In humans, when BC is administered at a routine dose of 7.5 mg/day (0.15 mg/kg, b.wt), the effective concentration attained is approximately 1×10^{-9} M[19]. If we assume that rats have a similar metabolism of this drug, extrapolation to a dose 10 times greater would yield an effective concentration of 10^{-8} M, which is similar to the inhibition constant estimated by us. Di Carlo et al.[20] have reported a biphasic in vitro effect of BC on the ER from rat uterus. They found that BC was inhibitory at high doses, whereas at low concentrations the binding capacity of the receptor protein increases. This finding is in accordance with the results detected by us in adrenal tissue, using high doses of this drug[10]. Some evidence for a noncompetitive inhibition by BC on the estrogen binding activity in rat uterus has been presented[21]. However, the method of Lineweaver-Burk was developed for measurements of the effect of the concentration of substrates on enzymes, and not for binding studies. Best-Belpomme and Dessen[12] developed calculations which adapt classical inhibition studies to binding assays for receptors or antibodies. Using the Best-Belpomme and Dessen equations, apparent noncompetitive curves found with Lineweaver-Burk analysis, may in fact be of a competitive nature.

We cannot at present explain how drugs with chemical structures different from that of estradiol, can inhibit the binding of this hormone to its own receptor. Nevertheless, a clear inhibition was demonstrated by Lineweaver-Burk analysis. A more reliable approach to the relationship between ER in adrenal gland and dopamine-related drugs could be the measurement of the binding activity in isolated or cultured adrenal cells.

1 Consejo Nacional de Investigaciones Cientificas y Técnicas.

2 Acknowledgments. The authors are deeply indebted to Mrs Diana Bas for her skillful technical assistance, and Mrs Elaine Leclerc, Mrs Dora Beatriz Destéfano for typing the manuscript, and to Dr James G. Chafouleas (CHUL, Quebec) and Dr Lawrence Crim (Newfoundland, Canada) for their helpful criticism.

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0014-4754/86/020136-03$1.50 + 0.20/0

Proton and potassium fluxes in rat red blood cells incubated with sugar phosphates

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Summary. Fructose-1,6-diphosphate counteracts potassium ejection and proton uptake induced in rat red blood cells by valinomycin and an uncoupler. The effect on potassium ejection is reduced in the presence of ouabain and divalent cations.
Key words. Rat erythrocytes; K^+-flux; H^+-flux; fructose-1,6-diphosphate; potassium ejection.

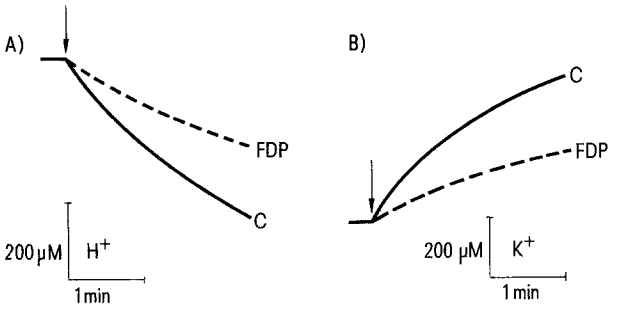
A previous study[1] showed that fructose-1,6-diphosphate (FDP) induces an uptake of K^+ and an extrusion of H^+ ions detectable, in rat erythrocytes, both in the presence and absence of valinomycin. Further work[2] showed that FDP increases the internal pH and K^+ concentration, and acts as an activator of Ca/Mg-ATPase of human red blood cells. This seems to be a rather specific effect of FDP, which is also bound by the red cell membrane.

An increase of the internal FDP concentration has been observed when FDP is incubated with human red blood cells[3], and FDP is more active than other sugars in protecting the mouse from potassium toxicity[4], since it enhances the uptake of K^+ by the tissues and therefore reduces the hyperkaliemic state[5].

This paper reports the results obtained by measuring K^+ and H^+ fluxes in rat erythrocytes incubated with FDP, fructose + phosphate (F + P), fructose-6-phosphate (F6P) fructose-2,6-diphosphate (F2,6P), an endogenous stimulator of phosphofructokinase[6], and cyclic FDP (FDPc), an intermediate in the chemical synthesis of F2,6P.

Materials and methods. FDP trisodium salt (Biomedica Foscama, Roma), F + P (Boehringer, Mannheim), F6P, FDPc and F2,6P (Sigma, St Louis) were used without further purification. Proton flux was measured with a Beckman glass electrode and potassium flux with a Beckman K^+-sensitive electrode, connected to a Beckman Expandomatic pH-meter and an Omni-Scribe recorder (Houston Instrument). All measurements were carried out at 25 °C, under constant magnetic stirring.

