$$\mathcal{H}_{ij} = \frac{g^2\beta^2}{r_{ij}^3}\left\{-\frac{1}{2}\mathbf{S}_i\cdot\mathbf{S}_j(1-3z_{ij}^2/r_{ij}^2)+\frac{3}{2}S_{iz}S_{jz}(1-3z_{ij}^2/r_{ij}^2)\right.$$
$$\left.-\frac{3}{2}(S_{ix}S_{jx}-S_{iy}S_{jy})\left(\frac{x_{ij}^2-y_{ij}^2}{r_{ij}^2}\right)\right\}. \quad (13)$$

In the density matrix formalism, the first-order dipolar interaction energy is

$$(\Psi\,|\,\mathcal{H}_d\,|\,\Psi) = \frac{N(N-1)}{2}\int' \mathcal{H}_{ij}\rho d\tau_i d\tau_j, \quad (14)$$

where the prime on the integration sign in equation (14) indicates that the primes on the primed variables are dropped before the integration operation. The first term in the braces in equation (13) is isotropic in the spins and obviously does not contribute to a zero-field splitting. The term $S_{iz}S_{jz}$ can be written thus:

$$S_{iz}S_{jz} = {}^1/_2(S_{iz}+S_{jz})^2 - {}^1/_2S_{iz}^2 - {}^1/_2S_{jz}^2 \quad (15)$$

$$S_{iz}S_{jz} = {}^1/_2(S_z+S_{iz})^2 - {}^1/_4. \quad (16)$$

The last term on the right in equation (16) is constant and does not contribute to a zero-field splitting. The first term on the right in equation (16) annihilates ρ_4 so that only components of ρ antisymmetric in the space variables can contribute to the splitting. Similar remarks apply to the last term in braces in equation (13). The theorem is therefore demonstrated.

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† Alfred P. Sloan Fellow.

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*ON THE ASYMMETRICAL DISSOCIATION OF HUMAN HEMOGLOBIN**

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INTRODUCTION

Human adult hemoglobins (Hb) have been shown to dissociate reversibly in mildly acid solution into approximately half-molecules.[1-3] We reported recently[3] the interesting finding that when mixtures of the CO-compounds of HbA and HbS, HbA and HbC, and HbS and HbC were dissociated at acid pH, then reassociated near neutral pH and subjected to electrophoresis, no "hybrid" molecules, that is, molecules with electrophoretic mobility intermediate between those of the parent molecules, were detectable.

Of the several possible explanations which were advanced at the time to account for these observations, one is of particular interest here. It is known[4] that the

HbA molecule has four polypeptide chains of only two types and may therefore be represented by the formula a_2u_2. The results obtained by Ingram[5] strongly indicate that HbS differs from HbA only in the substitution of a single valine residue for a glutamic acid residue in one of these two types of chains. Assuming that this is correct, we may therefore represent the HbS molecule as s_2u_2, the u chains of HbS and HbA being taken as identical. If these molecules were to dissociate into nonidentical fragments, HbA into $a_2 + u_2$ and HbS into $s_2 + u_2$, then it is clear that, on recombination, no electrophoretically distinguishable hybrids would be produced, in accord with the experimental facts. On the other hand, recombination would result in an exchange of u_2 fragments, which we suggested might be detected by appropriately labeling these units. Similar considerations apply to HbC. To test this hypothesis, the experiments reported in this paper were undertaken.

