it has a high oxidative activity, it is very low in catalatic activity and is devoid of peroxidatic activity.

SUMMARY

1. The iron, total nitrogen, total amino-nitrogen and α -amino-nitrogen contents of PMc were determined and the amino-acids present were identified.

2. The adsorption spectra of the oxidized and reduced forms of this pigment resemble closely those of ordinary cytochrome *c*.

3. This pigment combines with cyanide, methyl-carbarylamine, carbon monoxide, nitric oxide and nitrosobenzene. It is autoxidizable and shows properties similar to type V of ordinary cytochrome *c* (i.e. cytochrome *c* at pH 13) on the one hand and haemoglobin on the other.

4. It is inactive in both the succinic oxidase system and the cytochrome oxidase system with *p*-phenylenediamine as substrate, but strongly catalyses the oxidation of ascorbic acid. As an artificial ascorbic acid oxidase, it has a Q_{10} of 11,500. This catalysed oxidation of ascorbic acid is inhibited by cyanide, methylcarbarylamine, nitrosobenzene and carbon monoxide, the latter inhibition being somewhat light sensitive.

5. The pigment shows little catalatic activity and is devoid of peroxidatic activity.

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The Haemoglobins of the Human Foetus and Infant. Electrophoretic and Spectroscopic Differentiation of Adult and Foetal Types

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The differences between human adult and foetal haemoglobin have been summarized recently by Jope & O'Brien (1949) and by Kendrew (1949). Apart from the difference in the rate of denaturation by alkali, these differences were not sufficiently great to allow the determination of the small amounts of adult haemoglobin in the haemoglobin of the human foetus during early development and no such attempt has been reported. The statements on the relative amounts of the two types of haemoglobin at and after birth are not in good agreement. Most of the evidence is based on the difference in the resistance to denaturation by alkali, the foetal

haemoglobin being the more resistant type. According to Haurowitz (1929, 1930) the blood of the newborn contains 80% resistant and 20% non-resistant haemoglobin. Jonxis (1949), at variance with a previous statement (Jonxis, 1948), reports that at birth the percentage of 'later' haemoglobin in healthy newborn infants ranges between 2 and 25% and that after 22 weeks all foetal haemoglobin disappears. Brinkman & Jonxis (1935) find that the foetal haemoglobin disappears entirely at 7 months after birth. The two types of haemoglobin in the newborn are immunologically different (Darrow, Nowakovsky & Austin, 1940). In the haemoglobin

of a 2.5 months premature infant a small amount of adult haemoglobin was present (titre with adult haemoglobin antiserum 1:16, with cord-haemoglobin antiserum 1:512). A difference in electrophoretic mobility between adult and foetal carboxyhaemoglobin was found by Andersch, Wilson & Menten (1944), but these authors studied cord blood only and they did not report the proportions of the two components.

In the present work the difference in mobility between adult and foetal oxyhaemoglobin was determined and the proportions of adult oxyhaemoglobin in foetal and post-natal red-cell extracts were studied by electrophoresis at low ionic strength, that is, under conditions resulting in large boundary anomalies. In addition, the ultraviolet absorption spectra were recorded. The position of the tryptophan fine structure band (the tryptophan 'notch') is 2898 Å. for the foetal and 2910 Å. for the adult haemoglobin (Joep, 1949) and it was anticipated that the spectra of mixtures of the two types might serve as a method of estimating their relative proportions.

EXPERIMENTAL

Preparation of oxyhaemoglobin solutions. Foetal samples were taken from the heart or from the umbilical cord, blood samples at birth from the placental end of the cord, those of the infant from the heel and those of the adult from the antecubital vein. The cells were washed with physiological saline, lysed with 2-4 vol. of distilled water and centrifuged. Addition of 0.1 vol. of 0.3M-Na₂HPO₄ or of phosphate buffer, pH 7, to the supernatant brought down the remainder of the stroma. The solutions were put up for dialysis immediately. Samples of the concentrated solutions were diluted with 0.02M-phosphate buffer, pH 7, to an appropriate concentration (about 0.02-0.03 g./100 ml.) and examined spectroscopically, usually without delay, or after storage in the refrigerator for not more than 2 hr.

Electrophoresis. The mobilities in phosphate at pH 7.1 and 8.0 were compared by the differential method (Hoch, 1949). The buffer at pH 7.1 was the same as used by Andersch *et al.* (1944) and at pH 8.0 an ionic strength of 0.05 was chosen.

