Journal of Steroid Biochemistry, 1972, Vol. 3, pp. 161–165. Pergamon Press. Printed in Great Britain

ALDOSTERONE INDUCED MORPHOLOGICAL CHANGES IN AMPHIBIAN EPITHELIA *IN VIVO**†

C. L. VOÛTE, S. HÄNNI and E. AMMANN
The Laboratory of Experimental Nephrology, Dept. of Medicine, University of Basel, Switzerland

SUMMARY

In the epithelium of the frog skin and the toad bladder only one type of cell, the mitochondria-rich cell, responds in a characteristic and similar way to variations in sodium load and to stimulation by parenteral aldosterone. These observations support the view that these cells are in a way involved in the osmoregulation controlled by aldosterone.

INTRODUCTION

In a previous communication we have discussed the functional and structural relation in the aldosterone induced moult in the epithelium of the frog skin *in vitro* [1]. We called attention (Fig. 1) to the characteristic changes observed in a specialized epithelial cell, the mitochondria-rich cell, MR cell or flask cell, changes occurring in parallel with the moulting cycle. With earlier investigators [2, 3] we shared the opinion that these cells must be involved somehow in the desquamation process of the amphibian epidermis.

Our studies concerning these cells were extended to other epithelia not subject to cyclic changes, namely the epithelium of the toad and frog urinary bladder.

The question raised for this study consisted in whether or not the mitochondria-rich cells of other epithelia responding to aldosterone would show similar changes to those we had observed in the moulting epithelium of the frog skin under the influence of this hormone. This was critical in order to enable us to separate the two superimposed aldosterone effects observed in the frog skin epithelium: The effect on the moulting and the effect on sodium transport. Also it was important to know whether we were dealing with the same type of cells even if their source was different.

For this purpose three groups of *Bufo marinus* and *Rana temporaria* were used. The animals were kept for six days in shallow distilled water. NaCl-Ringers or in NaCl-Ringers but receiving 10 micrograms aldosterone(aldocorten CIBA) per day during the last 3 days. The animals were kept as usual: the frogs at 4–6°C and the toads at room temperature. After this period the animals were

*Dedicated to my father Hans Voûte M.D., deceased 13.7.71.

†This study was supported by grant No. 3.343.70 of the Swiss National Foundation for Scientific Research.

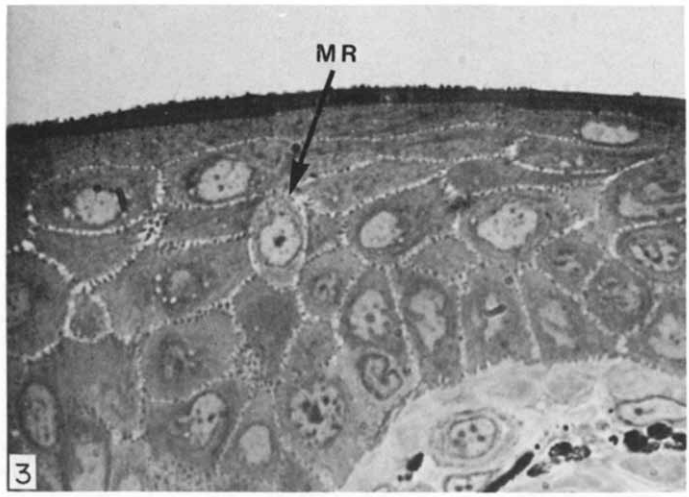

Fig. 3. Epidermis of a frog kept in an NaCl-rich medium. Arrow indicates unstimulated MR cell. Magnif. × 1100.

