at low temperature may mean that the DNA particles are enabled to sustain one ionization within their structures, but no more. Increasing the ion density by a factor of two could then give the observed increase in cross-section. Another possible extension of the work would be the bombardment of active DNA before the separation from protein.

Summary.—Pneumococcus transforming principle DNA has been bombarded with 2-m. e. v. electrons and 3.8-m. e. v. deuterons with indication by assay of activity remaining of a molecular weight of about 6,000,000.

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*STUDIES ON THE HEMOGLOBIN OF COOLEY'S ANEMIA AND COOLEY'S TRAIT**

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The diseases sickle cell anemia and Cooley's anemia (also known as thalassemia or Mediterranean anemia) have associated with them "minor" or "trait" forms of the disease in which the symptomatology is minimal, and the abnormalities in the red cells are correspondingly less. Extensive genetic studies carried out on these diseases[1-4] have led to the conclusion that in each case the trait is inherited as a Mendalian dominant, the person

being heterozygous in the sickle cell gene or Cooley's gene. Homozygosity in either of these genes leads to the corresponding anemia.

Our understanding of sickle cell anemia has improved considerably with the discovery of an abnormal hemoglobin and a molecular interpretation of the pathological behavior of the red cell.[5] In that disease it was possible to demonstrate heterozygosity and homozygosity directly, by observing varying amounts of the abnormal hemoglobin.

Liquori[6] has recently reported the presence of fetal hemoglobin in cases of Cooley's anemia. We have carried out a number of experiments which support his findings, and have extended the investigation to include cases of Cooley's trait, for which no fetal hemoglobin was found. A hypothesis is advanced to explain these facts.

Methods.—Blood from eleven individuals, representing five different families, was investigated (table 1). All of the five patients with Cooley's

TABLE 1

PATIENT	AGE	SEX	RELATION	DIAGNOSIS
S. F.	18 mos	F	...	Cooley's anemia
D. F.	3 yrs	M	Brother of S. F.	Cooley's trait
M. F.	Adult	F	Mother of S. F.	Cooley's trait
V. P.	3 yrs	M	...	Cooley's anemia
J. P.	5 yrs	M	Brother of V. P.	Cooley's trait
J. C.	Adult	F	Maternal Aunt of V. P.	Cooley's trait
P. M.	Adult	F	Maternal Aunt of V. P.	Cooley's trait
M. L.	19 mos	F	...	Cooley's anemia
J. L.	Adult	M	Father of M. L.	Cooley's trait
B. S.	20 mos	F	...	Cooley's anemia
W. B.	18 mos	F	...	Cooley's anemia

anemia were typical in that they came from families of Italian extraction and displayed the characteristic hematological, roentgenographic and physical findings.[7]

None of the patients with Cooley's anemia had been transfused within three months. Patients B. S. and W. B. had never received transfusions; M. L. had been transfused 101 days before our blood specimen was taken, and the other two had not been transfused for over four months.

Blood was collected from these patients using sodium citrate as an anticoagulant, and stroma-free concentrated solutions of hemoglobin were prepared by the method of Drabkin.[8] Blood samples from new born infants were taken from the placental end of the cord and prepared by the same method. The carbon monoxide derivative was used for both the electrophoretic and spectrophotometric studies.

Electrophoresis.—Two types of electrophoresis experiments were undertaken. Those at 0.1 ionic strength (potassium phosphate buffer) and *p*H

range 5.9 to 7.0 were carried out in an attempt to separate components without introducing large boundary anomalies. Another group of experiments were performed in a 0.03 ionic strength sodium phosphate buffer at *p*H 8.2, in which the ratio of protein concentration to buffer ions is large and the resultant boundary anomalies permit the detection of components which differ only slightly in mobility.[9] The latter experiments were modifications of a procedure described fully by Beaven, Hoch and Holiday[10] in an investigation of adult and fetal hemoglobins.

