Disproportional Synthesis of the Adult Duck's Two Hemoglobins during Acute Anemia

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ABSTRACT Concurrent synthesis of two or more hemoglobins occurs in normal man, the human hemoglobinopathies, and certain animal species. Duck erythrocytes produced in response to acutely induced anemic hypoxia (hemolysis or blood loss) contained reciprocally altered proportions of Hb I ($\alpha_2^I \beta_2^I$) and Hb II ($\alpha_2^{II} \beta_2^{II}$); the relative proportion of Hb II was 50–100% increased. Relative rates of synthesis of the two hemoglobins remained proportional to their new concentrations throughout erythroid maturation. This information favors the proposal that relatively increased activity, not delayed decay, of biosynthetic processes responsible for net synthesis of Hb II had occurred. These studies support the concept that the individual biosyntheses of multiple hemoglobins, presumably under genetic control, are potentially manipulable, and they provide evidence for one mechanism leading in a reproducible fashion to alterations in net synthesis in vivo.

INTRODUCTION

The erythrocytes of man and various other animals contain two or more physicochemically distinct hemoglobins (1), the relative proportions of which are presumably under genetic control (2). The ability to induce changes in relative proportions of hemoglobins, or proportions within any species of protein, would carry conceptual and practical significance. One area of clinical application, for example, would include the management of human hemoglobinopathies.

In search of information bearing on the manipulability of hemoglobin proportions, we selected the duck as an experimental animal. The hemoglobin of both duck and chicken consists of two distinct components (3–5). In the maturing duck (6) the relative proportions of the two adult hemoglobins (Hb I and Hb II) gradually change in reciprocal fashion and ultimately stablize at a Hb I : Hb II ratio of approximately 80 : 20. A third hemoglobin present at hatching in both duck and chicken eventually disappears during maturation (6, 7) in a manner reminiscent of human Hb F.

This report describes a consistently reproducible means of inducing disproportional changes in net synthesis of the adult duck's two hemoglobins in vivo. An explanation of these changes is presented which evolved from studies on relative rates of synthesis of the two hemoglobins both in vivo and in vitro.

METHODS

Acute anemia, i.e., packed cell volume reduced to less than half of control value, was produced in adult white Peking ducks by daily subcutaneous injections of 2–4 ml of a 2.5% solution of acetylphenylhydrazine in 47.5% ethanol, or by removal of a volume of blood equal to 8% of body weight over 3 days through cardiac puncture.

Blood samples were drawn from wing veins, jugular veins, or hearts into syringes wet with heparin. Subsequent procedures in the preparation of whole hemoglobin were performed at 4°C. The erythrocytes, washed three times in a low magnesium saline solution (8), were frozen and thawed once: avian erythrocytes resist simple hypotonic hemolysis. Lysis was completed in hypotonic Tris buffer, pH 7.4 (8), and cell debris and nuclei were sedimented at 105,000 *g* for 1 hr. The supernatant hemo-

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globin was converted to cyanmethemoglobin and dialyzed for 24 hr against 0.005 M phosphate buffer, pH 8.6, containing 100 mg of KCN/liter.

Preparative separation of hemoglobins was accomplished by starch block electrophoresis (9), column chromatography on carboxymethylcellulose [CM-cellulose (10)], and column chromatography on diethylaminoethylcellulose (DEAE-cellulose) by a modification (11) of Huisman's technique (12). Samples of individual hemoglobins were used to confirm the identities of fractions obtained by the various separatory techniques. Optical density measurements were made on eluted hemoglobins at 280, 415, and 540 mμ. Since barbiturate buffer absorbs significantly at 280 mμ, eluates from starch block were not measured at that wave length. Individual hemoglobins were concentrated from their dilute solutions onto small columns by minor modifications of techniques of Huisman, Dozy, and Meyering: Hb I on CM-cellulose (13) and Hb II on DEAE-cellulose (12). In later experiments hemoglobins were concentrated on Sephadex G-25 (14).

After vertical electrophoresis of replicate samples of hemoglobin on polyacrylamide gel (15) in Tris-borate-EDTA buffer, pH 9.2, the unstained gel strips were scanned at 420 mμ in a recording densitometer. Simultaneous integration of the optical density recordings was used to compute hemoglobin proportions. The technique yielded a precision of ± 5.0% (1 SD). Its accuracy was proven by comparison with proportions determined by starch block electrophoresis which in turn agreed with proportions obtained from DEAE-cellulose chromatography. The most efficient medium for separating nonheme protein (13, 16) from Hb I and II was CM-cellulose, but recoveries of total Hb were often less than 90%. Consequently, specific activities cited in these investigations are derived from CM-cellulose column chromatography, and hemoglobin proportions are derived from vertical gel electrophoresis, in which the assay technique (densitometer scanning) guarantees 100% recovery.

Labeling of hemoglobin in vivo was accomplished with a single intravenous injection of 80–100 μc of ^{59}Fe (as ferrous citrate), or 100–500 μc of a mixture of amino acids–^{14}C (algal hydrolysate). When acutely anemic ducks were studied, the isotope was given immediately after the final bleeding. Blood was obtained for analyses 24–72 hr later.

