density of the colour formed, after the addition of copper acetate and diethylamine, by means of a photoelectric absorptiometer. The graphs showed considerable deviations from linearity outside the range 0.5–5.0 p.p.m. CS₂.

Table 1 shows a comparison of the two methods when used for the analysis of three benzene samples.

Table 1. SULPHUR AS CARBON DISULPHIDE (P.P.M. BY WEIGHT)
S.T.P.T.C. standard method G.L.C. method

Sample 1	6.3	6.4
Sample 2	0.4	<0.1
Sample 3	0.3	<0.1

A good agreement is obtained in the case of sample 1 having a relatively high CS₂ content.

The gas-liquid chromatography (G.L.C.) method is probably more accurate than the S.T.P.T.C. method with samples 2 and 3, since these results were based on the absence of any CS₂ peak, in contrast with a calibration sample containing 0.1 p.p.m. of this compound.

Table 2

Injection number	1	2	3	4	5	6	7	8	9	10
Peak ht. (cm)	10.50	10.76	11.21	10.95	10.65	10.95	10.75	11.15	11.35	11.15
	Mean peak height = 10.94 cm									
	Standard deviation = 0.274 cm									
	Per cent standard deviation = 2.5									

Table 2 gives the results of ten repeat injections of a 10-μl. sample of benzene containing 1.0 p.p.m. sulphur as CS₂.

The results indicate a relatively high degree of repeatability for this type of analysis.

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Separation of Coumarins

By sorption on ion exchange resins¹⁻³ it is possible to separate some coumarins in dilute solution. A column of the strongly basic anion exchange resin, 'Amberlite IRA-400' (−40, +60 mesh; Rohm and Haas Co.), in the chloride form (bed length, 50 cm; bed volume, 25.8 c.c.) has been used for this purpose.

The coumarins studied were of three groups. Group A includes coumarin (I), 3-Me(I), 7-Me(I), 3,4-di Me(I), 6,7-di MeO-4-Me(I) and 7,8-di MeO(I); group B includes 7-OH(I) and 8-OH(I); group C includes 6,7-di OH-4-Me(I) and 7,8-di OH-4-Me(I).

In a run, 50 ml. 5.636 × 10^{−4} M solution of one of the above coumarins in 10 per cent methanol (by volume) was passed through the column at 5 ml./min and the sorbed solute on the column was eluted. The effluent was collected in samples and estimated for the solute content by ultra-violet absorption⁴. Group A compounds were eluted by 10 per cent methanol. Group B compounds were not eluted by 10 per cent methanol, but were slowly eluted by N/10 HCl in 10 per cent methanol. Group C compounds were not eluted by 10 per cent methanol or by N/10 HCl in 10 per cent methanol, but were eluted by N/100 HCl in methanol. It is therefore possible to separate binary and ternary mixtures containing not more than one compound from each group.

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BIOCHEMISTRY

Altered Electrophoretic Mobilities of some Erythrocytic Enzymes as a Function of their Age

A NUMBER of recent reports^{1,2} have indicated that the *in vitro* storage of human red blood cells leads to an increased electrophoretic mobility of some enzymes (for example, glucose-6-phosphate dehydrogenase, acid phosphatases) when compared with the mobility of the same enzyme obtained from fresh blood. This finding parallels that previously reported for haemoglobin derived from fresh or stored red cells³.

The availability of several methods for the separation of young and old red blood cells or of their lysates from a red blood cell population⁴⁻⁶ prompted us to investigate the electrophoretic behaviour of some enzymes derived from erythrocytes of different ages. This communication presents results of preliminary experiments indicating an altered electrophoretic mobility of some erythrocytic enzymes as a function of the age of the red cell from which they are derived.

It was also found that this altered electrophoretic behaviour is not shared by all erythrocytic enzymes, nor even necessarily by enzymes having the same substrate specificities but originating in different aged erythrocytes of other species. The implications of these experiments are briefly discussed.

100 ml. of human blood, collected in EDTA or acid-citrate-dextrose solutions, were washed six times with isotonic saline, great care being taken to remove the buffy white blood cell layer. The washed red cells were then subjected to the serial osmotic haemolysis procedure of Simon and Topper⁶ in which lysates from young and old erythrocytes are obtained separately. The smallest possible lysing volumes were used so as to have a high enzyme content per unit volume. Lysates were centrifuged at high speed (13,000g for 10 min) to remove the stroma. The haemolysates corresponding to the reticulocytes were not examined.

Control experiments on the behaviour of white blood cells in the serial osmotic haemolysis procedure indicated that the contribution of enzymes by residual, contaminating white blood cells in the red blood cells fractionated was small and most probably did not affect the results described here. Further experiments, however, using other separation procedures for young and old erythrocytes⁵ are in progress to confirm our results by another, independent method.

Glucose-6-phosphate dehydrogenase (G-6-PD) and glutamic-oxaloacetic transaminase (GOT) activities were assayed by standard spectrophotometric methods⁷⁻⁹ on the lysates of the youngest and older human erythrocytes. One unit of enzyme is defined as that amount required for a ΔO.D._{340 mμ} of 0.001/min./ml. of lysate. For comparative purposes samples of blood were stored at 4°–5° C for up to four weeks. The latter samples were washed with saline after storage, lysed and enzyme activities determined as indicated above.

Starch-gel electrophoresis of G-6-PD was carried out according to the method of Fildes and Parr¹ except that, at these authors' suggestion, a 0.01 M phosphate buffer at pH 7.5 was substituted for the *tris* buffer described in their paper. GOT was electrophoresed following the method of Schwartz *et al.*¹⁰, but using a 0.01 M phosphate buffer at pH 7.3.

In all the electrophoretic runs the number of enzyme units of each lysate applied was identical to that of the fraction to which it was compared. About 10 units (as defined here) were applied for both G-6-PD and GOT.

Fildes and Parr¹ reported that G-6-PD has an increased electrophoretic mobility when the red blood cells from which it is derived have been stored. We have repeated these experiments and confirm their findings. The