Whole blood was collected from Wistar male rats (280 g), after decapitation, and 5 ml of it was diluted with 30 ml of 125 mM NaCl, 30 mM Tris-HCl pH 8.0 and 10 mM EDTA. After 10 min centrifugation at 2000×g, supernatant and white cells were discarded and the sedimented red cells washed three times with 30 ml of the diluting solution. Hemoglobin (Hb) content was measured according to Beutler[7]. 3–4 mg/ml of Hb were used in each assay and concentration of sugar phosphates ranged between 1 and 4 mM. The experiments were carried out in the presence of 0.3 µg/ml valinomycin and 2 µg/ml carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (Sigma, St Louis), used together to induce outward K^+ and inward H^+ movements[8] and also in the presence of 66 µM ouabain (Siguama, St Louis), 4 mM $MgCl_2$ and 0.5 mM $CaCl_2$, to inhibit Na/K-ATPase[9] and activate Ca/Mg-ATPase[10]. H^+ translocation was measured in an



Tracing of H^+ uptake, with disappearance of H^+ from the medium (A) and K^+ ejection, with increase of K^+ in the medium (B), after addition of valinomycin/FCCP (arrow). Control (C) and 4 mM FDP (FDP).

incubation medium of 100 mM NaCl, 30 mM KCl, 5.5 mM glucose and 2.5 mM Tris-HCl pH 8.0; K^+ translocation in a medium of 250 mM sucrose, 5.5 mM glucose and 5 mM Tris-HCl pH 8.0, by adding 1 mM KCl before the addition of valinomycin/FCCP.

Results and discussion. The figure shows typical tracings of H^+ and K^+ movements obtained in the presence and absence of FDP: the inhibitory effect of FDP on both fluxes, as documented in table 1 and table 2, seems rather specific.

A blank with NaCl was carried out to see whether the effect was due to the added Na^+ counterions or to osmotic factors. The samples with added NaCl showed a difference of less than 5% from the controls.

The only sugar with an action comparable to, albeit lower than that of FDP is F2,6P. If one considers the K^+/H^+ ratio (table 3), with an active Na/K-ATPase only FDP lowers the ratio by increasing the external H^+ and decreasing the external K^+. In the presence of Ca/Mg/ouabain, only FDP increases the ratio.

The presence of Ca/Mg/ouabain modifies the homeostasis of K^+ and H^+ fluxes induced by valinomycin/FCCP, and only FDP seems to be able to counteract such a modification.

The demonstrated interaction of FDP with the red cell membrane[2] might be related to this phenomenon and FDP could act as a trigger of a stimulus-response-recovery cycle of the red cell according to Roth et al.[11]. The clinical effects of FDP cannot, in fact, be explained on the basis of a penetration of the compound through the cell membranes, which is not possible, and FDP must therefore exert its intracellular effects by acting from outside. The present data, together with those obtained with human red cells[2] indicate that, by affecting ion translocation, FDP influences phosphofructokinase and hence glycolysis on one side and membrane polarization on the other.

Table 1. Inhibitory effect of 1.5 mM sugars, in the presence and absence of Ca/Mg/ouabain (CaMgOu), on the valinomycin/FCCP-induced K^+ ejection from rat red blood cells

		CaMgOu
Control	79.6 ± 1.1 (100)	44.7 ± 0.8 (100)
FDP	44.3 ± 0.9** (55)	31.5 ± 0.7* (70)
F2, 6P	64.0 ± 0.8* (80)	35.1 ± 0.4* (78)
F6P	67.0 ± 0.6 (84)	40.1 ± 0.6 (90)
F + P	68.5 ± 0.7 (86)	38.0 ± 0.5 (85)
FDPc	69.6 ± 0.8 (87)	37.6 ± 0.4 (84)

Mean ± SE of six experiments. Values expressed as nion/min/mg Hb. Percent in brackets. ** Statistically significant by Student's t-test ($p < 0.01$). * Statistically significant by Student's t-test ($p < 0.05$).

Table 2. Inhibitory effect of 1.5 mM sugars, in the presence and absence of Ca/Mg/ouabain (CaMgOu), on the valinomycin/FCCP-induced H^+ uptake by rat red blood cells

		CaMgOu
Control	46.7 ± 2.1 (100)	58.0 ± 1.8 (100)
FDP	29.0 ± 1.8** (62)	30.0 ± 1.6** (51)
F2, 6P	32.8 ± 1.6* (70)	43.0 ± 1.5* (74)
F6P	40.6 ± 1.4 (87)	56.0 ± 1.8 (96)
F + P	38.3 ± 1.2 (82)	49.8 ± 2.1 (86)
FDPc	38.5 ± 1.6 (83)	49.6 ± 1.3 (85)

Mean ± SE of six experiments. Values expressed as nion/min/mg Hb. Percent in brackets. ** Statistically significant by Student's t-test ($p < 0.01$). * Statistically significant by Student's t-test ($p < 0.05$).

Table 3. K^+/H^+ ratio calculated from tables 1 and 2 data, in the presence and absence of Ca/Mg/ouabain (CaMgOu)

		CaMgOu
Control	1.70	0.77
FDP	1.52	1.05
F2, 6P	1.95	0.81
F6P	1.65	0.71
F + P	1.78	0.76
FDPc	1.80	0.75

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0014-4754/86/020138-02$1.50 + 0.20/0

Differential effect of silybin on the Fe^{2+}-ADP and t-butyl hydroperoxide-induced microsomal lipid peroxidation[1]

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Summary. We have observed a differential effect of silybin dihemisuccinate on rat liver microsonal oxygen consumption and on lipid peroxidation induced by NADPH-Fe^{2+}-ADP and t-butyl hydroperoxide. These results are ascribed to the antioxidant properties of the flavonoid. The differences observed in the effect of the catalysts may be a consequence of the different capacity of silybin to act as a scavenger of free radicals formed by NADPH-Fe^{2+}-ADP or t-butyl hydroperoxide.
Key words. Flavonoids; antioxidants; liver microsomes; lipid peroxidation.