to flow through the cell constantly during the course of the titration. The effluent solution from the appropriate delivery tube was then allowed to flow into the solution containing the sample.

Typical results which have been obtained with this technique are summarized in the table. The generator electrolyte which, on electrolysis, furnished the hydrogen and hydroxyl ions for the acid-base titrations was 1.0 *M* sodium sulfate and that which furnished the iodine for the arsenious oxide titration was 1.0 *M* potassium iodide. A constant generation current of 0.2500 amp. was maintained in all titrations. The reproducibility of the analyses is excellent. The finite time which is required for mixing apparently causes a significant delay in indicator response at the equivalence point. This delay is believed to be the primary source of the small but consistent positive error which was observed in all determinations. Automatic devices which will anticipate the approach of the end-point and thus permit greater accuracy in the determination of the generation time are now being studied in the hope of eliminating this systematic error.

Further studies on the applicability of this technique to a wide variety of titrations, including precipitation titrations, are now in progress in this Laboratory.

[1] Farrington, P. S., and Swift, E. H., *Anal. Chem.*, **22**, 889 (1950). References to previous work by Swift and his coworkers are given in this paper.

[2] Cooke, W. D., and Furman, N. H., *Ibid.*, **22**, 896 (1950).

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*A NEW INHERITED ABNORMALITY OF HUMAN HEMOGLOBIN**

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In the course of a study of the inheritance of the sickling phenomenon[1] two families were encountered in which there occurred one or more children with a hematological picture which resembled that of sickle cell disease but was of less severity. The situation further differed from that usually encountered in sickle cell disease in another important respect. It has been shown that in the great majority of instances both parents of a child with

sickle cell disease exhibit the sickle cell trait.[1, 2] In each of these two families, however, the erythrocytes of only one parent could be induced to sickle.

Electrophoretic studies[3, 4] have demonstrated the presence of an inherited abnormality of hemoglobin in sickle cell disease and sickle cell trait. Similar studies of various members of these two anomalous families have now been carried out and have led to the recognition of a new inherited abnormality of hemoglobin. The detailed results of the electrophoretic analyses are given in table 1. In family A the hemoglobin of the propositus was found to consist of three electrophoretic components.

TABLE 1

	AGE	SICKLING TEST	PATTERN (FIG. 2)	HEMOGLOBIN COMPONENT,[a] % NORMAL	SICKLE	NEW COMP.
Controls						
Normal	..	−	*a*	100	−	−
Sickle cell anemia	..	+	*b*	−	100[b]	−
Sickle cell trait	..	+	*c*	55–76	24–45[b]	−
Family A						
Father (P. C., Sr.)	29	−	*d*	64.7	−	35.3
Mother (E. M. C.)	28	+	*c*	66.5	33.5	−
Brother (P. C., Jr.)	6	−	*d*	66.4	−	33.6
Brother (R. G. C.)	4	−	*a*	100	−	−
Propositus 1, ♀ (P. A. C.)	3	+	*f*	13	39	48
Family B						
Father (J. W.)	33	−	*d*	69.8	−	30.5
Mother (D. F. W.)	31	+	*c*	68.9	31.1	−
Propositus 2, ♀ (R. W.)	12	+	*e*	−	47	53
Propositus 3, ♂ (T. W.)	10	+	*e*	−	50	50
Brother	8	−	*a*	100	−	−

[a] The authors are indebted to Dr. Ibert C. Wells for carrying out these computations.

[b] Wells, I. C., and Itano, H. A., in press. In some cases the hemoglobin of individuals with the clinical diagnosis of sickle cell anemia contains a small fraction (5 to 20 per cent) of normal hemoglobin.

The mobilities of two of the components correspond to those of the hemoglobins from normal and sickle cell anemic individuals, respectively. The other component, which migrates as a more positive ion than either normal or sickle cell anemia hemoglobin, has hitherto not been encountered. In family B the hemoglobin of each of the two propositi was found to be a mixture of two types, namely, that characteristic of sickle cell disease and the new fraction. Electrophoretic studies of the remaining members of the families revealed that in each family the hemoglobin of the parent whose erythrocytes sickled exhibited the electrophoretic findings typical of sickle cell trait,[4,5] while the hemoglobin of the other (non-sickling) parent was a mixture of two types, normal and the new component detected in the pro-

positi. The families involved in the study are pictured in figure 1. The propositi through whom the study was initiated are indicated by arrows.

Carbonmonoxyhemoglobin solutions were prepared and analyzed electrophoretically in the Tiselius apparatus; exact details of the preparation and analysis have recently been reported.[5] The carbonmonoxyhemoglobin solutions were diluted to 1.0 per cent concentration and dialyzed against cacodylate-sodium chloride buffer[5] of ionic strength 0.1 and pH 6.50 for eighteen hours. The final scanning diagrams were taken after fifteen hours of electrophoresis at 1.5°C. at a potential gradient of 3.49 to 3.60 volts per centimeter. The mobilities in the ascending boundaries of the carbonmonoxyhemoglobins from normal and sickle cell anemic individuals

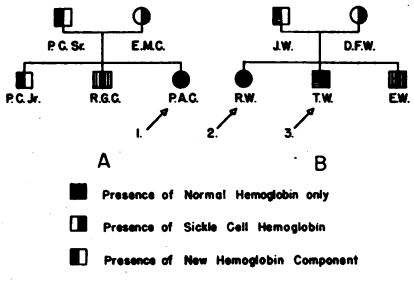

FIGURE 1

The two families under study.