All the experiments which served to analyse mixtures were made in 0.01M-Na₂HPO₄ by the usual technique. The haemoglobin concentration was about 1 g./100 ml. After the ascending boundaries had migrated some three-quarters of the length of the compartment, the electrophoresis was continued while the boundaries were kept in position by compensation. The ascending boundaries only were observed. The descending boundaries were disturbed by convection. It has been pointed out (Hoch, 1950) that conditions resulting in pronounced boundary anomalies (low ratio of ionic strength:protein concentration) may be favourable for showing up small amounts of a faster component, the mobility of which differs little from that of the main component. These conditions apply to the analysis of foetal red-cell extracts which contain little only of the faster adult haemoglobin.

Evaluation of the electrophoretic patterns. Equation 1 (Hoch-Ligeti & Hoch, 1948) was used to calculate the

corrected proportion of adult haemoglobin in the total and equation 2 to calculate the corrected relative difference in mobility.

$$C_1' = \frac{C_1'}{1 + \frac{V_1'}{S} K(C_1' - C_1')}, \quad (1)$$

$$\frac{V_1' - V_2'}{V_1'} = \frac{S}{V_1'} \frac{C_1'}{C_1'} \frac{1}{1 + KC_1'}. \quad (2)$$

(For significance of symbols see Hoch-Ligeti & Hoch (1948).)

These equations were derived on the assumption that the two components do not specifically interact with each other and that K is constant over the range of protein concentrations used. If the two components interact the corrected values for the proportion of adult haemoglobin represent minimum values and those for the relative difference in mobility maximum values. The neglect of the variation of K with the haemoglobin concentration is not of great consequence as can be judged from equations 1 and 2. For example, if K at 1.2 g./100 ml. were 0.135 instead of 0.123, the value for $C_1'/C_1' \times 100$ in the fifth analysis of Table 2 would be reduced from 25 by only 1.3. The haemoglobin concentration in the adjusted solution, C_1' , was determined at the end of the run by the Kjeldahl method or colorimetrically. The coefficient K has previously been determined under identical experimental conditions to be 0.123 (Hoch, 1950) for adult oxyhaemoglobin at a concentration of 1.1 g./100 ml. and it was assumed that it is the same for foetal oxyhaemoglobin. The migration velocities were measured from the position of the original boundary and V_1' from $V_1' = V_{s1}'/(1 + KC_1')$ (Hoch-Ligeti & Hoch, 1948).

Spectroscopic methods. Logarithmic-cam moving-plate spectrograms were obtained with the improved version (Holiday, 1950) of the apparatus previously described (Holiday, 1937) by the same author. The wavelength of the tryptophan notch on each plate was determined by two or three observers, whose results did not normally differ by more than ± 1 Å. from the mean value.

The wavelength scale of the spectrograph was frequently checked by taking moving-plate spectrograms of a mercury resonance lamp, and using in particular the lines at 2803, 2894 and 2967 Å. to check the scale in the 2900 Å. region of the tryptophan notch. The wavelength scales of the actual haemoglobin spectrograms were also checked, using the hydrogen line at 3680 Å., which occurs in the spectrum of the hot-cathode hydrogen arc used as the continuous light source.

A Unicam photoelectric spectrophotometer was used to obtain the data listed in Table 4.

Spectrograms of most of the samples in the ultraviolet were obtained with an automatic recording spectrophotometer to be described in detail elsewhere (Holiday & Sutton, 1951).

Stock solutions of adult and foetal oxyhaemoglobins for preparing the mixtures listed in Table 2 were made from fresh concentrated stock solutions by appropriate dilution with buffer as calculated from colorimetric analyses for oxyhaemoglobin, using the visible absorption bands, and the dilutions checked for equal haemoglobin concentration on the Unicam spectrophotometer.

RESULTS

Electrophoresis

Comparison of mobilities. In Table 1 are summarized the experimental conditions and the results of the differential electrophoresis. The adult oxyhaemoglobin was found to have a greater anodic mobility at both pH 7.1 and 8.0. The difference in mobility was higher at the higher pH.

least 6% adult haemoglobin. The pH in these experiments, which were made in 0.01 M-Na₂HPO₄, was about 8.8 and the difference in mobility between the two components was 0.3×10^{-5} sq.cm. V.⁻¹ sec.⁻¹, which is greater than found at pH 8. Two full-term cord-blood samples were analysed at two concentrations of oxyhaemoglobin. The agreement in the results is taken as evidence that equations 1 and 2 are applicable.