The carbonmonoxyhemoglobin solutions were diluted with the phosphate buffer preparation to a concentration of 0.5 to 1.0%, and were then

TABLE 2

ELECTROPHORETIC MOBILITY AS A FUNCTION OF *p*H

PATIENT	DIAGNOSIS	*p*H OF BUFFER	MOBILITY × 10^5 CM^2 $VOLT^{-1}$ SEC^{-1}[a]
S. F.	Cooley's anemia	5.93	+1.88
	Cooley's anemia	6.9	−0.27
V. P.	Cooley's anemia	6.50	+0.45
		6.50	+0.50
		6.92	−0.14
		6.98	−0.29
D. F.	Cooley's trait	6.91	−0.33
M. F.	Cooley's trait	5.93	1.81
		6.9	−0.20
		6.91	−0.19
J. P.	Cooley's trait	5.93	1.84
		6.88	−0.20
		6.91	−0.19
J. C.	Cooley's trait	5.93	1.78
		6.75	0.04
		6.88	−0.19
		6.90	−0.16

[a] Average of ascending and descending limbs.

Buffer = 0.1 ionic strength phosphate; gradient = 3.8–6.7 volts cm^{-1}; protein concentration = 0.8–1.0 g per 100 ml.

dialyzed against the buffer at 4°C. for 12 to 24 hours in a carbon monoxide atmosphere. Values of *p*H were read on samples of the dialyzate after they had come to room temperature.

The modified Tiselius apparatus described by Swingle[11] was used. The experiments at 0.1 ionic strength employed potential gradients of 3.8 to 6.7 volts per cm. and those at 0.03 ionic strength employed gradients of 17.5 to 18.7 volts per cm. In the latter experiments, the boundaries were kept within the cell compartment by counter compensation. Before each electrophoresis the diluted carbonmonoxyhemoglobin solution was run in the preparative ultracentrifuge at 40,000 g for 40 minutes. It was found that this procedure effectively removed small amounts of colorless (presumably stromal) contaminants.

Alkaline Denaturation.—Some experiments were performed to determine the resistance of the hemoglobin from Cooley's anemia patients to alkaline denaturation. A small volume (0.05 ml) of the blood sample was diluted with 3 ml of N/12 NaOH to produce a 0.2% carbonmonoxyhemoglobin solution. This was read spectrophotometrically at 650 mμ, as suggested by Jonxis,[12] and the formation of the brown alkaline hemochromogen was recorded as a function of time.

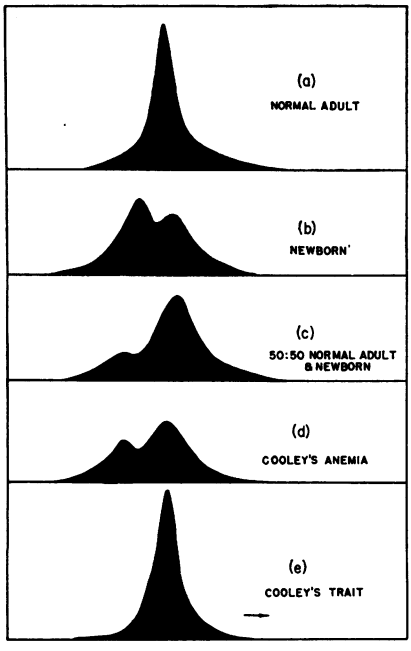

FIGURE 1

Electrophoretic patterns of the ascending boundary of carbonmonoxyhemoglobins. (*a*) Normal adult hemoglobin. (*b*) Newborn cord blood. (*c*) 50:50 mixture of (*a*) and (*b*). (*d*) Cooley's anemia hemoglobin, patient M. L. (*e*) Cooley's trait hemoglobin, patient J. L. Sodium phosphate buffer at pH 8.2; ionic strength 0.03; carbonmonoxyhemoglobin concentration 1.08 g per 100 ml.; time 280 to 300 min. The boundary is moving to the right.

Spectrophotometry.—The concentrated carbonmonoxyhemoglobin solutions were diluted with carbon monoxide-saturated distilled water and were then read immediately in a Beckman spectrophotometer, model DU. The slit width was 0.25 mm, which corresponds to a spectral width at half maximum of 0.88 mμ. The dilutions were always made to give a standard optical density, 0.430 at 290 mμ.

For the determination of small differences in optical density, multiple readings were made and the standard error was determined.

Results.—In table 2 are listed the mobilities of some of the hemoglobin preparations at different *p*H values. Special emphasis was placed upon examination of the hemoglobin of individuals with Cooley's trait, since it was felt that these might represent cases with mixtures of different types of molecules, in analogy with sickle cell trait. However, only one peak was consistently evident. The mobility of the trait blood between the *p*H values 6.88 and 6.92 was -0.21 ± 0.05 cm^2 volt^{-1} sec^{-1}, and the average value for the two anemia samples also was -0.21. A comparison of these values with the mobility-*p*H results cited for normal carbonmonoxyhemoglobin[5] shows that they are equivalent within experimental error.