Initial experiments with cell suspensions in vitro with either ^{59}Fe or amino acids–^{14}C as radioactive precursors showed that the specific activity ratio of the two hemoglobins was independent of the use of either duck serum, duck plasma, or Krebs-Ringer phosphate buffer, pH 7.4 (17), as suspending medium. The unsaturated iron-binding capacity of duck serum was found to be approximately 100 μg/100 ml, and this capacity was never exceeded in in vitro experiments employing added ^{59}Fe. When ^{59}Fe was used with phosphate buffer, sufficient conalbumin (Worthington Biochemical Corporation, Freehold, N. J.), recrystallized five times, was added for complete iron binding (18). The specific activity ratio of the two hemoglobins, using ^{59}Fe as precursor, was also independent of the presence of amino acids and succinate [1] added to a final concentration each of 0.25 μmole/ml of suspension medium. The following method of short-term incubation was therefore used routinely: erythrocytes after removal of buffy coat were suspended in autologous plasma to a packed cell volume of approximately 50%, 5–10 μc of ^{59}Fe/ml of cells or 10 μc of algal hydrolysate–^{14}C/ml of cells were added, and the suspension was incubated at 37°C in a Dubnoff shaker for 90 min under room air. The pH of the suspension remained within 7.3–7.4. At termination of incubation the suspension was chilled to 4°C, and unbound radioactivity was removed by repeated washing of the cells in low magnesium saline solution (8). Solutions of individual hemoglobins were assayed for radioactivity, for ^{59}Fe in a well-type scintillation counter, or for ^{14}C in a liquid scintillation counter after decolorization (less than 10 mg of Hb/sample) with H_2O_2 and suspension in a scintillating gel (19). The precision of assaying samples decolorized by this method was ± 3.3% (1 SD). Constant volumes of solutions of known hemoglobin concentration were used, and sufficient counts were collected to yield within 3% probable error.

Incubations of longer duration were carried out under pH-stat control (20). A mixture (4/5/3, v/v/v) of washed erythrocytes, autologous serum, and Krebs-Ringer bicarbonate buffer, pH 7.4 (17), containing 0.1 mg of streptomycin and 0.2 mg of penicillin/ml was placed in a siliconized Erlenmeyer flask. An indwelling electrode led to a pH-stat (Radiometer titrator, model TTTla) set for pH 7.4, and a 0.077 M glucose–0.154 M sodium bicarbonate solution was delivered on demand into the cell suspension. The flask was rotated within a 37°C incubator, and its contents were exposed to a flowing, humidified atmosphere of 20% O_2–5% CO_2–75% N_2. Samples of cell suspension removed periodically over 24 hr were incubated separately for 90 min in the presence of amino acids–^{14}C or ^{59}Fe as described above.

Differential centrifugation (21) was used to obtain erythrocytes of low density and high density from the peripheral blood of ducks with acute (blood-loss) anemia. Centrifuge tubes with a length: width ratio of 10:1 were filled with a 1:1 suspension of cells in autologous plasma and centrifuged (room temperature) at 200 *g* for 10 min followed by 2000 *g* for 10 min. Top (low density) and bottom (high density) halves of the packed cell mass were resuspended in autologous plasma and centrifuged as before. The top half of the low density fraction and the bottom half of the high density fraction were used for subsequent determinations.

Calculations of cell volume and cell content of individual hemoglobins were made from the following determinations: erythrocytes were enumerated with a Coulter counter model A; packed cell volumes were

[1] Alanine, arginine, asparagine, aspartic acid, citrulline, cysteine, cystine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophane, tyrosine, valine, and succinic acid: 1 μmole each/ml of isotonic sodium chloride.

measured after centrifugation at 10,000 *g* in capillary tubes (22); the absorption coefficient of human hemoglobin was used to compute hemoglobin concentrations (23); and proportions of individual hemoglobins were determined as described above.

Purine-bound pentose was assayed by the orcinol reaction (24) on lysate centrifuged at 10,000 *g* and extracted by the Ogur-Rosen procedure (25). No diphenylamine-reacting material was found.

RESULTS

Effect of acute anemia on relative proportions of hemoglobins. Chronological changes in packed cell volume and relative proportions of Hb II during hemolytic anemia produced by subcutaneous injections of acetylphenylhydrazine are charted in Fig. 1 A; the relative proportion of Hb II nearly doubled. When the injections were stopped, the packed cell volume returned rapidly to normal, and hemoglobin relative proportions returned nearly to base line levels within 2 wk. The addition of acetylphenylhydrazine to whole blood in vitro with consequent hemolysis of 60% of the cell mass did not significantly alter hemoglobin relative proportions.

