Die oben beschriebenen Reaktionen stellen somit die erste Partialsynthese von Aldosteron aus 18-unsubstituierten natürlichen Steroiden dar.

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Summary

The preparation of the (18 → 11β)-lactone of Δ^5-3-ethylenedioxy-11β-hydroxy-21-acetoxy-20-oxo-pregnen-18-oic acid (XV) is reported. Since this compound has previously been transformed into aldosterone, the reactions described represent the first partial synthesis of this important hormone from readily available 18-unsubstituted natural steroids.

The Antitoxic Effect of Aldosterone

Adrenal hormones — including those produced by the medulla, such as nor-adrenaline, and those emanating from the cortex, such as aldosterone — are essential for certain cardiovascular effects of endogenous amines. Aldosterone constitutes an exception among the corticosteroids inasmuch as no other adrenocortical substances have been found to display an effect analogous to that of aldosterone [1–3]. The experiments described below confirm the importance of the role of aldosterone in homeostasis.

Bacterial toxins are liable to give rise to a lethal syndrome characterised chiefly by hemorrhages, involving also the adrenals in particular, and by circulatory failure. We therefore investigated what effect aldosterone[4], as one of the genuine cortical substances[5], has upon this syndrome.

Our experiments were carried out on cats anaesthetised with Dial-urethane (Dial, Ciba, 30 mg/kg i. p. and 30 mg/kg s. c.). The endotoxin employed should, on the one hand, be such as to produce death within the time limits imposed by the acute nature of the experiment, but, on the other hand, it should not have such an acute action that it becomes impossible to analyse the resulting circulatory failure. Consequently, we selected endotoxins which, given in comparatively small intravenous doses, were capable of killing all the anaesthetised but otherwise untreated cats within an average of $1^1/_2$–2 h[4].

The preparations used were of the polysaccharide type obtained from *Proteus* — Prep. 19338/7, 20117/1, and 23871/5 (KAHNT) — and from *Serratia*, i.e. Prep. 22148/E (SHEAR). The observation time lasted 6–7 h following administration of the endotoxin.

Table I
Cat: PPS No. 20117/1: 0·03 mg/kg i.v.

	mg/kg i.v. 30 min bef. PS	Number of animals surviving	dying
Controls		0	6
D,L-Aldosterone (free)	0·01	0	1
	0·03	3	0
	0·1	3	0
Cortexone (-glycoside)	30·0	0	1
	50·0	0	3
	150·0	0	1
Hydrocortisone (Na salt of the hemitetrahydrophthalate)	20·0	0	1
	30·0	4	0
Prednisolone (Na salt of the hemitetrahydrophthalate)	1·0	0	1
	3·0	0	1
	10·0	0	1
	20·0	5	0
SEF (-Na-dihemisuccinate)	1·0	0	2
D,L-Aldosterone 0·03 + SEF	0·03	2	0
0·03 + SEF	0·1	2	0
0·03 + SEF	1·0	0	3
0·1 + SEF	1·0	1	1
PPS No. 23871/5: 0·1 mg/kg i.v.			
Controls		1	6
D,L-Aldosterone	0·03	1	3
	0·1	5	0
Hydrocortisone	30·0	2	1
Prednisolone	20·0	4	0
Corticosterone (free)	1·0	0	1
	10·0	0	1
	30·0	0	1
PPS No. 19338/7: 0·2 mg/kg i.v.			
Controls		0	3
D,L-Aldosterone	0·03	0	2
	0·1	2	0

From Table I it will be seen that aldosterone affords protection against shock elicited by *Proteus* polysaccharides (PPS) and that the amount required for this purpose is to some extent related to the size of the dose of PPS administered and to the type of polysaccharide involved. 3β, 16α-dihydroxy-allopregnan-20-one (referred to here under the abbreviation SEF)[6,7], exerts a competitive antagonistic effect on this protective action of aldosterone. Administered prophylactically, other corticosteroids, e.g. hydrocortisone and prednisolone, also afford protection. On the other hand, corticosterone and cortexone, even in very large doses, are ineffective. In this experiment, aldosterone not only proved over 100 times more active than hydrocortisone and prednisolone, but, in contrast to prednisolone, it also afforded protection in advanced stages of endotoxin poisoning (Table II).