The Hb molecules were labeled in two independent ways. The first method may be referred to as the "heme-labeling method" and is based on the experiments of Itano and Robinson.[6] If a Hb molecule with all four hemes in the CO form is mixed at neutral pH with a ferri-Hb molecule with all four hemes in the ferric state, in the absence of a small-molecule oxidizing or reducing agent no electron exchange occurs, i.e., the hemes remain in their original states. The hemes are thereby effectively labeled. The utilization of this heme labeling in exchange experiments is best illustrated by an example. Let us represent the fully saturated CO-HbS molecule by the symbol $[(s - CO)_2(u - CO)_2]^{+2}$, where the superscript $+2$ denotes the net charge of the molecule relative to a reference charge of zero on CO-HbA. Similarly, ferri-HbC may be represented by $[(c^{\oplus})_2(u^{\oplus})_2]^{+8}$, where the superscript $\oplus$ indicates that the heme is in the ferric state, and the charge $+8$ results from the fact that CO-HbC has a net charge of $+4$ units greater than CO-HbA, and each of the four ferriheme groups contributes an extra $+1$ charge. Now, if identical subunits were produced on acid dissociation of HbS and HbC (symmetrical dissociation), these would be $[(s - CO)(u - CO)]^{+1}$ and $[(c^{\oplus})(u^{\oplus})]^{+4}$, respectively. In a dissociated and recombined mixture of these two molecules, there should appear *three* electrophoretic components, the two original parent molecules and a new species, $[(s - CO)(c^{\oplus})(u - CO)(u^{\oplus})]^{+5}$. On the other hand, if two nonidentical subunits (asymmetrical dissociation) were produced on acidification (each containing two hemes, see discussion), these would be $[(s - CO)_2]^{+2}$ and $[(u - CO)_2]^{0}$ for the HbS; and $[(c^{\oplus})_2]^{+6}$ and $[(u^{\oplus})_2]^{+2}$ for the HbC. (The possible difference in net charge of the a and u chains in HbA cancels out for our purposes and is omitted here.) Recombination of a dissociated mixture should yield *four* electrophoretic components, the two original parent molecules and two new species, $[(s - CO)_2(u^{\oplus})_2]^{+4}$ and $[(c^{\oplus})_2(u - CO)_2]^{+6}$. Analogous experiments with other pairs of CO-Hb and ferri-Hb may be analyzed similarly.

The second method of labeling was with C^{14} incorporated in vivo into HbA by the injection of C^{14} glycine into genetically normal individuals. Mixtures of C^{14}-HbA with unlabeled HbS or with unlabeled HbC, all in the CO form, were dissociated, allowed to reassociate, and then separated by starch-block electrophoresis. The specific activity of the fractions was then determined.

The results of both types of experiments show that exchange of fragments of Hb molecules occurs in acid solution and that each type of Hb molecule must dissociate into nonidentical subunits.

MATERIALS AND METHODS

Heme-Labeling Experiments

Preparation of CO-Hb and Ferri-Hb Solutions.—Stock solutions of CO-Hb A, S, and C were prepared as previously described.[3] The stock solutions were diluted to 3 per cent Hb with 0.2 *M* NaCl and were dialyzed against at least three changes of 0.2 *M* NaCl under CO. To prepare ferri-Hb, the stock solutions of CO-Hb were diluted to 3 per cent with phosphate buffer, pH 6.8, $\Gamma/2$ 0.2, were oxidized with $K_3Fe(CN)_6$[6], and were then dialyzed against 0.2 *M* NaCl under N_2. The dialyzed solutions of CO-Hb and ferri-Hb were stored under CO and N_2, respectively, at 5° C.

Recombination Experiments.—The nine possible combinations of CO-Hb and ferri-Hb from the three hemoglobins A, S, and C, were dissociated and recombined. Equivalent amounts of CO-Hb and ferri-Hb solutions prepared as described above were mixed and were diluted to yield 6–7 ml. of 1 per cent CO-Hb and 1 per cent ferri-Hb in 0.2 *M* NaCl. To the mixture was added an equal volume of Na acetate buffer, pH 4.7, $\Gamma/2$ 0.2, and after 20 minutes at 22°–23° C., the solution was set to dialyze against K phosphate buffer, pH 6.8, $\Gamma/2$ 0.02 at 5° C. on a shaker. Neutralization was accompanied by loss of part of the hemoglobin as a precipitate, which was removed by centrifugation. The supernatant solution was then subjected to moving boundary electrophoresis, as previously described.[6] Only in the ascending limb was adequate resolution of the components obtained.

Electrophoretic analyses of recombined mixtures were compared with analyses of untreated mixtures of the same original composition and of controls prepared by mixing the separately acidified and neutralized CO-Hb and ferri-Hb. The net charge of any new component was usually estimated by visual comparison of patterns from the recombined and control samples. In some cases, an untreated sample of known identity was added to an aliquot of the recombined mixture, and an electrophoretic analysis was performed to aid in the identification of the components.

