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ALDOSTERONE: MECHANISM OF ACTION ON ISOLATED SODIUM-TRANSPORTING EPITHELIA

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SUMMARY

The present conception regarding the biochemical mechanism of action of aldosterone on target cells is summarized: while the initial step—interaction of aldosterone with supposedly specific "receptor" proteins, cytoplasmic and nuclear—is well documented, unequivocal proof for hormonal influence on ribonucleic acid and protein metabolism is still lacking.

On the other hand, analysis of the changes brought about by aldosterone in the function of responsive epithelia specialized in transcellular sodium transport suggests that synthesis of (hypothetical) protein is induced by the hormone; this protein might operate as a sodium "permease" at the apical cell border in target tissues; furthermore there are arguments indicating that it has distinct properties, resulting chiefly in much more efficient sodium transport when external sodium concentration is low. Thus aldosterone is well suited for defending our "milieu intérieur" against undue sodium losses.

Aldosterone is the main sodium-retaining hormone in mammals; its role consists in protecting our sodium capital, hence, it helps maintain adequate irrigation of our cells. This goal is met by means of reduction of sodium losses by the organism: under the influence of this steroid hormone, sodium concentration in the lumen of the distal parts of nephron and intestine, and in the lumen of excretory ducts of sweat glands, is decreased, sometimes to negligible values.*

Amphibian epithelia made it possible to progress significantly in our endeavour to unravel the mode of action of aldosterone, because the physiological effect of the latter can be reproduced *in vitro* on such tissues [1, 2]—a rather privileged state of affairs when it comes to steroid hormones.

Expression of the action of aldosterone is thought to involve nucleic acid and protein metabolism. The general concept was formulated by Karlson [3] from the case of ecdysone; it has been successfully extended to most steroid hormones [4].

The sequence of events is currently understood as follows:

(1) The first step seemingly consists of an interaction between aldosterone and a cytoplasmic protein exhibiting a specific affinity for this steroid hormone; a complex would result that migrates into the nucleus where it undergoes some transformation before combining with chromatin [5].

Two points deserve further consideration here: does aldosterone act *per se*? There are indications that recovery of the steroid might not be quantitative after prolonged exposure to target tissues [1]. Whether a chemical change of aldosterone is required for it to acquire full biological activity intracellularly, should be assessed properly.

Furthermore, what is the significance of the cytoplasmic "receptor" proteins alluded to above? Is their presence sufficient for aldosterone to exert an effect on

*The well documented action of aldosterone on saliva, also due to enhanced transport of sodium along the ducts of the glands, is of lesser importance in such a perspective.

sodium movement? This could be questioned, were it only because these proteins can be extracted in relatively large amounts from the duodenum and the small intestine[5], which structures are doubtful targets for the "mineralocorticoid" action of aldosterone[6. 7]. The latter has non-negligible "glucocorticoid" properties[8]; as there now is evidence that distinct receptors exist for these 2 types of corticosteroid effects[9], the nature of the proteins serving as receptors in different tissues deserves attention. It would also be worth while examining to what extent recent observations of Steggles *et al.*[10] apply to this steroid hormone: these investigators have produced experimental arguments leading them to ascribe a critical role in the expression of gonadal steroid hormone action on target tissues to acidic proteins coating chromatin.

(2) The steroid-protein complex would act as a "de-repressor", thereby triggering the synthesis of DNA-dependent RNA, dubbed messenger-RNA; the latter, after migration into the cytoplasm, would direct synthesis by ribosomes of protein(s) eventually responsible for the aldosterone effect.

This scheme is backed essentially by indirect arguments such as the fact that the aldosterone-dependent sodium retention does not set in when RNA, or protein, synthesis is inhibited[11. 12]. A less indirect approach consisted in demonstrating that the specific activity of RNA in competent tissues could be enhanced by aldosterone[13. 14].

But difficulties arise for rigorous interpretation of such data when evidence for net *synthesis* of the labelled material is sought; an attempt was therefore made to look for changes of polysomes in ribonucleic acids when toad bladder tissue is exposed to, and stimulated by, aldosterone. No change in the polysome profile could be detected. Furthermore, analysis by double labelling of RNA radioactivity in polysomes, so as to catch *m*-RNA at work as it were, revealed only discrete hormone-induced shifts in radioactivity distribution, of questionable biological significance[15].

It is pertinent to recall here that with rat hepatoma cells in culture—a biological system intensively studied by Tomkins—there is a large selective increase in the rate of synthesis of a given protein (tyrosine aminotransferase) as a result of exposure to dexamethasone; yet no clearcut change in uridine incorporation into RNA could be demonstrated[16].

