Inhibition of P^{32}-Orthophosphate Exchange by Sodium Fluoride in Erythrocytes from Patients with Hereditary Spherocytosis.* (22589)

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To elucidate the mechanism underlying the abnormal metabolic behavior of inorganic phosphate previously demonstrated(1,2) in red cells from patients with hereditary spherocytosis (HS), experiments were carried out to determine whether any difference existed in the effect of fluoride on the orthophosphate-P^{32} exchange reaction between the extra- and intra-cellular compartments. The rate of disappearance of orthophosphate-P^{32} from the plasma at 37°C was used as an index of this isotope exchange reaction(3). As illustrated in Fig. 1, rbc from HS patients are more sensitive to fluoride than normal rbc with respect to the orthophosphate-P^{32} exchange reaction. Results obtained with blood from splenectomized patients did not differ significantly from those obtained with blood from non-splenectomized patients. Reticulocytosis in the latter group did not appear to affect the results significantly.

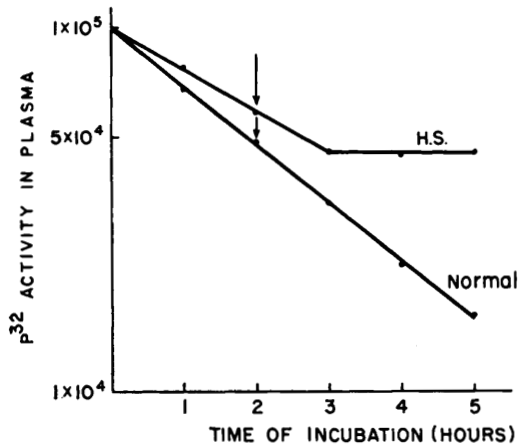

FIG. 1. Orthophosphate-P^{32} exchange by normal and HS red cells in presence of 10^{-3} M NaF; time of addition of NaF indicated by the arrow. P^{32} activity in plasma (ordinate plotted logarithmically) expressed as counts/min. (c.p.m.)/ml of plasma corrected to an initial standard addition of 10^5 c.p.m./ml of plasma. Results presented here, obtained with single specimens of normal and HS blood, are representative of those obtained with blood samples from 5 hematologically normal persons and from each of 6 patients with hereditary spherocytosis; 3 of patients had been splenectomized.

It is noteworthy that in both normal and HS cells, adenosine causes a reversal of the inhibitory effect of fluoride and of iodoacetate (4), suggesting that certain products of adenosine metabolism are effective in removing the fluoride block.

Inhibition of orthophosphate-P^{32} exchange by fluoride implicates enolase as a point of blockage. This interpretation is supported by the enhancement of fluoride inhibition in the presence of additional unlabeled orthophosphate (final concentration, 10^{-3} M)(5). The finding that adenosine counteracts not only the effect of fluoride but also that of iodoacetate suggests that the effective products of purine riboside metabolism may be contributing directly to the formation of phosphoenol-pyruvate, perhaps by way of hydroxypyruvate, without prior conversion to triose phosphates.

It was deemed worthwhile to ascertain whether HS cells also differed from normal rbc with respect to the effect of fluoride on the rate of exchange of intracellular P^{32}-labeled phosphate with unlabeled extracellular phosphate. To this end blood from hematologically normal donors and from patients with HS was incubated with orthophosphate-P^{32} for 1 hour. The rbc were then separated by centrifugation and washed twice with fresh autogenous plasma. The labeled cells were finally mixed with fresh plasma in proportion to the original hematocrit and reincubated for 4 hours with or without added fluoride. Re-

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TABLE I. Comparison of Effect of Fluoride on Rate of Exchange of Intracellular P^{32}-Phosphate in Labeled Red Cells from Normal Donors and from Patients with Hereditary Spherocytosis (HS).

	Normal cells			HS cells		
Hr of incubation	Control,* No. additives c.p.m./ml plasma‡	NaF added† c.p.m./ml plasma‡	Control: NaF ratio	Control,* No. additives c.p.m./ml plasma‡	NaF added† c.p.m./ml plasma‡	Control: NaF ratio
1	8680	5120	1.69	4320	7780	.55
2	8460	6080	1.39	5720	9400	.60
3	8060	6380	1.26	6800	9780	.69
4	10340	7160	1.41	7900	10960	.72

These data, obtained with single specimens of normal and HS blood, are representative of those obtained with blood samples from 3 hematologically normal donors and from 3 previously splenectomized HS patients.

* P^{32}-labeled cells, incubated with fresh plasma.

† *Idem* and 5×10^{-3} M NaF (final concentration).

‡ c.p.m. = counts/min. obtained with a Geiger-Müller tube. The count was obtained from 0.05-ml aliquot of plasma which had been dried onto a small spot on filter paper.

sultant P^{32}-activity of the plasma was determined at hourly intervals (Table I). The data show that the addition of fluoride caused an increase in the rate of release of P^{32} from the labeled HS cells, whereas fluoride had the opposite effect on normal rbc. The data presented indicate that orthophosphate-P^{32} exchange in HS cells differs in a characteristic manner from that in normal human rbc. Although more information is needed for an adequate explanation of these findings, it seems likely that enzymes activated by Mg^{++}, such as enolase and ATPase, are involved. Whether the formation of calcium fluoride has any significant relation to the observation reported, possibly by bringing about changes in electrolyte distribution between rbc and plasma(6), cannot be stated at this time.

Summary. 1. The exchange of orthophosphate-P^{32} by normal human red blood cells (rbc) and rbc of patients with hereditary spherocytosis (HS) has been studied in the presence of NaF. 2. The flux of orthophosphate-P^{32} into HS cells could be inhibited by 10^{-3} M NaF whereas the flux into normal rbc remained unaffected by this concentration of fluoride. 3. The exchange of intracellular phosphate-P^{32} was studied similarly in normal P^{32}-labeled rbc and in P^{32}-labeled rbc from patients with HS. Addition of NaF caused an increase in the rate at which P^{32} was released from HS cells and caused a decrease in the rate of release of P^{32} from normal rbc.

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