this paper, and to Dr. R. W. Riddell for the complement-fixation tests.

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In 1959 the Society of Public Health Educators in America published three additional monographs in their series, Numbers 4, 5, and 6. Number 4, by Dr. W. Griffiths (University of California), is "Health Workers' Attitudes toward Community Development Programs"; in Number 5, Dr. Cora DuBois (Harvard University and Radcliffe College) reconciles the theories of anthropologists, sociologists, and psychologists as they relate to socio-cultural change. Also in Number 5 is an appraisal of operational research in health education by Dr. K. D. Benne (Boston University). Number 6 contains three articles: "Motivation—Some Basic Psychological Issues," by Dr. Richard S. Lazarus (University of California); "Motivation in Small Groups," by Dr. Edmund H. Volkart (Stanford University); and "Power and Participation in the Local Community," by Dr. William Kornhauser (University of California). Further details are available from the Society of Public Health Educators, Office of the Monograph Committee, 121, East 11th Street, Oakland, California, U.S.A.

STUDIES ON THE HAEMOGLOBIN OF NEWBORN NIGERIANS

BY

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In studies of diseases caused by inheritance of the abnormal haemoglobins, in particular haemoglobin S, the presence of foetal haemoglobin in the erythrocytes during the first few months of life interferes with early determination of the haemoglobin genotype of individuals (using the standard techniques as present in general use). As a result sickle-cell anaemia and its genetic variants have not been detected at birth or during the first three months of life, and consequently this gap in our knowledge of the natural history of these diseases remains to be filled. It has also been impracticable to include the neonatal period in investigations on the population dynamics of the sickle-cell gene. It is not inconceivable that factors which operate during this period might lie behind some of the riddles presented by sickling frequencies in populations.

In March, 1958, it was decided to investigate the haemoglobins of babies at birth and to follow up these cases for six months in order to get an idea of the changing pattern of the haemoglobin content of the erythrocytes. We hoped that the pattern of results of the standard tests employed might point a way to earlier definitive diagnosis of diseases caused by the abnormal haemoglobins.

The object of this communication is to present the results of some of our investigations, in particular our findings on filter-paper electrophoresis of the haemoglobin, which in a number of cases yielded unexpected and unusual results.

Material and Methods

The subjects in this investigation were 100 babies born at University College Hospital, Ibadan. The only criterion for inclusion was that a sample of cord blood was obtained at delivery. Subsequently, samples of blood were collected monthly at the post-natal clinic. Unfortunately, a large proportion of cases defaulted from follow-up, and in many cases our investigations are incomplete. Table I shows the follow-up achieved.

TABLE I.—*Number of Cases Attending Monthly Follow-up*

	Birth	1 Month	2 Months	3 Months	4 Months
No. of cases ..	100	50	29	18	4

(1) Sickling tests were done using freshly prepared 2% sodium metabisulphite. (2) Foetal haemoglobin

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estimations were done using the one-minute alkali-denaturation technique of Singer *et al.* (1951). (3) Filter-paper electrophoresis of the haemoglobin was carried out with the hanging-strip method, using barbitone buffer at *p*H 8.6 ionic strength 0.05. (4) Erythrocyte osmotic fragility tests were done using technique described by Dacie (1954).

Results

Sickling was detected at birth in only five cases, the foetal Hb content being 50%, 70%, 89%, 80%, and 66.4% respectively. In six other cases sickling, while undetected at birth, became evident during the next two months. The foetal haemoglobin at birth in this group ranged from 72 to 86%. All sickling preparations were checked by at least two and usually three of us. Of these 11 sicklers, seven have proved to be sickle-cell-trait carriers, one appears to be a case of sickle-cell haemoglobin C disease (sickling was first detected at 2 months of age in this case), and in the remaining three the genotype could not be accurately determined owing to lack of follow-up.

