HEMOGLOBIN INHERITANCE IN INBRED MOUSE STRAINS, I. STRUCTURAL DIFFERENCES

BY JOHN J. HUTTON, JOHN BISHOP,† RICHARD SCHWEET, AND ELIZABETH S. RUSSELL

UNIVERSITY OF KENTUCKY COLLEGE OF MEDICINE, LEXINGTON, AND ROSCOE B. JACKSON MEMORIAL LABORATORY, BAR HARBOR, MAINE

Communicated by Sewall Wright, June 22, 1962

Studies of the nature and manner of inheritance of differences in the primary molecular structure of mouse hemoglobins are of both theoretical and practical interest. The mouse system may yield results very helpful in understanding certain aspects of human hemoglobin variation which are not open to direct experimental test, since planned genetic crosses and studies of developmental changes can be made in the mouse. Although up to the present no studies of primary chain structure, based on the "fingerprint" method, have been reported for mice, variation in hemoglobin structure has been suspected from the existence of inherited differences in electrophoretic pattern.

Ranney and Gluecksohn-Waelsh[1] used paper electrophoresis to investigate the hemoglobins of several inbred mouse strains and found two distinct electrophoretic patterns. One of these, the "single" pattern, showed one discrete electrophoretic component. The second, the "diffuse" pattern, showed a trailing edge on either side of a major component, suggesting the presence of one or more minor components. Subsequent breeding experiments[2] showed the single and diffuse hemoglobins to segregate as though controlled by allelic genes. More recently, the locus for single versus diffuse has been shown to be linked to the gene for albinism in linkage group I.[3] Single and diffuse hemoglobins of inbred mouse strains have now been characterized by several different electrophoretic techniques.[4–8] In each case, single hemoglobins contained a single major component, while diffuse hemoglobins have been observed to have two,[5] three,[2] or more[6] significant components. It was not possible to decide in each case whether all the components were real or whether denaturation could account for some of them.

To provide a basis for future genetic studies using the *structure* of each chain as marker, the differences between the hemoglobins of several inbred mouse strains were studied by various chemical methods. The results show that a number of different inbred strains have structurally different hemoglobins and that diffuse hemoglobins consist of a mixture of two different hemoglobins.

Materials and Methods.—All the mice used in this investigation were produced at the Roscoe B. Jackson Memorial Laboratory and came from inbred colonies regularly maintained there. The inbred strains used were C57BL/6J, SEC/1-*Se*Re, AKR/J, DBA/2J, and C3H/HeJ. These are referred to in the text as C57BL, SEC, AKR, DBA, and C3H, respectively. In addition, mice from a stock (FL/Re) carrying the flexed gene, inbred through 17 generations of brother-sister mating, were used. These are referred to as FL. C57BL and SEC are single-hemoglobin strains, electrophoretically, while AKR, DBA, C3H, and FL carry diffuse hemoglobin.[3–9]

Preparation of hemoglobin: The brachial artery was severed and the blood which accumulated between the rib-cage and the skin was drawn into a heparinized pipette. The erythrocytes were sedimented (3,000 × *g*, 15 min) and washed 3 times with isotonic saline. The cells were lysed with 4 volumes of ice-cold distilled water, and all subsequent operations were carried out at 4°. To this suspension, 0.05 vol. of 0.2 *M* $MgCl_2$ and 0.05 vol. of 3 *M* KCl were added. After 15 min

the stroma were removed by centrifugation (50,000 × *g*, 30 min). The supernatant was dialyzed immediately, as described below, or stored at 5°. Hemoglobin prepared in this way from single strains was homogeneous in the ultra-centrifuge. Radioactive hemoglobin was prepared by incubating reticulocytes[10] with C^{14}-leucine *in vitro*,[11] followed by the treatment described above.