This is one of the arguments that led these investigators to suggest that hormonal control of protein synthesis in higher organisms is exerted at a post-transcriptional stage[17]. Korner had also pointed at such a possibility[18] on the basis of the fact that steroid hormones in general merely modify the *amount* of cell constituents.*

(3) In the case of aldosterone however, it has not so far been possible to demonstrate unequivocally a stimulation of protein synthesis either. Actually, toad bladder polysomes did not incorporate more labelled amino acids into polypeptide chains, when the tissue was under the influence of aldosterone[15].

The data just summarized concerning the "molecular" events leading to the expression of this sodium-retaining activity of aldosterone on competent cells, were largely collected on amphibian epithelia. The latter represent a privileged tool for studies of this kind because such organs perform rather specifically active transcellular sodium transport, and this at room temperature for long

*A notable exception is provided by progesterone which induces specifically the synthesis of avidin by estrogen-primed chick oviducts[4].

periods of time with minimal substrate requirements—as will be illustrated; furthermore, net sodium movement can conveniently be expressed in electrical units[19]. Finally, ventral skin and urinary bladder of anouran Amphibia display an enhanced sodium-transporting activity when incubated in the presence of physiological concentrations of aldosterone.* The behaviour of toad (*Bufo marinus*) skin and bladder seems essentially identical in this respect: fresh preparations react after a latency period of 1–2 h (Figs. 1 and 2) and they reach a steady state of hyperactivity after approximately another 2 h.

A prolonged preincubation before addition of aldosterone seems advantageous since it renders baseline activity more stable and more uniform from animal to animal; but there are indications that the response to aldosterone of both bladder and skin appears more slowly in such conditions and that it takes a longer period of time, especially in the case of toad skin, before a steady state obtains (Figs. 3 and 4).

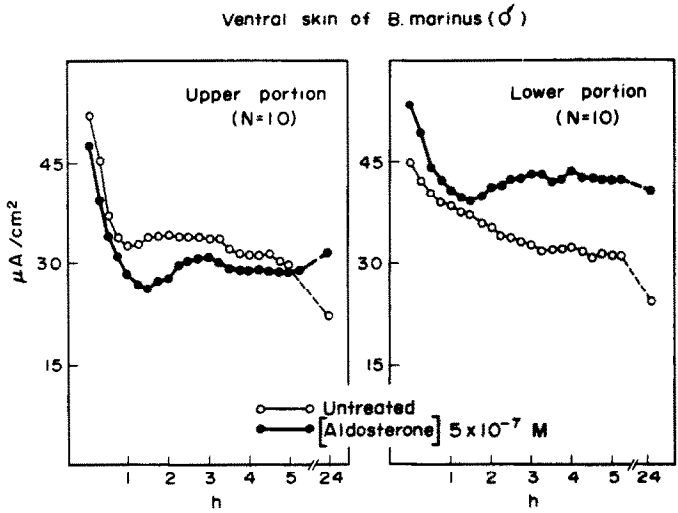

Fig. 1. Response of toad skin to aldosterone. Ventral skin of male *Bufo marinus* was divided into an upper (cephalic) and a lower (caudal) portion, each of which in turn was cut along the vertical axis. All 4 tissue pieces were incubated simultaneously as described by Ussing and Zerahn[19]; one piece of the upper half and one of the lower half were exposed from the outset to aldosterone, 5×10^{-7}M, added to Ringer's on the inside. After 90 min incubation (at room temperature), the hormone-treated tissue displayed a progressive increase in activity—short-circuit current reflecting quantitatively net, active sodium transport. After the 3rd h of incubation, the hormone-induced effect had stabilised.

Although this was more apparent with the lower portion of the ventral skin preparation, for neither series was the hormonal effect statistically significant before the following morning (glucose and antibiotics were added after the initial 5 h of incubation). Final levels of activity were in the case of untreated preparations (μA/cm^2 ± S.E.M.), 23·3 ± 5·7 and 25·6 ± 7·5 for upper and lower portions, respectively; corresponding values for aldosterone-treated pieces were 32·3 ± 5·1 and 44·1 ± 8·2, respectively. Statistically, there was no difference between the latter values (Mean Δ: 11·8; S.E. Mean Δ: 5·8; $P > 0{\cdot}05$). These observations are largely similar to data recently published by Porter[20].

*It should be kept in mind that plasma aldosterone concentration in Amphibia is 2 orders of magnitude higher than in man[1].