The effect of endogenous amines on the blood pressure is altered in characteristic fashion in animals suffering from endotoxin shock: that of nor-adrenaline is suppressed, and that of adrenaline reversed. Aldosterone restores the original action of adrenaline and nor-adrenaline on the

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[3] R. MEIER and H. J. BEIN, Helv. physiol. Acta *12*, C 83 (1954).
[4] We should like to express here our thanks to Dr. J. SCHMIDLIN for the preparations of D,L-aldosterone and to Dr. F. W. KAHNT and Dr. M. J. SHEAR for the endotoxins with which they so kindly supplied us.
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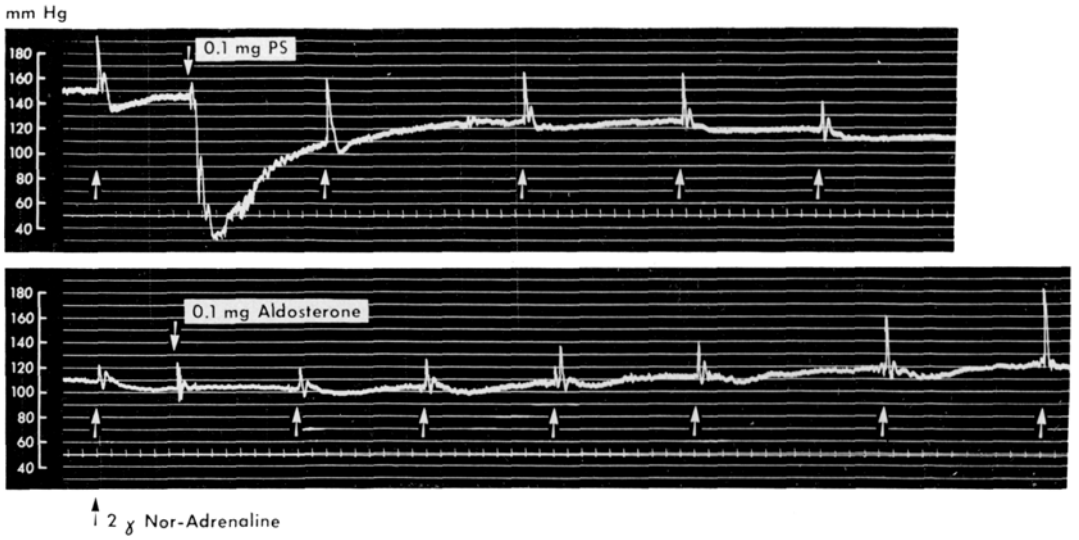

Fig. 1.—Cat. Blood pressure. Inhibition of the pressor response to i.v. nor-adrenaline (2·0 γ/kg) after *Proteus* polysaccharide (PS) 0·1 mg/kg i.v. 'Normalisation' following aldosterone (0·1 mg/kg i.v.). Time indicated in units of 1 min.

arterial pressure (Fig. 1 and 2); in response to aldosterone, the arterial pressure rises again. Where administration of threshold levels by inhibiting the formation or release of nor-adrenaline. Despite this, however, the animal's death

Table II
Cat; PPS No. 23871/5: 0·1 mg/kg i.v.

	mg/kg i.v.	Corticosteroid inj. Time after PPS (min)	Number of animals surviving	Number of animals dying	Death after PPS (min)
D,L-Aldosterone	0·1	30, 40	2	0	
		55, 60	3	0	
	20·0	30	0	2	45, 60
Prednisolone .	30·0	35	0	3	50, 65, 140
	50·0	50	0	1	140

Table III
Adrenalectomised rat; SPS* No. 22148/E: 0·1 mg/kg i.v.

	mg/kg i.p.	Time before SPS (min)	Number of animals surviving**	Number of animals dying
Controls . . .			3	15
D,L-Aldosterone	0·3	30	2	7
	1·0	30	7	3
Hydrocortisone	10·0	180	7	2
Prednisolone .	10·0	180	9	1

* *Serratia* polysaccharide.
** Observation time: 40 h.