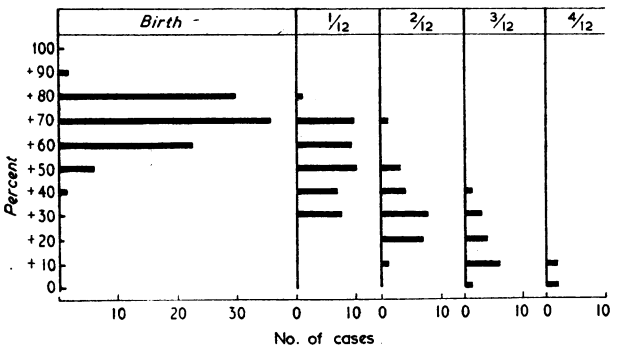

FIG. 1.—Foetal haemoglobin estimations in 100 cases at birth and in a proportion of these at monthly intervals for four months.

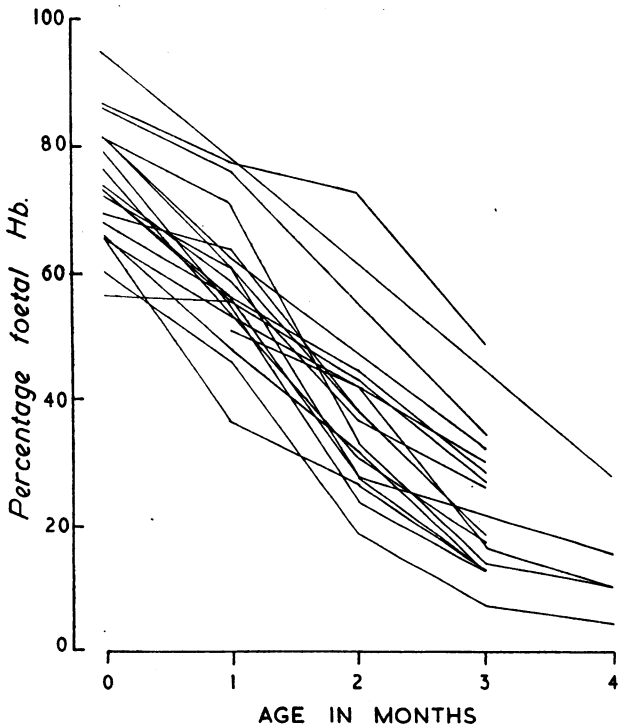

FIG. 2.—Serial foetal haemoglobin estimations in 18 cases, plotted to show the rate of fall in individual cases.

Foetal Haemoglobin Estimations.—Fig. 1 shows all the estimations carried out on the first series of 100 cases. It will be seen that at birth most of these cases had a foetal haemoglobin percentage of 70 or more; however, there was a wide range from 45 to 96%. In subsequent months there was a progressive fall, and by four months some patients had lost most of their foetal haemoglobin. Fig. 2 shows the rate of fall of foetal haemoglobin in 18 cases month by month. It will be seen that in general the decline of individual cases follows the pattern of fall indicated in Fig. 1.

Filter-paper Electrophoresis

As was to be expected, the presence of foetal haemoglobin obscured the true adult haemoglobin pattern at birth and during the early months. Haemoglobin A moves faster than F and the latter faster than S at *p*H 8.6, but the difference in their mobility is not great enough to get clear separation on electrophoresis. Haemoglobin C, however, moves very much slower than A, F, or S, and even at birth can be identified with certainty. Case 27, with 60% foetal haemoglobin, showed haemoglobin C at birth. As things stand at present, using the standard techniques, it is not always possible to diagnose the adult genotype at birth except possibly in the case of sickle-cell haemoglobin C disease. When sickle cells are present at birth haemoglobin S is certain to be found; if the same case shows a percentage of haemoglobin C on electrophoresis, the diagnosis of sickle-cell haemoglobin C disease can be made with certainty.

A completely unexpected finding on electrophoresis was the presence of fast-moving haemoglobin components in 11 of the 100 cases investigated. Out of 40 new cases recently investigated 4 were found to have such a component.

Among the cases showing fast-moving haemoglobins were uniovular twins. Fig. 3 shows the electrophoretic pattern in these children at birth and subsequently.