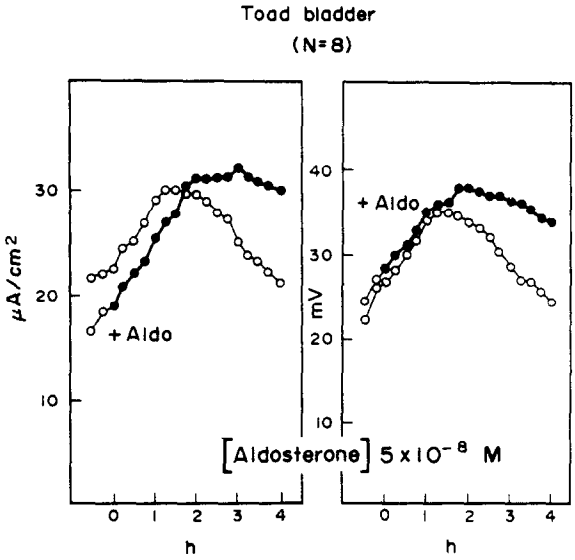

Fig. 2. Response of toad bladder to aldosterone. Both lobes of urinary bladders of male *Bufo marinus* were incubated simultaneously according to Ussing and Zerahn[19]. One lobe was exposed from the outset to aldosterone, 5×10^{-8} M, added to Ringer's on the inside.

As was the case with toad skin, the hormonal effect began to be apparent after approximately 90 min. It was statistically significant during the 4th h of incubation (Mean Δ: 12·1 μA/cm²; S.E. Mean Δ: 4·7; $P < 0{\cdot}02$).

Not indicated on the graph is the fact that incubation went on overnight, antibiotics but no glucose being added. The following morning the effect of aldosterone was quite definite since activity of untreated preparations averaged (in μA/cm² ± S.E.M.) 6·0 ± 1·1, while it was 23·1 ± 7·2 for matched hormone-treated ones. Transmembrane potentials were (mV ± S.E.M.) 19·4 ± 4·2 and 44·5 ± 11·3, respectively. There thus was an increase in tissue conductance associated with prolonged response to aldosterone, in keeping with a recent report[21]; the acute effect of this hormone on fresh bladder tissue also results in increased conductance[1].

Incidentally, Fig. 4 reemphasizes the point that the latency period is not a function of the concentration of aldosterone in the incubation fluid[1].

These data point to a satisfactory activity and responsiveness of toad bladder and skin for long periods of time, and to a large increase in sodium-transporting activity of aldosterone-treated preparations after incubation had gone on all night in the presence of the hormone. Furthermore, activity both of the control and of the treated preparations eventually stabilized in such conditions. Glucose is not required for this although it enhances the hormonal effect.

When investigation is not primarily concerned with the initiation of the aldosterone effect, it is obviously advantageous to have as clearcut a hormonal stimulation as possible. Therefore, attempts to localize the site of action of aldosterone on sodium-transporting epithelia were made with fragments of the ventral skin "fully" stimulated, i.e. exposed to aldosterone for 15–18 h before the actual experiments were performed.

Sodium transport by structures such as toad bladder and skin requires that the ion move across two main barriers, corresponding in all likelihood to the apical and the basal poles of the cells carrying out active transepithelial move-

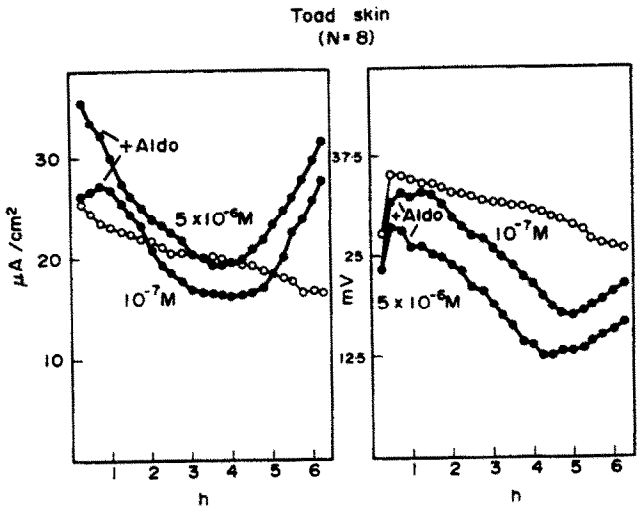

Fig. 3. Response of toad skin to aldosterone after a prolonged preincubation. Incubation of 3 randomly selected pieces of the ventral skin of male *Bufo marinus* was started in the evening according to Ussing and Zerahn[19], and it proceeded through the night in the presence of glucose, 10 mM, and antibiotics. The following morning, i.e. approximately 16 h later, solutions were renewed and antibiotics withdrawn. Thereafter, one fragment of each "triplet" served as a control while the 2 other ones were exposed to aldosterone at 2 concentrations differing by a factor of 50 as indicated.

