Short Communications

Haemolysis of erythrocytes from vitamin E-deficient chickens

GYÖRGY AND ROSE[1] found that erythrocytes of vitamin E-deficient rats became haemolysed *in vitro* after addition of a dilute solution of dialuric acid. This haemolysis could be prevented by incubating a suspension of the erythrocytes of a vitamin E-deficient rat with a tocopherol emulsion, prior to the dialuric acid haemolysis test. Red blood cells of rats, receiving a diet adequate in vitamin E, did not show *in vitro* haemolysis after addition of the dialuric acid solution.

CHRISTENSEN *et al.*[2] reported that strong *in vitro* haemolysis of vitamin E-deficient rat erythrocytes could also be observed when the cells were suspended in physiological saline (0.9 %) and kept at 37° or 47° C for some time ("saline haemolysis"). According to these authors erythrocytes of vitamin E-deficient chickens did not show dialuric acid and saline haemolysis; CHRISTENSEN *et al.* stated that the greater resistance of chick red cells could be explained by the fact that the cells are nucleated.

In view of the fact that haemolysis is primarily a phenomenon of the cell membrane, it seemed interesting to us to study the haemolysis test with erythrocytes from chickens that had been fed the vitamin E-deficient basal diet mentioned recently by SCOTT *et al.*[3]; according to the authors this diet should be suitable for use in the study of uncomplicated vitamin E deficiency in chickens.

Day-old chickens (North Holland Blues), hatched from breeding hens that had received a commercial ration, were fed with the basal diet alone and supplemented with vitamin E (*dl*-α-tocopherol-acetate). Blood was taken by puncture and the erythrocytes suspension for the haemolysis test was prepared as described by GYÖRGY AND ROSE[1]; after removing the saline-citrate solution by centrifuging the red cells were washed once with physiological saline before making the 5 %-suspension. The test was carried out according to ROSE AND GYÖRGY[4]; after the addition of the dialuric acid solution to the red cell suspension the tubes were shaken, placed in the incubator at 37° C for 45 minutes and then kept at room temperature for at least 4 hours. At the end of this period the percentage haemolysis was determined by measuring the red color at 540 mμ with a Coleman Universal Spectrophotometer.

It was found that erythrocytes of chickens that had received the basal diet for about three weeks, haemolysed completely *in vitro* after the addition of dialuric acid solution, while chickens that had received the basal diet plus 25 mg *dl*-α-tocopherol-acetate per kg, did not show *in vitro* haemolysis of any significance.

Incubation of a suspension of the red cells from vitamin E-deficient chickens with a tocopherol emulsion (*dl*-α-tocopherol emulsified with Tween 80) for one hour, followed by removal of the emulsion by centrifuging, resulted in complete prevention of dialuric acid haemolysis. Erythrocytes of chickens that had received the basal diet supplemented with vitamin E for about three weeks and that showed a negative dialuric acid haemolysis test at that time, became about four weeks later completely haemolysed by dialuric acid, after the same chickens had been fed during that period with the deficient diet instead of the supplemented one. On the other hand, the red cells of chickens that had received the deficient diet for some time and that showed a positive haemolysis test, were no longer haemolysed by dialuric acid after the chickens were given a large dose of vitamin E for three days.

The erythrocytes of the day-old chickens used in our experiments did not haemolyse *in vitro* with dialuric acid.

When the red cells of vitamin E-deficient chickens, showing positive dialuric acid haemolysis, were suspended in physiological saline and kept at 37° or 47° C for varying periods (1–20 hours), there was no difference in percentage haemolysis, compared with the erythrocytes of chickens receiving the vitamin E-supplemented basal diet.

The experiments described above, show that the dialuric acid haemolysis test, previously reported by GYÖRGY AND ROSE[1] for rats, is also valid and applicable for chickens under the reported conditions. The saline haemolysis, observed by CHRISTENSEN *et al.*[2] with erythrocytes of vitamin E-deficient rats, could not be observed by us with vitamin E-deficient chickens.

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