The approximate minimal inhibitory concentrations of obtusastyrene, dihydroobtusastyrene II, 2-methyl-4-cinnamylphenol III, and 2-cinnamyl-4-ethylphenol IV, were determined by using a low density algal inoculum, containing 7.6 absorbance units at 664 nm in 100 ml culture solutions. After 44 h the chlorophyll content of recovered algae was measured. Control cultures gave 40 absorbance units. Compounds I, II, III, and IV slightly inhibited growth at concentrations as low as 0.1 µg/ml. As shown in Figure 1, however, complete inhibition of growth occurred with each of these compounds at concentrations of 5–15 µg/ml. The inhibitory effect of these cinnamylphenols was also observed with *Scenedesmus obliquus*. While a control culture of this organism (initial density 5.5 absorbance units in 100 ml) increased to give 143 absorbance units in 110 h, growth in the presence of obtusastyrene at 20–60 µg/ml was completely inhibited, these cultures becoming colorless with the loss of all viable cells.

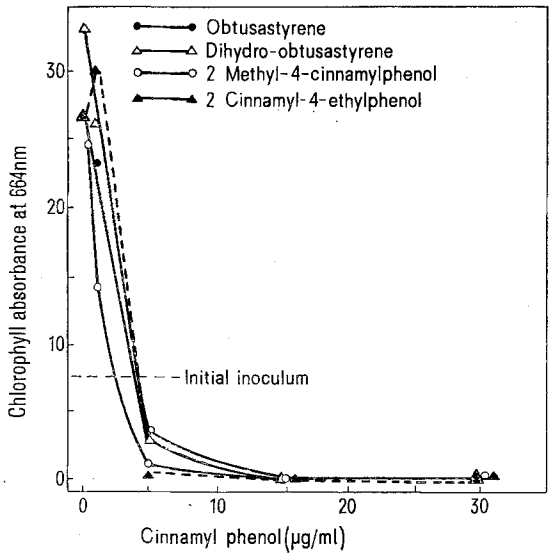


Fig. 1. Chlorophyll absorbance measured in *Chlorella pyrenoidosa* ATCC 11469, after treatments with the cinnamyl-phenols for 44 h. The control culture had chlorophyll absorbance of 40 units after 44 h.

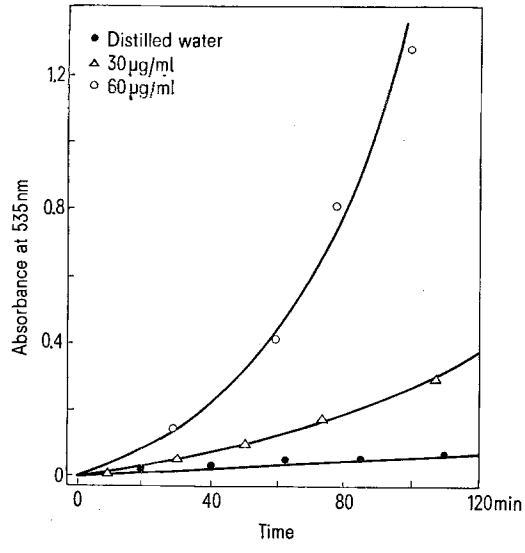


Fig. 2. The rate of betacyanin leakage from 30 discs (5.9 g) of *Beta vulgaris* root into 50 ml solution after treatment with obtusastyrene.

The antialgal properties of the 6 cinnamylphenols I–IV were compared with that of phenol itself as a standard against a dense initial population of *C. pyrenoidosa* (108 absorbance units in 100 ml test medium). Phenol, violastyrene and isoviolastyrene were not inhibitory at concentrations of 100–400 µg/ml. The cinnamylphenol I–IV were not inhibitory at concentrations of 5 µg/ml with this algal density. At concentrations of 15–30 µg/ml, however, I–IV rapidly killed the algal cells. After 20–45 h of exposure they precipitated in brown aggregates, and growth did not occur when these aggregates were washed and transferred to fresh nutriten solutions.