C^{14}-Labeling Experiments

Preparation of C^{14}-Labeled HbA.—In connection with other studies carried out by Nathan and Berlin,[7] 100 microcuries of glycine-2-C^{14} were injected intravenously into subjects with myeloid metaplasia. Heparinized samples of blood were drawn periodically following injection and analyzed for C^{14} activity in hemoglobin. A number of such samples of O_2-Hb were obtained which had been drawn during and shortly after the period of maximum specific activity. The samples had been frozen, and several samples from each of two patients were thawed and separately pooled. The resultant two samples were referred to as "C^{14}-HbAI" and "C^{14}-HbAII" in our experiments. They were reduced with Na dithionite under CO, saturated with CO, and dialyzed against barbital buffer, pH 8.60, $\Gamma/2$ 0.05.

The HbS and HbC samples were conventional unlabeled materials.

Recombination Experiments.—After preliminary experiments were carried out to define the appropriate conditions, three pairs of experiments were performed. Each pair consisted of a recombined sample and its control. In the former, a mixture was made of about equal parts of C^{14}-HbAI or II and HbS or HbC in the

CO form at a total protein concentration of about 6 per cent in barbital buffer, pH 8.60, Γ/2 0.05, and two volumes of this mixture were diluted with 1 volume of acetate buffer, pH 4.25, Γ/2 0.60 at 0° C. The final pH was close to 4.35. After about 1 hr. the mixture was set to dialyze against the CO-saturated barbital buffer. For the control, the same Hb components were separately brought to pH 4.35 with the acetate buffer and were then dialyzed back into the barbital buffer. Then they were mixed to give a solution as similar in composition as possible to the recombined sample. The details are given in Table 1.

TABLE 1

C^{14}-LABEL EXCHANGE EXPERIMENTS

Expt. No.	Mixture	Hb Type	Hb Solution* Conc. (Per Cent)	Hb Solution* Vol. (Ml.)	Current (Ma.)	Time (Hr.)	Recovery† (Per Cent)	Wt. Sample Counted	Counts/Min.‡	Specific§ Activity
5.....	Control‖	C^{14}-HbAI	2.18#	2.2	27	59	69	7.16	393/30	4.7
		HbS	4.15	2.0			66	6.77	22/30	0.2
6.....	Recomb.	C^{14}-HbAI	3.02#	3.8	27	60	62	7.19	296/30	3.5
		HbS					59	7.29	123/30	1.4
7.....	Control**	C^{14}-HbAII	3.25	1.6	37	71	59	7.06	2008/75	9.5
		HbS	2.69	1.8			70	7.13	105/50	0.6
	Recomb.	C^{14}-HbAII	2.92	3.6			60	7.20	1559/75	7.1
		HbS					71	7.15	362/50	2.4
8.....	Control**	C^{14}-HbAII	3.05	2.0	39	45	59	6.95	1206/40	10.5
		HbC	3.28	2.0			76	6.78	49/30	0.5
	Recomb.	C^{14}-HbAII	3.52	4.0			67	7.19	926/40	8.0
		HbC					67	6.68	200/30	2.3

* Refers to the solution applied to the starch block.
† 100 × Hb eluted from starch block/Hb originally applied to block.
‡ Corrected for background of 2.5 c.p.m.
§ Corrected for self-absorption.
# Sample applied to block 12 hours after mixing the two Hb at pH 8.6.
‖ Approximately half of the C^{14}-HbAI precipitated upon reneutralizing the acid-treated protein. In all other cases, less than 10 per cent of the protein precipitated.
** Sample applied to block within 1 hour after mixing.

These samples, after a denatured residue was removed by centrifugation, were then subjected to parallel starch-block electrophoretic separations (see next section). After elution from the starch block, the fractions were made 0.5 *N* in NaOH by the addition with agitation of 5 *N* NaOH. After 15 minutes standing at room temperature, the denatured Hb was precipitated with one-third saturated $(NH_4)_2SO_4$. The reddish-brown precipitates were centrifuged, washed, and centrifuged successively with one-third saturated $(NH_4)_2SO_4$, 33 per cent ethanol, 33 per cent ethanol, 95 per cent ethanol, and ether. The powdery precipitates were air-dried. They were then counted by the procedure described below.