Resolution of components of mixed hemoglobins: The hemoglobin solution was dialyzed (18 hr at 4°) against "Developer No. 2" of Allen *et al.*[12] at 9/10 concentration. Columns of Amberlite CG-50, Type II resin, were equilibrated and developed at 4° with the same developer. Hemoglobin (10–15 mg) was applied to an analytical column (0.9 × 40 cm) and eluted with 300–400 ml of developer in 36 hr. Fractions of 5.0 ml were collected and the optical density read at 280 and 415 mμ. Hemoglobin (300–600 mg) was applied to preparative columns (5.2 × 35 cm) and eluted with 7 liters of developer in 100 hr. Selected tubes were pooled, dialyzed against 15 volumes of distilled water for 18 hr at 4°, and then concentrated by stirring for 2 hr at 4° with 0.005 volume of a slurry of Amberlite CG-50 resin (previously equilibrated with Developer No. 2[12] at 2/10 concentration). After allowing the resin to settle, most of the clear supernatant was decanted and the resin was collected by centrifugation. The hemoglobin was eluted by stirring 3 times with 0.5 volume of 0.5 *M* potassium phosphate buffer, pH 7.0, at 4° and centrifuging. The recovery by this procedure was approximately 80%.

Polypeptide chain separation: Globin was prepared by the method of Rossi-Fanelli *et al.*[13] with the omission of the bicarbonate dialysis step. The polypeptide chains were separated on columns of CG-50 Type II resin by a method slightly modified from that of Wilson and Smith.[14–16]

Characterization of the C- and N-terminal groups: The N-terminal amino acid of intact hemoglobin was studied by the dinitrofluorobenzene method.[17] The penultimate amino acid at the N-terminus of each chain was determined by a procedure slightly modified from that of Wilson and Smith.[18] To identify the C-terminal peptide of each chain, 5 mg of separated chain was digested with carboxypeptidases A and B in a final volume of 0.45 ml according to the procedure of Guidotti,[19] except that the total digestion time was 1 hr. After digestion, the residual protein was precipitated by the addition of 20 volumes of acid acetone (0.6 ml of 4 *N* HCl per 100 ml acetone), washed with ether, and dried. The carboxypeptidase-treated chain was then digested with trypsin and fingerprinted.

Fingerprinting: The sample (hemoglobin, globin, or polypeptide chain) was adjusted to 5–10 mg/ml in 0.2 *M* ammonium bicarbonate, pH 8.0, denatured by heating and digested with TCA-treated trypsin (Worthington Biochemical Co.) according to Hill *et al.*,[20] except that the time of digestion was extended to 4 hr at 37°. The insoluble material was removed by centrifugation and the supernatant was stored at −15°. In some cases, the "tryptic" core was isolated and digested with chymotrypsin.[21] Fingerprints were prepared by the method of Katz *et al.*[22] After electrophoresis, the papers were dried at 90° for 30 min and stained either with ninhydrin or with special stains for arginine, histidine, tyrosine, and tryptophan. The procedures used were those collated by Chernoff and Liu.[15] The peptide patterns of the α- and β-chains of adult human hemoglobin prepared by these methods were used as standards for comparison with various mouse hemoglobins (Fig. 1). The human peptides were numbered by comparison with the data of Baglioni.[23]

Results.—Chromatographic analysis: Each of the single hemoglobins (C57BL and SEC) showed a single major hemoglobin peak upon chromatography on Amberlite CG-50 resin (Fig. 2*a*). A very minor component in all cases which was not retained on the column was also observed but has not been studied. All four diffuse hemoglobins (AKR, FL, C3H, and DBA) showed a major peak in the same region as the single hemoglobin plus an additional minor component (Fig. 2*b*), which was approximately 20% of the total hemoglobin. It was of some interest to determine whether the major hemoglobin component of diffuse strains was identical to the single hemoglobin of C57BL. When mixtures of C57BL and AKR hemoglobins were chromatographed, a single *major* peak and one minor peak of optical density were observed (Fig. 2*b*). When C^{14}-labeled C57BL hemoglobin (labeled by incubating C57BL reticulocytes[10] with C^{14}-leucine *in vitro*[11])

was mixed with unlabeled AKR hemoglobin and chromatographed, the peaks of optical density and radioactivity in the major component did not coincide perfectly (Fig. 2*b*). Essentially the same result was obtained when labeled C57BL and unlabeled FL hemoglobin were employed. In a control experiment, when labeled and unlabeled C57BL hemoglobins were employed, the peaks of optical density and radioactivity coincided perfectly (Fig. 2*a*). This asymmetric distribution of specific activity across the major peak in diffuse-single mixtures suggests that the major component of the diffuse strains is not identical with hemoglobin of the single mouse, C57BL.