Chronological changes produced by acute blood-loss anemia, charted in Fig. 1 B, contrast with those produced by hemolytic anemia in that the return of per cent of Hb II to normal coincided with the life span of duck erythrocytes, known to be approximately 40 days [(26) confirmed by us]. In both types of acutely produced anemia the rise in per cent of Hb II paralleled the appearance of young erythroid cells in the peripheral blood characterized by a loose chromatin pattern and intense cytoplasmic basophilia. At the peak of the Hb II response these young cells regularly constituted from 30 to 50% of circulating erythrocytes. The rapid return of hemoglobin relative proportions to normal after drug-induced hemolytic anemia (Fig. 1 A) suggested early removal from the circulation of damaged young erythrocytes, whereas a significant number of erythrocytes produced in response to acute blood-loss anemia were able to survive normally (Fig. 1 B).

Fig. 2 shows electrophoresis patterns on polyacrylamide gel (benzidine stain) of hemoglobin obtained during the control period and at the peak of response of per cent of Hb II to hemolytic anemia, and densitometer (optical density) recordings of the unstained gel. A hemoglobin traveling in the position marked Hb "X" appeared consistently, in small amounts, in preparations from duck erythrocytes during anemia produced either by hemolysis or blood loss and has not been investigated further.

Analyses of mature and immature erythrocytes from acutely anemic ducks. The production of acute anemia through repeated bleedings over a 3 day period, reducing packed cell volumes to less than half of control values, consistently yielded an increased proportion of Hb II. Hemoglobin relative proportions in low density and high density erythrocytes, separated by differential centrifugation, from seven consecutive bleeding experiments are shown in Table I. In experiments designed to determine age-related cell characteristics, blood from an acutely anemic duck was incubated for 90 min in vitro with ^{59}Fe. The cells were washed free of unbound radioactivity, resuspended in autologous plasma, and fractionated by differential centrifugation. Analyses were performed on three fractions: low density cells, high density cells, and unseparated mixture. The results are summarized in Table II. Cell content of ribonucleic acid and the hemoglobin specific activities in the low density cells are measures of their immaturity. In addition, examination by light microscopy demonstrated that the low density fraction (Fig. 3 A) was composed almost entirely of the primitive erythroid cells described above, while erythrocytes of the high density fraction (Fig. 3 B) were morphologically adult. Thus the technique of differential centrifugation for separating old and young erythrocytes of the human is also applicable to nucleated erythrocytes of the duck. The same information was obtained when either ^{59}Fe or amino acids–^{14}C were used as cohort labels for hemoglobin in vivo (e.g., see Table III).

Cell content of the individual hemoglobins in adult nonanemic ducks is approximately 37 pg of Hb I and 8 pg of Hb II (6). Calculations comparing these figures with the amounts of Hb I and II in the immature cells of Table II reveal that synthesis of Hb I was 32% complete, whereas synthesis of Hb II was 50% complete. In the immature cells of a similar experiment where the average cell population was older, the corresponding figures were 62 and 77%.

The identity of each hemoglobin from immature cells was confirmed by concentrating the hemo-

