can produce the whole range of pathological changes that have so far been described for the 2 series of more complex natural compounds derived from plant sources. As such they are potentially useful tools in the investigation of the means by which the latter compounds produce some of the interesting and unusual lesions.

Zusammenfassung. Nachweis, dass die verschiedenen Schädigungen in Leber und Lungen von Ratten und Mäusen nach Vergiftung mit einigen Furanosesquiterpen-Naturstoffen mit synthetischem 3-substituiertem Furanderivat nachzuahmen ist. 3-Hydroxymethylfuran-N-Aethylcarbamat (IV) hat ähnliche Giftwirkungen wie Ipomeanin (V) und Ipomeanol, während das NN-Diäthylcarbamat (VII) mit Ngaion (I) und Ipeomeamaron vergleichbar ist.

A. A. SEAWRIGHT and A. R. MATTOCKS

Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton (Surrey, England), 7 May 1973.

Effect of Inhibitors of RNA and Protein Synthesis on the Aldosterone Na^+ Transport Response in Toad Bladder

The machnism of aldosterone action in toad bladder has been described[1] in terms of a predominant effect on mucosal permeability to sodium ions and a second effect on mitochondrial enzyme activity[1,2] allowing for an increased supply of high energy intermediate as the ion pump becomes rate limiting. Dose response characteristics of the hormone response[3] have enabled us to separately describe a two stage mechanism in terms of the saturation of 2 types of receptor site[4] having K_a values of the order 10^8 l/mole and 10^{10} l/mole respectively. We have proposed that the role of 2 receptors in the mechanism of steroid hormone action may be to allow control of protein synthesis at the transcriptional and translational level[5]. The present work has involved a study of the effect of actinomycin D and cycloheximide on the aldosterone stimulated Na^+ transport across the isolated toad bladder.

Materials and methods. All toads (*Bufo marinus*) used in this work were soaked in 0.6% saline for at least 24 h before each experiment in order to reduce the release of endogenous mineralocorticoid. They were rapidly pithed and the half bladders excised and stretched across a double chamber[3]. The bladders were preincubated for 1 h in aerated frog Ringer's solution and aldosterone (10^{-9} or 10^{-7} M) added to the serosal surface of each section of bladder with actinomycin D (10^{-6} M) or cycloheximide (10^{-6} M) added at various times after the hormone. The short circuit current (SCC) was measured at intervals over a 5 h period. In other experiments the bladders were preincubated in the presence of 1 mM pyruvate for 90 min before addition of aldosterone (10^{-7} M). The effect of actinomycin D (10^{-6} M), added 5 and 10 min after or 10 min before the hormone, on the SCC was measured over a 4 h period. The effect of cycloheximide (10^{-6} M) added 10 min after the hormone was studied in a further series of experiments.

Substrate depleted bladders[6] were treated with aldosterone (10^{-7} M) and the synergistic effect of 1 mM pyruvate on the SCC measured in the presence and absence of actinomycin D (10^{-6} M) or cycloheximide (10^{-6} M). In a final series of experiments we have measured the maximum percentage increase in SCC following 1 mM pyruvate addition 90, 180, 270 or 330 min after treatment of substrate depleted bladders with 10^{-7} M aldosterone, in the presence and absence of actinomycin D (10^{-6} M) added 10 min after the hormone.

Results and discussion. As shown (Figure 1), the low dose of aldosterone has little effect on the measured SCC, but an immediate increase was observed following actinomycin D treatment. This stimulatory effect of actinomycin D on Na^+ transport across the isolated toad

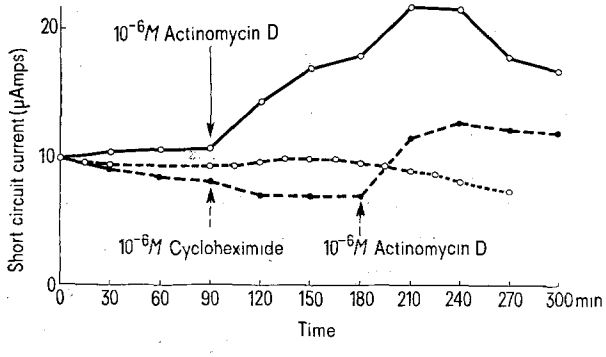

Fig. 1. Increased short circuit current across the isolated toad bladder stimulated by 10^{-6} M actinomycin D, additions as indicated, in the presence of 10^{-9} M aldosterone added at time zero to the serosal surface of both sections of bladder. The results represent the mean of 2 experiments.

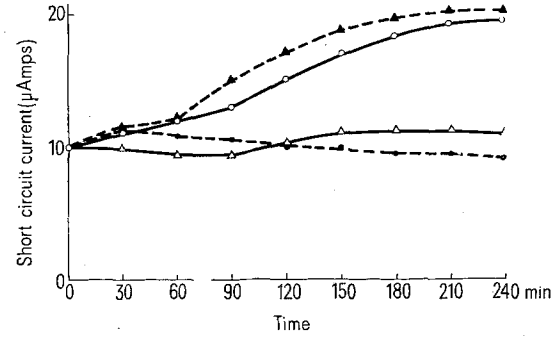

Fig. 2. Short circuit current across the isolated toad bladder stimulated by 10^{-7} M aldosterone in the absence (–○—○–) and presence of 10^{-6} M actinomycin D added 10 min after (--▲--▲--), 5 min after (–△—△–), and 10 min before (--●--●--) the hormone. The results represent the mean of 8, 4, 2 and 2 experiments respectively.