Table 1. *Comparison of electrophoretic mobilities of adult and foetal oxyhaemoglobins by the differential method*

(In columns (2) and (8) A = adult, F = foetal; in column (4) asc. = ascending, desc. = descending. The minimum difference in mobility of components (column 7) is calculated from equation 2 of Hoch (1949).)

Exp. no.	pH of buffer	Haemoglobin concentration in top layer (g./100 ml.)	Potential gradient (V. cm. ⁻¹)	Total change in distance (cm.) between the two ascending or the two descending boundaries	Total time (min.)	Difference in migration velocity of boundaries (10 ⁻⁵ cm. sec. ⁻¹)	Minimum difference in mobility of components (10 ⁻⁵ sq.cm. V. ⁻¹ sec. ⁻¹)	Faster component
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	8.0	A 0.09 F 0.12	14	Asc. 0.47	320	2.4	0.17	A
2	7.1	A 0.22*	8.7	Asc. 0.10	140	1.2	0.14	A
3	7.1	A 0.5† F 0.5	8.7	Asc. 0.12 Desc. 0.17	300 300	0.7 0.9	0.09	A

* One experiment only with adult haemoglobin in top layer on ascending side.

† One experiment only with adult haemoglobin in top layer on ascending side and foetal haemoglobin in top layer on descending side.

Table 2. *Electrophoretic analyses of foetal and infant haemoglobin*

Source	Apparent proportion of faster component, % of total ($C'_1/C'_1 \times 100$)	Apparent relative difference in velocity, % of V'_{a1} ($S/V'_{a1} \times 100$)	Haemoglobin concentration in adjusted solution (g./100 ml.)	Corrected proportion of faster component, % of total ($C'_1/C'_1 \times 100$)	Corrected relative difference in mobility, % of V'_1 ($(V'_1 - V'_2)/V'_1 \times 100$)
(1)	(2)	(3)	(4)	(5)	(6)
Foetus, 20 weeks	26	2.2	0.72	6.5	8.7
Foetus, 20 weeks	34	1.7	0.86	6.9	7.9
Premature infant, 36 weeks	43	2.0	0.87	11	7.5
Full-term infant	55	3.0	0.96	21	7.6
Full-term infant, a	68	2.5	1.21	25	6.6
Full-term infant, b	39	3.1	0.65	15	7.5
Full-term infant, a	50	2.1	1.01	13	7.4
Full-term infant, b	36	2.6	0.74	16	8.5
Infants, 5 weeks (pool of two samples)	55	3.0	0.96	21	7.6
Infants, 2 months (pool of two samples)	79	4.8	0.88	54	6.8
Infants, 4 months	94	8.2	About 0.9	88	8.8

Foetal and infant haemoglobin. Fig. 1 and Table 2 show that there was a progressive increase in the relative area of the faster component as one passed from the 20-week foetus to 4-month infants, and that the correlated relative difference in mobility between the two components was constant over the whole range. The fast component was therefore assumed to represent adult oxyhaemoglobin in all cases. If this assumption is correct, then at 20 weeks the haemoglobin of the foetus already contained at

Artificial mixtures. To 20-week foetus oxyhaemoglobin different amounts of adult oxyhaemoglobin were added and the mixtures analysed by electrophoresis and spectrographically (Table 3 and Plate 4, Fig. 1 h). It was found that the proportions of adult oxyhaemoglobin, corrected with the aid of equation 1, agreed better with those calculated from the amounts used in preparing the mixtures than the proportions as apparent from the patterns. However, the error in drawing the partition line between the two peaks

Table 3. *Electrophoretic analyses of artificial mixtures of adult and foetal haemoglobin*

Exp.	Proportion of adult haemoglobin added to 20-week foetus haemoglobin (%)	Haemoglobin concentration of adjusted solution (g./100 ml.)	Faster component found (%)		Faster component (%) calculated on basis of foetus haemoglobin containing	
			Apparent	Corrected	Apparent adult haemoglobin	Corrected adult haemoglobin
(1)	0	0.72	26.1	6.5	—	—
	22.4	1.05	57.1	22.4	42.7	27.4
	48.5	0.96	79.1	56.0	61.9	57.9
(2)	0	0.86	33.6	6.9	—	—
	34.0	0.90	64	35.1	56.2	38.6
	50.8	0.90	74	50.6	67.3	54.2

and in locating the boundaries appeared to result in an uncertainty of up to ± 5 in the figure given for the corrected percentage of adult oxyhaemoglobin. Because a small percentage of adult haemoglobin produces an exaggerated boundary peak in the pattern of foetus haemoglobin, a foetal sample in which no adult haemoglobin is present would have been better suited for proving the validity of equation 1, but no such material was available for study, since the amount of blood yielded by 12-week foetuses was far from sufficient for electrophoresis and a foetus of an age of about 16 weeks, which possibly would have been free from adult haemoglobin, was not available.