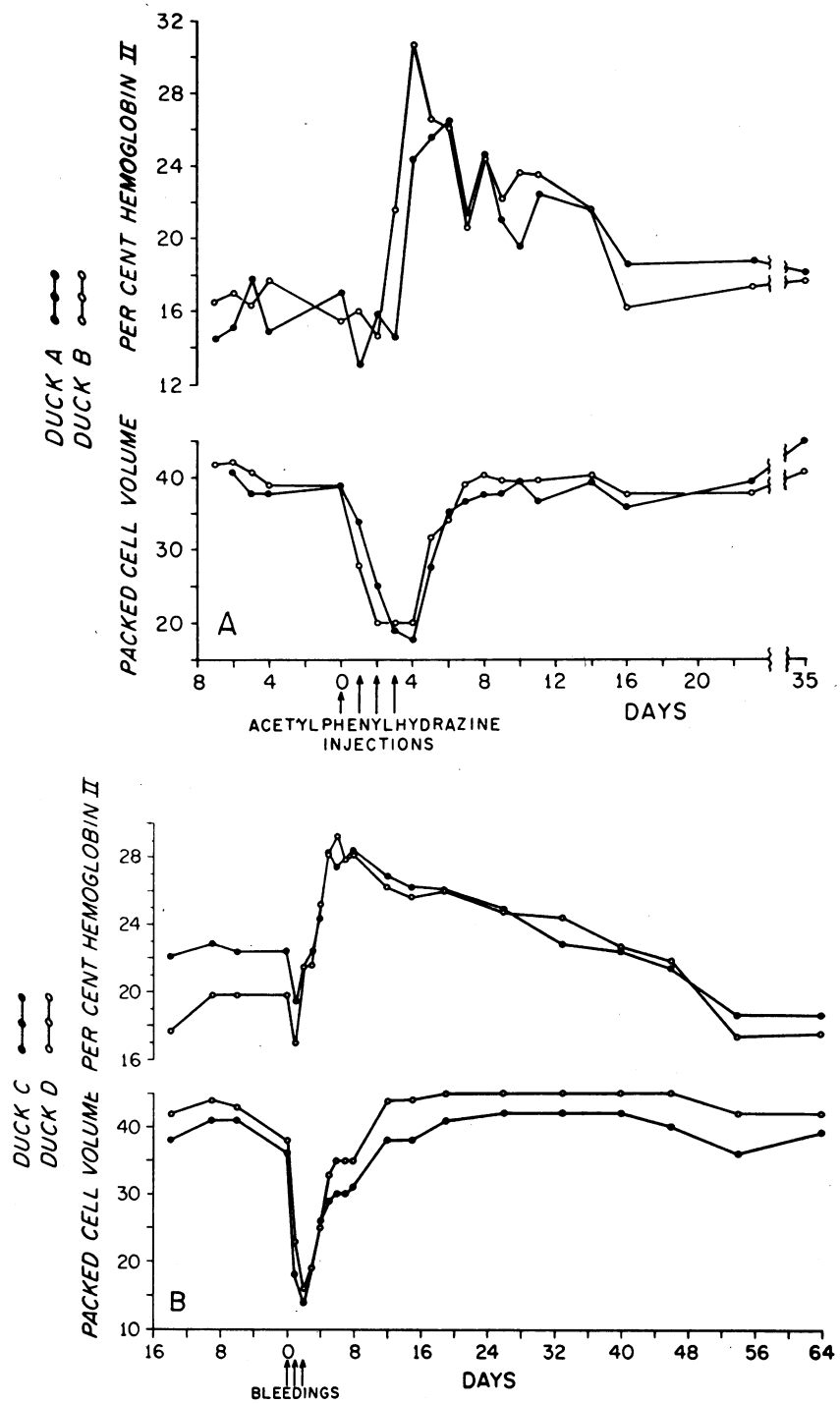

FIGURE 1 Changes in packed cell volume and relative proportion of Hb II in ducks responding to (*A*) hemolytic anemia and (*B*) acute blood loss. The proportion of Hb II in bled ducks returned to base line at approximately the known survival time of duck erythrocytes (40 days).

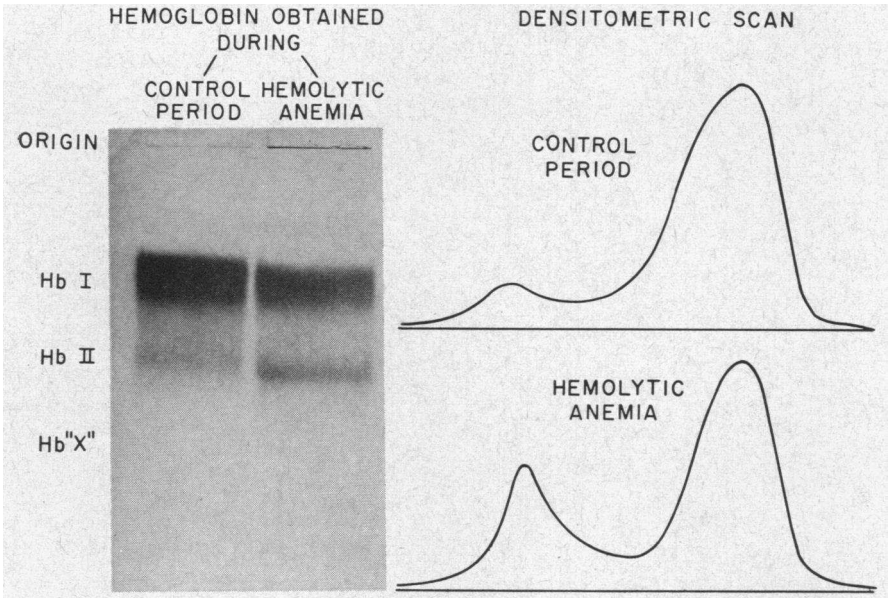

FIGURE 2 Effect of acute anemia on the relative proportions of duck hemoglobins. Polyacrylamide gel electrophoresis pattern (left), stained with benzidine, and corresponding optical density (420 mμ) scans (right) of unstained gel, of duck hemoglobin obtained before and during hemolytic anemia. Electrophoresis was conducted at 300 v for 4 hr at 10°C. Concentrations of total hemoglobin in the two samples were adjusted to give approximately equal amounts of Hb I. An increased proportion of Hb II during acute anemia is evident in the stained gel pattern, and is demonstrated quantitatively by the optical density scan. A hemoglobin traveling in the position marked Hb "X" appeared consistently in preparations from acutely anemic ducks and has not been investigated further.

globin from pooled peak tubes after separation on CM-cellulose columns, dialyzing it against the buffer used for column equilibration, and rechromatographing on CM-cellulose with nonradioactive whole hemoglobin (from nonanemic ducks) as carrier. The peak of radioactivity was found to be coincident with the appropriate nonlabeled hemoglobin. A minor peak of radioactivity also traveled with the nonheme protein fraction when Hb II was tested in this fashion.

