Changing blood picture in sickle-cell anaemia from shortly after birth to adolescence

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SYNOPSIS The blood picture of children with sickle-cell anaemia was found to change with age. The changes were most marked in the first year but the mean level of haemoglobin, haemoglobin F, and target cells fell until adolescence, and irreversibly sickled cells rose. Reticulocytes, Howell Jolly bodies, and normoblasts altered little after one year. The fall in haemoglobin F suggested a delayed changeover from fetal to adult haemoglobin production. It was concluded that the blood changes in sickle-cell anaemia were progressive throughout childhood.

Previous authors have shown that some features of the blood in sickle-cell anaemia can vary or be interrelated. Schneider (personal communication) showed that haemoglobin F fell during childhood. Serjeant (1970) showed an inverse relationship between the level of haemoglobin F and the number of irreversibly sickled cells in adults. Davis (1972) showed variations in the number of target cells. Pearson *et al* (1969) discussed Howell Jolly bodies in relation to functional asplenia. The present study was planned to see how the blood picture of sickle-cell anaemia developed from infancy and whether a steady state was reached. In addition to the haemoglobin F, irreversibly sickled cells, target cells, and Howell Jolly bodies, the haemoglobin, reticulocytes, and the presence or absence of normoblasts were considered.

Patients studied

These were 75 children attending the sickle-cell clinic, who were free from symptoms attributable to sickle-cell anaemia and had no intercurrent infections. Elimination of children who were sick meant that any changes that were found might be attributed to age. Capillary samples of blood were collected at yearly intervals or more frequently under the age of 1 year. A total of 174 samples was obtained in three years from children aged 4 days to 16 years. To enable the results on the youngest infants to be compared with the changes in haemoglobin and haemoglobin F found in normal infants of similar age a control population was introduced. This

consisted of the results of these measurements on 134 infants aged 1 to 7 months. These were hospital patients and therefore not strictly normal; however, it was considered that their clinical conditions were unlikely to affect the results significantly. The haemoglobin F levels on 17 normal cord blood samples were also included in the control findings.

Methods

Haemoglobin was estimated as cyanmethaemoglobin and haemoglobin F by the one-minute alkali denaturation method of Singer *et al* (1951) modified for the use of capillary samples of blood. The percentage of irreversibly sickled cells (Jensen *et al*, 1960), target cells (Davis, 1972), and Howell Jolly bodies were determined by examining 1 000 cells on each blood film. The presence or absence of normoblasts was noted. One thousand cells were also counted to determine the percentage of reticulocytes. To minimize personal variation all the counts were done by the author.

Results

The results on all the children with sickle-cell anaemia are summarized in table I, and on the control group of infants in table II. The mean of each group of results is plotted against age in the figure, and inspection suggested that the results might be considered in two parts, namely, below and above 1 year of age.

During the first year there were marked fluctuations in some of the means. Comparison of the

Figure Diagram to illustrate the changes in the mean haemoglobin (Hb), haemoglobin F (HbF), irreversibly sickled cells (ISC), target cells (TC), Howell Jolly bodies (HJB), and reticulocytes (R). The solid line indicates the mean for infants and children with sickle-cell anaemia and the interrupted line that for the control group of infants.

patients with the controls showed that the former showed a fall and rise in the mean haemoglobin level similar to the latter though the mean reached a lower level and the subsequent rise was less marked. After five months the mean haemoglobin of the patients fell again whereas it would have been maintained in the control infants. The mean haemoglobin F was higher in the patients than in the controls by 1 month of age and was above the range of the controls by 4 months. The fall in the mean haemoglobin F was, therefore, slower in the patients than in the controls. Irreversibly sickled cells and Howell Jolly bodies were uncommon under 1 year of age, but target cells appeared to reach a maximum level at about the end of the first year, and reticulocytes were raised (greater than 2%) from 1 month onwards.

Under 1 year normoblasts were more frequently absent than present (72%).

