Mechanical Fragility of Thalassaemic Erythrocytes

ERYTHROCYTIC fragmentation, produced by the incessant buffeting in the circulation, has been considered by several authors^{1,2} to be the most important factor in the destruction of erythrocytes. This fragmentation was thought to be much increased in thalassaemia major (Cooley's anaemia), where poikilocytosis and schizocytosis are very evident³⁻⁵. Nevertheless, there are few experimental records of mechanical fragmentation *in vitro*.

I have tested the *in vitro* fragility of normal and thalassaemic erythrocytes by means of a very simple method described elsewhere⁶: small quantities (2 ml.) of oxalated blood are shaken with steel globules for 20 min. in horizontal tubes in a Kahn shaker, specimens being removed periodically for both an erythrocyte count and photometric determination of solubilized haemoglobin. Using ten normal and ten thalassaemic samples, the following results were obtained.

(1) Traumatization does not produce fragmentation of erythrocytes, but haemolysis; the increase with time of solubilized haemoglobin being proportional to the decrease of erythrocytes (see graph).

(2) Thalassaemic erythrocytes do not show increased mechanical fragility to *in vitro* traumatization; in fact, considering the half-time period of the destruction of erythrocytes, I found for normal erythrocytes a mean value $m = 8.91 \pm 0.43$ min., and for thalassaemic erythrocytes a mean value $m = 11.52 \pm 0.98$ min. There is greater variability for thalassaemic erythrocytes ($\sigma = 3.11 \pm 0.69$) than for normal erythrocytes ($\sigma = 1.38 \pm 0.30$). In spite of this greater variability for thalassaemic erythrocytes, the difference between the mean values of half-time periods for normal and thalassaemic erythrocytes are significant according to the Student-Fisher *t*-test (calculated $t = 2.40$; given t for $P = 0.05: 2.10$). It may then be concluded that thalassaemic erythrocytes show decreased fragility against *in vitro* traumatization.

There is consequently no reason for considering osmotic and mechanical fragilities as antithetic

properties (as suggested by some authors⁵) since both are decreased in thalassaemia. Moreover, it appears unlikely that the increased fragmentation (schizocytosis) of thalassaemic erythrocytes will be due to the buffeting in the circulation; it seems more probable to be a spontaneous phenomenon, caused by intrinsic factors (possibly a changed haemoglobin-iron linkage, as suggested by Perosa⁷). This view is supported also by the existence of fragments of very young erythrocytes (reticulocytes) in the blood and bone-marrow of children affected by the disease.

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Formation of 2-keto-D-Gluconic Acid, 5-keto-D-Gluconic Acid, and Tartronic Acid by *Acetobacter* Species

THE production of 2-keto-D-gluconic acid from glucose and also from gluconic acid by *Pseudomonas* species was at one time considered to be a feature which differentiated these organisms from *Acetobacter* species. Bernhauer and co-workers¹⁻³ showed, however, that twelve species of *Acetobacter* produced from glucose a strongly reducing acid which formed a soluble calcium salt. They assumed that this substance was either 2-ketogluconic acid, or aldehydo-gluconic acid, or a mixture of both, because they were able, from cultures of *A. gluconicum* and of *A. suboxydans*, to isolate and to characterize 2-keto-gluconic acid, and because aldehydo-gluconic acid was isolated from cultures of *A. gluconicum* and *A. xylinum*.

Four years ago, one of us (D. K.) examined the glucose metabolism of seventeen species of *Acetobacter* in a medium consisting of glucose (10 per cent w/v) in yeast-water in presence of calcium carbonate (3 per cent w/v) at 27-30°. All produced a reducing acid which was trapped as a soluble calcium salt. From the metabolism solutions of cultures of *A. orleanense*, *A. pasteurianum*, *A. acetigenum* and *A. turbidans*, 2-keto-D-gluconic acid was isolated and was characterized as the potassium salt, as the quinoxaline derivative and as the phenylhydrazide of its phenylhydrazone.

We have also established the formation of 5-keto-D-gluconic acid in addition to that of the above-mentioned reducing substances in glucose cultures of *A. acetigenum*, *A. orleanense*, *A. viscosum*, *A. kützingianum*, *A. pasteurianum* and *A. turbidans*. This acid was identified by study of its properties and behaviour and by ultimate analysis. The production of the 5-keto-acid by these species has not hitherto been reported.

In more recent work we have observed that when *A. acetosum* is cultivated in the same medium it produces first calcium gluconate; then, if the period of incubation is prolonged, a reducing substance appears and accumulates. In one experiment the cultures of *A. acetosum* were incubated for one month at 30° and afterwards allowed to stand for five months at room temperature. Neither glucose nor gluconic acid could then be detected in the medium.