Four hours were required for the hormone-treated preparations to display signs of enhanced sodium-transporting activity, irrespective of aldosterone concentration.

Noteworthy was the marked drop in both short-circuit current and electrical potential that took place during the latency period. This phenomenon was not seen with fresh preparations (cf. Fig. 1). Whether it has the same significance as the phenomena described and discussed by Hviid Larsen[22] cannot be answered.

ment of the ion. Whereas the first step (penetration of sodium at the apical cell border) is a saturable one, it is exergonic in standard incubation conditions. Extrusion of sodium from the cell at the basal border, and also at borders facing interspaces, requires instead the expenditure of energy – hence the term "pump". Yet the latter would operate far from capacity under most circumstances.

Thus, two impacts can be imagined for aldosterone: it could facilitate sodium movement apically, or it could improve the affinity of the "pump" for the ion.

The issue, stated in this most simplified way, was approached experimentally; 3 sets of data seem relevant.

(a) The repercussions on matched treated vs. untreated preparations of reductions in the concentration of sodium in the solution on the outside were examined. Strikingly, "affinity" of the tissue for sodium was much higher after treatment with aldosterone[24].

If the saturable step in transepithelial sodium transport is indeed due to a specialized structure located at the apical cell border, such data suggest the latter as the site of action of aldosterone; they indicate in addition that the properties of this saturable structure are modified by the hormone.

(b) Another approach consisted in assessing the changes in sodium-transporting rate brought about by amiloride, an acylguanidine that seemingly prevents sodium from crossing the apical border of the epithelia studied[25. 26]. This

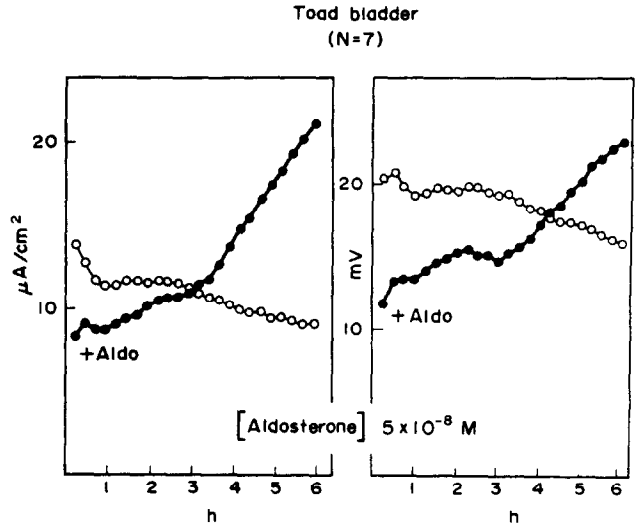

Fig. 4. Response of toad bladder to aldosterone after a prolonged preincubation. Preincubation of both lobes of the urinary bladder took place overnight in the presence of glucose and antibiotics. The following morning, after replacement of the solutions (from which antibiotics were withdrawn), one preparation of each pair was exposed to aldosterone, 5×10^{-8}M.

Rise in short-circuit current and transmembrane potential ensued after 1–2 h, and the hormonal effect became increasingly apparent as time went by, so that no steady state was achieved even after 6 h of incubation. Such data are reminiscent of those originally reported by Porter and Edelman [23].

drug was *less* active on aldosterone-stimulated preparations [27], which is again interpreted as an indication of an increased affinity of the apical structure for sodium relative to that for amiloride under the influence of this hormone.

(c) A third argument could be drawn from the fact that aldosterone failed to modify the relationship between sodium transport rate and pool size in stimulated bladder tissue [28]. In other words, assuming that the "pool" is located to a significant extent before the "pump", the fact that pool size and transport rate increased in proportion is taken to indicate that the kinetics of the "pump" were not significantly influenced by aldosterone. Therefore, if the "pump" operates far from capacity, increased availability of sodium results in increased rate of disposal by the "pump". Thus the conclusion is arrived at that aldosterone facilitates sodium access to the "pump" by acting at the apical cell border.

