



Fig. 3 Photomontages may be made from overlapping negatives. The torn, overlapping lines are outlined in black.  
 — × 12 Bar = 1 mm

Reference

<sup>1</sup> Johannessen JV. Photographic techniques. In: Johannessen JV ed. *Electron microscopy in human medicine*. Vol 1. New York: McGraw-Hill, 1978:311-27.

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## Letters to the Editor

### An aqueous mounting medium

Many types of cytochemical study, whether on exfoliated smears, isolated cells, or on cryostat sections, involve the precipitation of a water-insoluble coloured reaction product that by its nature can be dissolved by lipid solvents. Thus, for inspection under a coverslip such specimens have to be mounted in an aqueous mountant, such as glycerine jelly or Farrants' medium. The former is messy to use; the latter often gives only temporary retention of the exact localisation of some of the coloured reaction products, such as the formazan of neotetrazolium chloride. It may not be common knowledge that a formulation for mounting media, based on polyvinyl alcohol (that is used in many cytochemical reactions) was described long ago.<sup>1</sup> Its lack of popularity may have been due to the crude preparations of

polyvinyl alcohol then available, and to the fact that acetone was included in such formulations (probably to dissolve the crude polyvinyl alcohols of that time).

We have reinvestigated this formulation, excluding acetone, and using one of the more water-soluble grades of polyvinyl alcohol. Of several we have tried, the aqueous mounting medium (designated Z5), which seems satisfactory, is prepared as follows: 12 g of the G18/140 grade of polyvinyl alcohol (obtained from Wacker Chemicals Ltd, Bridge Street, Walton on Thames, Surrey) is dissolved at 60°C in 50 ml of a 0.4 M sodium acetate:acetic acid buffer, pH 6.5. We normally leave this to stand for one day at room temperature to ensure complete solution. To this are added 20 ml glycerine and 20 ml lactic acid. These add to the clarity of the medium and to its ability to penetrate the specimen, and they also help to plasticise

the polyvinyl alcohol against cracking when the film of mountant, under the coverslip, dries. The solution is then made up to 100 ml with the 0.4 M sodium acetate:acetic acid buffer of pH 6.5. One crystal of thymol is included to stop growth of micro-organisms.

The pH can be varied according to the pH-characteristics of the coloured reaction product. Calcium can be included in the medium if the tissue is fatty.

The refractive index of the fluid mounting medium, measured with a Leitz Jolley refractometer, is close to 1.5 (1.498). Thus this medium gives a clear image of the tissue and a preparation that remains permanent at least for several months. It is easier to use than glycerine jelly and could ease the lot of the cytochemically-minded cytologist or histopathologist who cannot avail himself of conventional dehydration and embedding

in a xylol-based or alcohol-based mountant.

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#### Effect of hyperglycaemia as assessed by glycosylated haemoglobin concentrations on red cell enzyme activities in diabetes mellitus

Our study was initiated to study the effects of hyperglycaemia on the key catalytic proteins of erythrocytes, namely, glucose-6-phosphate dehydrogenase, phosphohexose isomerase, and pyruvate kinase, in an attempt to understand known abnormalities that have been demonstrated in the erythrocytes of diabetics. These include reduced red cell deformability,<sup>1</sup> and increased erythrocyte half-life, along with other haematologic parameters, namely, increased leucocyte adherence, and change in platelet function in response to epinephrine as measured in a group of diabetics before and after achievement of glucose control.<sup>2</sup>

Interest in explaining the pathophysiology of the sequelae of diabetes mellitus has been sparked by the recognition that tissues which are not dependent on insulin for glucose transport are altered when exposed to excess glucose in diabetes. Changes resulting from the incorporation of glucose or its metabolic products have been observed in erythrocytes, as raised glycosylated haemoglobin concentrations,<sup>3</sup> in the accumulation of sorbitol in lens, sciatic nerve, and renal papilla, via the conversion of glucose by aldose reductase,<sup>4</sup> and an increase in glycosylation of hydroxylysine in the glomerular basement membrane in diabetics.<sup>5</sup> The latter effect has been postulated to account for the increased permeability of glomerular basement membrane in diabetics. In our study the glycosylated haemoglobin concentrations served as a measure of the degree of hyperglycaemia.

Glycosylated haemoglobins are synthesised throughout the life span of the mature erythrocyte by attachment of glucose to N-terminal valine of the beta-globin peptide chains by a post-translational nonenzymatic reaction.<sup>6</sup> This condensation process is practically irreversible under physiological conditions and depends on the circulating concentration of blood glucose; thus, the concentration of the glycosylated haemoglobin fraction reflects the plasma glucose concentration integrated over an extended period of time determined by the life span of the erythrocyte.<sup>7</sup> Glycosylation is an indiscriminate process and can affect all proteins containing free amino groups.

Blood samples were obtained from fasting diabetic patients managed as outpatients and in the emergency room and non-diabetic outpatients at the Kaiser Foundation Hospital in Honolulu. Assays were performed on fresh blood in batches consisting of equal numbers of diabetic and non-diabetic subjects.

Whole blood was used for the measurement of glycosylated haemoglobin, glucose-6-phosphate,<sup>8</sup> pyruvate kinase,<sup>9</sup> and phosphohexose isomerase<sup>10</sup> activities. Glycosylated haemoglobin was measured by cation exchange chromatography.<sup>11</sup>

As shown in the Table glycosylated haemoglobin concentrations were significantly raised in the diabetic group over the non-diabetic control. The three enzymes demonstrate insignificant variation in activity between diabetic and normal groups as indicated by the Student's *t* test. Plasma glucose concentrations among fasting diabetic outpatients ranged from 82 to 305 mg/100 ml (4.55–17 mmol/l). The red cell enzyme activities in two patients, who were initially seen in the emergency room, with highly raised plasma glucose concentrations (650 and 804 mg/100 ml (36 and 44 mmol/l)) and glycosylated haemoglobin

concentrations (17.9 and 24.9 g/dl), were in the normal range—clearly demonstrating lack of effect of extreme hyperglycaemia on the activities of these enzymes. Although exposure to excess glucose has deleterious effects on some tissues, glucose-6-phosphate dehydrogenase, phosphohexose isomerase, and pyruvate kinase do not appear to be functionally altered in hyperglycaemia. However, the possibility remains that these proteins may be glycosylated at residues which do not affect the active site. McMillan *et al.*<sup>1</sup> postulate that observed reduction in erythrocyte deformability is caused by increased cytoplasmic viscosity due to glycosylation of haemoglobin. If this is the case, it is tempting to suggest that glycosylation of other soluble proteins in the red cell may contribute to this effect.

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#### Enzyme activities and glycosylated haemoglobin concentrations the erythrocytes of normal and diabetic subjects

	Normal		Diabetic		Significance
	No.	Mean ± SD	No.	Mean ± SD	
Glycosylated haemoglobin (g/dl)	41	7.97 ± 0.89	42	10.96 ± 3.32	p < 0.001
Glucose-6-phosphate dehydrogenase (IU/g Hb)	41	7.0 ± 1.0	42	6.8 ± 1.1	NS
Phosphohexose isomerase (IU/g Hb)	27	33.2 ± 5.5	30	34.1 ± 7.7	NS
Pyruvate kinase (IU/ml RBC)	27	2.60 ± 0.43	25	2.44 ± 0.52	NS

NS = not significant.