Thus, the chromatographic evidence shows that hemoglobin of each of four diffuse strains contains two components (in addition to the chromatographic "front") while hemoglobin from each of two single strains has only one component. Furthermore, the hemoglobin of C57BL is different in chromatographic properties from both components of AKR and FL.

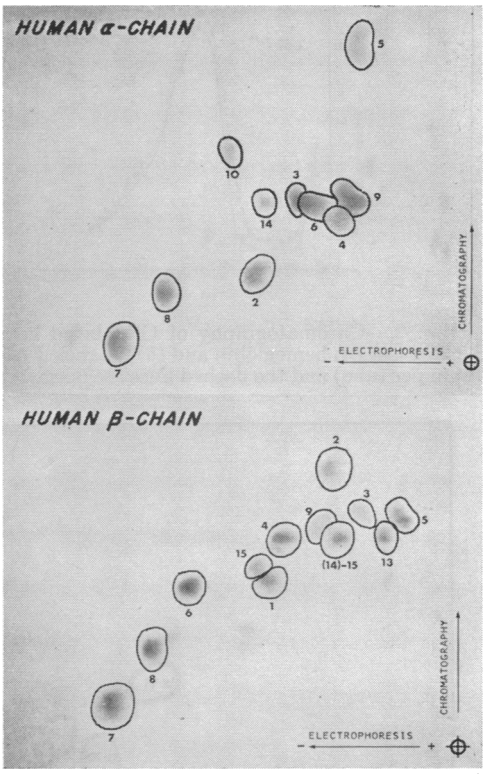

FIG. 1.—Peptide patterns of the alpha and beta chains of adult human hemoglobin. See the text for description of solvent systems used. Peptides were numbered by comparison with the data of Baglioni,[23] and based on position and staining characteristics. It will be noted that several peptides have not been identified as yet.

Peptide patterns of hemoglobins: Four inbred strains were studied by the "fingerprinting" technique. The individual, separated components of AKR and FL and the single hemoglobins of C57BL and SEC were used for these studies. The differences observed were confirmed in most cases (see below) by studies of "fingerprints" of the separated α- and β-chains.[16] All of the six hemoglobins of these strains were found to be different (Table 1). The α-chains of FL-1 and FL-2 were the same, but differed from the α-chains of the other four hemoglobins. Four different β-chains were found. These were (1) C57BL, (2) SEC, (3) AKR-1 and FL-1, and (4) AKR-2 and FL-2. A composite tracing showing all the peptides studied in the four strains of mice is given in Figure 3. Most of the peptides were clearly separated, but identification was confirmed where possible by specific staining reactions.[16]

In order to assign peptide differences to the proper chain, the α- and β-chains of each strain of mice were separated as described.[16] For AKR and FL, the separated chains contained the components of both hemoglobin peaks. No new spots were found in any of the separated α-chains, compared to the intact hemoglobins. Peptide $\alpha 4$ showed the presence of histidine in fingerprints of FL hemoglobin. The new peptide (α4H) was considered to be present in both FL-1 and

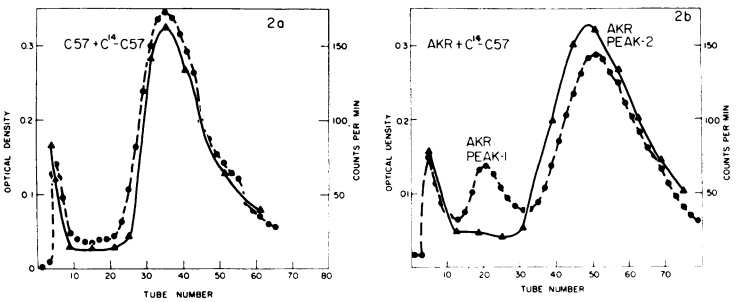

FIG. 2.—Chromatography of C^{14}-labeled hemoglobin from strain C57BL mixed with (*a*) unlabeled C57BL hemoglobin and (*b*) unlabeled AKR hemoglobin. The solid lines are radioactivity (cpm per tube) and the dashed lines are optical density at 415 mμ.