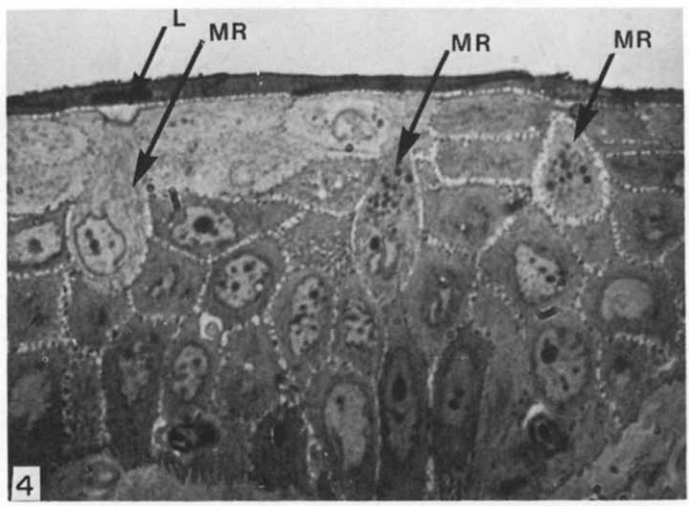

Fig. 4. Skin epithelium of a frog kept in distilled water. Note the alterations in MR cells (arrows). L = "Lake formation" at tip of neck in subcorneal space. Magnif. × 1000.

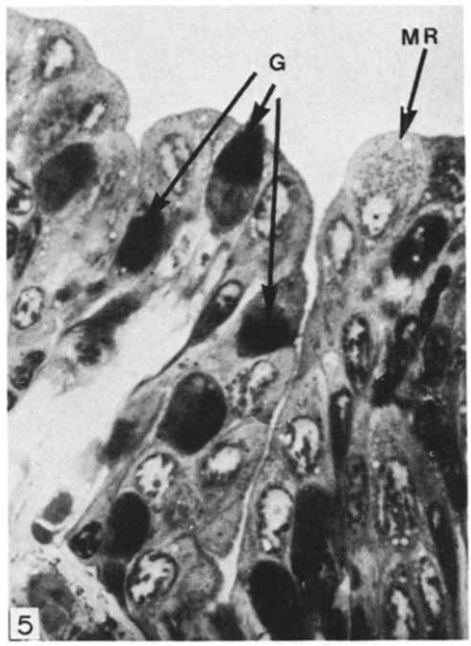

Fig. 5. Same situation as Fig. 3 but for the epithelium of the toad urinary bladder. Arrow indicates unstimulated MR cell. Cells with very dark content are goblet cells(G). Magnif. × 1000.

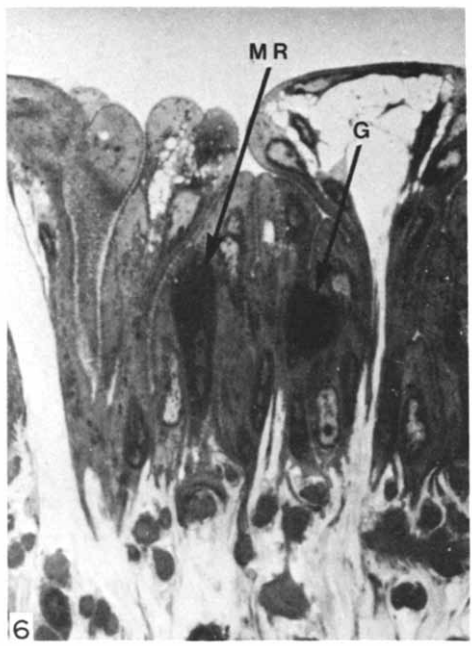

Fig. 6. Epithelium of a toad urinary bladder, animal kept in distilled water. Note stimulated MR cell (MR) with granular dark cytoplasm as compared to a goblet cell(G) with black content(mucus). Magnif. × 1000.

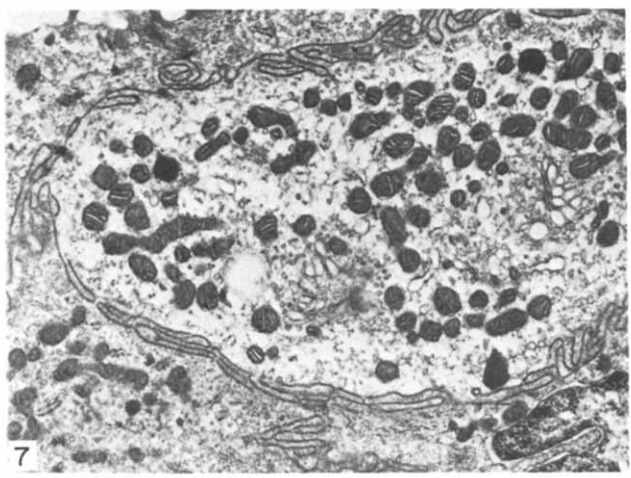

Fig. 7. Electronmicrograph of an unstimulated MR cell. Note loose cytoplasm including various organelles (Golgi complex, vesicles and modest amount of ribosomes). Magnif. × 17,000.