Spectroscopic

In discussing spectrographic results, it is important to appreciate that the wavelengths found for the tryptophan notch are subject to error in the spectrograph wavelength scale and to plate-reading errors. In the period during which the present results were obtained, wavelength calibrations indicated the spectrograph scale to be in error by -1.5 to -3 A. so that measurements on identical samples would be expected to vary over a range of 1.5 A. With respect to reading errors, for three observers the range of disagreement for individual plates is not normally greater than ± 1 A. for the tryptophan notch. If both these sources of error operated together in the most unfavourable sense, identical samples would be expected to give readings differing by 3.5 A. In practice, over short periods, measurements for example on normal adult samples, fall in the range 2909–2911 A., that is a range of 2 A., and it seems reasonable to assume that only wavelength differences greater than this are likely to be significant. The present discussion will be based on this assumption.

Considering first the samples of foetal origin which extend over the foetal age range 10–40 weeks, the latter being full-term cord bloods taken at birth, these all fall in the wavelength range 2897–2901 A. with 2898 A. as the most frequently observed value (mode) and 2898.3 as the mean (Table 4). The mode, 2898 A., agrees with the figure given by Jope (1949) for foetal haemoglobin. Apart from the sources of

error discussed above, it is of interest to note that the five samples giving 2897 A. for the wavelength of the tryptophan notch all gave resolved maxima for this feature when examined on the automatic recording spectrophotometer at a spectral band width of about 7.5 A., while the other foetal samples gave well

Table 4. *Wavelength of tryptophan notch in foetal and adult haemoglobin at pH 7 in 0.02M-phosphate buffer*

No. of samples	Description	λ (A.)
1	Foetal, 10 weeks	2898
2	Foetal, 12 weeks	2900 2900
5	Foetal, 16 weeks	2898 2898 2898 2897 2898
2	Foetal, 20 weeks	2898 (a) 2901* (b) 2900*
1	Foetal, 26 weeks	2898
6	Foetal, 36 weeks	2897 2897 2897 2898 2899
3	Full term, 40 weeks	2898 2899 2899
1	Infant, 2 months (pool of two samples)	2904
2	Infant, 4 months (pool of two samples)	2908
	Infant, 5 months (pool of two samples)	2905
12	Adult	2909 2910 2909 2910 2910 2909 2908 2910 2910 2912 2911

* Two separate preparations from whole blood.

defined inflexions, which were not resolved, although easy to distinguish from the poorly defined inflexions shown by adult samples. These differences are evident in Fig. 2. For foetal samples the difference between a well defined inflexion and a resolved maximum appears to be a question of instrumental performance, as it has been found that moving-plate spectrograms of foetal samples which did not give

the mean 2909.8 Å., in good agreement with the value (2910 Å.) given by Jope (1949) for adult human haemoglobin. The range of values, extending over 4 Å., is wider than would be expected from the sources of error discussed above, and arises from the single figures at the extreme of this range. One of these extreme values (2912 Å.) was obtained at the same time that a 5-month foetal sample gave 2900 Å., which is near the upper limit of the values for foetal haemoglobin, so that both these figures may be associated with a temporary instrumental error of +1 to +2 Å., which later disappeared, as shown by the subsequent reversion to figures at or nearer the mean values for foetal and adult samples.

Table 5. *Wavelength of tryptophan notch in artificial mixtures of adult and 20-week foetal human oxyhaemoglobins*

Nominal composition*		λ (Å.)
Foetal (%)	Adult (%)	
25	75	2905
50	50	2902.3
75	25	2901.2
50	50	2901.3
40	60	2902.5
30	70	2905.7
20	80	2906.8
10	90	2907.8

* Not corrected for 7% adult type haemoglobin in 20-week foetal samples.