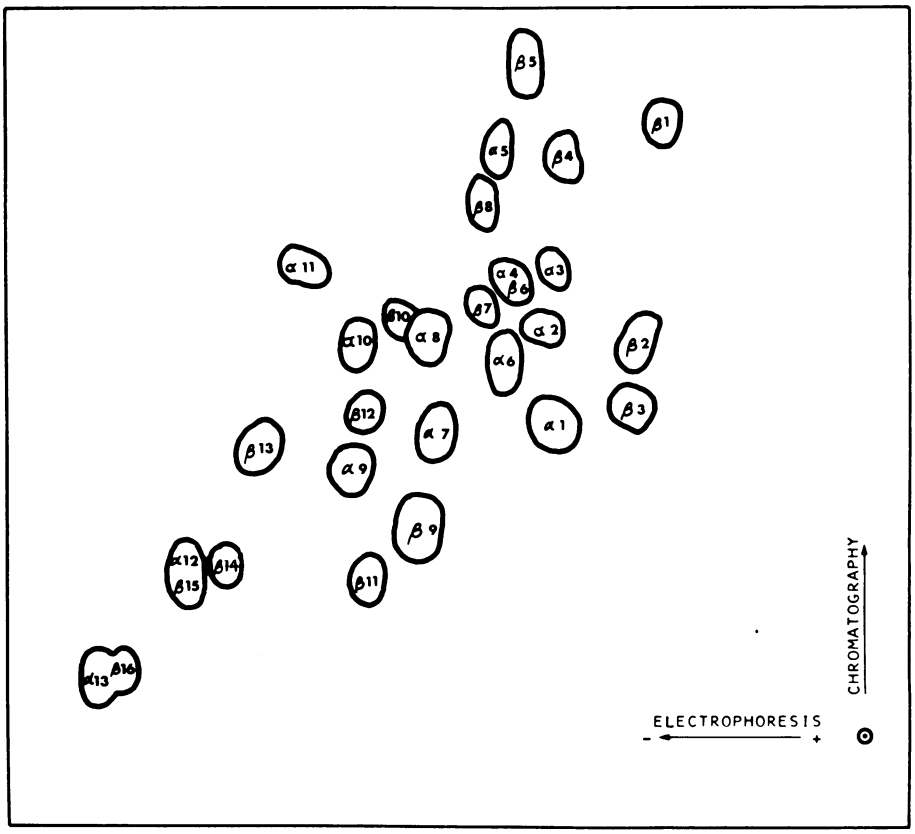

FIG. 3.—Composite tracing containing all of the peptides studied. See the text and Table 1 for details of the assignment of peptides to various mouse strains and alpha or beta chains. Peptides α2 and α6 were detected by the spray for *his*, since the ninhydrin color at the concentration used was faint. Peptides α12 and β15 are lysine.

FL-2 as this histidine-containing spot was found on fingerprints of both *intact* separated FL-1 and FL-2 hemoglobins.

The fingerprints of SEC (single) hemoglobin contained peptide β6 and lacked peptide β7 (Table 1), in contrast to C57BL hemoglobin, which contained β7

TABLE 1

DISTRIBUTION OF PEPTIDES IN VARIOUS MOUSE HEMOGLOBINS

	Peptide number	Source of Hemoglobin: C57BL	SEC	AKR-1	AKR-2	FL-1	FL-2
α-chain	4	+	+	+	+	−	−
	4H	−	−	−	−	+	+
β-chain	2	+	+	+	−	+	−
	3	−	−	−	+	−	+
	4	+	+	−	+	−	+
	5	−	−	+	−	+	−
	6	−	+	+	−	+	−
	7	+	−	−	+	−	+
	8	+	+	−	−	−	−

The basis for these assignments is given in the text. Peptides which are not specifically mentioned in this table were found in all of the α- or all of the β-chains studied.

and lacked β6 (Table 1). With respect to these two peptides, AKR-1 and FL-1 were like SEC, while AKR-2 and FL-2 followed the C57BL pattern.