From 1 year onwards changes in the means still appeared to be occurring but at a slower rate. It was considered appropriate to examine the regression coefficient for each of the measurements with age and to determine the significance (P) of its standard error. This showed that the haemoglobin level, haemoglobin F, and target cells fell significantly from 1 to 16 years ($P < 0.02$, < 0.001 , < 0.02 , respectively). On the other hand, the irreversibly sickled cells rose significantly ($P < 0.001$). There was no significant change in the Howell Jolly bodies or reticulocytes although the means of both were raised. Normoblasts were more commonly present than absent from 1 year onwards (91%).

Age	Number of results	Haemoglobin (g/dl)		Haemoglobin F (%)		Reticulocytes (%)		Irreversibly sickled cells (%)		Target cells (%)		Howell Jolly bodies (%)		Normoblasts	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Present	Absent
4 d	1	(15.4)	—	(81)	—	(0.1)	—	(0.0)	—	(0.2)	—	(0.0)	—	0	1
1 mth	5	9.78	1.49	64.40	4.96	2.82	1.12	0.10	0.11	0.68	1.02	0.06	0.42	2	3
2 mth	6	9.30	0.71	44.67	4.86	5.27	1.45	0.33	0.20	1.07	1.26	0.00	—	1	5
4 mth	2	10.00	1.20	34.00	2.00	3.10	1.30	0.15	0.15	0.40	0.40	0.00	—	0	2
5 mth	4	10.60	0.30	29.50	2.06	4.25	1.64	0.48	0.39	1.45	1.23	0.00	—	1	3
6 mth	4	8.65	1.02	24.75	3.49	8.55	4.88	0.68	0.54	0.25	0.29	0.03	0.49	1	3
7 mth	3	9.30	0.50	22.67	2.84	3.40	1.10	0.60	0.77	1.63	0.66	0.03	0.05	0	3
8 mth	3	9.30	1.10	17.67	1.18	4.23	1.68	0.43	0.54	2.07	0.62	0.03	0.05	2	1
9 mth	4	9.85	0.37	18.25	4.02	4.40	2.30	0.45	0.15	4.90	2.30	0.05	0.05	1	3
10 mth	2	8.15	0.75	21.50	8.50	10.35	4.35	0.90	0.50	0.55	0.79	0.00	—	2	0
11 mth	3	9.77	0.78	16.67	4.48	3.27	0.68	0.17	0.17	2.43	1.18	0.00	—	0	3
1 yr	16	8.07	1.00	13.80	6.20	7.65	3.90	2.44	3.20	1.04	1.03	0.22	0.32	10	6
2 yr	14	7.99	1.30	9.36	6.16	9.61	3.79	3.79	2.32	0.93	0.98	0.17	0.12	14	0
3 yr	11	8.18	0.90	9.00	3.96	10.54	3.62	4.62	2.13	1.34	1.49	0.16	0.11	11	0
4 yr	12	8.35	1.28	8.67	5.00	10.49	4.04	5.46	3.36	1.58	1.68	0.21	0.16	12	0
5 yr	8	8.18	0.67	5.88	2.93	9.78	3.77	5.43	2.75	1.08	0.91	0.31	0.21	7	1
6 yr	14	7.85	1.27	5.64	4.21	11.51	4.18	8.36	5.45	0.39	0.35	0.34	0.36	14	0
7 yr	15	8.26	0.79	9.20	5.85	10.66	3.13	4.70	3.51	0.95	0.76	0.31	0.30	14	1
8 yr	11	7.59	1.39	8.73	6.24	9.63	6.58	4.65	3.88	1.03	1.29	0.28	0.16	11	0
9 yr	12	7.58	1.00	7.67	5.35	12.33	5.53	5.43	4.15	0.59	0.72	0.28	0.17	12	0
10 yr	8	7.70	1.07	6.38	4.26	7.90	2.17	9.24	5.61	0.49	0.63	0.43	0.20	7	1
11 yr	8	7.54	1.08	6.13	2.61	8.38	4.18	9.04	6.71	0.46	0.38	0.18	0.12	7	1
12-16 yr	8	7.29	0.62	5.13	1.89	7.50	4.06	7.14	4.01	0.41	0.39	0.28	0.25	6	2

Table I Findings in cases of sickle-cell anaemia

SD = standard deviation. Results in parentheses indicate single case aged 4 days.