Starch-Block Electrophoresis.—The technique followed was essentially that of Kunkel and Wallenius.[8] In the first pair of experiments, with C^{14}-HbAI and HbS (Expts. 5 and 6, Table 1), the recombination experiment and its control were separated on two different starch blocks 30.5 × 20 × 0.80 cm., whereas in subsequent experiments each pair of experiments was resolved on different halves of the same starch block, 30.5 × 29 × 0.80 cm. An example of the degree of separation achieved is given in Figure 1. The clear CO-Hb samples, in barbital buffer, pH 8.60, Γ/2 0.05, were made into starch pastes and deposited in troughs about 12 cm. long by 0.70 cm. wide in the buffer-equilibrated starch block. The separations were carried out at 5° C.; other conditions of the experiments are given in Table 1.

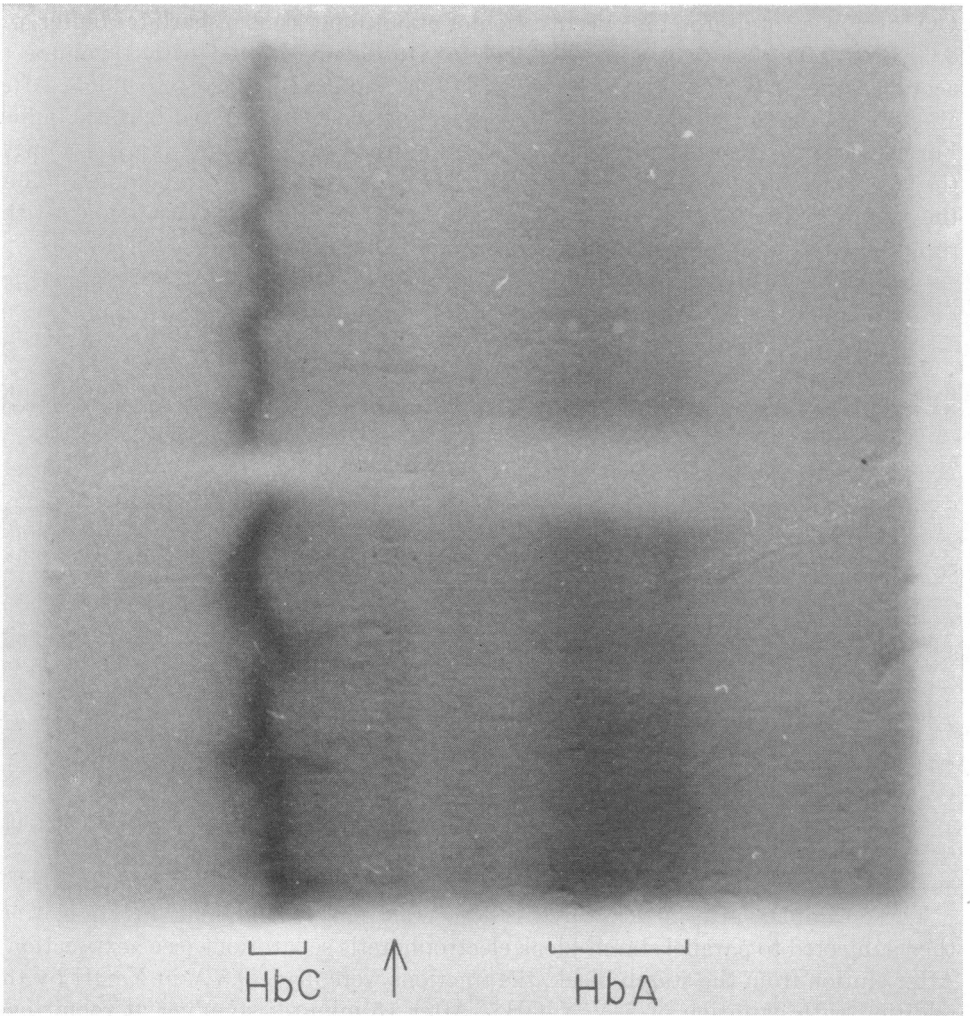

FIG. 1.—Starch-block electrophoresis of the CO-forms of C^{14}-HbAII and HbC, Expt. 8 of Table 1 of the text. Two parallel separations were performed: *top*, the control mixture; *bottom*, the recombined mixture. Electrophoretic migration was to the right for HbA, to the left for HbC, the origin designated by the arrow. The brackets demarcate the zones which were eluted to recover the Hb. The optical density in this reproduction is due to CO-Hb absorption.

