) total globin (as 1). It is evident, as mentioned in the text, that while Hb 1 and Hb 2 are pure, Hb 1a is slightly contaminated by Hb 2 and thus some β globin chain is present (4).

globins purified by Amberlite seem to have 1 globin chain in common (α) and 2 different counterpart globin chains, β for Hb 2 and γ for both Hb 1 and Hb 1a.

The denomination of globin chains does not imply any similarity with human globins, and the γ chain has no relation with fetal-like properties. The fact that Hb 1 and Hb 1a have different chromatographic properties, but seem to have the same globin composition ($\alpha_2 \gamma_2$), might be due not to differences in amino acids, but to the presence on Hb 1 of acidic extra charges. Acetyl groups at the NH_2 terminal have been reported for chicken Hb by SATAKE et al.[13]: the presence of such groups can markedly influence the migration of Hb on Amberlite, as found by SCHROEDER et al.[14] for Hb F I from human cord blood.

In conclusion, our results suggest that in adult chicken, 2 main Hb types are present, Hb 1 ($\alpha_2 \gamma_2$), and Hb 2 ($\alpha_2 \beta_2$); the chromatography on Amberlite can separate Hb 1 into Hb 1 and Hb 1a, which anyway seem to have the same globin chains ($\alpha_2 \gamma_2$). Work is in progress for a more detailed characterization of hemoglobins and globins from adult chicken[16].

Riassunto. La emoglobina del pollo adulto è eterogenea e sembra essere formata da 2 frazioni principali, Hb 1 e Hb 2. Le 2 frazioni sembrano avere una catena in comune α, e 2 altre catene globiniche, β e γ, diverse fra loro. Sono discusse alcune proprietà delle emoglobine e delle globine.

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[16] This work was supported by a grant from the Italian CNR, Impresa di Enzimologia, by the Grant No. 5 RO1 HDO2834-02 from the National Institute of Health, USA, and by a grant from the Cooley's Anemia Blood and Research Foundation for Children, Inc., USA.