Although the mode of action of the cinnamylphenols on algal cells is uncertain, they do appear to rupture the cellular membranes with subsequent leakage of the cellular constituents. JUDIS[17] has previously suggested that the toxicity of *p*-chloro-*m*-xylenol to *Escherichia coli* is due to a similar lysis of the bacterial cell, and observations on the effects of obtusastyrene on red beet tissue support this interpretation. Thus, when discs of red beet tissue were suspended in aqueous solutions of obtusastyrene, the water-soluble, red betacyanin pigments were rapidly leached into the surrounding medium. As shown in Figure 2, beet discs treated with aqueous obtusastyrene (60 µg/ml) lost 38 % of their total betacyanin pigments to the external solution within 100 min. Significant loss of betacyanin pigment did not occur when the beet tissue was suspended in distilled water.

Zusammenfassung. Der phenolische Inhaltsstoff von *Dalbergia* Arten, Obtusastyrol und verwandte, zimtsäureartige Phenole hemmen das Wachstum der Algen *Chlorella pyrenoidosa* und *Scenedesmus obliquus*.

B. G. CHAN and L. JURD

Western Regional Research Laboratory,
Agricultural Research Service,
United States Department of Agriculture,
Berkeley (California 94710, USA), 19 March 1973.

[17] J. JUDIS, J. Pharm. Sci. *52*, 126 (1963).

The Toxicity of Two Synthetic 3-Substituted Furan Carbamates

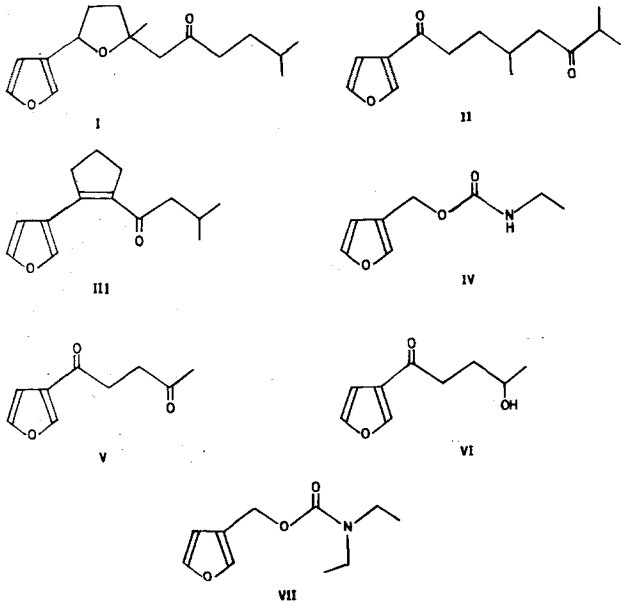
Poisoning of livestock through consumption of foliage of various species of plants within the family Myoporaceae is well known in Australia and New Zealand[1]. The toxic compounds involved are furanosesquiterpene essential oils, the best known of which is ngaione (I)[2,3]. Ngaione is of

[1] M. D. SUTHERLAND and R. J. PARK, in *Terpenoids in Plants* (Ed. J. B. PRIDHAM; Academic Press, London 1967), p. 147.

[2] B. F. HEGARTY, J. R. KELLY, R. J. PARK and M. D. SUTHERLAND, Aust. J. Chem. *23*, 107 (1970).