At the end of the experiment, the zones of the two hemoglobins were readily demarcated (see Fig. 1). The zones were cut out and eluted with an equal volume of H_2O. The recovery of each fraction was determined spectrophotometrically and was of the order of 60 per cent or better of the protein applied to the block.

Counting Procedure.—The activity of these Hb samples was too low to permit reliable counting in the usual type of windowless counter. Dr. M. V. Simpson generously made available to us the low-background Geiger counter constructed in his laboratory.[9] By means of heavy shielding and an anticoincidence circuit, the background of this counter could be lowered to 2.5 c.p.m. for routine use.

To plate the samples for counting,[9] the surface of an aluminum planchet was slightly roughened with carborundum. An estimate quantity of the dry protein

powder was transferred to the weighed planchet, and enough water was added to make a slurry which could be evenly distributed over an area of 2 cm^2. The planchet was then dried under a heat lamp and reweighed. Close to 7 mg. of protein was usually deposited. The specific activity values obtained were corrected for self-absorption to zero sample thickness.

RESULTS

Heme-Labeling Experiments

Stability of Heme-Label.—Acidification and neutralization of either CO-Hb or ferri-Hb alone did not result in any new electrophoretic components. Any combination of CO-Hb and ferri-Hb prepared by mixing separately acidified and neutralized components yielded the same electrophoretic patterns as did untreated mixtures of the same components. These results demonstrate that no electron exchange, with consequent loss of the heme-label, occurs in these experiments. In other words, a particular heme group initially labeled by binding with CO or by oxidation to the ferric state remains in this condition throughout the recombination experiments.

Recombination of CO-Hb and Ferri-Hb of Same Hb Type.—The three mixtures, CO-HbA and ferri-HbA, CO-HbS and ferri-HbS, and CO-HbC and ferri-HbC, were studied. In each pair the net positive charge of the ferri-Hb is four units greater than that of the CO-Hb at acid pH, and the two forms are easily resolved by electrophoresis.[6] Recombination in each case resulted in three electrophoretic components (see Fig. 2)—the two original ones and a single new component with

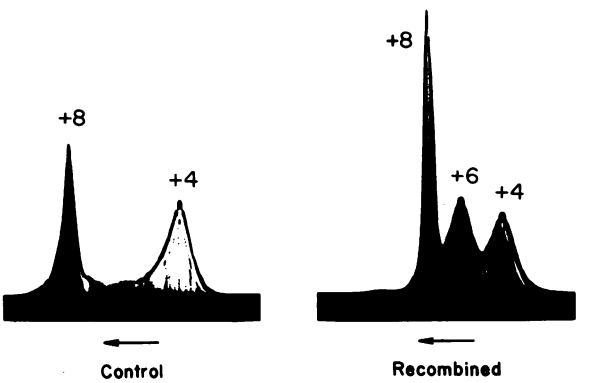

FIG. 2.—Moving-boundary electrophoresis of control and recombined mixtures of CO-HbC and ferri-HbC. The arrows indicate the direction of migration.

mobility about halfway between those of the parent species. Although these results do not distinguish between symmetric or asymmetric dissociation of Hb, they demonstrate that the new component contains two CO-hemes and two ferri-hemes. Each of the subunits produced by acid dissociation of Hb must therefore carry heme groups. Furthermore, the absence of the two other components with mobilities one-fourth and three-fourths the way between the parent molecules demonstrates that no appreciable dissociation into single chains occurs under these conditions.

Recombination of CO-Hb and Ferri-Hb of Different Hb Type.—The six possible mixtures of COHb and ferri-Hb of different pairs of HbA, S, and C were examined.

