. Sodium- and Potassium-Activated ATPase in Uptake of Fe^{59} By the Rabbit Reticulocyte.* (29930)

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Iron enters the reticulocyte during a phase of its maturation to an erythrocyte. There is evidence that the mechanism is dependent upon metabolic energy-producing processes and is blocked by low concentrations of metabolic inhibitors, such as 2,4-dinitrophenol and iodoacetic acid(1). Furthermore, the transfer does not seem to be linked to heme synthesis, and uptake of Fe^{59} by human reticulocytes is moderately increased by glucose.

Dowdle, Schacter and Schenker studied the transport of Fe^{59} by everted segments of rat duodenum(2). They reported that transport took place from the mucosal to the serosal surface against a concentration gradient *in vitro,* and was dependent on oxidative metabolism and the generation of phosphate bond energy.

Sodium- and potassium-activated adenosine triphosphatase activity in human erythrocytes is inhibited by a relatively low concentration of ouabain(3). Other investigations indicated a relationship between Na-K activated ATPase and the active movement of both electrolytes (Na^+ and K^+)(4) and non-electrolytes (glucose and its analogs)(5) against their respective concentration gradients.

In light of these findings, evidence was sought and found which suggested that there is a link between sodium- and potassium-activated ATPase and uptake of Fe^{59} by the rabbit reticulocyte.

Methods. Reticulocytes were obtained from phenylhydrazine-treated rabbits. New Zealand white rabbits were given 1 ml of a 2.5% aqueous phenylhydrazine solution intraperitoneally for 4 consecutive days. Blood was obtained by cardiac puncture on the seventh day, using heparin as the anticoagulant. After centrifugation, the packed cells were washed with 2 volumes of isotonic NaCl solution and recentrifuged. Five ml of packed cells, containing approximately 60% reticulocytes, were suspended in 5 ml of isotonic NaCl solution and mixed with 20 ml NaCl solution containing 9×10^{-9} mole of Fe^{59},† 50 ml Eagle's MEM growth medium,‡ and 10 ml commercial rabbit serum.‡ This suspension was separated into 3 equal aliquots, and duplicate samples were immediately taken from each aliquot. The average radioactive counts of all the duplicate samples, after processing, were assumed to represent instantaneously-bound membrane iron, released by the cells upon hemolysis, and this count was subtracted from each subsequent count. The aliquots were then incubated at 37°C in a water bath shaker. After 30, 60, 90, 120, and 180 minutes, duplicate samples were taken from each aliquot and processed. After 60 minutes' incubation, 0.5 ml 6×10^{-3} M ouabain was added to one flask, 0.5 ml 6×10^{-5}M ouabain was added to the second, and 0.5 ml isotonic NaCl solution was added to the third, which served as control.

Each sample was processed as follows. The cells were washed with isotonic NaCl solution containing non-radioactive $FeCl_3$ (1×10^{-7} M) to minimize cellular iron loss; after centrifugation, the cells were hemolyzed with water and the supernatant was assayed for radioactivity. The assumption was made that uptake above the initial count represented intracellular iron.

Results and discussion. It can be assumed from Fig. 1 that the cells were undergoing a steady uptake of Fe^{59} for the first 60 minutes, prior to addition of ouabain. Subsequent measurements of uptake showed that ouabain inhibited Fe^{59} uptake. Both concentrations of the alkaloid were equally effective, an indication that the inhibition was of an enzymatic nature. Similar results have been ob-

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† $Fe^{59}Cl_3$ from Oak Ridge Nat. Laboratory, Oak Ridge, Tenn.

‡ Microbiological Associates, Inc., Bethesda, Md.

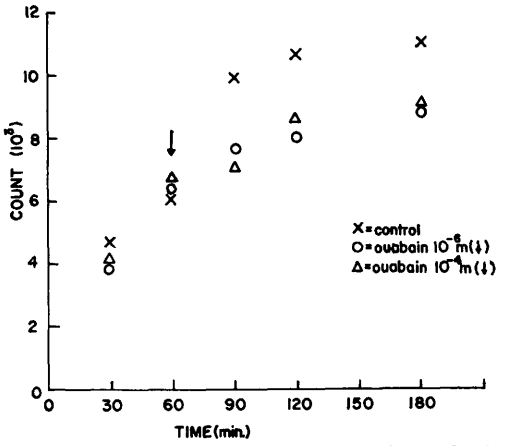

FIG. 1. Effect of ouabain on Fe^{59} uptake by rabbit reticulocytes.

tained in 10 experiments. Consequently, it is concluded that the Na-K ATPase system is probably involved in iron transport in rabbit reticulocytes, thus providing further evidence of the widespread function of this system in transport processes.

Summary. Uptake of Fe^{59} by rabbit reticulocytes in the presence of ouabain was inhibited. From such evidence it is concluded that the Na-K ATPase system is probably involved to some degree in iron transport in rabbit reticulocytes.

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Serum Cholesterol Reduction by Para-Aminosalicylates in Man. I-131 Triolein Absorption Studies.* (29931)

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It has been reported from this laboratory that oral administration of neomycin reduced significantly the concentration of serum cholesterol in man(1,2,3). Following this finding, the effect of 17 additional antibacterial drugs on the serum cholesterol level of patients has been studied. Several antibacterial drugs were found to affect serum cholesterol to varying degrees(3,4). Of these, oral administration of para-aminosalicylic acid at daily doses of 8 to 12 g was most effective, and reduced serum cholesterol concentrations significantly (average fall: 25%) in each of 15 patients(3). Similar results from other laboratories have been published(5,6,7), and Tygstrup, Winkler and Jorgensen reported that oral administration of para-aminosalicylates resulted in significantly lower serum cholesterol levels than intravenous administration of the drug in 9 patients(7,8). They also noted a moderate increase of fecal triglyceride excretion following oral administration of the drug. The possibility that the serum cholesterol-lowering effect of PAS may depend on its local action in the gastrointestinal tract had to be considered, particularly in the context of evidence reported earlier from this laboratory, showing that the cholesterol-reducing effect of neomycin depends exclusively on its activity within the gut(3).

Oral administration of para-aminosalicylic acid (acid PAS) has been reported to cause more severe gastrointestinal symptoms than sodium para-aminosalicylate (sodium PAS) (9), and it has been shown that absorption of the acid compound is slower than that of the sodium salt(9). In the present study the effect of acid and sodium PAS on serum cholesterol concentration of a group of patients is reported. The effect of administration of

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