TABLE I

Relative Proportion of Hb II in Low Density and High Density Erythrocytes from Acutely Anemic (Blood-Loss) Ducks

Duck	% of Hb II	
	Low density erythrocytes	High density erythrocytes
1	24.4	19.4
2	24.2	21.4
3	25.9	20.5
4	27.0	22.7
5	23.6	19.6
6	31.2	19.1
7	28.2	20.0

Relative rates of synthesis and survival of Hb I and II in vivo. Data in Table III are representative of experiments on hemoglobin synthesis in vivo. Immature and mature erythrocytes were obtained by differential centrifugation of peripheral blood from acutely anemic (blood-loss) ducks. Specific activity ratios were 1.0 in all in vivo experiments with nonanemic ducks using ^{59}Fe or amino acids–^{14}C. It is notable that the specific activities of Hb I and II from the immature erythrocytes are approximately equal despite the increased proportion of Hb II.

Relative rates of survival of Hb I and II in vivo were determined after the intravenous injection of 500 μc of amino acids–^{14}C (algal hydrolysate) into a nonanemic duck. Results are charted in Fig. 4. Specific activities of the two proteins remained equal to each other during their intravascular life

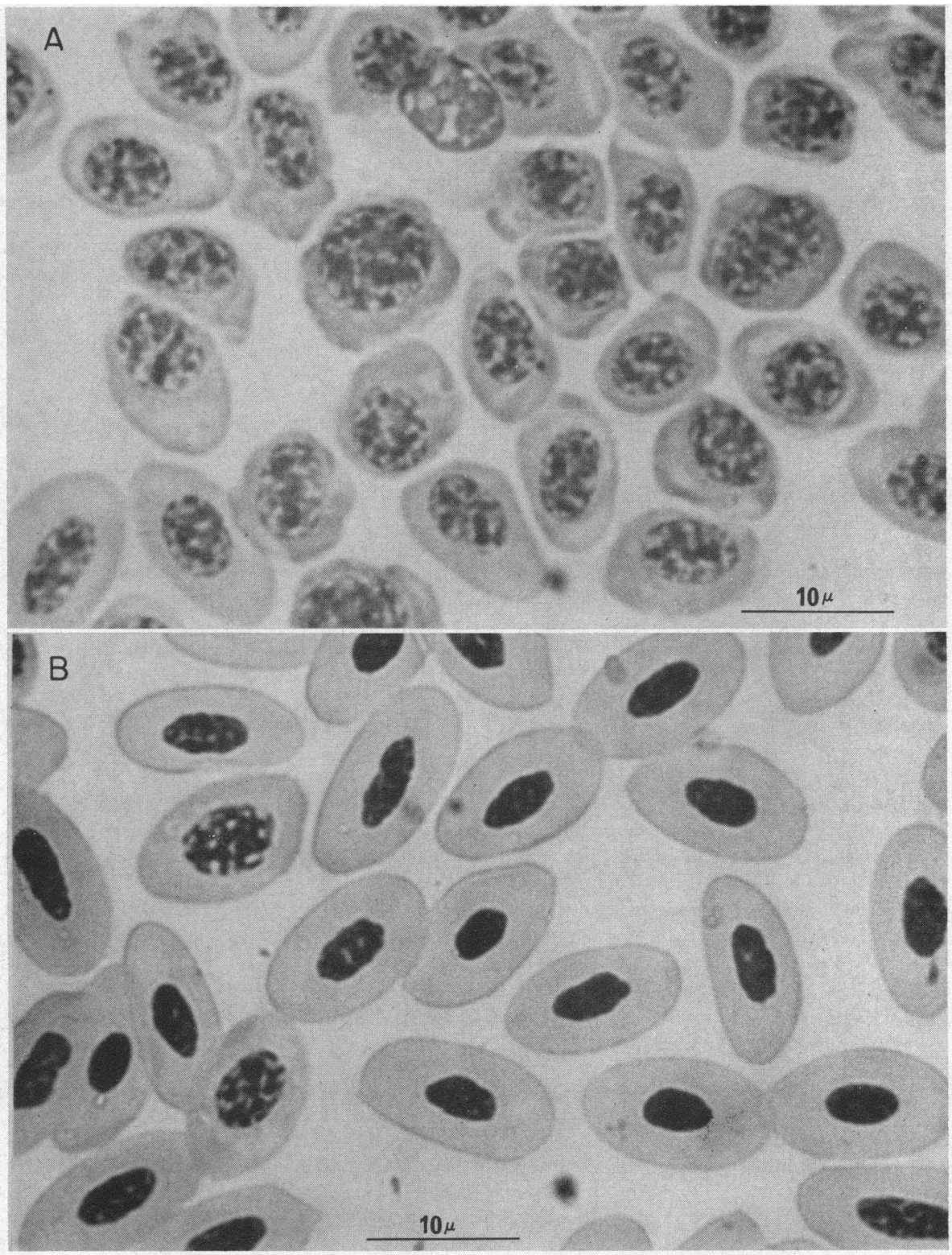

FIGURE 3 Photomicrographs of (*A*) low density erythrocytes and (*B*) high density erythrocytes separated from the blood of an acutely anemic duck by differential centrifugation. The low density erythrocytes show the cytoplasmic staining and loose chromatin pattern characteristic of primitive erythroid cells, whereas the high density erythrocytes are morphologically adult.