[3] F. A. DENZ and W. G. HANGER, J. Path. Bact. *81*, 91 (1961).

particular interest because it causes zonal injury in the liver of sheep which may in different animals be either strictly centrolobular or periportal in location[4] and in mice is consistently midzonal in distribution[3,5] (Figure 1). In addition, the trans isomer of ngaione, epingaione[2] as well as the enantiomorph ipomeamarone, a phytoallexin derived from spoiled sweet potatoes (*Ipomaea batatas*)[6–8] both have an LD_{50} of about 200 mg/kg by i.p. administration and produce the same liver lesion in mice[9]. The furanosesquiterpenes, myoporone[10] (II) and myodesmone (III)[11], which are also derived from various strains of the plant *Myoporum deserti*, are toxic to mice and both produce the unusual midzonal necrosis of the liver even though their structures are quite different from that of



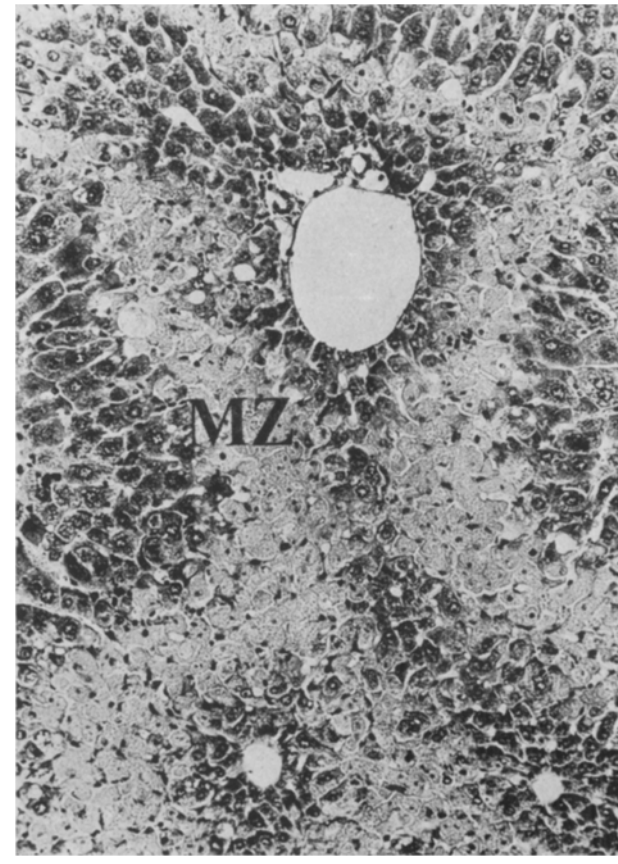


Fig. 1. Midzonal necrosis of the liver (MZ) of the mouse caused by dosing with furanosesquiterpene essential oils isolated from *Myoporum spp.* This lesion is also caused by 3-hydroxymethylfuran N, N-diethyl carbamate when administered to male mice at 60 mg/kg after pretreatment of the animals with sodium phenobarbitone.

ngaione. The structural feature common to all these molecules is the furan ring with a substituent side chain at the 3 position.

The location of the zone of injury in the liver after ngaione can be consistently varied from the midzonal to either the periportal or centrolobular locations depending on whether the animal is pretreated with either phenobarbitone or β-diethylaminoethyl phenylpropyl acetate (SKF 525A)[12,13]. These compounds enhance and depress respectively the activity of the hepatic microsomal mixed function oxidases[14]. Accordingly it was suggested that ngaione may require metabolism by these enzymes before it becomes toxic to the liver and that injury to particular hepatocytes depended on the activity of the microsomal oxidative enzymes within them[12].

In preliminary investigations of the structural features required of the side chain at the 3 position of the furan ring for the molecule to cause zonal hepatic necrosis which could be varied in location, simple analogues such as 3-hydroxymethylfuran, 3-carboxyfuran and the methyl ester of the latter were tested. These compounds administered to mice by i.p. injection at the LD_{50} level (150 to 380 mg/kg) all produced centrolobular necrosis of the liver which could not be altered to periportal by pretreatment with phenobarbitone. In an initial attempt to enlarge the substituent side chain, 3-hydroxymethylfuran N-ethyl carbamate (IV) was prepared. This compound given i.p. was much more toxic for mice and rats with $LD_{50's}$ of 32 and 15 mg/kg respectively. The main lesions were pulmonary oedema and renal tubular necrosis, liver injury being slight or entirely absent. When (IV) was dosed to mice pretreated with phenobarbitone, lung and renal injury was less severe but centrolobular liver necrosis occurred more consistently, particularly at the higher dose levels of 50 to 60 mg/kg which such pretreated animals could then tolerate. Compound (IV) is similar in its toxic effect to those of the natural furanoterpenes ipomeanine[8,15] (V) and ipomeanol[16,17] (VI)