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bladder in the presence of aldosterone has been confirmed in several experiments. It was found that actinomycin D (10^{-6} *M*) inhibited the response to aldosterone (10^{-7} *M*) when added 10 min before or 5 min after the hormone (Figure 2). However, when added 10 min after the hormone the normal aldosterone response was developed. This failure of actinomycin D to inhibit the hormone response is in contrast to the inhibitory effect observed with 10^{-6} *M* cycloheximide.

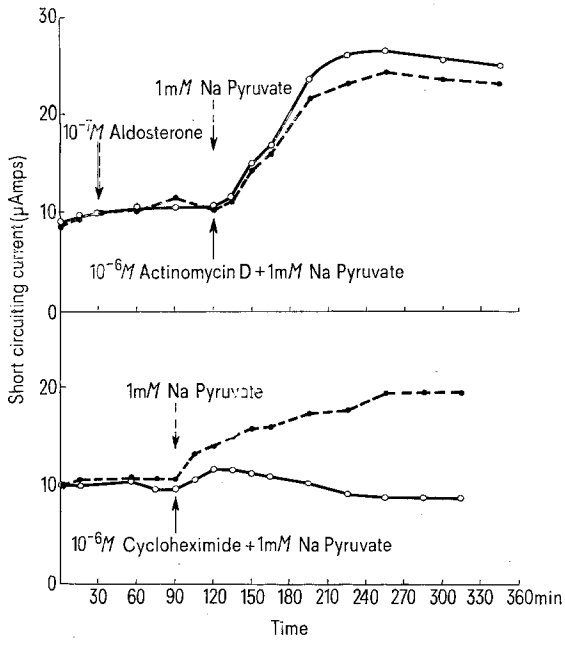

Fig. 3. (Lower) Shows the synergistic effect of 1 m*M* pyruvate added to substrate depleted bladders in the absence (--●--●--) and presence (–○—○–) of 10^{-6} *M* cycloheximide.
(Upper) Shows the synergistic effect of 1 m*M* pyruvate added to substrate depleted bladders in the absence (--●--●--) and presence (–○—○–) of 10^{-6} *M* actinomycin D. The results represent the mean of 2 experiments.

In substrate depleted bladders we fail to get an aldosterone (10^{-7} *M*) response in agreement with the observations of LEAF and SHARP[6]. We have found (Figure 3) that actinomycin D fails to inhibit the synergistic effect of pyruvate in contrast to the inhibitory effect obtained with 10^{-6} *M* cycloheximide. The pyruvate synergistic response, expressed as a percentage increase in SCC did not vary significantly over the 6 h period either in the absence or presence of 10^{-6} *M* actinomycin D added 10 min after the hormone. The simulatory effect of actinomycin D on Na^+ transport in the presence of 10^{-9} *M* aldosterone is taken as evidence for translational control of protein synthesis as part of the mechanism of aldosterone action in toad bladder. It is suggested that all transcriptional effects of the hormone may be associated with the highest affinity binding sites. The second set of binding sites characterised as cationic by agar gel electrophoresis[4] may be involved with repression of post transcriptional inhibitors.

Zusammenfassung. Die biochemischen Wirkungen von Aldosteron in der Krötenblase wurden untersucht und der Mechanismus der Hormonwirkung diskutiert.

D. A. FRITH and R. S. SNART

Department of Zoology, The University, Sheffield S10 2TN (England), 14 May 1973.

Intraspecific Variation of Lactate Dehydrogenase (LDH) Isoenzymes in Some *Belone belone* Populations from the Adriatic and Tyrrhenian Seas

In recent years, information on LDH isoenzymes (E.C. 1.1.1.27) in Teleost fish has greatly expanded. These studies have demonstrated that the electrophoretic pattern of this tetrameric enzyme, which is ubiquitous in all organs and tissues, is considerably more complex than that found in higher vertebrates. In fact, besides the A and B (or M and H) loci, characteristic of mammals and birds and probably homologous to these[1], 2 more loci have been described in teleosts. The first locus, postulated by MARKERT and FAULHABERT[2], designated E, has a high tissue specificity and is almost exclusively active in retina, lens and brain, although it may sometimes be active in the heart muscle[3–5]. The second locus, designated L, was described by LUSH[6] and is most active in the liver. By contrast, no description is available of a locus corresponding to the C locus, which is characteristic of the earliest spermatogenetic stages in birds and mammals[7–9].

A number of cases of intraspecific polymorphism, depending on mutation of some of the above listed loci, have been reported. Among the teleosts, which are of particular interest here, some cases of intraspecific polymorphism have been described in *Clupea arengus*[10], in various Salmonid species[11–14], in some cod populations[2, 6, 10, 15, 16] and in *Lepidorhombus whiff-iagonis*[17].

This paper describes a peculiar pattern of intraspecific LDH polymorphism in *Belone belone* (Teleostea, Belonidae) populations from different geographic areas.

Materials and methods. The electrophoretic behaviour of LDH from 2 separate *Belone belone* populations was

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