Fig. 2. Ultraviolet absorption spectra of adult and foetal human oxyhaemoglobins. (a) Adult, showing unresolved inflexion. (b) 36-Week foetal, showing unresolved inflexion. (c) 36-Week foetal, showing resolved tryptophan notch at 2897 Å. The curves are direct tracings of spectrograms obtained with the automatic recording spectrophotometer at a constant spectral band width of 7.5 Å. The curves correspond to approximately equal concentrations of oxyhaemoglobin, but for clarity have been displaced on the vertical co-ordinate which is linear in optical density (D).

resolved maxima in the automatic recording spectrophotometer, do show resolution of the notch in densitometer records of the plates (Plate 4, Fig. 3). In spite of this partial discrepancy between the automatic recorder and the moving-plate spectrograms, the densitometer records from plates of adult samples confirm the lack of resolution of the tryptophan notch in these samples and the relatively poorer definition of the resulting inflexion.

In the light of these comments, the wavelengths found for samples of differing foetal age cannot be regarded as showing any significant variation. As will be shown below, the position of the tryptophan notch in mixtures of foetal and adult haemoglobin is at, or very near, the foetal position (2898 ± 1 Å.) if the proportion of adult type is not greater than 50%.

The twelve adult samples all fall within the wavelength range 2908–2912 Å.; the mode is 2910 Å. and

The spectroscopic results obtained with known mixtures of foetal and adult haemoglobins are shown in Table 5. For the mixtures containing 50% adult type, the displacement of the tryptophan notch from the foetal position is barely significant if spectrograph errors of 1–2 Å. have to be considered. For the mixture containing 10% foetal and 90% adult types the position is not experimentally distinguishable from the figure for the adult type itself. For the mixtures of intermediate composition the displacements from the foetal position are roughly consistent with their compositions, the positions of the notch moving away from the foetal to the adult position as the proportion of the latter is increased. From a graphical treatment of the results (Fig. 4), however, it appears that the position of the tryptophan notch is not a linear function of the relative proportions of the two forms, but that very little shift from the foetal position occurs until the proportion of adult type is nearly 50%, so that almost the whole of the wavelength difference of some 12 Å. between the foetal and adult types occurs in the composition range 50–90% adult. It is not difficult to understand this behaviour when it is recalled that in the foetal type the tryptophan notch is much better resolved than in the adult type and will therefore tend to dominate the notch profile in

mixtures of the two types, so that relatively large proportions of the adult type will be required to influence the profile in mixtures.

Fig. 4. Relation between the position of the tryptophan notch and the composition of artificial mixtures of adult and 20-week foetal human oxyhaemoglobins. The compositions have been corrected for the proportion (7%) of the adult type in 20-week foetal haemoglobin.

This dominant effect of foetal-type haemoglobin on the location of the tryptophan notch unfortunately precludes the use of this technique to check the estimates of adult type haemoglobin in foetal samples by the electrophoretic method (Table 2). As pointed out below, the two methods are in fair agreement in estimating the small proportions of foetal type haemoglobin in early infant blood samples. These are in the composition range in which the spectroscopic method is of definite value.

It may be noted at this point that in haemoglobin the tryptophan absorption is superimposed on that of β -phenylalanine and tyrosine, of which the latter makes an appreciable contribution to the total absorption of the globin moiety of the molecule. It is clearly of importance to the present work to ascertain if the superposition of an absorption band affects the observed wavelength of the fine structure. This situation has been studied by using mixtures of β -phenylalanine and diphenyl, in which the fine-structure bands of β -phenylalanine are superimposed on the smooth rising absorption of the longwave side of the absorption band of diphenyl. The results obtained by the moving-plate method are listed in Table 6 and show that over a wide range of relative contributions by these two compounds to the total absorption, the wavelengths of the β -phenylalanine fine-structure bands are constant to ± 1 Å.

The intensities of the total aromatic amino-acid absorption bands in foetal and adult haemoglobins were compared by measuring the ratios of the densities of solutions of the two types adjusted to nearly equal concentration, at the wavelengths of the visible maxima and at selected points on the globin band. The results (Table 7) show that, relative to the haem absorption, the globin absorption is identical in the two types.

Some additional spectroscopic evidence is available regarding the state of the β -phenylalanine residues in foetal and adult human haemoglobins. The fine-structure bands of this aromatic amino-acid are clearly visible in moving-plate spectrograms of many proteins, including haemoglobin, and are found to be displaced to long wavelengths, in comparison with the free amino-acid, for reasons discussed below.