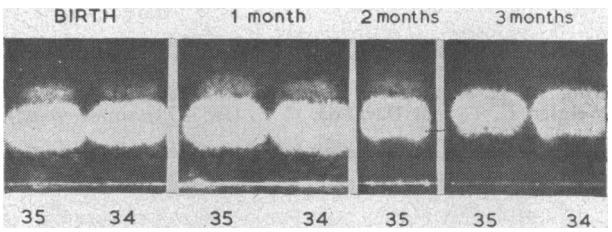

FIG. 3.—Serial paper electrophoresis of the haemoglobin at monthly intervals in Cases 34 and 35, showing the gradual disappearance of the abnormal fast-moving haemoglobin component.

It will be seen that the abnormal fraction persisted for the first two months and then disappeared at the third month.

Table II shows sex, birth weights, foetal haemoglobin estimations, and genotypes in the children concerned and also the maternal blood groups, genotypes, and tribal origin. It would appear that "fast-moving" haemoglobin is unrelated to any of the details listed. It is of interest to note that erythrocyte osmotic fragility tests performed on two of the cases showed a greatly reduced osmotic fragility in both. Fig. 4 shows the osmotic fragility curves in these cases.

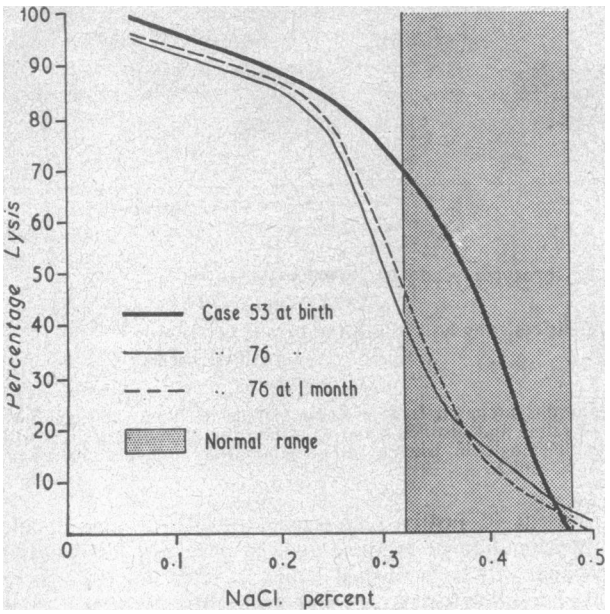

FIG. 4.—Erythrocyte osmotic fragility curves obtained in two cases which showed the abnormal fast-moving haemoglobin component.

Discussion

Our failure to detect sickling at birth in some infants who later showed it accords with the experience of others. Diggs *et al.* (1933) found sickling in only 6 out of 159 newborn American negroes, an incidence of 3.8%, compared with an incidence of 8.3% among 2,539 negroes of all ages. Sharp and Schleicher (1936) report an instance in which sickling was not detected at birth but was present at 4 months. On the other hand, Watson *et al.* (1948), in their series of 226 mothers and babies, found sickling in 8% of the mothers and 8.4% of the babies. Our foetal haemoglobin estimations show that the failure to demonstrate sickling in some cases was not related to the quantity of foetal haemoglobin present. The range of values obtained in these cases was similar to that found among cases which showed sickling. The reason for our failure sometimes to detect sickling might be technical, but we think this is unlikely, as scrupulous attention to detail was always observed. Sickling preparations were made from cord blood, while in the series reported by Watson *et al.*, heel-prick samples were used. It seems reasonable to postulate that differences in the results of sickling tests at birth in their series and ours might be related to the source of origin of the samples tested.

Our results of foetal haemoglobin estimation are in agreement with those reported by others (Jonxis, 1948; Chernoff and Singer, 1952). Chernoff and Singer have shown that, while there is a sharp decline of foetal haemoglobin during the first six months of life, substantial amounts may persist until the end of the first year of life, and traces may persist for three to four years. In some cases the mechanism for production of foetal haemoglobin may never be lost. This usually happens in certain congenital haemolytic disorders, notably thalassaemia and sickle-cell anaemia (Liquori, 1951; Chernoff and Singer, 1952; Itano, 1953). Occasionally foetal haemoglobin may reappear during the course of some acquired blood dyscrasia (Singer *et al.*, 1951; Itano, 1953) and has also been detected in molar pregnancies (Bromberg *et al.*, 1957).