Because of Liquori's report,[6] mobilities were measured of mixtures of

hemoglobin from Cooley's anemia patients with hemoglobin from normal adults and newborns. In no case could two peaks be resolved, and the mobility results were similar to those cited above. Fetal hemoglobin was found to migrate effectively with the same mobility as the adult form in the *p*H range at 0.1 ionic strength.

Additional experiments were then carried out at the higher *p*H values and lower ionic strengths in which it has been possible to resolve fetal and adult hemoglobins electrophoretically.[10]

In figure 1 are reproduced some of the patterns obtained from various blood samples. It can be seen that normal adult carbonmonoxyhemoglobin (*a*) produces a nearly symmetric, rapidly moving peak, in contrast to cord blood (*b*), for which two peaks are observed. The pattern for a mixture of the two (*c*) permits identification of the faster component as adult hemoglobin and of the slower as fetal hemoglobin.

TABLE 3

ELECTROPHORETIC ANALYSES OF COOLEY'S ANEMIA AND FETAL CARBONMONOXYHEMOGLOBINS

BLOOD	APPARENT % OF FASTER COMPONENT	CORRECTED % OF FASTER COMPONENT
1. B. S.—Cooley's anemia	6.5	2.8
2. W. B.—Cooley's anemia	5.6	2.0
3. M. L.—Cooley's anemia	63.4	42.
4. Newborn	41.0	18.
5. Newborn and normal adult (50:50 mixture)	83.7	62.

Ascending boundary only; *p*H 8.2, ionic strength 0.1, concentration 1.08 g per 100 ml, time 280–300 min.

Hemoglobin from Cooley's anemia patients (*d*) produces a two-peak pattern, with mobilities equal to those for a newborn and adult mixture, while the hemoglobin from Cooley's trait (*e*) produces one peak, indistinguishable from the normal adult (*a*).

This analysis has been carried out on the hemoglobin from three cases of Cooley's anemia (table 3) and three cases of Cooley's trait. Each of the anemia patterns shows the two components described above, while the trait patterns show only one. These results for Cooley's anemia substantiate the observations of Liquori.

In a buffer medium of low ionic strength in which there are two components with only slightly differing mobility, the faster component tends to concentrate itself ahead of the boundary of the slower component to balance the decrease in migration velocity. By using Svensson's equation[13] describing the conductivity change across a boundary, Hoch-Ligeti and Hoch[14, 9] have derived an expression relating the areas in the electrophoretic pattern of a two-component system to the concentrations. They assume

absence of interaction between the two components and linear dependence of migration velocity on concentration. These approximations have been justified for the hemoglobin system in 0.03 ionic strength.[9] We have utilized their equation to calculate the amounts of fetal and adult hemoglobins in the cases of Cooley's anemia. The apparent and calculated percentages of the faster moving (presumably normal adult) component are given in table 3.

Patients B. S. and W. B. have a very small amount of the faster moving material. It is interesting to note that the specimens B. S. and W. B. represent unusually pure samples of fetal hemoglobin. Newborn infants have 15 to 20% of adult hemoglobin, as observed electrophoretically, while even the 20-week fetus is found to have 6%.[10] This nearly pure preparation of fetal hemoglobin can be used for the calibration of spectrophotometric readings.

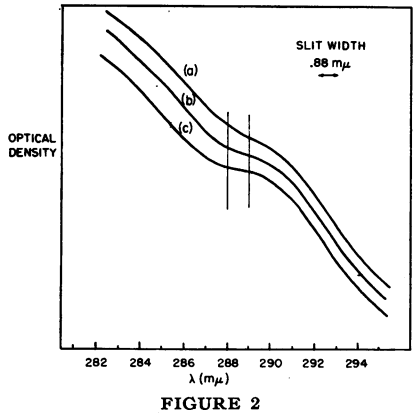

FIGURE 2

Ultra-violet absorption spectrum of carbon: monoxyhemoglobins. (*a*) Adult; (*b*) 50:50 adult-newborn mixtures; (*c*) newborn. The variation in the difference in optical density between 288 and 289 mμ can be readily seen. The curves actually all go through the same point at 290 mμ but are displaced vertically here for clarity.