Table 6. Wavelengths of β -phenylalanine fine-structure bands superimposed on diphenyl absorption band

Solvent: 95% ethanol
(R = concentration of β -phenylalanine:concentration of diphenyl.)

	$\lambda_{\max}$ (Å.)							
β -Phenylalanine alone	2365*	2419	2472	2523	2583	2613	2640	2678
β -Phenylalanine + diphenyl, $R=5.6$	2368*	2418	2473	2522	2582	2613	2640	2678
β -Phenylalanine + diphenyl, $R=1.4$	—	—	—	2522	2583	2613	2640	2678
β -Phenylalanine + diphenyl, $R=0.78$	—	—	—	—	—	2612	2641	2678

* Very faint band.

Table 7. Comparison of 20-week foetal* and adult haemoglobins in visible and ultraviolet light

(Approx. 0.033% solutions in 0.02M-phosphate buffer, pH 7; 1 cm. cells.)

λ (Å.)	5760	5410	2900	2800	2700
$D_{5760}^{1\text{cm. foetal}}/D$	0.326	0.331	0.62	0.76	0.82
	(1.00)	1.05	0.525	0.43	0.40
$D_{5760}^{1\text{cm. adult}}/D$	0.336	0.316	0.62	0.77	0.85
	(1.00)	1.06	0.54	0.435	0.395

* Containing 7% of adult type haemoglobin.

Measurements of these band positions in foetal and adult human haemoglobins, which are reported in detail elsewhere (Beaven, Holiday & Jope, 1950), show that their positions are identical in the two types within the limits of experimental error, which range from ± 2 to ± 6 Å. depending on the band resolution.

DISCUSSION

From the experiments presented here it is apparent that the change from foetal to adult haemoglobin is a gradual process starting not later than midway

through pregnancy. In the earliest foetal sample examined (20 weeks) there is 6–7 % of adult type haemoglobin. At birth about 20 % adult haemoglobin was found, in agreement with the experiments of Haurowitz (1929, 1930). In infants 4 months of age only about 10 % foetal haemoglobin was present.

Adult and foetal haemoglobin dissociate into smaller molecules at low concentrations, but the two do not differ in the degree of dissociation (Gutfreund, 1949, quoted by Jope, 1949). In the present experiments dissociation must have been slow or suppressed since there was no evidence of the formation of mixed molecules with an intermediate mobility due to recombination of the fragments; two peaks only were seen in all instances.

The spectroscopic results for samples from infants of age 2 and 4 months respectively show progressive shifts from the foetal to the adult position of the tryptophan notch, and suggest that replacement of the foetal type of haemoglobin is very nearly complete 4 months after birth. It may be pointed out that this period is in good agreement with the average life of a red blood corpuscle as estimated by several concordant methods (Jope, 1946*a, b*). The proportion of adult-type haemoglobin 2 months after birth is 54 %, and 4 months after birth 88 %, by electrophoretic analysis (Table 2); from the measurements on synthetic mixtures of foetal and adult types the spectroscopic estimates of adult type in the 2- and 4-month infant samples are 60 % and about 80 %, respectively, in fair agreement with the electrophoresis figures. The rate of replacement of foetal by adult type haemoglobin after birth is thus of the same order when estimated by two independent methods.

The results given in Table 7 suggest that foetal and adult types of haemoglobin do not differ with respect to their total aromatic amino-acid composition. Porter & Sanger (1948) find by end-group analysis that the adult type contains 5 valine end groups compared with 2.6 for foetal type. As their sample of foetal type was taken from a 30-week foetus, with a possible adult type content of 10 %, the end-group analyses correspond more nearly to 2 valyl end groups in the pure foetal type.

The spectroscopic results confirm Jope's finding that the tryptophan fine-structure maximum occurs at a longer wavelength in adult-type human oxyhaemoglobin than in the foetal type, and further reveal that this shift is accompanied by a loss in resolution. For mixtures of the two types containing 50 % or more of the adult type, the position of the maximum can be used to estimate the proportions of the two types and the results are in fair agreement with the electrophoretic estimates of composition. No other differences in absorption spectra, either qualitative or quantitative, between

the foetal and adult types of human oxyhaemoglobin, have been found.