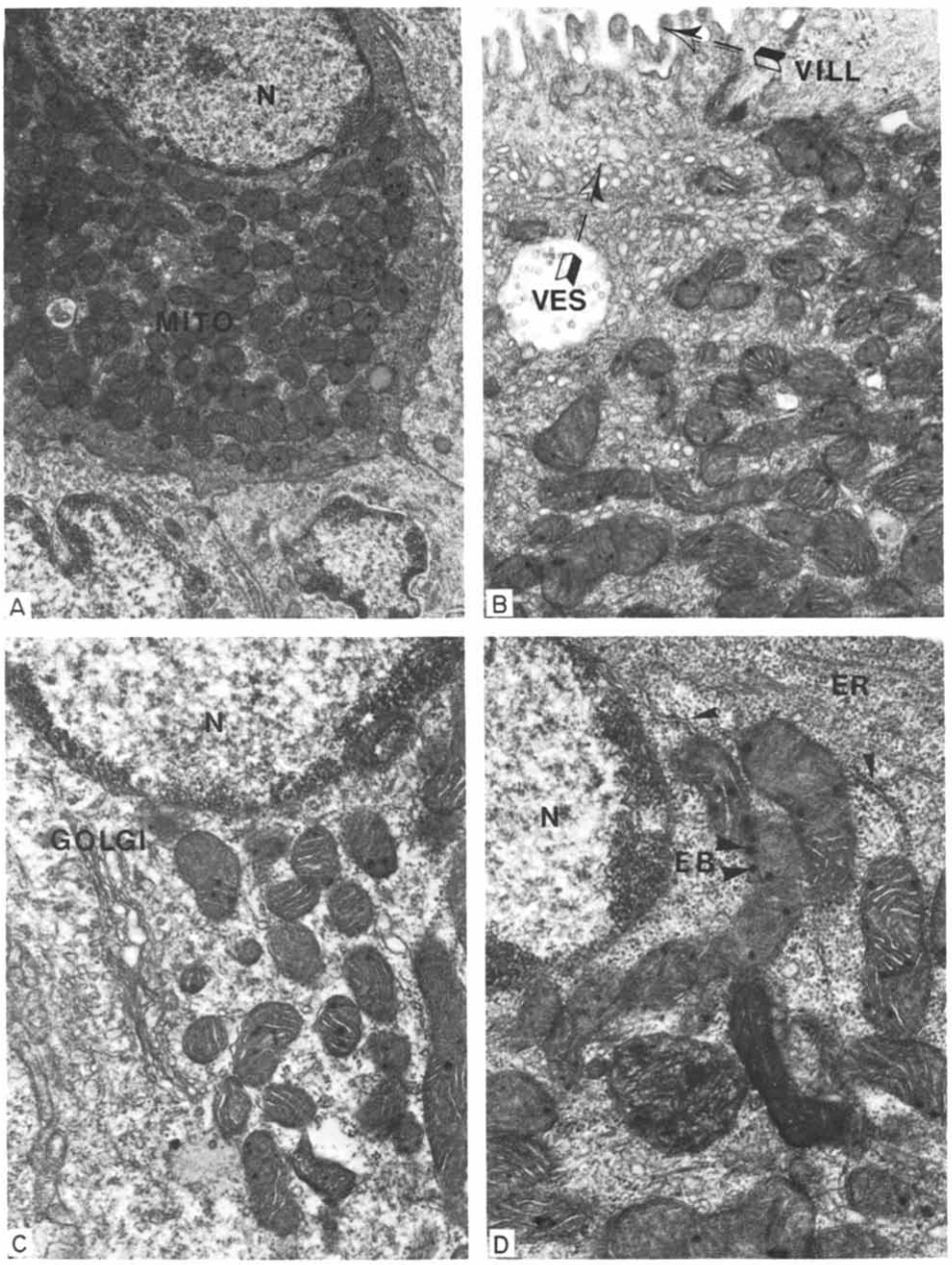

Fig. 8. Various parts of a stimulated MR cell. A: Basal and midportion of cell. MITO = mitochondria, N = nucleus. Magnif. × 14,000. B: Luminal pole of cell. VILL = microvilli extending into the lumen, VES = vesicles. Magnif. × 28,000. C: Perinuclear zone with Golgi complex. Magnif. × 28,000. D: Higher magnification illustrating various forms of mitochondria with electrondense bodies(EB). Highly increased density of ribosomes. partially lined by a membrane(ER). Magnif. × 35,000.