TABLE 2

HEME-LABEL EXCHANGE EXPERIMENTS

Components of Original Mixture CO-Hb Ferri-Hb	Components of Recombined Mixture (Probable Identity) Nominal Charge 0	+2	+4	+6	+8
a_2u_2 ..... s_2u_2	$[(a - CO)_2(u - CO)_2]^0$	$[(a - CO)_2(u^*)_2]^{+2}$	$[(s^*)_2(u - CO)_2]^{+4}$	$[(s^*)_2(u^*)_2]^{+6}$	..
a_2u_2 ..... c_2u_2	$[(a - CO)_2(u - CO)_2]^0$	$[(a - CO)_2(u^*)_2]^{+2}$	...	$[(c^*)_2(u - CO)_2]^{+6}$	$[(c^*)_2(u^*)_2]^{+8}$
s_2u_2 ..... a_2u_2	...	$[(a^*)_2(u - CO)_2]^{+2}$ $[(s - CO)_2(u - CO)_2]^{+2}$	$[a^*)_2(u^*)_2]^{+4}$ $[(s - CO)_2(u^*)_2]^{+4}$	...	...
s_2u_2 ..... c_2u_2	...	$[(s - CO)_2(u - CO)_2]^{+2}$	$[(s - CO)_2(u^*)_2]^{+4}$	$[(c^*)_2(u - CO)_2]^{+6}$	$[(c^*)_2(u^*)_2]^{+8}$
c_2u_2 ..... a_2u_2	...	$[(a^*)_2(u - CO)_2]^{+2}$	$[(a^*)_2(u^*)_2]^{+4}$ $[(c - CO)_2(u - CO)_2]^{+4}$	$[(c - CO)_2(u^*)_2]^{+6}$	...
c_2u_2 ..... s_2u_2	...	...	$[(s^*)_2(u - CO)_2]^{+4}$ $[(c - CO)_2(u - CO)_2]^{+4}$	$[(s^*)_2(u^*)_2]^{+6}$ $[(c - CO)_2(u^*)_2]^{+6}$	...

The results are summarized in Table 2. Consider the case of the CO-HbS and ferri-HbC mixture, discussed in the introduction. The four electrophoretic components observed upon dissociation and recombination (Fig. 3) are exactly those expected for asymmetric dissociation of Hb and are inconsistent with symmetrical dissociation. The same conclusion is derived from the five other experiments.

C^{14}-Labeling Experiments

The results of the parallel experiments listed in Table 2 clearly indicate that exchange of some of the C^{14} activity of CO-HbA occurs to CO-HbS and CO-HbC upon acid dissociation and recombination.[10] In each experiment the specific activity of the originally unlabeled Hb rose appreciably and that of the HbA decreased by about the same amount. These differences were well outside the experimental errors involved in the starch-block separations and in the counting procedures.

That exchange occurs, but no electrophoretic hybrid molecules form, on dissociation and recombination of pairs of different CO-Hb proves that the Hb molecules dissociate asymmetrically. If the HbA is assumed to have been uniformly labeled in the a and u chains and complete equilibration of the Hb fragments occurred, the ratio of the specific activity of the recovered HbS or HbC fraction to that of the original C^{14}-HbA would be given by $(M_{u_2}/M_{Hb})p$, where M_{u_2} and M_{Hb} are the molecular weights of the u_2 fragment and of the intact Hb molecule, respectively, and p is the ratio of C^{14}-HbA to total Hb in the original mixture. If the u_2 fragment is assumed to represent about half the Hb molecule and p = 0.5, the specific activity of the recovered HbS and HbC fractions should have been about 25 per cent of the original C^{14}-HbA activity. In the three experiments, 5-6, 7, and 8, this activity was 25, 19, and 17 per cent, respectively, in reasonable agreement with expectations.

DISCUSSION

The Structure of Hemoglobin.—Two major structural conclusions are required by these results: (*a*) in mildly acid solution, the Hb-molecule dissociates into nonidentical subunits, each containing two identical polypeptide chains, and therefore in aqueous solution the binding between identical chains is stronger than between unlike chains; (*b*) each subunit contains two hemes, and therefore a single heme is associated with a single polypeptide chain, and a dyad axis of symmetry[11, 3] relates the two heme-chain units within the subunit.