PPS has caused the action of adrenaline on the pressure to be reversed, this reversal can also be counteracted by infusing small doses of nor-adrenaline (0·1 γ/kg per min); this would seem to suggest that the endotoxin reduces the nor-adrenaline concentration in the effector organs to sub- cannot be prevented by repeatedly administering, at regular intervals of 10 min, either adrenaline (2·0 γ/kg), nor-adrenaline (2·0 γ/kg), a combination of both, or hypertensin. It is conceivable that in endotoxin shock aldosterone may play an important part not only as regards

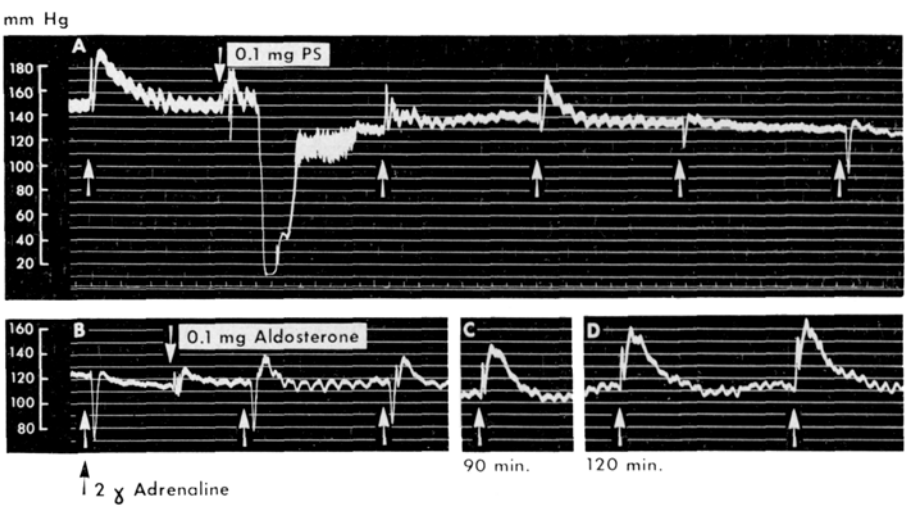

Fig. 2.—Cat. Blood pressure. Reversal of the pressor response to adrenaline, 2 γ/kg i.v., after *Proteus* polysaccharide (PS) 0·1 mg/kg i.v., and gradual 'normalisation' of the pressor response by aldosterone (0·1 mg/kg i.v.).

the effect produced by nor-adrenaline but also as regards its formation or release.

The endotoxin has no clear-cut influence on the effect caused by histamine on the blood pressure, and its influence on that of serotonin is only erratic, insofar as it diminishes or reverses the pressor effect of serotonin, the latter can be restored by administering aldosterone. We were unable to determine whether prednisolone also has some influence on the alterations provoked by endotoxin in the pressor action of adrenaline and nor-adrenaline, because the animals – despite large doses of prednisolone – died too soon (Table II).

The results obtained by Halberg, Spink, and Bittner[8] in experiments on adrenalectomised mice are corroborated and extended by our finding that, in rats adrenalectomised 20–24 h prior to the administration of endotoxin, aldosterone not only prolongs the survival time but affords protection from death even when given in only one single dose (Table III). Here, once again, aldosterone proves far more effective than other corticosteroids, although the difference is not so pronounced as in the cat.

In guinea-pigs, on the other hand, all the corticosteroids exert a protective action, though one that is less clear-cut; here, however, in comparison with other animal species (cat, rat, mouse), larger doses of endotoxin have to be given and death also ensues more slowly, so that a single injection of steroid can hardly be expected to afford prolonged protection. It is therefore all the more surprising that animals pretreated with BCG can be protected by an intravenous dose of aldosterone (1 mg/kg) given 10 min before PPS, whereas doses of prednisone or prednisolone 10 to 30 times larger are required to achieve a comparable protective effect.

Prophylactic treatment with aldosterone in doses of 1–3 mg/kg also protects mice against the lethal action of *E. coli* or *S. typhimurium* toxin as well as against that of hemorrhagic snake venoms (*Vipera aspis*, *Crotalus adamanteus*), but not against the neurotoxic venom of *Naja tripudians*. Prednisolone, which is effective to roughly the same degree in the mouse as in the rat, has to be administered in doses some 10–30 times larger, whereas hydrocortisone and cortexone display no protective action, even in high dosage.