The production of foetal haemoglobin is under a different genetic control from adult haemoglobin. This is shown by the fact that foetal haemoglobin is present in all infants irrespective of their adult haemoglobin genotype. Foetal haemoglobin differs from adult haemoglobin in its crystal form, solubility, immunological behaviour, electrophoresis, spectral absorption, amino-acid composition, and resistance to alkali denaturation (Wintrobe, 1956).

It remains to be shown whether foetal haemoglobin occurs in a mixture with adult haemoglobin in individual erythrocytes or whether there are two distinct cell populations. The fact that at birth only a small percentage of red cells show sickling indicates that there is a qualitative difference in the haemoglobin of individual cells. Further, the rate of increase in the percentage of sickled cells observed during the first four months of life (Watson *et al.*, 1948) bears an inverse relation to the percentage of foetal haemoglobin found during the same period in our cases and in those reported by others (Chernoff and Singer, 1952). This observation has been confirmed by Shields *et al.* (1958). These observations imply that there are two cell populations—one containing foetal haemoglobin and the other adult haemoglobin, the latter gradually replacing the former during the first few months of life.

Case 2 in the present series showed sickling at birth, and we attempted to separate S-containing cells from F-containing cells by lysing the erythrocytes in varying

TABLE II.—*Some Particulars Relating to Babies Showing Abnormal "Fast-moving" Haemoglobin at Birth*

Baby							Mother		
No.	Sex	Birth Weight lb. oz.	Birth Weight g.	Sickling	% Hb F	Genotype	Blood Group	Genotype	Race
9	F			—	72·2	A/A	A.Rh+	A/A	Yoruba
29	F	6 15	3,175	?	84·3	?	O.Rh+	A/A	Ibo
33	M			—	69·0	A/A	B.Rh+	A/A	Yoruba
34*	F	6 7	2,920	—	76·7	A/A	A.Rh+	A/A	Yoruba
35*	F	6 8	2,950	—	73·5	A/A			
40	M			—	78·8	A/A	O.Rh+	A/A	
43	F	6 7½	2,935	—	65·7	A/A	A.Rh+	A/A	"
47	F			—	78·9	A/A	B.Rh+	A/A	"
53†	M	Premature		—	94·6	A/A	A.Rh+		Tsekiri
63	F	5 11	2,580	+ at 2/12	86·0	S/C			Yoruba
76†	F			—	70·1	A/A	B.Rh+		"
				New Series Started in November, 1958					Ghanaian
107	F	6 5	2,865	+	66·6		O.Rh+	A/S	Yoruba
106	F	7 13	3,545	—	65·0		O.Rh+	A/A	"
125	F	6 1½	2,764	—	77·2		O.Rh+		"
137	F	5 1	2,295	—	75·0		O.Rh+	A/S	"

* Twins. † Erythrocyte osmotic fragility reduced.

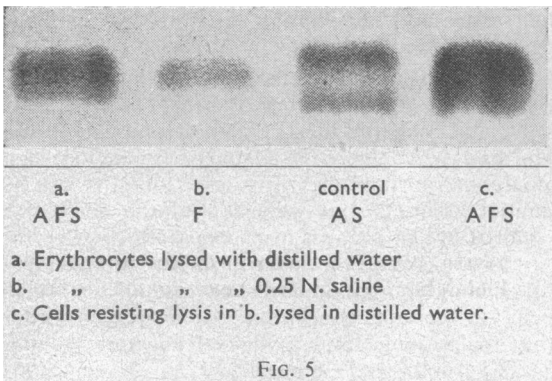

FIG. 5

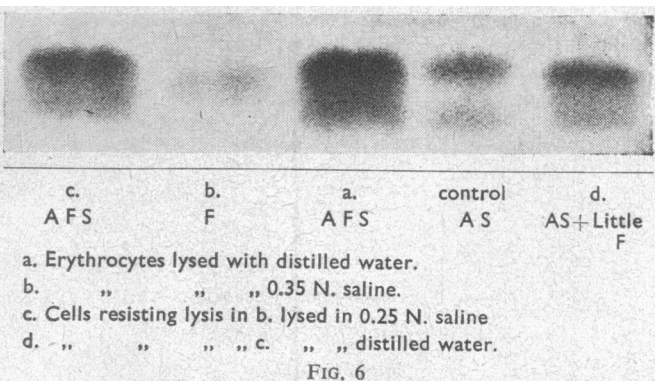

FIG. 6

FIGS. 5 and 6.—Showing paper electrophoresis on haemoglobin solutions prepared from a single sample of blood of Case 2 at one month (left) and two months (right), using different strengths of saline and distilled water to obtain the haemolysates. It will be seen that solution (b) in each case consists almost entirely of foetal haemoglobin, whereas the other solutions contain a mixture of haemoglobins A, S, and F.

