Effect of aldosterone on cation transport through the membrane of cold-stored human erythrocytes incubated for 3 h at 37°C

Δ Na (mEq/l of cells ± S.D.[a])		P[b]	Δ K (mEq/l of cells ± S.D.[a])		P[b]
Control	Aldosterone		Control	Aldosterone	
−15.80 (±0.50)	−17.90 (±3.56)	> 0.05	+2.60 (±0.10)	+2.25 (±0.85)	> 0.05
−4.75 (±0.65)	−5.30 (±1.42)	> 0.05	+1.08 (±0.32)	+0.33 (±0.17)	> 0.05
−8.05 (±1.35)	−9.07 (±0.56)	> 0.05	+3.70 (±0.10)	+3.79 (±0.18)	> 0.05

[a] Standard Deviation.
[b] Significance of the difference of cell cation concentration changes in samples incubated with or without aldosterone (Student's 't' test).

However, the lack of an *in vitro* action of aldosterone on cation transport in cold-stored erythrocytes is not sufficient evidence against a direct action *in vivo* as a possible cause of the increased sodium content of human red cells in the diseases with increased blood levels of the hormone. (See following paper II.)

A different effect of adrenal steroids *in vivo* and *in vitro* on the cation content of erythrocytes has been already described[6,11].

STREETEN and SOLOMON[11] found that ACTH and cortisone, administered intravenously in man, produced an increase of the K erythrocyte content, whereas no measurable effect was noted by adding the same hormonal agents during the *in vitro* incubation of the cells. SHERWOOD JONES[6] showed that the *in vitro* changes produced by DOC glucoside on human red cell erythrocytes are at variance with the erythrocytic changes induced *in vivo* by the administration of large doses of DOC acetate to rats.

An increase of the sodium content of red cells has recently been reported[12] after administration of aldosterone to a patient with Addison's disease.

Riassunto. L'aldosterone non esercita alcun effetto sugli scambi attivi di Na e di K che si manifestano *in vitro* attraverso la membrana di eritrociti umani incubati a 37°C dopo 6 giorni di conservazione a 4°C.

II. Effect of a Steroidal Antagonist of Aldosterone (SC 9420) on Sodium and Potassium Transfer

Several investigators showed that cardiac glycosides and related steroids with lactone rings completely inhibit the active phase of cation transport through the membrane of human erythrocytes, both *in vitro*[7,9,10,13,14] and *in vivo*[15]. This effect has recently been referred to a direct action of the drugs on the specific enzymes which control cation transport in the red cell[16,17].

GANTENBEIN et al.[18] demonstrated that strophanthidin acts as an antagonist to desoxycorticosterone acetate (DCA) on sodium reabsorption mechanism in the renal tubule: they think that cardiac glycosides may have a competitive action with aldosterone-like steroids for a crucial locus of action within the body cells (acting on the enzymatic mechanisms which control active cation transport through the cell membranes). A similar mechanism has been postulated by the same investigators[7] to explain the effect of aldosterone on the changes induced by strophanthidin in cold-stored erythrocytes *in vitro:* the hormone can antagonize the strophanthidin-induced inhibition of cation exchanges through the membrane of cold-stored human red cells during incubation at 37°C, re-establishing a partial flux of Na from the cells and of K to the cells.

Effect of SC 9420 and Ouabain on cation transport through the membrane of cold-stored human erythrocytes for 3 h at 37°C

Δ Na (mEq/l of cells ± S.D.[a])		P[b]	Δ K (mEq/l of cells ± S.D.[a])		P[b]
Control	SC 9420		Control	SC 9420	
−22.8 (±3.15)	−21.4 (±0.82)	> 0.05	+2.25 (±0.54)	+2.40 (±0.30)	> 0.05
−26.0 (±1.20)	−25.7 (±1.92)	> 0.05	+8.20 (±0.10)	+8.40 (±0.11)	> 0.05
−11.1 (±0.10)	−5.0 (±1.06)	> 0.05	+6.70 (±0.24)	+6.77 (±0.29)	> 0.05
−9.2 (±2.03)	−7.4 (±0.88)	> 0.05	+5.22 (±0.21)	+5.27 (±0.30)	> 0.05
	Ouabain			Ouabain	
[c] −10.55 (±1.07)	−0.38 (±1.04)	< 0.01	+5.83 (±0.40)	−2.19 (±0.98)	< 0.01

[a] S.D. = Standard Deviation.
[b] Significance of the difference of cell cation concentration changes in samples incubated with and without SC 9420 or Ouabain (Student's 't' test).
[c] Average of 5 experiments.

Using the same method previously described[19], we studied the effect of a steroid with a lactone ring, recently synthesized, SC 9420 or Spironolactone[20], on cation transport in cold-stored human erythrocytes incubated for 3 h at 37°C. As the Figure shows, this drug, which acts as antagonist to the sodium-retaining action of aldosterone, competing for a crucial locus of action within the renal tubular cells[21–23], has a structure which, besides being similar to that of aldosterone, is also similar to that of ouabagenin and related steroids with lactone rings studied by KAHN[14] as inhibitors of cation transport in cold-stored erythrocytes. Therefore, it is suggested that steroidal antagonists of aldosterone can affect cation transport through the membrane of body cells with a mechanism of competition with aldosterone-like steroids for a crucial locus of action within the cells, such as that postulated by SULSER and WILBRANDT[7] for cardiac glycosides.