In cats anaesthetised with Dial-urethane, aldosterone – in a dose of 1 mg/kg – also affords protection against acute 'shock' produced by a lethal intravenous dose (4–6 mg/kg) of trypsin. In this case, too, other protective corticosteroids, such as prednisone and prednisolone, display no action unless given in much larger doses (30 mg/kg). The protective activity of aldosterone thus includes an ability to antagonise 'shock states' induced by various means.

In anaesthetised cats, aldosterone – like the other corticosteroids – is ineffective against the typical depressor action exerted on the blood pressure by the histamine liberator Compound 48/80.

In addition to the reasons of a pharmacodynamic character which have been suggested to account for the protection afforded by aldosterone against endotoxin shock, the antitoxic action of certain steroids may also possibly be partly due to some form of direct interference. This would seem to be borne out by our observation that, in guinea-pigs, mixtures of aldosterone or prednisolone and PPS produced *in vitro* have an appreciably less toxic and pyrogenic effect than untreated PPS.

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Research Laboratories of the Pharmaceutical Department of CIBA Limited, Basle, October 14, 1959.

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Zusammenfassung

Aldosteron schützt bei verschiedenen Tierarten – als wirksamstes aller untersuchten Corticosteroide – gegenüber verschiedenartig ausgelösten «Schockzuständen». Bei der Katze normalisiert es die durch Endotoxine veränderte vaskuläre Reaktivität gegenüber Adrenalin und Nor-Adrenalin. Es ist möglich, dass im Endotoxin-Schock die Konzentration von Nor-Adrenalin unterschwellig wird, und dass seine Wirkung, eventl. auch Freisetzung, von Aldosteron abhängt.

Adsorption de protéines par les plaquettes sanguines

On sait que les plaquettes sont entourées d'une couche de plasma, appelée «atmosphère plasmatique périplaquettaire» par Roskam[1]. Bounameaux[2] a pu y doser divers facteurs de la coagulation. Utilisant des techniques immunochimiques[3], il nous a été possible de mettre en évidence de nombreuses protéines plasmatiques dans les lysats de plaquettes abondamment lavées. Ces protéines plasmatiques paraissent bien adsorbées à la surface des plaquettes: c'est dans le but de confirmer cette hypothèse que nous avons réalisé le présent travail, tout en utilisant une protéine marquée d'un élément radioactif.

Techniques. Les réactifs suivants ont été utilisés: plaquettes humaines lavées 7 fois en solution aqueuse de NaCl à 0,9%, additionné de 0,1% d'éthylène-diamine tétra-acétate. Ces plaquettes parfaitement isolées sont finalement suspendues en NaCl aq. à 0,9% au pH 7,4, et leur concentration ajustée à 2000000 d'éléments par mm³. Ces plaquettes sont utilisées dès leur isolement.

Solution d'albumine marquée à l'iode radio-actif, renfermant 0,3 mg de protéine par ml.

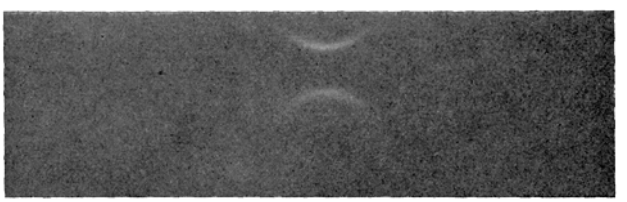

Fig. 1. Autoradiographie d'une immunoélectrophorèse obtenue avec un lysat de plaquettes incubées en albumine radio-active et révélée au moyen de sérum anti-sérum humain.

La suspension plaquettaire a été divisée en 3 lots: le premier est immédiatement congelé; les plaquettes du second sont incubées dans la solution d'albumine marquée, pendant 2 h à 37°C, puis pendant une nuit en glacière; le troisième lot servant de témoin subit le même traitement que le second, mais en NaCl aq. à 0,9%. Après incubation, les plaquettes des 2e et 3e lots sont à nouveau lavées 7 fois dans le liquide de lavage décrit ci-dessus, et leur concentration amenée à 2000000 d'éléments par mm³. Le temps suivant consiste en la lyse des 3 suspensions plaquettaires par congélation et décongélation successives dans la neige carbonique. Les lysats ainsi obtenus sont ensuite concentrés 10 fois par évaporation.

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