One of the most interesting properties of Hb is the sigmoid character of its O_2-binding curve, showing that interactions exist among the heme groups of a Hb molecule. Wyman and Ingalls,[12] studying the O_2 binding of *horse* Hb in strong urea solutions, in which the Hb molecule is split into two fragments, concluded that the four hemes occur in pairs, with strong interaction between members of

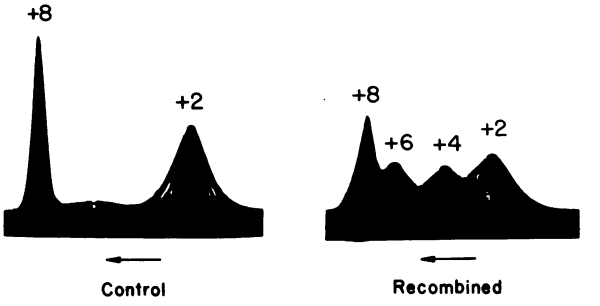

FIG. 3.—Moving-boundary electrophoresis of control and recombined mixtures of CO-HbS and ferri-HbC. The arrows indicate the direction of migration.

the same pair and weaker interaction between members of different pairs. However, we may ask the question, Which hemes are paired? If horse Hb in urea dissociates, as does human Hb in mild acid, into nonidentical subunits, then we conclude that the strong interactions exist between equivalent hemes, that is, those related by the dyad axis of symmetry in the intact molecule, and the weak interactions are between the nonequivalent hemes.

St. George and Pauling[13] suggested, on the basis of experiments on the binding of alkyl isocyanides to Hb, that the hemes are somehow buried within the Hb molecule. It has been found by Reichmann and Colvin[14] that heme tends to stabilize the horse globin molecule against the dissociation into single chain fragments which occurs at low pH. A structure consistent with these and our results might have the two equivalent hemes sandwiched between the two identical chains of each subunit on the Hb molecule, perhaps contributing to the free energy of binding of the two identical chains. The binding of O_2 or some other ligand by one of the equivalent hemes might separate the chains slightly[13] or might otherwise alter the environment around the other member of the heme pair, to permit the latter to bind the second ligand more readily. Conversely, however, the binding of a ligand such as *t*-butyl isocyanide to Hb might facilitate the dissociation of the Hb subunits into single chains, an effect which, to our knowledge, has not yet been investigated.

The Biosynthesis and Genetics of Hemoglobin.—The currently accepted working hypothesis for the biosynthesis of proteins is that a polypeptide chain is in some manner synthesized on a ribonucleic acid (RNA)-containing nucleoprotein template,

organized into microsomes, the RNA having its structure in some way determined by deoxyribonucleic acid (DNA)-containing nucleoprotein, organized into genes and chromosomes. Since there are two different kinds of polypeptide chains in each Hb molecule, the question arises as to what biosynthetic and genetic relationships exist between them. Are the two kinds of chains synthesized on two contiguous templates, or on two entirely independent ones? In what state are the synthesized chains released from the template: as single chains, as subunits, or as intact Hb molecules? Are the two kinds of chains under the control of a single genetic locus or of two entirely independent ones?

Our experimental observations bear most directly on the second of these three questions. It appears unlikely that single chains are synthesized and then released into the cytoplasmic solution of the red cell. If this were the case, then within sickle trait cells there should be formed some *as* subunits through collisions of single *a* and *s* chains.[15] This would result in the production of some asu_2 hybrid molecules, which would be detectable electrophoretically. In fact, such hybrids are not observed.[16] On the other hand, our findings indicate that if, after synthesis, two identical chains were to combine on or near the template to form a subunit[17] and the subunits were afterward released into the cell, then, by combination of two unlike subunits, intact Hb molecules would form spontaneously at the pH of the red cell. So long as the amount of u_2 subunits in the cytoplasmic solution of a sickle trait cell, for example, was equivalent to the sum of the amounts of a_2 and s_2, the intact molecules HbA and HbS would be produced without the intervention of further biosynthetic processes. In other words, it is not required, from this point of view, that intact Hb molecules be synthesized before being released into the cell. It follows, then, that the two types of chains might conceivably be synthesized entirely independently and might be under independent genetic control.

However, the postulate of independent synthesis of u_2, on the one hand, and of a_2 and its mutant analogues, on the other, is not adequate to explain the apparent balance which exists in the net synthesis of the two types of chains. For example, the amount of total Hb per cell is usually low in HbC disease. Here subnormal synthesis of c_2 subunits, also evident in HbC trait,[18] is balanced by a corresponding decrease in net synthesis of u_2. If u_2 synthesis were independent and normal, an excess of u_2 should result, and the apparent absence of half-molecules in hemolyzates at physiological pH[19] suggests either that the excess subunits are destroyed or that the sites of synthesis of the two chains interact in such a way as to produce subunits in equal amounts.