In fact, the β-chains of AKR-1 and FL-1 were identical, as were the β-chains of AKR-2 and FL-2 (Table 1). Therefore, discussion of further β-chain differences in AKR-1 and AKR-2 will be understood to apply also to FL-1 and FL-2. Two peptides, β3 and β4, were present in AKR-2 and not in AKR-1, while β2 and β5 were present in AKR-1 and not in AKR-2. It is probable that β2 and β3 are from homologous regions of the chains since where one was present, the other was absent (SEC and C57BL contained β2, but not β3) and both contained arginine. Peptides β4 and β5 may also be homologous (SEC and C57BL contained β4, but not β5), but β4 contained histidine, while β5 did not. This was true also for the β6 and β7 pair which may be homologous, but only β7 contained histidine. Finally, peptide β8, a tryptophan peptide, was present in C57BL and SEC but absent from both components of AKR and FL.

These differences have been checked many times in fingerprints of both intact hemoglobins and separated chains.[16] No discrepancies were found in comparing fingerprints of separated chains with those of intact hemoglobins. However, the identities of peptides with the same mobilities, and especially the homologies noted, must be considered tentative until confirmed by amino acid analysis of these peptides. In particular, no peptide has been found in AKR or FL to correspond to peptide β8 present in SEC and C57BL. As a further check on these results, chymotryptic digests of the tryptic "cores" of all the hemoglobins were electrophoresed in one dimension. No tryptophan peptides were found and the patterns obtained were the same for all four strains. A number of fingerprints with butanol-pyridine-water as the chromatographic solvent were run. These confirmed the results reported here, but were less satisfactory than the solvent used generally.

Characteristics of the isolated chains: Only valine was found as the N-terminal amino acid of intact C57BL hemoglobin studied by the dinitrofluoro-benzene method, although this must be confirmed since quantitative studies were not done. Each of the separated chains of C57BL globin was analyzed for the penultimate amino acid. One chain appeared to have leucine as the penultimate amino acid and is termed the α-chain, whereas the β-chain may contain glycine following valine.[16]

After carboxypeptidase digestion, fingerprints of the α-chain of C57BL did not contain peptide α10. No new peptides were seen, and no other peptide was

TABLE 2

CHROMATOGRAPHIC COMPARISON OF MOUSE AND HUMAN PEPTIDES

	Peptide Number		Staining reaction	Sequence of the human peptide†
	mouse	human*		
α-chain	6	T-4	arg, his, tyr	val-arg, 15 residues
	8	T-3	tryp	ala-ala-tryp-gly-lys
	10	T-14	arg, tyr	tyr-arg (C-terminal)
	11	T-10	arg	leu-arg
	12	T-8	—	free lysine
	13	T-7	his	gly-his-gly-lys
β-chain	8	T-2	tryp	ser-lys, 9 residues
	12	T-15	his, tyr	tyr-his (C-terminal)
	13	T-6	—	val-lys
	15	T-8	—	free lysine
	16	T-7	his	ala-his-gly-lys

* Nomenclature proposed at the Hemoglobin Structure Workshop, Boston, December, 1960. Numbers were assigned to the human peptides using the data of Baglioni.[23]

† Sequences were derived from data summarized by Watson and Kendrew.[26]

distinctly affected. This peptide contains arginine and tyrosine (Table 2). Upon digestion of the β-chain, peptides β12 and β10 disappeared. In some digestions, β10 was present when β12 was absent, suggesting that β12 is C-terminal and β10 is adjacent to it. Several new peptides appeared after carboxypeptidase digestion of this chain, but these were not characterized.

Comparison of mouse and human hemoglobins: At the present time, there is a great deal of interest in the comparative chemistry of homologous proteins from various species of animals.[24, 25] The peptide patterns of mouse (C57BL) and human hemoglobins were compared by fingerprinting digests of each and *mixtures* of mouse and human chains. Several peptides moved to identical locations and reacted identically with the various specific amino acid sprays which were used (Table 2). For most of the mouse-human peptide pairs which moved to the same position, the peptide from human hemoglobin[26] is known to be relatively small (Table 2). The criteria for identity which have been employed do not establish definitively that the peptides in question are common to both organisms. It will be of interest to investigate the amino acid composition of these mouse peptides for comparison with the homologous human peptides.