TABLE II

Analyses of Mature and Immature Erythrocytes, from an Acutely Anemic (Blood-Loss) Duck, Labeled In Vitro with ^{59}Fe before Separation by Differential Centrifugation

Erythrocyte age	Volume/cell	Hemoglobin/cell			Specific activity		Ribonucleic acid/cell
		Hb I	Hb II	Hb "X"	Hb I	Hb II	
	μ^3		pg		cpm/OD, 415 mμ		10^{-10} μmole of purine-bound pentose
Immature, low density	174	12.0	4.0	1.0	3830	4320	7.64
Unseparated mixture		23.4	6.4	1.2	1010	1350	2.82
Mature, high density	145	33.8	8.4	0.7	NS	NS	2.27

NS, not significant.

span. The curves resemble survival plots of duck erythrocytes labeled with glycine–^{14}C (27).

Relative rates of synthesis of Hb I and II in vitro. Cumulative labeling of Hb I and II in vitro, during the incubation of erythrocytes from an acutely anemic (blood-loss) duck with amino acids–^{14}C, is shown in Fig. 5. Here and in other in vitro experiments, when whole blood and either isotope was used, the ratio of specific activities remained constant but unequal during the incubation. 90 min was arbitrarily chosen as the duration of subsequent short-term incubations.

Data in Table III are representative of short-term experiments on hemoglobin synthesis in vitro in which the erythrocyte populations were separated into immature and mature fractions by differential centrifugation after incubation with isotope. In the immature cell fractions, where synthesis was most active, the specific activity ratios approached 1.0.

The possibility that disproportional decay in the rates of synthesis of the two hemoglobins during erythroid maturation might explain the altered proportions of hemoglobins was examined by measuring rates of synthesis during duck reticulocyte maturation in vitro over 24-hr periods. Six separate incubations of erythrocytes from acutely anemic ducks are shown in Fig. 6. Each point represents the specific activity ratio (Hb II: Hb I) of hemoglobins from a sample of cell suspension after its removal from the pH-stat–controlled flask and incubation for 90 min with a radioactive precursor

TABLE III

Specific Activity of Hemoglobin Labeled with ^{59}Fe or Amino Acids–^{14}C and Chromatographed on CM-Cellulose

Radioactive precursor	Experiment	Duck	% of Hb II	Specific activity		Specific activity ratio, Hb II/Hb I
				Hb I	Hb II	
				cpm/OD, 415 mμ		
	In vivo	Nonanemic		637	635	1.0
		Anemic*				
^{59}Fe	In vivo	Immature cells	31.2	5690	6050	1.1
		Mature cells	19.1	589	653	1.1
	In vitro	Immature cells	28.2	9550	11,800	1.2
		Mature cells	20.0	226	357	1.6
	In vivo	Nonanemic		138	140	1.0
		Anemic*				
Amino acids–^{14}C	In vivo	Immature cells	28.8	389	380	1.0
		Mature cells	18.8	43	54	1.3
	In vitro	Immature cells	31.1	7324	8098	1.1
		Mature cells	20.0	684	1360	2.0

* After intravenous injection of isotope (in vivo studies) or incubation with isotope (in vitro studies), erythrocytes from acutely anemic (blood-loss) ducks were separated into immature and mature fractions by differential centrifugation.

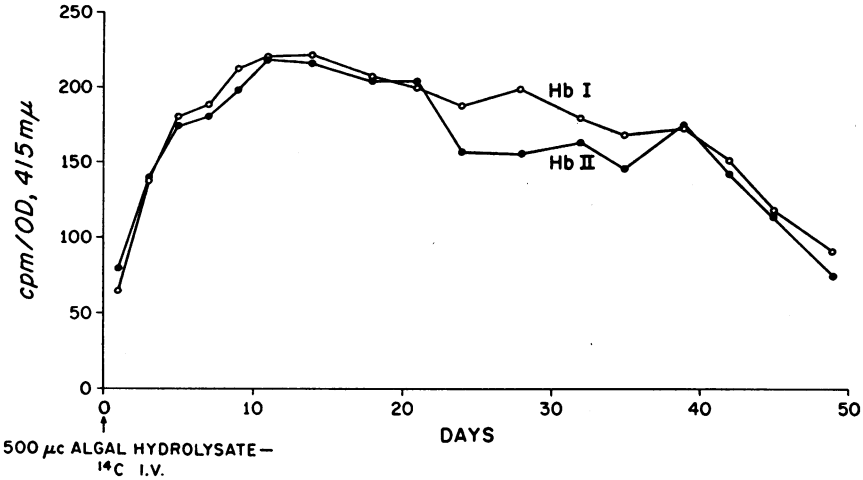

FIGURE 4 Specific activities Hb I and II in erythrocytes removed periodically after a single intravenous injection of amino acids–^{14}C (algal hydrolysate) into a nonanemic duck. The specific activities remained equal to each other throughout their intravascular life span.