Blood from human subjects was drawn, stored and handled as previously described[9,19]. SC 9420 was added to one half of the aliquots, before incubation, at a con-

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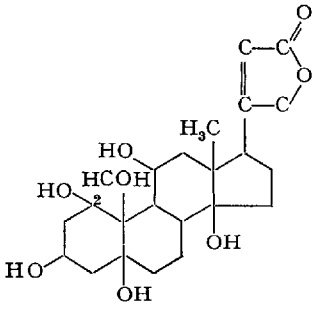
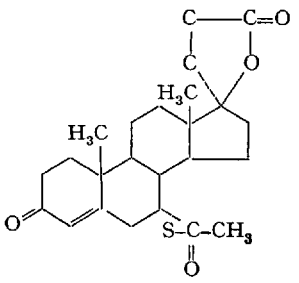
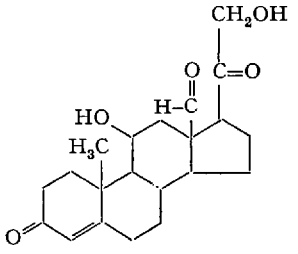


Ouabagenin



SC 9420 (Aldactone)



Aldosterone (Aldehyde form)

centration (3×10^{-3} *M* in 0.1 ml of ethanol) similar to that used for the steroids with lactone rings, which KAHN and ACHESON found to be active on cation transport in their experiments in cold-stored erythrocytes[10]. The same amount of pure ethanol was added to the control aliquots. Ouabain was added at the concentration of 2.5×10^{-5} g in 0.1 cm^3 of distilled water; the same amount of pure water was added to the control samples.

The quantities of potassium removed from the incubation medium and the quantities of sodium set free in the same medium during incubation are shown in the Table. SC 9420 had no measurable effect on these changes under the conditions of the experiment. On the contrary, Ouabain blocked potassium uptake by, and sodium output from, incubated cold-stored human erythrocytes.

Our experiments do not support the assumption that steroidal antagonists of aldosterone compete with aldosterone-like hormones for the enzymatic mechanism which controls cation transport not only within the renal tubular cells, but also within the blood red cells and possibly within the body cells in general.

Riassunto. Nonostante l'affinità strutturale con l'uabaina, lo spirolattone antialdosteronico SC 9420 non esercita la stessa azione del glicoside cardiocinetico sugli scambi attivi che si manifestano *in vitro* attraverso la membrana di eritrociti umani incubati a 37°C dopo 6 giorni di conservazione a 4°C. Non trova quindi conferma sperimentale l'ipotesi che entrambe queste sostanze competano con l'aldosterone in qualche fase del processo enzimatico di trasporto degli elettroliti a livello di tutte le membrane cellulari dell'organismo.

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On the Mechanism of Action of Some 4-Aminoanalogues of Folic Acid

The biochemical mechanism of action of the antileukemic active 4-amino-analogues of folic acid, aminopterin and amethopterin has been found to be in a strong inhibition of the folic acid hydrogenases[1–6]. In this way, the formation of the coenzymatically active tetrahydrofolic acid may be prevented and therefore the biosynthesis of thymidylate and purines inhibited. In order to prepare less toxic antimetabolites of folic acid with another mechanism of action, some 4-amino-analogues of coenzymatically active folic acid derivates have been prepared in our laboratory[7]. This paper presents the inhibiting action of these substances on some enzyme systems converting folic acid into coenzymatically active single carbon carriers. Besides the folic acid hydrogenases, the serine-aldolase[8] and the hydroxymethyltetrahydrofolic acid dehydrogenase[9,10] from pigeon liver were studied as the hydroxymethylation coenzyme generating systems. From the enzymes participating in the formation of formylation coenzymes, the ATP-activated formylase of tetrahydrofolate[11], using formate as the formyl source, the formiminotransferase and deaminase, described recently by TABOR and WYNGARDEN[12], and the aerobic formylase[13] were examined. The enzyme system leucovorin-cyclodehydrase[14] converting leucovorin into its coenzymatically active imidazoline-derivate was included in the enzymes studied. Table I shows the inhibitory properties of several 4-amino-analogues of folic acid on the above-mentioned enzyme systems achieved from acetone powders of mammalian and avian liver in a partially purified form.

The inhibiting power of the 4-amino-analogues mentioned could be observed neither on the aerobic formylase of folic acid nor on the tetrahydrofolic acid formylase using formate as the formyl donor with the inhibitor concentrations equimolecular to the substrate concentration. The most strongly inhibited enzyme systems are the folic acid hydrogenases which are inhibited by 8.6×10^{-9} *M* aminopterin, or 6.2×10^{-9} *M* amethopterin. The substitution of the nitrogen atom N^{10} by the formyl or hydroxymethyl group, and the hydrogenation of the pyrazine ring, diminish the inhibition activity more than 10–100 times.

Of the enzymes formylating the tetrahydrofolate, the system formiminotransferase + cyclodeaminase only was inhibited by all 4-amino-analogues tested, but only concentrations 10^{-4} *M* caused the 50% inhibition of the enzymes. As tested by the Lineweaver-Burk test, the

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