The effect of isoniazid and its methanosulphonate derivative on the brain of the dog appears to be the production of oedema in the cerebral white matter, leading to loss of myelin, and accompanied by glial proliferation. These changes are very similar both morphologically and in distribution to those produced previously with phenelzine and indanyl carbethoxy hydrazine, but as isoniazid and its methanosulphonate derivative are not monoamine oxidase inhibitors, it would suggest that the changes in the myelin produced by all four drugs are not due to monoamine oxidase inhibition. The histological changes are reminiscent of those induced in rats by administration of triethyl tin compounds by Magee *et al.*[2], in which there is widespread oedema of myelin, with spaces between the fibres, but axons and myelin are intact; later work has shown that the oedema accumulates in large clefts within myelin sheaths[3].

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HAEMATOLOGY

Relationship between Activity of Pyruvate Kinase and Age of the Normal Human Erythrocyte

Mature erythrocytes of human beings derive energy chiefly through anaerobic glycolysis. Pyruvate kinase catalyses a key[1,2], adenosine triphosphate-generating glycolytic reaction. We have examined the relationship between the activity of pyruvate kinase and the age of the normal human red cell.

Freshly drawn venous blood, anticoagulated with heparin, was obtained from each of six normal adult males. A modification[3] of a method of serial centrifugation[4] was used to separate erythrocytes into fractions containing relatively young and relatively old packed red cells. Reticulocyte, erythrocyte, leucocyte, and platelet counts of these fractions were determined. Haemolysates were prepared by freezing and thawing 1 ml. of each fraction of red cells three times and by subsequent addition of distilled water. Activities of both pyruvate kinase and glucose-6-phosphate dehydrogenase (G-6-PD) were measured. Available evidence indicates that the activity of G-6-PD in the normal human red cell decreases as the cell ages *in vivo*[5,6].

Results are shown in Table 1. Reticulocyte counts and activities of G-6-PD provide evidence that centrifugation effected significant separation of young and old cells. The mean activity of pyruvate kinase in haemolysates of relatively old red cells proved 45 per cent less than that in haemolysates of relatively young red cells. The mean platelet count of fractions of relatively young red cells (78,000/mm³) was less than that of fractions of relatively old cells (103,000/mm³); contamination by platelets, therefore, does not account for the difference detected between the mean activities of pyruvate kinase in haemolysates of young and old red cells. The mean leucocyte count of fractions containing relatively young red cells was 840/mm³ and that of fractions containing relatively old cells was 340/mm³. We investigated the activity of pyruvate kinase in lysates of leucocytes purified by the method of Fallon *et al.*[11]. These investigations indicated that less than 6 per cent of the activity of pyruvate kinase in any of the haemolysates examined (Table 1) was attributable to contamination by leucocytes. Contamination by leucocytes accounted for approximately 3–4 per cent of the mean activity of pyruvate kinase in haemolysates of relatively young red cells and for approximately 2 per cent of the mean activity of this enzyme in haemolysates of relatively old red cells. The results indicate that the activity of pyruvate kinase in the normal human red cell decreases markedly as the cell ages *in vivo*. These findings coincide with the results of investigations reported by Tanaka, Valentine and Miwa[9]. The results suggest that a decrease in activity of pyruvate kinase sustained during ageing of the normal human red cell may be important in senescence of the cell.

Table 1. Normal Human Red Cells separated by Serial Centrifugation into Fractions containing Relatively Young and Relatively Old Cells

Subject	Reticulocytes (%)		Activity of G-6-PD*		Activity of pyruvate kinase†	
	Young cells	Old cells	Young cells	Old cells	Young cells	Old cells
1	1·5	0·2	362	256	17·0	9·2
2	2·1	0·4	267	190	15·9	10·3
3	2·9	0·4	286	199	17·4	10·1
4	2·2	0·5	277	183	17·1	10·1
5	2·8	0·3	275	149	13·7	6·0
6	5·7	0·1	319	187	19·4	10·1
Mean	2·9	0·3	298	194	16·8	9·3
Significance‡	$0·001 < P < 0·01$		$P < 0·001$		$P < 0·001$	

* Activity of glucose-6-phosphate dehydrogenase, assayed by a modification of the method of Glock and McLean (ref. 7) similar to that used by Zinkham and Lenhard (ref. 8), expressed as μmoles nicotinamide-adenine dinucleotide phosphate reduced/g haemoglobin/h.

† Activity of pyruvate kinase, assayed by a modification of the method described by Tanaka, Valentine and Miwa (ref. 9) (modifications consisted of doubling the concentration of adenosine diphosphate and trebling the concentration of phosphoenolpyruvate in the assay mixture), expressed as μmoles nicotinamide–adenine dinucleotide formed/g haemoglobin/min.

‡ Evaluated by Student's *t*-test as outlined by Fisher (ref. 10).

It has not been established, however, that activity of pyruvate kinase falls below a rate-limiting level during the life span of the normal red cell. Hexokinase has been considered a pacemaker of glycolysis in the human red cell and evidence has been presented indicating that activity of hexokinase decreases substantially as normal red cells age *in vivo*[3]. Many other age-related changes in the human erythrocyte have been reported[12]. Considerable additional information will be required for clear delineation of the factors which limit the survival of the normal human red cell.

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