of hemoglobin (amino acids–^{14}C in Experiments 1 and 4, ^{59}Fe in the others). Cell content of RNA (as purine-bound pentose) was determined during Experiments 3 and 5 as a measure of reticulocyte maturation; a net disappearance of over 50% was observed in both. Specific activities also declined approximately 80–90% in every experiment. The specific activity ratio during each maturation experiment remained relatively constant, whether or not a volume of serum (Experiments 3–5) or plasma (Experiment 1) sufficient to double the extracellular phase was added to each sample of cell suspension, before addition of isotope, to provide a fresh supply of amino acids, iron, and other factors possibly essential for cell

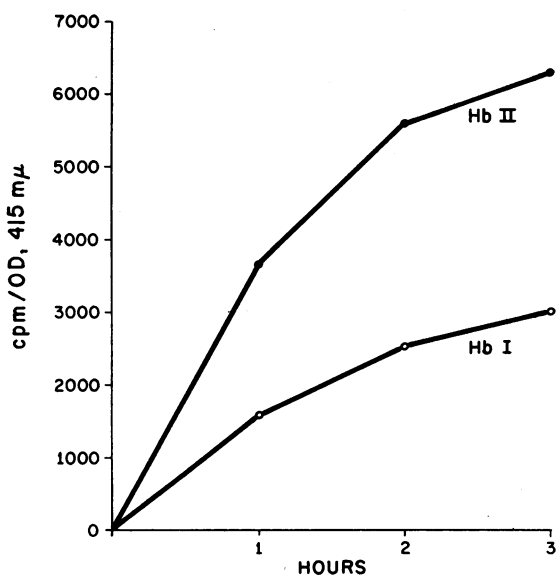

FIGURE 5 Cumulative isotopic labeling of Hb I and II in vitro. 10 μc of amino acids–^{14}C (algal hydrolysate)/ml cells were added at zero time to a suspension of erythrocytes from an acutely anemic (blood-loss) duck. The specific activity ratio remained constant throughout the incubation.

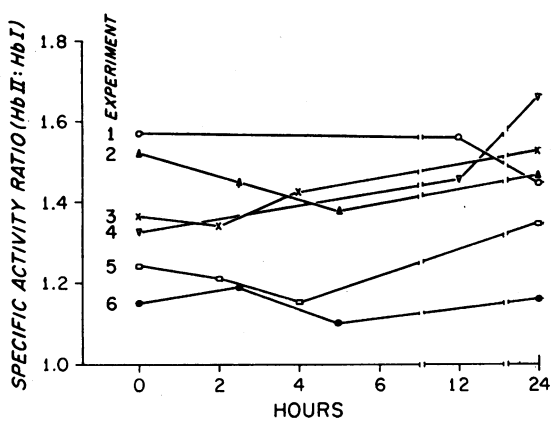

FIGURE 6 Relative rates of synthesis (isotopic labeling) of Hb I and II during duck reticulocyte maturation in vitro. At each point a sample of cell suspension from an incubation mixture under pH-stat control was removed and separately incubated for 90 min with a radioactive precursor of hemoglobin (amino acids–^{14}C in Experiments 1 and 4, ^{59}Fe in the others). A volume of serum (Experiments 3–5) or plasma (Experiment 1) sufficient to double the extracellular phase was added to each sample of cell suspension before addition of isotope.

metabolism. Thus there was no evidence that the rate of synthesis of Hb I was decelerating faster, in proportion to the amounts of the two hemoglobins present, than that of Hb II.

DISCUSSION

In the experiments reported here, acutely produced anemia led to altered relative proportions of the duck's two adult hemoglobins. We are unaware of any other dual hemoglobin system in which disproportional changes in net synthesis can be induced at will in vivo. Reproducible alteration of relative rates of in vitro labeling of human fetal and adult hemoglobins with isotopic precursors has occurred under the influence of hypoxia (28, 29), glucose deprivation (28), and erythropoietin (30). Iron deficiency (31, 32) and folic acid deficiency (33, 34) appeared to be associated with disproportionally altered net synthesis of the two hemoglobins in humans with Hb AS disease or Hb AE disease. These sparsely reported occurrences do not presently lend themselves to experimental manipulation. There is a heterogeneous distribution of Hb F within the erythrocyte population itself of individuals with Hb SS disease which reflects a variability of gene expression from cell to cell (35), but attempts to alter relative proportions of Hb F and S have not been consistently successful. The effect of blood-loss anemia on relative proportions of sheep hemoglobins has proved to be a replacement of one (type A) of the two hemoglobins by a third [type C (36)].