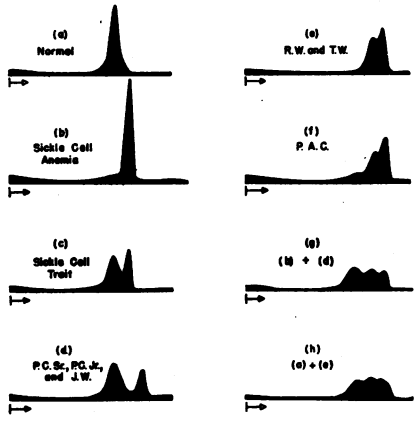

FIGURE 2

The Longsworth scanning diagrams of the carbonmonoxyhemoglobins of the individuals under study compared to the scanning diagrams of the carbonmonoxyhemoglobins of individuals known to be hematologically normal or to have sickle cell anemia or sickle cell trait.

under these conditions are 2.4×10^{-5} and 2.9×10^{-5} cm./sec. per volt/cm., respectively. The mobility of the new component is 3.2×10^{-5} cm./sec. per volt/cm.

The Longsworth scanning diagrams of the ascending boundaries are shown in figure 2. The various components are visible in the scanning diagrams of the descending boundaries, but the resolution of the peaks is poor.[5] Patterns (*a*) and (*b*) are those of the hemoglobins from normal and sickle cell anemic individuals, respectively, obtained under the experimental conditions described above. The hemoglobins of two of the siblings (R. G. C. and E. W.) of the propositi showed the normal pattern. Pattern (*c*), a typical sickle trait pattern containing both the normal and sickle cell

anemia hemoglobins, is similar to those of the hemoglobins of the sickling parents (E. M. C. and D. F. W.) in this study. Pattern (*d*) shows two components, normal hemoglobin and the new component, but lacks the sickle cell anemia component. This pattern was found in both the non-sickling parents (P. C., Sr., and J. W.) and in a brother (P. C., Jr.) of one of the propositi. The hemoglobins of two of the propositi (R. W. and T. W.) gave the two-component pattern, (*e*), which appears to contain sickle cell anemia hemoglobin and the new component but not normal hemoglobin. The hemoglobin of the third propositus (P. A. C.) resolved into all three components, as shown in pattern (*f*). The analysis of a solution prepared by the addition of one part of sickle cell anemia hemoglobin to two parts of P. C., Sr.'s hemoglobin resulted in pattern (*g*), which clearly shows three components. A similar result, pattern (*h*), was obtained by the addition of one part of normal hemoglobin to two parts of R. W.'s hemoglobin.

The foregoing results lead to the conclusion that a previously unreported protein component, differing in electrophoretic mobility from the hemoglobins of normal and sickle cell anemic individuals, is present in considerable amounts in the erythrocytes of certain individuals. Other observations indicate that this component is indeed another abnormal hemoglobin. The presence in the erythrocytes of a protein other than hemoglobin to the extent of from one-third to one-half of the total protein contents of the cells would cause a markedly low mean corpuscular hemoglobin concentration[6] instead of the normal values which have been observed. Dilution of the different hemoglobin preparations to 1.0 per cent concentration was based on a spectrophotometric determination using a standard curve obtained at 540 mμ with normal hemoglobin.[5] Subsequent electrophoretic analyses of these diluted solutions revealed that the total area of the scanning diagram was in each case equal to that obtained with a 1.0 per cent solution of normal hemoglobin. This indicates that within the error of this method the molecular extinction coefficient at 540 mμ is the same for all three components, assuming equal molecular weights. It may be noted that the color of the fast moving component in the ascending boundary was observed to be the same as that of normal carbonmonoxyhemoglobin.

The data which have been presented suggest that the tendency to form the new hemoglobin component is inherited as if due to a single dominant gene. The effect of this gene in the homozygous condition is not yet known; it is possible that it corresponds to some already recognized hematological syndrome. The relationship of this gene to the sickle cell gene is not clear at the present time. The hematological picture in the individuals who may be postulated to have received both genes is explicable either on the basis of the factor interaction on the part of two independent genes, or as a consequence of multiple allelism.

A detailed hematological delineation of this new entity is in progress. Physicochemical investigations to further characterize the new hemoglobin component are also in progress.

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‡ Contribution No. 1457.

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[5] Wells, I. C., and Itano, H. A., *J. Biol. Chem.*, in press.
[6] Perutz, M. F., *Nature*, **161**, 204 (1948).

BIOELECTRIC POTENTIALS AS A MEASURE OF RADIATION INJURY

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The criteria used to measure the biological effects of radiation by most workers as well as by the writer are in a sense purely biological in character, such as the size of the plant or embryo, the number of surviving colonies and the like. In 1942, Professor A. L. Romanoff of Cornell University worked with the writer on the effects of x-rays on the diameter of the developing blastoderm of the chick egg and also on the effects of x-rays on the bioelectric potential of the blastoderm.[1, 2] Dr. Romanoff and the writer were greatly impressed by the similarity of the curves representing the variation of the two parameters with dosage. The experiments seemed to indicate that the bioelectric potentials may be as reliable a measure of the effectiveness of radiation as the size of the embryo.

Because of the War, work was not resumed in this field until recently. In 1947 Jones[3] measured the variation of the bioelectric potential of seeds of corn and beans with x-ray dosage. The shape of the curves was exponential, typical of the variation of many relevant parameters with dosage. He also confirmed the results of Nelson and Burr[4] that the more vigorous strains of seeds have a higher potential. Since radiation affects the vigor of a plant, these results again indicated that potentials may be used as a measure of the effectiveness of radiation. Hunter[5] using wheat seedlings, which have easily measurable coleoptiles, found in some of his