Discussion.—The present investigation, based on "fingerprinting," combined with chain separation and isolation of hemoglobins by column chromatography, provides direct evidence of differences in primary molecular structure of mouse hemoglobins. Six chemically distinct hemoglobins have been isolated from the erythrocytes of adult mice from four inbred strains. This sample may suggest too high an estimate of the frequency of mouse hemoglobin variations, since the inbred strains used in this study were selected in part on the basis of previous investigations suggesting they might show differences in hemoglobin structure.[1–9, 27] Alternatively, since some structural variations may be "silent" under the analytical methods used, mouse hemoglobins may vary even more than our results suggest. There is little doubt that some hemoglobin variation exists in nature.[3, 28] One generalization we can make is that no hematological abnormalities have been found in adult mice carrying any of the varying hemoglobin types which we have observed. This is different from the current human situation, where practically all hemoglobin deviations which have been described seem to be hemoglobinopathies.

In two of the tested inbred strains, AKR and FL, previously described as having

electrophoretically diffuse hemoglobin,[4, 9] it was possible by column chromatography to separate two distinct hemoglobins, present in decidedly unequal proportions (roughly 80% and 20%). Erythrocytes from each of the other two inbred strains tested, C57BL and SEC, known to have electrophoretically homogeneous (single) hemoglobin,[1, 4, 28] contained only one kind of hemoglobin, but a different hemoglobin was found in each strain. In all, four different beta chains have been identified by fingerprints, β^{C57} in C57BL, β^{SEC} in SEC, β^{AKR-1} in the minor component of AKR and FL, and β^{AKR-2} in the major component of AKR and FL. Alpha chain fingerprints of hemoglobins from three of the inbred strains, AKR, C57BL, and SEC, appeared identical, but that from the fourth inbred strain, FL, showed a difference in the staining properties of one peptide.[16]

The mouse hemoglobins, like other mammalian hemoglobins, are made up of two distinct species of polypeptide chains. Because of their striking similarities to the alpha and beta chains of human hemoglobin, these are also called alpha and beta. The mouse and human alpha chains are eluted first from CG-50 resin in a urea gradient.[16] Both appear to have the sequence valine-leucine at the N-terminus and probably the same peptide (tyrosyl-arginine) at the C-terminus. Similarly, mouse and human beta chains are eluted second, have valine *not* followed by leucine at the N-terminus, and probably have the same dipeptide (tyrosyl-histidine) at the C-terminus. In addition, several identical sequences appear to occur in each pair of chains (Table 2). The interspecies correspondence of alpha and beta is considerably greater than the intraspecies correspondence of alpha versus beta chains. Thus, there is considerable evidence for homology, or origin from a common ancestral gene, for the alpha-chain determining genes of mouse and man, and similarly for the beta-chain determining genes. Presumably, the hemoglobin of the common ancestor of mouse and man already had differing alpha and beta chains. Such recent homology does not, of course, rule out the possibility of an ultimate common origin for genes at the alpha- and beta-determining loci. Present evidence suggests that in the mouse, as in man, the beta-chain is more variable than the alpha-chain, which may mean either that mutations affecting the latter occur less frequently, or that they are selected against more stringently.

In the mouse, as in man, hemoglobins have been found which appear to differ by position or staining properties of a single peptide and possibly, therefore, by a single amino acid. It should be remembered, however, that further differences may be found on more complete analysis of these globin chains. The alpha-chain of FL differs from that of the other three inbred strains examined by the presence of histidine in peptide $\alpha 4$.