Alkaline Denaturation.—We have carried out experiments on alkaline denaturation, with results in agreement with those of Liquori. Cooley's anemia hemoglobin was found to denature more slowly than normal adult hemoglobin; samples W. B. and B. S., with only 2 to 3% of the faster component, denature more slowly than samples of hemoglobin from newborns, in comformity with the electrophoretic analyses.

Spectrophotometry.—Jope[15] discovered a difference in the ultraviolet absorption spectra of fetal and adult hemoglobins. The inflection point of the tryptophan fine structure band is found at 2910 A in fetal and at 2898 A in normal adult hemoglobin. Beaven, Holiday and Jope[16] have measured the position of the inflection point in a series of fetal and infant bloods and have correlated it with the ratio of fetal and adult hemoglobin. Beaven, Hoch and Holiday[10] have shown that the position of the inflection point for artificial fetal-adult mixtures is not a linear function of concentration, since a small amount of fetal hemoglobin has a marked effect in producing a shift in the inflection point. Thus, they find their spectral method only of limited usefulness.

In the course of checking their work, we have devised a method which

we believe is of somewhat greater utility both in instrumentation and range of applicability. Curves such as those illustrated in figure 2 were obtained on the Beckman Model DU spectrophotometer. Curve (*a*) is the absorption spectrum of normal adult carbonmonoxyhemoglobin when examined with a slit width of 0.88 mμ, curve (*c*) is the spectrum for a sample of newborn carbonmonoxyhemoglobin, and curve (*b*) is the spectrum of a 50:50 mixture of the adult and newborn hemoglobins, the total concentration being constant. The increased prominence of the tryptophan shoulder can be seen in curve (*c*), although there is no resolution of a separate peak. Instead of attempting to determine the position of the inflection point, we measure the change in optical density for the interval 288 to 289 mμ.

If Beer's law is valid, the difference in optical density over this interval should be a linear function of the concentration of fetal or adult hemoglobin.

TABLE 4

SPECTROPHOTOMETRIC AND ELECTROPHORETIC ANALYSES OF COOLEY'S ANEMIA AND COOLEY'S TRAIT CARBONMONOXYHEMOGLOBIN

BLOOD	Δ288–289 $\times 10^3$	% ADULT HEMOGLOBIN	
		SPECTROPHOTOMETRIC	ELECTROPHORETIC
1. B.S.—Cooley's anemia	0	2.5	2.8
2. W. B.—Cooley's anemia	0.5	7.5	2.0
3. M. L.—Cooley's anemia	4.5	35	42
4. J. L.—Cooley's trait	12.5	95	100
5. J. C.—Cooley's trait	13.0	101	100

To test the assumption of Beer's law, artificial mixtures of adult and newborn hemoglobins were prepared, and the differences in optical density between 288 and 289 mμ (Δ288–289) were measured. All of these mixtures were prepared to a constant total concentration initially, and the percentage of adult hemoglobin in the newborn sample used was determined electrophoretically as cited above. The results, plotted in figure 3, show a linear dependence of Δ288–289 upon the concentration of adult hemoglobin.

Determination of small differences in optical density is possible provided one exercises great care in the measurements. By taking multiple measurements in the region near 0.400, optical densities can be determined to ± 0.0005. This corresponds to an error of $\pm 6\%$ in the determination of the amount of the adult component.

The accuracy of the curve in figure 3 was tested by determining Δ288–289 for various samples of hemoglobin, the fetal-adult compositions of which had been determined electrophoretically. The percentages of adult hemo-

globin as obtained by the two methods are given in table 4. They are found to be in agreement to within 7%.

Discussion.—Hoch[9] has described a faster moving component present in normal adult hemoglobin in concentrations of 2 to 3%. Our cases B. S. and W. B. have 2 to 3% of a component which migrates faster than fetal hemoglobin and could be either normal adult hemoglobin or the small component described by Hoch. The mobility data are not sufficiently accurate to settle this question.

