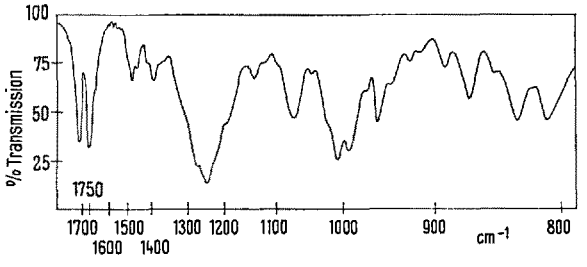
permis de le purifier suffisamment pour obtenir un spectre IR interprétable avec sécurité.

Le Tableau rapporte les résultats obtenus chez six patientes. Dans un cas de tumeur maligne surrénalienne, nous avons identifié avec certitude le sulfate de 7-oxo-DHEA dans la tumeur. Il était aussi vraisemblablement présent dans les deux autres tumeurs (identification par une chromatographie). Les trois autres malades sont atteintes de virilisme. Chez des femmes normales, avec des prises d'essai de plasma du même ordre de grandeur, nous n'avons pas détecté de S-7-oxo-DHEA.

Composés de référence. La 7-oxo-DHEA a été obtenu à partir de son acétate (F = 183–185°)[8] par saponification par la potasse dans le t-butanol. Cet acétate provenait de l'oxydation de l'acétate de DHEA par le chromate de potassium en milieu anhydride acétique-acide acétique[9]. Le spectre IR de la 7-oxo-DHEA était conforme à celui de DOBRINER et al.[10].

Le S-7-oxo-DHEA a été préparé par la technique de l'un de nous[11]. Son spectre IR est publié ci-contre (Fig.)[12].



Ester-sulfate de 7-oxo-DHEA (Na)

7-oxo-DHEA: F = 232–235°
théorique C% 75,47 H% 8,66 pour $C_{19}H_{26}O_3$
trouvé C% 75,93 H% 8,80

S-7-oxo-DHEA (sel de sodium): F = 162–165°
théorique C% 51,57 H% 6,55 pour $C_{19}H_{25}O_6SNa,2H_2O$
trouvé C% 52,10 H% 6,40

Commentaires. L'isolement du S-7-oxo-DHEA pose le problème de son origine. En premier lieu, la 7-oxo-DHEA ne semble pas un artefact d'oxydation[13]; en effet, il n'y a pas de parallélisme quantitatif entre la DHEA et la 7-oxo-DHEA aussi bien dans les résultats rapportés ici que dans nos mesures urinaires; de plus, l'amphénone et le SU-4885 font varier à l'inverse les deux stéroïdes dans les urines[14,15].

L'isolement de la 7-oxo-DHEA dans le sang d'une tumeur surrénalienne et dans le tissu tumoral lui-même peut indiquer, confirmant l'opinion de GALLAGHER[14], la biosynthèse surrénalienne de la 7-oxo-DHEA. Pourtant, après administration de DHEA par voie orale ou intramusculaire à des adultes et enfants normaux, nous avons isolé de la 7-oxo-DHEA dans les urines, ce qui tend à en faire un produit du métabolisme de la DHEA. En réalité, peut-être la surrénale partage-t-elle avec d'autres parenchymes la capacité d'oxyder la DHEA sur le carbone 7.

Signalons que dans aucun des milieux étudiés, nous n'avons trouvé de 7-oxo-DHEA libre ou glucuro-conjuguée; nous l'avons toujours isolée directement à l'état de sulfate ou après une solvolyse par l'acétate d'éthyle[7].

Summary. The isolation of the ester-sulfate of 5-androstene 3β-ol 7,17-dione is described. This sulfate has been found in the peripheral venous blood plasma of several virilized women. Moreover, it has been isolated in 3 adrenal tumors and in the adrenal venous plasma of one of them.

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Studies on Cation Transport in Cold-Stored Human Erythrocytes. I. Effect of Aldosterone on Sodium and Potassium Transfer

An increase of the sodium content of the erythrocytes has recently been demonstrated by one of us[1–3] in several diseases with oedema, in essential hypertension and in one case of primary hyperaldosteronism. This increase has been confirmed, in the oedematous states, by RIECKER and BUBNOFF[4].

Since all these diseases are characterized by an increased secretion of aldosterone, a direct effect of the hormone on cation transport through the membrane of erythrocytes could explain the increased sodium content of the cells in these conditions.

A direct action of desoxycorticosterone (DOC) on cation transport in cold-stored erythrocytes has already been demonstrated by SHERWOOD JONES[5,6]: the hormone blocked the Na and the water output from human red cells incubated at 37°C after 6 days of cold storage.

Further experiments with aldosterone *in vitro* were carried out more recently. SULSER and WILBRANDT[7] found that aldosterone (10^{-7} g/cm³) has not measurable effect on sodium and potassium transfer through the membrane of cold-stored human erythrocytes incubated at 37°C. On the contrary, FRIEDMAN and FRIEDMAN[8] have been able to block with aldosterone (10^{-7} g in 5 cm³) the Na output which takes place in human red cells incubated at 37°C after 2–24 h of cold storage.

The experiments described in this paper were carried out to study if aldosterone modifies the cation transport in human cold-stored erythrocytes incubated at 37°C.

Blood from human subjects was drawn, stored and handled as previously described[9]. After 6 days of cold storage, eight 5 ml aliquots of each sample of blood were incubated in a Dubnoff shaker at 37°C, moving at the rate of 50 oscillations/min to avoid sedimentation of red cells. Aldosterone racemate (10 γ in 0.1 ml of ethanol) was added to 4 of the 8 samples before incubation; only 0.1 ml of ethanol was added to the remaining samples. Sodium and potassium were measured in the plasma with a Beckman DU flame photometer before and after incubation; sodium output and potassium uptake by the cells was calculated by difference, according to the method of KAHN and ACHESON[10]. The volume of cells was measured by means of hematocrit determinations before and after incubation, in duplicate.

The Table shows that erythrocytes removed measurable quantities of potassium from the incubation medium and lost measurable quantities of sodium in the same medium during incubation. Aldosterone had no measurable effect ($P > 0.05$) on these changes under the conditions of the experiment.

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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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