Recently, an increase in sodium content (and concentration) of isolated epithelial cells harvested from toad bladder exposed to aldosterone has been directly demonstrated [29. 30].

It is thus likely that aldosterone action on such preparations results in *improved* interaction of sodium with the tissue at the apical border of the latter. Furthermore, experimental data dealt with under (a) and (b) are best interpreted by assuming that the hormone induces new "permeases" [31], the properties of which are *different* from those of "permeases" supposedly involved in penetration of sodium at the apical cell pole in baseline conditions.

This conclusion is especially interesting in view of the theory according to which aldosterone acts on the genome: a stronger case could be made for aldo-

sterone setting off the synthesis of *m*-RNA if the (hypothetical) protein resulting from the process could be shown to have properties *different* from those of its kin. As said, there are arguments compatible with this conclusion. In this respect, it could be interesting to examine whether the changes in phospholipid metabolism recently described[32] for toad bladder tissue stimulated by aldosterone, occur at a given cell border.

However, some experimental facts cannot be easily reconciled with this interpretation. The effect of glucose already mentioned (see Fig. 5), seems incompatible with the contention that aldosterone stimulates sodium transport through an action exerted solely at the apical cell border since in all likelihood glucose merely provides fuel for the energy-requiring "pump".

Furthermore, Kirsten *et al.*[34] have demonstrated an increase in the activity of cellular enzymes under the influence of aldosterone, even when no sodium is transported; this makes it difficult to claim that increased metabolic expenditure incurred on account of stimulated active sodium transport under the influence of aldosterone, provides the explanation for this observation.

In order to shed additional light on the issue, complementary experiments were carried out (Fig. 6).

Paired pieces of ventral toad skin were incubated overnight, one piece of each pair being exposed to aldosterone from the outset. But energy consumption by the tissue was reduced by substituting sodium-free fluid to Ringer's on the outside through the night. The following morning, upon reintroduction of Ringer's on the outside, there was a short-lived burst of electrical activity, after which both preparations were treated with glucose. As can be seen, the aldosterone-treated piece of skin reacted to it, unlike the matched control.

In order to evaluate whether this response to glucose is specifically linked to

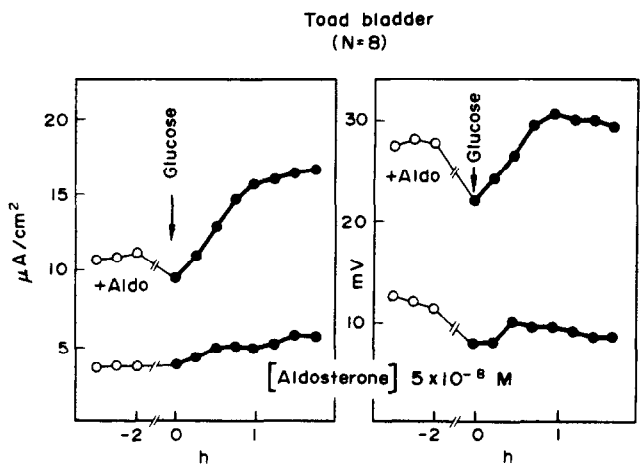

Fig. 5. Enhanced effect of glucose on the aldosterone-mediated stimulation of active sodium transport by the isolated toad bladder. Matched hemibladders were incubated according to Ussing and Zerahn[19], one preparation of each pair being exposed to aldosterone. After incubation overnight in the presence of antibiotics, solutions were replaced. Short-circuit current and electrical potential were recorded intermittently for 3 h during which activity was rather stable, yet moderate. After addition of glucose, 10 mM, there was a large response on the part of the hormone-treated membranes. The same observation has been made repeatedly on toad skin[33].