As B. S. and W. B. had never received transfusions, they have continued to live beyond the fetal stage with use of fetal hemoglobin alone as oxygen

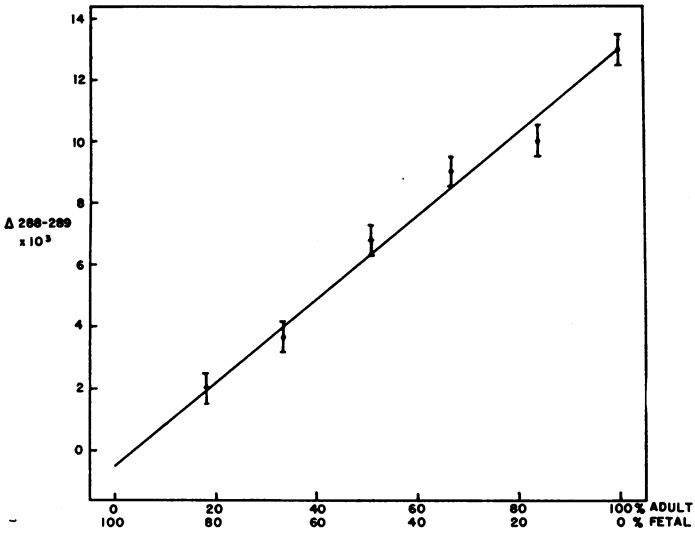

FIGURE 3

The change in the difference in optical density between 288 and 289 mμ as a function of adult hemoglobin concentration in fetal-adult mixtures.

carrier. To our knowledge, these are the first such cases reported in the literature.

Because M. L. had received a transfusion 101 days before the present sample was taken, we do not know with certainty what percentage of her normal adult hemoglobin was derived endogenously. Normal adult erythrocytes continue to live out their normal life span when transfused into patients with Cooley's anemia.[17] From a record of M. L.'s transfusion and hemoglobin levels, we estimate that one-half or less of her adult hemoglobin was obtained by transfusion. Liquori estimates that his two eight-year-old patients have 40 to 60% fetal hemoglobin.

We must explain why the homozygous patient with Cooley's anemia has large amounts of what we believe to be fetal hemoglobin, nearly 100%, while the heterozygous parent or sibling has no fetal hemoglobin.

Sickle cell disease differs from Cooley's anemia in that the presence of one mutant allele in the heterozygote results in the production of an altered hemoglobin molecule. Further, the homozygous patient with Cooley's anemia produces a molecule which appears not to be unique to the disease, but to be identical with a hemoglobin molecule normally produced at an earlier stage of development. The disease has prevented the cessation or has reactivated a metabolic process which normally stops at birth.

In order to bring this about, the Cooley's gene has both effectively to block the production of normal hemoglobin and to cause the continued production of fetal hemoglobin. We suggest that the primary effect of Cooley's gene is in some way to prevent the production of normal erythrocytes, and that there then occurs the continued production of erythrocytes containing fetal hemoglobin as a result of the consequent anemia. According to this interpretation fetal hemoglobin is not similar to sickle cell hemoglobin in being the aberrant product of an altered gene, but is instead the result of a compensatory response.

If this interpretation is valid, there should be other cases of severe anemia with which is associated the presence of fetal hemoglobin beyond the fetal stage. That this may be the case is suggested by the studies of Singer, Chernoff and Singer[18] in which they interpret a prolonged alkaline denaturation time as possibly indicating small amounts of fetal hemoglobin in various anemias.

The patients with Cooley's trait have one normal allele and one Cooley's allele, which together have the capacity to produce malformed, but stable erythrocytes. These patients have no severe anemia, and hence no strong hematopoetic stimulus, and it is observed that they do not produce cells containing fetal hemoglobin.

The exact nature of the defect in Cooley's anemia remains uncertain. The mutant gene may prevent some necessary step in the production of adult hemoglobin, or it may prevent the synthesis of normal adult stromal material, or in some other way impair normal red cell production. Any one of these defects would produce a severe anemia which, according to our postulate, would activate the production of cells containing fetal hemoglobin.

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THREONINE INHIBITION IN A STRAIN OF NEUROSPORA

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It is becoming increasingly apparent that metabolites normally present within the cell may act as inhibitors of some phases of metabolism while at the same time being necessary as intermediates in others.[1] This fact is of interest not only in the analysis of growth factor requirements, but also in the analysis of genetic blocks which prevent the production of cell metabolites. For it would follow that the inherited inability of an organism to synthesize a compound may not necessarily be due at all times to the absence of an enzyme catalyzing its synthesis, but rather to an inhibition of its synthesis by some other compound produced by the organism or supplied from the outside.

Work previously done in this laboratory on the pantothenicless mutants of *Neurospora crassa* has indicated that some such explanation as given above may apply to certain biochemical mutants of Neurospora, since the enzymes involved in the synthesis of pantothenic acid are present both in the pantothenicless mutants and the wild type strain.[2-4] Although the nature of the mechanism causing the enzyme system to be inactive in the