strengths of saline according to a technique previously described by one of us (Hendrickse, 1958). We presumed that the F- and S-containing cells would show differential lysis. Our results, shown in Figs. 5 and 6, offer direct support for the concept of separate cell populations. In sickle-cell anaemia, however, in which as much as 20% of the haemoglobin may be foetal in type, all the erythrocytes can be induced to sickle under optimum conditions (Singer *et al.*, 1951). It must be concluded, therefore, that in this condition sickle and foetal haemoglobin occur together in individual erythrocytes.

We have at present no indication of the significance of the fast-moving haemoglobin components we have found. The rate of disappearance indicates a relation to foetal haemoglobin. The occurrence in uniovular twins suggests an inherited abnormality. The fact that we are continuing to find fast foetal haemoglobin in about 10% of babies born in hospital suggests a similar incidence among all infants born locally. Similar fast-moving haemoglobin components have been reported in isolated cases by other workers. Fessas and Papaspyrou (1957) reported a fast-moving haemoglobin in a Greek infant. It was associated with thalassaemia. Its concentration fell with that of foetal haemoglobin and had almost disappeared at four months. It was not present in the parents. Ager and Lehmann (1958) reported a different fast-moving haemoglobin in a 4-weeks-old infant, and Vella (1959) observed the first haemoglobin in 63 out of 1,962 Chinese cord bloods in Singapore, and the second-haemoglobin " Bart's " in two.

After this investigation was concluded we sent three cord haemoglobins containing fast components to Dr. H. Lehmann, who kindly examined them with the help of Dr. J. A. M. Ager. One—No. 40-A—contained a haemoglobin with the electrophoretic and chromatographic properties of the abnormal foetal haemoglobin " Bart's." The two others—38-A and 44-A—were thought to contain the less well defined haemoglobin described by Fessas and Papaspyrou (1957). On electrophoresis as alkaline *p*H the fast component moved more slowly than " Bart's," and on chromatography at *p*H 6, where " Bart's " is eluted before H, the abnormal bands were seen to move behind H and in front of F.

It is tempting to postulate that our findings indicate hitherto unsuspected abnormal genes occurring in high frequency locally, but we have insufficient evidence at present to justify such speculation. A more detailed investigation of these cases is in progress, the results of which will be published later. It is of interest that Dr. Lehmann informed us that some time ago he had seen haemoglobin " Bart's " in the blood of a negro infant sent to him by Dr. R. Schneider from Galveston. Texas, U.S.A.

Summary

Results of sickling tests, foetal haemoglobin estimations, and paper electrophoresis of the haemoglobin in Nigerian infants at birth and during the first five months of life are presented.

" Fast " haemoglobin fractions were found at birth in 11 of 100 cases studied, and persisted for the first two months of life. Three of these were examined in detail and were found to resemble haemoglobin " Bart's " in one case and the haemoglobin of Fessas and Papaspyrou in two.

Our results are compared with those of other workers and their significance is briefly discussed.

We thank Dr. W. R. F. Collis for permission to undertake this investigation, Professor A. Brown and Dr. J. C. Edozien for use of certain laboratory facilities not available in our department, Dr. J. B. Lawson and the staff of the obstetrics department for supplying samples of cord blood, Dr. O. Ransome-Kuti for assistance with the follow-up, and Dr. C. Adi for technical assistance. We are grateful to Dr. H. Lehmann, of St. Bartholomew's Hospital, London, and to Dr. J. A. M. Ager, of St. Thomas's Hospital, London, for examining three of the " fast-moving " cord haemoglobins.

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