appearance and many forms of lysosomal origin. The endoplasmic reticulum is of the smooth type and not very abundant; Golgi zone and Golgi vesicles are prominent. With the aid of Fig. 8 we would like to demonstrate various parts of a stimulated MR cell. The cytoplasm now is gorged with mitochondria containing one to several electrondense bodies. Ribosomes have become very abundant and they are lined at rare occasions by a membrane. The Golgi complex has changed insofar as vesicles have become smaller but more frequent in its vicinity. The luminal pole of the cell is characterized by its large amount of small vesicles and the formation of microvilli extending into the lumen of the bladder or the subcorneal space in the skin epithelium.

All counts were done with reference to the total count of epithelial cells in the toad bladder and the count of the cells in the first living cell layer below the corneum in the frog skin. The last figure (Fig. 9) illustrates the results of these counts assembled in a graphic form to render the comparison easier for the two groups of different epithelial origin. The qualitative behaviour of the MR cells in both epithelia is similar, the total count however seems to vary in the frog skin epithelium only. This is due most probably to cyclic changes in this epithelium as compared to the steady bladder tissue. For obvious reasons further differentiation into secreting and non-secreting cells was possible in the epithelium of the frog skin only.

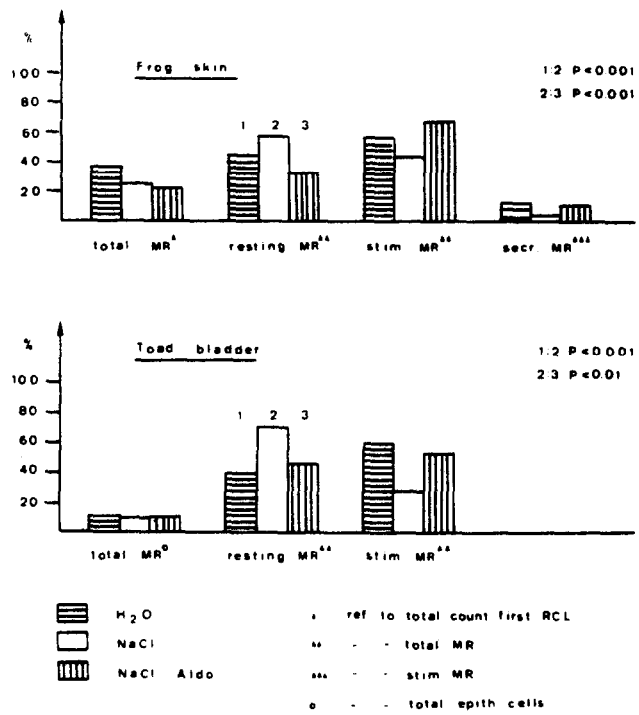

Fig. 9. Results of total and differential counts of MR cells in frog skin epithelium(top) as compared to toad bladder epithelium(bottom) under the three different experimental conditions. Statistical analysis: P in Fig. 9 indicates the statistical significance of the difference of the means of the different groups. Frog: N1 = 32, N2 = 32, N3 = 32, Toad: N1 = 20, N2 = 23, N3 = 16.

From these results we may conclude in summary, that in the epithelium of the frog skin and the toad bladder the mitochondria-rich cells respond in a similar way to variations in sodium load and to stimulation by parenteral aldosterone. In other words, endogenous hyperaldosteronism and exogenous stimulation with aldosterone in a low activity state produce the same differential picture of these cells.

Equally on the basis of these results the spectrum of our earlier statement, namely that these cells must be involved somehow in the moulting cycle, has to be enlarged by adding the possibility of a more general function of these cells: they might take active part in the osmoregulation controlled by aldosterone. Whether this would be through the intermediate step of hormone induced enzyme synthesis and secretion as, e.g. tentatively proposed in terms of a "permease" [5–7] or through some other mechanism remains, however, from the morphological point of view mere speculation.