Age (mth)	No. of results	Haemoglobin (g/dl)		Haemoglobin F (%)	
		Mean	SD	Mean	SD
Cord blood	17	—	—	75.24	6.89
1	26	11.64	1.84	56.81	11.50
2	28	10.39	1.22	26.68	16.29
3	26	10.87	1.07	13.69	6.61
4	27	11.22	1.05	7.78	5.84
5-7	27	11.50	1.33	3.96	2.18

Table II Findings in control group of infants

SD = standard deviation.

Discussion

The full blood picture of sickle-cell anaemia did not always appear before the age of 1 year. Under this age the blood showed a mixed picture in which the normal changes found in the first few months of life were more marked and were modified by the gradual emergence of a haemolytic picture. That haemolysis was present from an early stage was indicated by the presence of a raised mean reticulocyte count (greater than 2%). After 1 year the condition still progressed with a fall in the mean haemoglobin level, a rise in the irreversibly sickled cells, and a fall in the target cells. A steady state was not achieved during childhood. The level of haemoglobin F continued to fall until puberty, which confirmed the finding of Schneider (personal communication). Thus any

clinical advantage the presence of haemoglobin F might confer on the patient (Jackson *et al*, 1961) would not be lasting, and it might be suggested that in sickle-cell anaemia there was a prolongation of the normal changeover from fetal to adult haemoglobin production (in this case haemoglobin S), which might not proceed to completion during childhood.

Serjeant (1970) showed that high levels of irreversibly sickled cells were associated with low levels of haemoglobin F and *vice versa*. He excluded children from his studies because Schneider had reported that the most rapid fall in haemoglobin F occurred during childhood. The present study showed that the inverse relationship between the level of haemoglobin F and the percentage of irreversibly sickled cells applied to children aged 1 to 16 years. There was a very significant negative correlation ($p < 0.001$) which held even when the effects of age were eliminated by a partial correlation coefficient.

Target cells were found to decrease in number with increasing age. This was rather surprising because an increase might be expected as the result of auto-splenectomy during childhood. They showed a significant negative correlation coefficient with irreversibly sickled cells, but this disappeared when allowance was made for the effect of age. They were also negatively correlated with the percentage of reticulocytes, and this even held when allowance was

made for age ($P < 0.05$). It had been suggested (Davis, 1972) that the occasional target cells seen in normal films might be young cells which had just matured from reticulocytes because polychromatic cells (reticulocytes) not infrequently showed a target appearance. This did not appear to be the case in sickle-cell anaemia as the correlation was negative.

Reticulocytes and Howell Jolly bodies might be expected to rise with age as a result of autosplenectomy. That reticulocytes did not was probably a reflection of the maximum activity of the bone marrow in a chronic haemolytic anaemia. The findings of raised Howell Jolly bodies from the age of 1 year onwards when many of the children would still have had enlarged spleens was presumably illustrative of the functional asplenia described by Pearson *et al* (1969). In older children the presence of Howell Jolly bodies probably did indicate autosplenectomy. It should be noted that 19% of the results on children over the age of 1 year showed no Howell Jolly bodies, and in individual cases they were not necessarily present at every examination. Thus it seemed likely that splenic activity varied from time to time.

No attempt was made to enumerate the number of normoblasts present; it was merely recorded whether they were present or absent from the film. They were uncommon under the age of 1 year and very com-

mon over that age. Their presence or absence did not appear to be particularly associated with any of the other features which were studied.

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