An important distinction must be made between those heterogeneous hemoglobins which exist in every member of an animal species and those which segregate as allelomorphs within the population (polymorphic hemoglobins). Avian hemoglobins are examples of the former in which two or more genetic loci seem to be operating simultaneously. Primates, cattle, sheep, and goats have the latter (1). Certain examples of apparent hemoglobin heterogeneity in mammals actually represent the degradative development of an altered hemoglobin during erythrocyte aging in vivo (37–39). Our finding a temporally stable specific activity ratio of isotopically labeled duck hemoglobins in vivo (Fig. 4) excludes a precursor-product relationship of this type. Artifactual formation of polymers during preparative hemolysis can erroneously imply the presence of multiple hemoglobins in turtles and frogs (40) and mice (41). Analytical ultracentrifugation[2] of duck whole hemoglobin obtained under our experimenteal conditions revealed a single sharp homogeneous peak ($s^\circ_{20,w} = 4.5$) in good agreement with Svedberg and Hedenius (42); thus one hemoglobin is not simply a polymer of the other. The conclusive piece of evidence establishing duck Hb I and II as distinct proteins has been supplied by Saha: amino acid analyses of duck α^I- and β^I-chains (43) and α^{II}- and β^{II}-chains [3] have demonstrated that these are four different polypeptides. Data on hemoglobin molecular weight and chain size now permit duck Hb I and II to be written $\alpha_2^I\beta_2^I$ and $\alpha_2^{II}\beta_2^{II}$.

Itano, in discussing factors influencing relative proportions of structurally different hemoglobins, discriminated between cell rate of hemoglobin synthesis and over-all rate of hemoglobin production (2). Analogously, the increased proportion of duck Hb II might, at two extremes, be found in all erythroid cells produced under the influence of acute hypoxia, or confined to a circumscribed number of cells whose precursors were capable of responding to hypoxia with this shift in hemoglobin proportions. The data do not favor or exclude either possibility. In these and similar experiments where recognition of clonal growth is a fundamental issue, precise information may have to be derived from the application of techniques for analyzing the contents of single cells (44).

Studies with ^{59}Fe and amino acids–^{14}C showed that the two hemoglobins were synthesized in vivo at average rates proportional to their concentrations whether or not the duck was anemic, that is, the specific activity ratio of the two hemoglobins was equal to 1.0. Similarly when relative rates of synthesis of Hb I and II were tested in vitro in short-term incubations with erythrocytes from acutely anemic ducks, the specific activity ratio of hemoglobin from the immature (low density) cells was close to 1.0. The deviation away from 1.0 in the mature (high density) cells probably resulted from contamination of that fraction by small numbers of immature erythrocytes; i.e. synthesis of hemoglobin was occurring predominantly in cells

[2] We are grateful to Dr. Elliot Osserman for this determination.

[3] Saha, A. 1967. Personal communication.

containing approximately 30% Hb II, whereas the average proportion of Hb II in the entire fraction was approximately 20%. This occurrence would lead to a falsely elevated specific activity ratio (Hb II: Hb I).

Relative rates of synthesis, expressed as specific activity ratios, were then measured in sequential samples of reticulocytes maturing in vitro (Fig. 6). Similar incubation systems designed to analyze the progress of hemoglobin synthesis during erythroid maturation have been described by Marks, Rifkind, and Danon (45) and Boyer, Hathaway, and Garrick (46). The ratios remained constant during maturation, signifying no change in relative rates of decay of the two hemoglobins' biosynthetic processes. In other words, each hemoglobin continued to be synthesized at a rate proportional to its concentration in the young erythrocytes that had been produced under the stimulus of acute anemia.

Data presented here lead to speculations on the genetic and epigenetic control of duck hemoglobin synthesis and permit certain conclusions. The increased proportion of Hb II relative to Hb I in immature erythrocytes of acutely anemic ducks could result from (*a*) preferential destruction of Hb I in the marrow or immediately after cell release from the marrow; (*b*) production of Hb I and II in different cells, with Hb I cells preferentially destroyed in or immediately after release from the marrow; (*c*) a normally briefer residence of Hb II than Hb I in circulating blood; (*d*) a relatively reduced rate of loss (delayed decay) of biosynthetic processes responsible for synthesis of α^{II}- and β^{II}-chains from erythroid cells maturing during acute anemia; and (*e*) disproportionally increased net activity of these processes. Proposals *c* and *d* have previously been considered in studies on human heterogeneous hemoglobins (47, 48). Proposals *a* and *b* cannot be discarded but do not merit primary consideration. Stability of the specific activity ratio of ^{14}C-labeled Hb I and II during the life span of duck erythrocytes (Fig. 4) excludes proposal *c*. The data on hemoglobin synthesis presented in Table III and Fig. 6 make proposal *d* unlikely: no evidence of a changing specific activity ratio was found when relative rates of synthesis were compared in the chronology of erythroid maturation. The experimental data uniformly favor proposal *e:* disproportionally increased activity within the biosynthetic sequence leading to formation of α^{II}- and β^{II}-chains and their assembly into $\alpha_2^{II}\beta_2^{II}$.

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