Some comment on the difference in position of the tryptophan fine structure and in its degree of resolution in the foetal and adult types of human haemoglobin may be made on the basis of previous observations on synthetic peptides and proteins (Beaven *et al.* 1950). It is known that both shifts to longer wavelength and loss of resolution of fine structure occur in organic chromophores either by the introduction of non-absorbing substituent groups—the so-called weighting effect—or by interaction with polar, and especially hydroxylic, solvents (Brode, 1939; Morton, 1938). It appears unlikely, however, that the tryptophan residues in adult human globin contain such substituents and only environmental effects will be considered here. It is known from observations on tryptophan peptides that simple peptide combination shifts the tryptophan notch from 2880 Å. in the free amino-acid to about 2892 Å. with some loss of resolution, but this effect alone is inadequate to account for the shift to 2898 Å. in the foetal type and to 2910 Å. in adult-type human haemoglobins, or the conversion of the fine-structure maximum into a poorly defined inflexion in the latter type. In comparison with simple tryptophan peptides, the tryptophan notch may be shifted to 2910–2920 Å. in some proteins, and it has been shown that this further shift can be observed in tryptophan peptides if these are examined in an environment in which strong solute-solvent interaction may be expected. A convenient protein medium for such observations is a dried gelatin film. The following results for glycyltryptophan are typical:

	λ_{notch} (Å.)
Tryptophan (free acid in water)	2880
Glycyltryptophan (water)	2890
Glycyltryptophan (gelatin sol)	2890
Glycyltryptophan (gelatin film)	2917

Such an effect of a general protein environment on the absorption spectrum of tryptophan already combined in peptide linkage suggests that in adult haemoglobin a proportion of the tryptophan residues are oriented in a manner that leads to greater interaction with polar groups in the haemoglobin molecule itself or with water either as solvent or in the hydration layer, than for the foetal type. If this is indeed the case, the resulting additional longwave shift would tend to overlap and hence to obscure the resolved notch found in the foetal type, in which presumably the tryptophan residues are all subjected to sufficiently uniform environmental factors to give approximately equal longwave shifts and hence to show a resolved notch with a well defined maximum. A more detailed discussion of this suggestion would not be justified on the basis of present knowledge, but it is at least not inconsistent with the facts that

Fig. 1.

Fig. 3.

the foetal and adult types also differ with respect to speed of spreading in monolayers and in rate of alkaline denaturation, both of which may be plausibly related to different configurations of hydrophilic and alkali-sensitive groupings respectively in the globin molecules of the two types.

SUMMARY

1. Foetal and adult haemoglobins have been studied by electrophoretic and spectrophotometric methods.

2. The differences in their properties when examined by these two methods have been used to follow their distribution in human blood from early prenatal life.

3. Adult oxyhaemoglobin shows greater anodic mobility than foetal oxyhaemoglobin in phosphate at pH 7.1, 8.0 and 8.8.

4. The findings of Joep on the differences in spectroscopic characteristics between the two types of haemoglobin have been confirmed and extended.

5. At 20 weeks of pregnancy foetal oxyhaemoglobin contains at least 6% of 'adult' type; at birth about 20%, at 2 months after birth about 50%, and at 4 months between 80 and 90% 'adult' type.

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EXPLANATION OF PLATE

Fig. 1. Electrophoretic patterns of haemoglobins. (a) Foetus 20 weeks, 330 min. (b) Foetus 20 weeks, 325 min. (c) Premature baby 36 weeks, 337 min. (d) Baby full term, 307 min. (e) Babies 5 weeks, 303 min. (f) Babies 2 months, 217 min. (g) Baby 4 months, 119 min. (h) Artificial mixture, containing 58% adult haemoglobin, 155 min. All experiments in 0.01 M- Na_2HPO_4 at 15.7 V. cm.⁻¹ Ascending patterns; the direction of migration is from right to left. The haemoglobin concentration can be read from Table 2.

Fig. 3. Microphotometer records of logarithmic-cam, moving-plate spectrograms of human oxyhaemoglobin solutions. (a) Adult, showing unresolved inflexion at 2911 Å. (b) 20-Week foetal, showing resolved maximum at 2898 Å. (c) 12-Week foetal, showing resolved maximum at 2900 Å. All spectrograms taken on Hilger medium quartz spectrograph with slit of 0.15 mm. and hydrogen arc source, using Kodak B.10 plates. Samples (b) and (c) gave unresolved and resolved maxima respectively when examined on the automatic recording spectrophotometer at a spectral band width of 7.5 Å.