REFERENCES

1. Voûte C. L., Dirix R., Nielsen R. and Ussing H. H.: *Exptl. Cell Res.* **57** (1969) 448.
2. Fahrenholz C.: *Z. Mikr. Anat. Forsh.* **10** (1927) 297.
3. Spannhof L. and Humboldt Wiss. Z.: Univ. Berlin, Nat. R. **IX**(1959/60) 173.
4. Voûte C. L. and Ussing H. H.: *Exptl. Cell Res.* **62** (1970) 375.
5. Frazier H. S., Dempsey E. F. and Leaf A.: *J. Gen. Physiol.* **45** (1962) 529.
6. Edelman I. S., Bogoroch R. and Porter G. A.: *Proc. Nat. Acad. Sci. U.S.* **50** (1963) 1169.
7. Crabbé J. and de Weer P.: *Nature* **202** (1964) 298.

DISCUSSION

Leaf: Dr. Voûte, I think your very interesting results might come out a little better if you could perhaps resummarize them for us.

Voûte: With the help of the last slide (Fig. 9) the results may come out clearer. On top we have the counts for the frog skin and on the bottom the ones for toad bladder. The first block corresponds in both tissues to the counts in animals kept in distilled water. The second block demonstrates the counts in animals kept in NaCl Ringers and the last block indicates the results in both tissues of animals kept in a sodium rich medium but receiving aldosterone injections during the last three days. The first group of blocks gives us the total counts in the tissues, whereas the second group demonstrates the differential counts as expressed in % of the total counts. Thus we may see that both tissues, of different origin and animal species, behave in a similar manner when considering the activity degree of the MR cells under different conditions of endogenous or exogenous aldosterone effect. This is true for the differential picture only and not in the total count of MR cells. In the toad bladder the total counts vary insignificantly whereas in the frog skin exogenous aldosterone induces a further decrease of the total count. This is probably due to the fact that this hormone induces a moult and that during this period the MR cells of the outer layer empty their content completely into the subcorneal space and could, in contrast to the bladder MR cells, be looked at as a sort of suicidal cell. The differential counts, however, giving us the unstimulated versus stimulated state of these cells, are similar for both tissues under comparable conditions.

Edelman: What you call the "resting state" in the toad bladder under aldosterone decreases, and the fractional number of MR cells compared to the sodium chloride control decreases.

Voûte: Yes and no. In a high sodium medium we have the majority of the cells in the resting state; this would correspond to the lowest endogenous aldosterone level group. In the water group, however, the group corresponding to the high endogenous aldosterone effect, the differential situation is reversed: we find more stimulated MR cells than resting ones. The last group is the one in sodium-rich medium(endogenous aldosterone level low, the inhibited group) but receiving exogenous aldosterone during the last three days. This was done in order to see whether we were right in assuming that the functional state of the MR cells was controlled by aldosterone and not by some other unknown factor. These observed changes are early changes, within the first hour, when one observes no change in sodium transport yet.

Porter: Have you tried any of these experiments shorter than 6 days?

Voûte: No. They were all done under the same experimental conditions.

Crabbé: Is there any correlation between the "spontaneous" sodium-transporting activity of the preparations and their morphology, thus *irrespective* of hormone treatment? We all know that the activity of amphibian epithelia (in terms of sodium transport) is quite variable even when we try to define standard conditions of conservation of the animals.

Voûte: With respect to the MR cells I could not answer this question because we didn't do the necessary experiments (pair-correlations for example). If, however, we consider quantitative functional-morphological correlations, irrespective of the MR cells, I would like to refer to our last paper (Voûte *et al.*, *Exptl. Cell Res.* **62** (1970) 375), where most results came from preparations without any hormone treatment (some had received ADH).

Porter: The distilled water animals and the NaCl animals were done together if I understood correctly, whereas the aldo-treated ones were done separately?

Voûte: Only the frogs; the toads were done together. The skins in frogs (in toads it is probably very similar) undergo rather drastic seasonal variations and as we did the aldo experiments in the frogs during another season, the functional values of the skins were not comparable to those of the two other groups. The bladders. however, were functionally comparable in frogs and toads irrespective of the season. Because of lack of time we haven't counted the frog bladders so far.