migration is shorter than for the 'fast' esterase, more likely resembling their original migration in starch. Furthermore, the spot is better defined, although it is always less intense than the 'fast' esterase spot.

In another analogous experiment, the cuttings of E_1, E_3 and albumins of four animals were transferred to an agar plate. This was first incubated in carbonaphthoxycholine and the purple staining appeared only for E_1; the plate was then washed and incubated in indoxyl acetate, which coloured principally the E_3 spots in blue. No reaction was observed for albumin (Fig. 3).

The identification of E_1 with serum cholinesterase seems thus confirmed.

The comparison of the migration of four cholinesterases and three 'fast' esterases shows that the mobilities of those components in sera of horse, donkey, mule and hinny are the same.

These results show that the donkey serum lacks an esterase present in horse serum and that both their hybrids resemble the horse, whether it is the father or the mother.

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Estimation of Glucose-6-phosphate Dehydrogenase in Sheep Erythrocytes

Using three different methods, which all depend on dye reduction and the use of redox intermediates for the $NADPH_2$ formed in the assay, Budtz-Olsen *et al.*[1] have reported absence of glucose-6-phosphate dehydrogenase (G6PD)-activity in erythrocytes of sheep and goats. We have been estimating G6PD in sheep erythrocytes by a method which is not dependent on dye reduction, and although the values are lower than in human beings, there is still appreciable activity.

The method we have used for our investigations is based on that of Schrier *et al.*[2], using the reaction coupled to glutathione reductase (GSSGR):

$$\text{Glucose-6-phosphate} + \text{NADP} \xrightarrow{\text{G6PD}} \text{6-phosphogluconate} + \text{NADPH}_2$$

$$\text{NADPH}_2 + \text{Oxidized glutathione (GSSG)} \xrightarrow{\text{GSSGR}} \text{NADP} + \text{Reduced glutathione (2GSH)}$$

with determination of GSH as a measure of G6PD activity. This method, of course, depends on GSSGR activity being greater than that of G6PD, and so ensuring the rate of G6PD activity as the limiting factor. This appears to be so in the case of ruminant erythrocytes, where GSSGR activity is about twice that of G6PD, and also in the case of erythrocytes from drug-sensitive human beings, where the G6PD activity is less than 10 per cent of normal with GSSGR activity about 1·5-2 times normal[2,3]. This coupled reaction may not be strictly valid with normal human erythrocytes where G6PD activity is greater than that of GSSGR in terms of GSH production, and therefore the rate of the latter reaction is the limiting factor. Indeed, Jocelyn[4] used the same coupled reactions for the determination of GSSGR activity.

In order to clarify the problem, we have compared results on sheep and human hæmolysates by determining G6PD by three different methods: (*a*) coupling G6PD and GSSGR (GSSG method, Table 1); (*b*) rate of decolorization of dichlorophenolindophenol[5] by the continuous production of $NADPH_2$ (DCIP method); (*c*) continuous measurement of $NADPH_2$ at 340 mμ using the technique of Glock and McLean[6] for coloured samples, except that *tris* (hydroxymethyl)amino-methane buffer replaces that of glycylglycine (NADP method).

Table 1. Activity of Erythrocyte G6PD by Three Methods

Specimen		GSSG method *a*	DCIP method *b*	NADP method *c*
Sheep	1	1·38	0·112	1·45
,,	2	2·83	—	2·61
,,	3	1·41	0·130	1·43
Human	1	8·2	1·32	6·37
,,	2	6·35	1·58	7·99
,,	3	8·35	1·61	8·41

a, μmoles GSSG reduced/min/g hæmoglobin at 37°. *b*, μmoles DCIP decolorized/min/g hæmoglobin at 20°. *c*, μmoles $NADPH_2$ formed/min/g hæmoglobin at 37°.

All methods indicate that the G6PD activity of sheep erythrocytes is lower than that of human beings, as was found by Budtz-Olsen *et al.*

Since the spectrophotometric NADP method is generally accepted as the reference method, involving fewest reaction stages and hence having fewest variable factors, it would appear that the following conclusions could be drawn:

(1) The colorimetric coupled G6PD–GSSGR method works reliably in the case of sheep erythrocytes, although the method is probably not exact in the case of normal human erythrocytes.

(2) The method of Ells and Kirkman[5] utilizing the decolorization of DCIP gives lower values than the NADP-spectrophotometric method even making an allowance for the temperature difference. This discrepancy is very marked in the case of sheep erythrocytes and probably accounts for Budtz-Olsen *et al.* finding sheep erythrocytes to be deficient in this enzyme.

It is perhaps significant that the three methods used by Budtz-Olsen *et al.* involved the use of the redox intermediates methylene blue or phenazine methosulphate, which are not present in the coupled G6PD–GSSGR or NADPH-spectrophotometric methods.

It seems likely that methods involving addition of substances not normally found in the natural system for $NADPH_2$ production, that is, dyestuffs and redox intermediates, while their validity has been proved with human erythrocytes, may not be strictly applicable to red cells of other species. The possibility of co-factors which would affect the rate of a reaction occurring in some species and not in others is exemplified by Huennekens's[7] report that the methæmoglobin reductase of cattle erythrocytes does not require a redox-intermediate such as methylene blue whereas that from human erythrocytes does.

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In this mild controversy it is worth recalling that Somogyi[1] long ago found that the total glycolytic activity of sheep blood is only 20–30 per cent of that of human blood. Leng and Annison[2] have recently shown that glucose only in rare cases penetrates into sheep erythrocytes. But they also observed that of the low glucose metabolism which does take place in these red cells 15 per cent goes through the pentosephosphate pathway; when stimulated by methylene blue, the fraction rises to nearly 100 per cent. These findings implicate the presence of glucose-6-phosphate dehydrogenase in the cells, but the