The beta chain of SEC appears to differ from that of C57BL only by substitution of peptide $\beta 6$ for $\beta 7$. This difference in the β-chain may or may not be responsible for the marked difference in solubility properties of the hemoglobins from the two strains, described by Popp.[27]

Analysis of the "diffuse" characteristic: For some time, a puzzling feature of the genetics of mouse hemoglobin variation has been the apparent unit segregation of a gene controlling "diffuse/single" electrophoretic pattern. Proof of the existence of two distinct hemoglobins, with differing β-chains, in homozygous mice with diffuse hemoglobin pattern clarifies but does not solve the problem of the nature of action of genes at this locus. Two distinctly different β-chains are synthesized in these

mice, only a single β-chain in mice with single hemoglobin. Any explanation advanced for the diffuse pattern thus almost certainly will invoke the concept of duplication and differentiation of the locus which directly controls β-chain structure. There is a considerable difference (three peptide pairs) between the β-chain fingerprints of the two hemoglobins from AKR or FL mice, and a similar difference between each of these and the β-chain fingerprints of either C57BL or SEC.

The double hemoglobin system in a homozygous diffuse mouse is in some ways similar to the human A_1A_2 double hemoglobin system. The structural relation between major and minor hemoglobin components in diffuse mice approaches that between human A_1 and A_2. Mouse β^{AKR-1} and β^{AKR-2} differ by three peptide pairs, human β^A and delta^A by four peptide pairs.[29] The minor component comprises approximately 20% of the total hemoglobin in a homozygous diffuse mouse, 1–3% in a normal human.[30] In the mouse system, there is unit segregation of two β-chains versus a single β-chain. A tempting hypothesis is that house mice as a species are in the process of evolving toward a double-hemoglobin system analogous to that in the human. The observed frequencies of genes for diffuse/single would then be temporary, representing a particular stage in this evolution.

The structural differences reported here have been used as markers in genetic studies of hemoglobin inheritance.[31]

Summary.—Six different hemoglobins have been isolated from four strains of homozygous, inbred mice. Analysis of peptide patterns indicated that four different beta chains and two different alpha chains were present. Two strains, AKR and FL, contained two major hemoglobin types. The separated chains of mouse hemoglobin were similar in C-terminal, N-terminal, and certain internal peptides to the alpha and beta chains of human hemoglobin. Possible evolutionary implications of mouse hemoglobin variations were discussed.

* These investigations were supported by grant C(1074) from the U.S. Public Health Service and grants E-76 and E-162 from the American Cancer Society to the Roscoe B. Jackson Memorial Laboratory, and by grant H-5293 from the U.S. Public Health Service and grant G-11400 from the National Science Foundation to the University of Kentucky College of Medicine. The authors wish to thank Marcia L. Craig for her interest in and useful discussions of this manuscript.

† Present address, Institute of Animal Genetics, West Mains Road, Edinburgh, Scotland.

[1] Ranney, H. M., and S. Gluecksohn-Waelsh, *Ann. Hum. Genetics*, **19**, 269 (1955).

[2] Gluecksohn-Waelsh, S., H. M. Ranney, and B. F. Sisken, *J. Clin. Invest.*, **26**, 753 (1957).

[3] Popp, R. A., and W. St. Amand, *J. Heredity*, **51**, 141 (1960).

[4] Russell, E. S., and P. S. Gerald, *Science*, **128**, 1569 (1958).

[5] Welling, W., and D. W. van Bekkum, *Nature*, **182**, 946 (1958).

[6] Rosa, J., G. Schapira, J. C. Dreyfus, J. deGrouchy, G. Mathe, and J. Bernard, *Nature*, **182**, 947 (1958).

[7] Popp, R. A., and G. E. Cosgrove, *Proc. Soc. Exper. Biol. and Med.*, **101**, 754 (1959).

[8] Gluecksohn-Waelsh, S., *J. Cell. Compar. Physiol.*, **56**, Suppl. 1, 89 (1960).

[9] Craig, M. L., and E. S. Russell, unpublished data.

[10] Bishop, J., G. Favelukes, R. S. Schweet, and E. S. Russell, *Nature*, **191**, 1365 (1961).

[11] Bishop, J., J. Leahy, and R. Schweet, these PROCEEDINGS, **46**, 1030 (1960).

[12] Allen, D. W., W. A. Schroeder, and J. Balog, *J. Amer. Chem. Soc.*, **80**, 1628 (1958).

