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Effect of Ethanol on the Iron Uptake by Rat Reticulocytes

Iron-bound to transferrin is delivered to immature erythroid cells and directed to heme synthesis within the mitochondria (8). The process of iron uptake by these cells seems to be mediated by specific membrane receptors (1, 6), which allow the incorporation of iron followed by protein recycling, probably through a step linked to acid vesicles (10).

High levels of free heme in reticulocytes induce a drop in the amount of iron taken up by cells (7). In contrast, it appears that when iron is present, but heme sunthesis is otherwise depressed, a sideroblastic anemia will result (3). It has been described that ethanol exerts an inhibitory action on heme synthesis at the δ-aminolevulinic synthetase level (4), as well as other levels (2), which may provoke an anemic situation.

The aim of the present report is to observe the effect of this inhibition on the iron uptake by reticulocytes. Evidence is presented that under ethanol-induced heme synthesis inhibition the amount of iron taken up by immature erythroid cells is augmented.

Rat reticulocytes were obtained through repeated blood extraction from retroorbital plexus. Red blood cells (with 25-35 % of reticulocytes) were washed three times with Basal Medium of Eagle (BME) plus a 2 % of bovine albumin before the start of incubations. Rat plasma transferrin was purified and labelled with 59-Fe as in (5). Incubations were performed at 37° C, by mixing washed cells, resuspended in BME plus 2 % bovine albumin and 500 mg/ml glutamine (25 % final hematocrit) and labelled protein (100 μg protein/ml concentration). At different times, 0.5 ml aliquots were layered onto 1 ml of dibutyl phthalate and centrifuged for 30 seconds at 15,000 rpm. Corrections for trapped medium were made. The red blood cells pellet was lysed with 20 imosM (ideal milli osmolar) Tris-HCl pH 7.4 and one sample of lysate was mixed with cyclohexanone plus 0.1 N HCl for heme extraction. One sample was counted directly for total radioactivity. Samples for radioactivity counting were processed as in (9). The radioactivity was measured in a well type scintillation counter.

Fig. 1 shows the time course of total radioactive iron incorporated by reticulocytes, as well as the amount of iron incorporated to heme. It can be seen that the presence of ethanol induces a slight rise in the radioactivity taken up by cells. However, if cells were preincubated with 0.1 M ethanol for 15 minutes, the amount of radioactivity incorporated by cells is significantly higher than in the absence of ethanol. ($p < 0.001$ through a Student's t test for each pair of points). The presence of 0.1 M ethanol provokes a drop in the amount of iron incorporated to heme. It can be seen that both with or without preincubation, the inhibiting effect of ethanol is similar.

When the effect of different ethanol concentrations was studied (fig. 2), an increase in the amount of iron taken up by reticulocytes was initially observed, although this effect was not linear, perhaps due to the high ethanol concentrations used.

In conclusion, results presented show

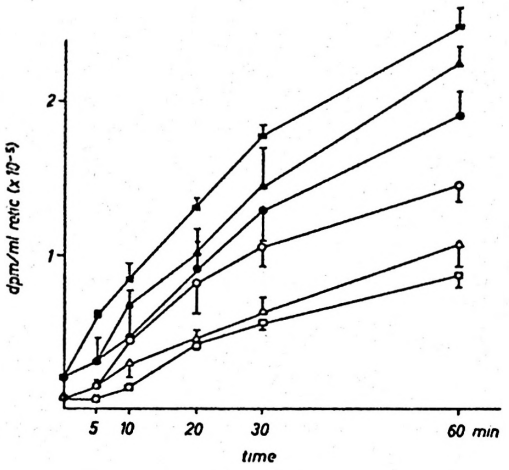

Fig. 1. *Effect of ethanol on the time course of iron (59-Fe) uptake (■ ▲ ●) and iron incorporation to heme (□ △ ○) by rat reticulocytes from fully saturated rat transferrin (100 μg protein/ml).*
Effect of 0.1 M ethanol (● ○) Controls, (▲ △) 0.1 M ethanol added at the same time than 59-Fe-transferrin. (■ □) 0.1 M ethanol added 15 minutes before 59-Fe-transferrin was added. Mean of three individual experiments ± standard deviation given.

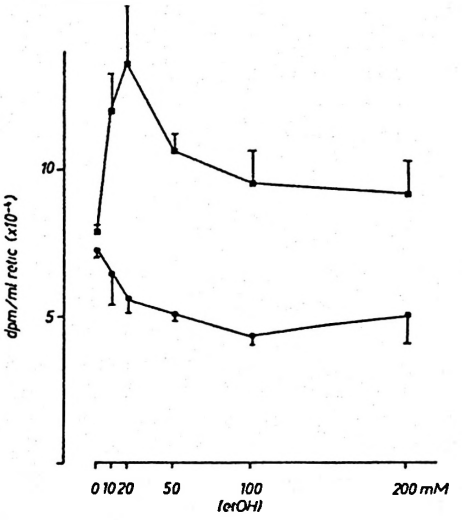

Fig. 2. *The effect of various ethanol concentrations on the incorporation of 59-Fe into reticulocytes and heme.*
Fully iron saturated transferrin concentration, 100 μg/ml. (■) Radioactivity in reticulocytes. (●) Radioactivity in heme. Mean of three individual experiments ± standar deviation given.

that ethanol may provoke an iron overload on immature red blood cells, not only by increasing the amount of metal in the mitochondria as aggregates, but increasing the actual amount of iron taken up by these cells.

Key words: Ethanol, Iron uptake, Reticulocytes, Transferrin.

Palabras clave: Etanol, Captación de hierro, Reticulocitos, Transferrina.

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