The simplest mechanism to account for such an interaction is that the two unlike subunits are synthesized on two contiguous templates and that the release of the subunits does not occur until they combine to form intact Hb molecules. In such a case, either two templates derived from independent genetic loci might unite in the cytoplasm, or a single locus (i.e., a contiguous segment of DNA) might contain information corresponding to a template on which both chains are synthesized.[20]

It is conceivable that two independent genetic mutations might occur in the same chromosome population corresponding to the two types of Hb chains, resulting in the synthesis of an abnormal *a*-type chain (denoted by *s*) together with an abnormal *u*-type chain (denoted by *v*). Some of the questions raised in this section

could be settled by the study of the Hb from a heterozygous cell bearing one normal and one such doubly abnormal chromosomal complement, synthesizing all four chains a, s, u, and v. If the subunits a_2, s_2, u_2, and v_2 were synthesized independently and released into the cell, four types of intact molecules—a_2u_2, a_2v_2, s_2u_2, and s_2v_2—should be formed within the cell. On the other hand, if the intact Hb molecules were synthesized on a contiguous template and then were released, only the molecules a_2u_2 and s_2v_2 should be found in the cell (provided that the rate of exchange of subunits in solution in the red cell were sufficiently slow), but on acidification and reneutralization of the Hb, all four types of Hb should appear.

Analogous phenomena may be involved, in at least some instances of heterocaryon complementation in *Neurospora*.[21, 22] Giles, Partridge, and Nelson[21] have reported that, with certain mutants unable to grow in the absence of adenine for lack of adenylosuccinase activity, combinations of pairs of mutants are able to form heterocaryons which do exhibit adenylosuccinase activity. In certain of the heterocaryons, about one-quarter of the enzyme activity of the wild type is produced. As the simplest postulate, we might assume that the molecule of adenylosuccinase contains two polypeptide chains and that the synthesis of each chain is controlled by a different genetic locus, say $\mathbf{Ad}^a$ and $\mathbf{Ad}^b$. Mutants might then be expected which were defective at one or the other locus. Such mutants singly would synthesize one chain which was normal, the other defective, and no active enzyme would result on combination of the two chains. In a heterocaryon, however, produced from one mutant defective at the $\mathbf{Ad}^a$ locus and one at the $\mathbf{Ad}^b$ locus, both normal and defective forms of both types of chains would be produced, and, on combination of these chains, one-quarter of the resultant molecules would be enzymatically wild type.

It was reported[21] that when extracts of each of the two complementing mutants grown independently were mixed, no adenylosuccinase activity was found. If the postulated two chains of each molecule did not dissociate appreciably under the conditions of the mixing experiment, no activity would be recovered. On the other hand, if conditions were found such that reversible dissociation was produced in such a mixture, active enzyme might be obtained, by analogy with the experiments we have performed with Hb mixtures.

SUMMARY

We have shown that human adult hemoglobin dissociates in mildly acid solution into two nonidentical subunits, by means of labeling experiments with mixtures of HbA, S, and C. The Hb was labeled either (1) in the heme groups by the binding of CO or by oxidation to ferri-heme or (2) in the polypeptide chains by incorporation of C^{14}. By both methods, exchange of label in the Hb mixtures was observed which was consistent only with asymmetrical dissociation of the Hb. The significance of these findings in connection with the structure of the Hb molecule and with problems related to the biosynthesis and genetics of Hb was examined.

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EFFECT OF ELECTRIC FIELD ON EQUILIBRIUM SEDIMENTATION OF MACROMOLECULES IN A DENSITY GRADIENT OF CESIUM CHLORIDE

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Recently, Meselson, Stahl, and Vinograd have reported on an ingenious method for determining the molecular weight of DNA and for separating DNA molecules of different molecular weight.[1] Their method involves the observation of the equilibrium distribution of DNA in a density gradient produced by a gravitational field, created by an ultracentrifuge, acting on a concentrated solution of cesium chloride. Their experimental results showed that at equilibrium the DNA formed a small band in the centrifuge cell. Within the band the DNA concentration was distributed in a Gaussian fashion with respect to distance along the centrifuge cell.