[13] Rossi-Fanelli, A., Antonini, E., and Caputo, A., *Biochim. Biophys. Acta*, **30**, 608 (1958).

[14] Wilson, S., and D. B. Smith, *Canad. J. Biochem. Physiol.*, **37**, 405 (1959).

[15] Chernoff, A. I., and J. C. Liu, *Blood*, **17**, 54 (1961).

[16] Hutton, J. J., J. Bishop, R. Schweet, and E. S. Russell, *Biochim. Biophys. Acta*, submitted for publication.

[17] Sanger, F., *Biochem. J.*, **39**, 507 (1945).
[18] Wilson, S., and D. B. Smith, *Federation Proc.*, **16**, 766 (1957).
[19] Guidotti, G., *Biochim. Biophys. Acta*, **42**, 177 (1960).
[20] Hill, R. L., R. T. Swenson, and H. C. Schwartz, *J. Biol. Chem.*, **235**, 3182 (1960).
[21] Hunt, J. A., and V. M. Ingram, *Biochim. Biophys. Acta*, **28**, 546 (1958).
[22] Katz, A. M., W. J. Dreyer, and C. B. Anfinsen, *J. Biol. Chem.*, **234**, 2897 (1959).
[23] Baglioni, C., *Biochim. Biophys. Acta*, **48**, 392 (1961).
[24] Hunt, J. A., *Nature*, **183**, 1373 (1960).
[25] Zuckerkandl, E., R. T. Jones, and L. Pauling, these PROCEEDINGS, **46**, 1349 (1961).
[26] Watson, H. C., and J. C. Kendrew, *Nature*, **190**, 670 (1961).
[27] Popp, R. A., submitted to *J. Hered.*
[28] Petras, M., personal communication.
[29] Ingram, V. M., *Nature*, **189**, 704 (1961).
[30] Ingram, V. M., and A. O. W. Stretton, *Nature*, **190**, 1079 (1962).
[31] Hutton, J., J. Bishop, R. Schweet, and E. Russell, these PROCEEDINGS, in press.

*DIFFERENCES IN FREE AMINO ACID CONTENT OF SEEDLINGS OF AWNED AND HOODED BARLEY, AND THEIR ALTERATION BY CHLORAMPHENICOL TREATMENT**

BY IGOR SARKISSIAN, S. S. SHAH, AND G. LEDYARD STEBBINS

UNIVERSITY OF WEST VIRGINIA AND UNIVERSITY OF CALIFORNIA, DAVIS

Communicated July 5, 1962

The antibiotic chloramphenicol has been used extensively in recent years for studies of protein synthesis. Hahn and Wisseman[1] have shown inhibition of formation of adaptive enzymes by chloramphenicol in *E. coli*. Wisseman *et al.*[2] have demonstrated suppression of incorporation of glycine-1-C^{14} into protein by chloramphenicol. Smith[3] pointed out that chloramphenicol inhibited utilization of certain free amino acids in *Staphylococcus aureus*. Studies of Gale and Folkes[4] and Wolff[5] have established that chloramphenicol inhibited protein synthesis in bacteria and beans respectively without altering the rate of synthesis of nucleic acids.

In some preliminary experiments of Stebbins and Khush (unpublished), chloramphenicol was used to study the effect of reduction of protein synthesis on flowering of two genotypes of barley. A significant decrease in the number of days to flowering in plants treated with 0.01 per cent chloramphenicol was observed under long day conditions and full illumination with natural daylight. Furthermore, preliminary analyses of protein and nucleic acid content in meristematic tissues indicate that at some stages of development the ratio of protein/DNA is lower in hooded (*KK*) than in awned (*kk*) plants (Stebbins *et al.*, unpublished). Consequently, an analysis of the specific effects of this antibiotic on the metabolism of the two genotypes should provide evidence for a better understanding of the action of the hooded (*K*) gene. The following is a report on the effects of chloramphenicol in altering the free amino acid content of hooded and awned seedlings.

Materials and Methods.—Seeds of the two genotypes were obtained from C. A. Suneson, Department of Agronomy, University of California, Davis. The *k* gene was represented by Atlas 46, a six-rowed spring variety. The hooded genotype was produced by seven generations of back-