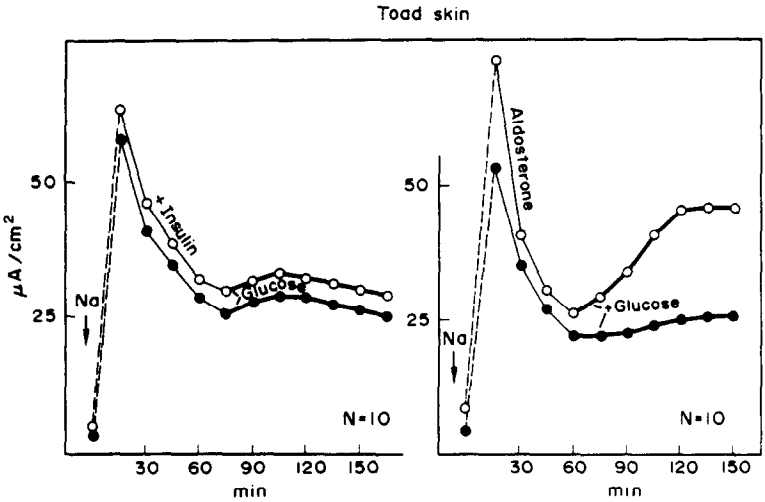

Fig. 6. Effect of glucose on sodium transport by toad skin stimulated by aldosterone. In 10 instances, aldosterone, 5×10^{-6}M, was present from the outset in the solution (on the inside) to which one piece of skin of each pair was exposed. Sodium-free Ringer's bathed the external surface of both pieces of each pair through the night. The following morning, this fluid was replaced by Ringer's after samples had been set aside for measurement of sodium concentration by flame photometry.

Given the low concentration of this ion—1·15 mEq/L on the average, against 115 mEq/L in Ringer's—little net sodium transport inward could have taken place through the night, even when the tissue was under the influence of aldosterone. Thus, metabolic expenditure related to transepithelial sodium transport was kept artificially low and—more significant yet—largely to the same level for both preparations of each pair. Nevertheless, shortly after resumption of incubation in standard conditions, glucose produced its classical effect, characterized by an increased activity of the steroid hormone-treated preparation only.

When the same protocol was adopted for experiments in which insulin (125 mV/ml) stimulated sodium transport instead of aldosterone, no such glucose effect could be demonstrated.

the use of aldosterone, advantage was taken of insulin that can also stimulate sodium transport by toad skin for protracted periods of time[35]. Obviously, energy-providing substrate is required for this hormonal response. Experiments were therefore conducted as above, but insulin replaced aldosterone. Yet, upon introduction of glucose into the medium, increase in sodium-transporting activity ensued for neither preparation.

It is likely, however, that the apical effect of aldosterone somehow conditions the action the hormone exerts at the vicinity of the "pump", because there are reasons to believe that aldosterone does not influence sodium transport by all cells—as would be expected otherwise in view of the quasi-ubiquitous presence of the sodium "pump" in cell membranes. It could be mentioned, in this context, that aldosterone fails to modify directly kalemia[36. 37]: a drop of the latter would be expected if this steroid hormone stimulated sodium transport in a widespread fashion—which apparently occurs with insulin or, as shown recently, with thyroid hormones[38].

Thus the physiological effect of aldosterone would be largely confined to tissues specialized in transcellular sodium movement, and still more specifically,

to those epithelia which are capable of efficient sodium transport (in terms of electro-chemical potential gains). This efficiency might reside in the existence of a saturable (for sodium) structure located at the apical cell border. It is pertinent to draw attention to the fact that, even among epithelia, only a few definitely react to aldosterone[1, 39–41]; they obviously should be given priority for studies concerning the intimate mechanism of action of this hormone.

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DISCUSSION

Adlercreutz: May I ask you, as a clinician, a question to a clinician: can you say whether there is any other mechanism by which aldosterone causes hypertension than through sodium retention? Do you think that aldosterone may have some other peripheral effect than on those 3 parts of the organism that you mentioned?

Crabbé: I think that the sodium retaining properties of aldosterone are sufficient to account for the hypertensive effect ascribed to this hormone. Investigators have come out with the conclusion that under the influence of this hormone the sodium content of smooth muscle in arterial wall is modified, but this might be a consequence of hypokalemia that eventually sets in, itself a result of events going on for extensive periods of time at the renal level. This betrays my bias, that aldosterone acts only on a few specialized structures, but I dare hope that I dealt fairly with the issue you raise.

Kellie: Do you have any explanation for the hour's delay in the effect?

Crabbé: When it became clear that aldosterone penetrates quickly into the tissue studied, the suggestion was made that the effect would be mediated via the synthesis of an intermediate that is critical in the expression of hormonal action. Now, one could suggest that the time lag be the consequence of the hormone proper undergoing some kind of transformation, as is the case with testosterone, which is transformed to DHT. Although there are indications that aldosterone cannot be recovered quantitatively from a target tissue such as the toad bladder incubated in its presence, exposure of this preparation to organic extracts that in all likelihood contain the metabolite(s) of the steroid hormone did not bring about a shortening of the latency period (unpublished). At the present stage, this latency period is considered as resulting from the time required for events involving RNA and protein synthesis. We don't have direct evidence for aldosterone-induced specific changes at these steps, but indirect evidence and much more convincing work done with other steroid hormones still favour this interpretation.