ketone: *tert*-butanol: glacial acetic acid: water: concentrated HCl (22.5: 22.5:35:19:1) (Solvent system III) was the most suitable for separating the hydrolytic cleavage products of ribonucleic acids. This mixture moved about 36 cm in 16 hours on Whatman No. 4 paper and had the advantage for 2-way chromatography that the positions of adenine and cytidylic acid were the reverse of those in solvent systems I and II.

The R_F values of bases, nucleosides and nucleotides in solvent systems I, II and III on Whatman No. 4 paper are shown in Table I.

TABLE I

	Rf values in the solvent systems		
	I	*II*	*III*
Guanine	0.18	0.20	0.19
Adenine	0.31	0.33	0.39
Cytosine	0.42	0.30	0.39
Uracil	0.62	0.58	0.67
Thymine	0.70	0.64	0.75
Guanosine	0.31	0.24	0.22
Adenosine	0.36	0.31	Trailed
Cytidine	0.48	0.40	0.29
Uridine	0.64	0.58	0.58
Guanosine-3′-phosphate	0.43	0.38	0.22
Adenosine-3′-phosphate	0.51	0.42	0.31
Cytidylic acid	0.60	0.53	0.26
Uridylic acid	0.77	0.70	0.54

This investigation was carried out during the tenure of a British Empire Cancer Campaign Research Fellowship and was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

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Received September 16th, 1955

Is foetal haemoglobin present in the blood of normal human adults?

The haemoglobin of the new-born child consists for about 80% of Hb-F and 20% of Hb-A. After four months the foetal component seems to have disappeared. BRINKMAN AND JONXIS[1] used the difference in the rate of alkali-denaturation, but did not succeed in demonstrating the presence of foetal haemoglobin in the blood of older children and adults. The method used, however, was not accurate below 5% of Hb-F present. Later, SINGER *et al.*[2] demonstrated with an altered alkali denaturation technique (starting with HbO_2) the presence of 1–3% of a foetal-like component in the blood of adults, while KÜNZER[3] with a similar procedure, but using cyanhaemoglobin, found 0.5–1% of such a component. With immunological methods CHERNOFF[4] demonstrated the presence of 0.05–0.5% of Hb-F, while moreover ROCHE AND DERRIEN[5] with a salting-out technique found small amounts of a component with the same solubility as that of Hb-F from cord blood. The amounts of this component found by these methods are so different that it seemed desirable to repeat the investigation concerning the problem of the possible presence of Hb-F in adult blood. Moreover it may be important to investigate whether the alkali-resistant fraction found was identical with foetal haemoglobin. For this purpose we used in our investigation the estimation of the amino acid composition of this fraction, as there are many differences between Hb-F and the other haemoglobins in this respect[6].

In the present study blood samples of the authors were used. The haemoglobin was prepared and purified by the method described previously[6]. Three samples of HbO_2 (no monocarboxyform) with a concentration of 6–8 mg % (total amounts 5320, 4615 and 7670 mg respectively) were exposed to the action of alkali (final pH 12.6) for two minutes. The denaturation process was then interrupted by adding an equimolar amount of hydrochloric acid. The denatured haemoglobin was salted-out by adding a 3.5 *M* phosphate buffer pH 6.5 (= 100%), described by DERRIEN[7], until a final concentration of 80% was reached. After filtration, the unaltered haemoglobin was precipitated by dissolving the calculated amount of pulverized KH_2PO_4 and K_2HPO_4

(equimolar quantities) to a final concentration of 98%. The precipitate was collected and dissolved in distilled water. The haemoglobin was first converted to haemoglobin by adding a small amount of $K_3Fe(CN)_6$ and then to cyanhaemoglobin with KCN. The exact haemoglobin concentration was determined by reading the optical densities at 4200° A and 5400° A in a Beckman DU spectrophotometer.

The amounts of the isolated haemoglobin in the three samples studied were 23 mg (= 0.4% of the original amount), 17 mg (= 0.35%) and 40 mg (= 0.5%) respectively. As there are relatively great differences in the amino acid composition between the Hb-F and the normal adult component some amino acid analyses are carried out in the isolated proteins. The method used is the same as that described previously[6]. The results given in Table I shows that the isolated fractions have a strong tendency to the foetal haemoglobin in view of the higher amounts of threonine, serine and isoleucine and the lowered amounts of valine, tyrosine and histidine. As the differences between the isolated haemoglobins and the adult component are always smaller than between the purified Hb-F and the Hb-A, it will be clear that next to the foetal haemoglobin

TABLE I

THE QUANTITIES OF DIFFERENT AMINO ACIDS IN 48-HOUR HYDROLYSATES OF THE ISOLATED HAEMOGLOBINS AND OF Hb-A AND Hb-F

(the values are given in g/100 g protein)

Amino acid	*Hb-A*	*Hb-F*	*Sample 1*	*Sample 2*	*Sample 3*
Aspartic acid	10.5	10.25	10.6	10.45	11.0
Threonine	5.5	6.5	6.4	6.05	5.95
Serine	4.45	5.85	5.65	5.85	5.2
Glutamic acid	7.25	7.65	7.95	8.15	7.45
Glycine	4.55	4.6	4.7	4.35	4.9
Alanine	9.95	9.7	9.6	9.8	9.9
Valine	10.9	9.5	9.8	9.6	9.2
Isoleucine	0.35	1.85	1.5	1.6	1.4
Leucine	15.15	15.3	15.8	15.5	15.15
Tyrosine	3.85	3.2	3.3	3.5	2.9
Phenylalanine	7.85	7.85	7.5	8.05	7.4
Lysine	9.9	9.85	9.55	9.9	9.7
Histidine	8.3	7.4	7.35	7.5	7.7

some Hb-A (about 25%) is also present. Hence it follows that the three haemoglobin samples studied contained 0.3–0.4% of Hb-F. During the procedure described here no notable quantities of Hb-F are precipitated together with the adult component. To prove this, small amounts of Hb-F from cord blood (8.5 mg and 21.5 mg respectively) were added to some haemoglobin samples of adults. The recovery was quite satisfactory (10 mg and 23.5 mg respectively).

From these data it will be clear that small amounts of alkali-resistant haemoglobin are demonstrable in the blood of normal adult individuals. The amino acid composition of this fraction makes it very likely that the alkali-resistant component is identical with the foetal haemoglobin. The percentages of 0.3–0.4 which we found are much lower then those described by SINGER *et al.*[2]; they are in correspondence with the small quantities estimated with quantitative immunologic techniques[4].

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Received September 30th, 1955