[4] I. J. CUNNINGHAM and C. S. M. HOPKIRK, N. Z. Jl. Sci. Technol. *26*, 333 (1945).
[5] A. A. SEAWRIGHT and R. M. O'DONAHOO, J. Pathol. *106*, 251 (1972).
[6] M. HIURA, Gifu Norin Senmon Gakko Gakujutsa Hokuku, *50*, 1 (1943).
[7] H. WATANABE and I. IWATA, J. agric. Chem. Soc. *26*, 180 (1952).
[8] T. KUBOTA and T. MATSUURA, J. chem. Soc. *1958*, 3667.
[9] M. D. SUTHERLAND, Aust. J. Chem., in press (1973).
[10] J. D. BLACKBURNE, R. J. PARK and M. D. SUTHERLAND, Aust. J. Chem. *25*, 1787 (1972).
[11] I. D. BLACKBURNE, R. J. PARK and M. D. SUTHERLAND, Aust. J. Chem. *24*, 995 (1971).
[12] A. A. SEAWRIGHT and J. HRDLICKA, Br. J. exp. Path. *53*, 242 (1972).
[13] G. J. ALLEN and A. A. SEAWRIGHT, Res. vet. Sci., in press (1973).
[14] A. J. CONNEY, Pharmac. Rev. *19*, 317 (1967).
[15] B. J. WILSON and M. R. BOYD, Proc. Fifth int. Conf. Pharmac. San Francisco, USA (1972), p. 253.

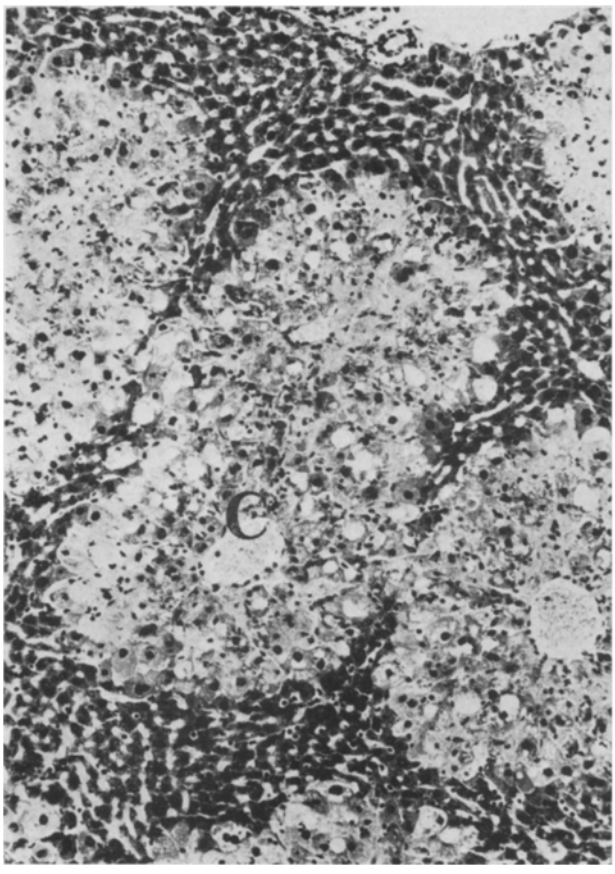


Fig. 2. Centrolobular necrosis of the liver (C) of a control mouse caused by a single i.p. dose of 3-hydroxymethylfuran, N,N-diethyl carbamate in arachis oil at 60 mg/kg.

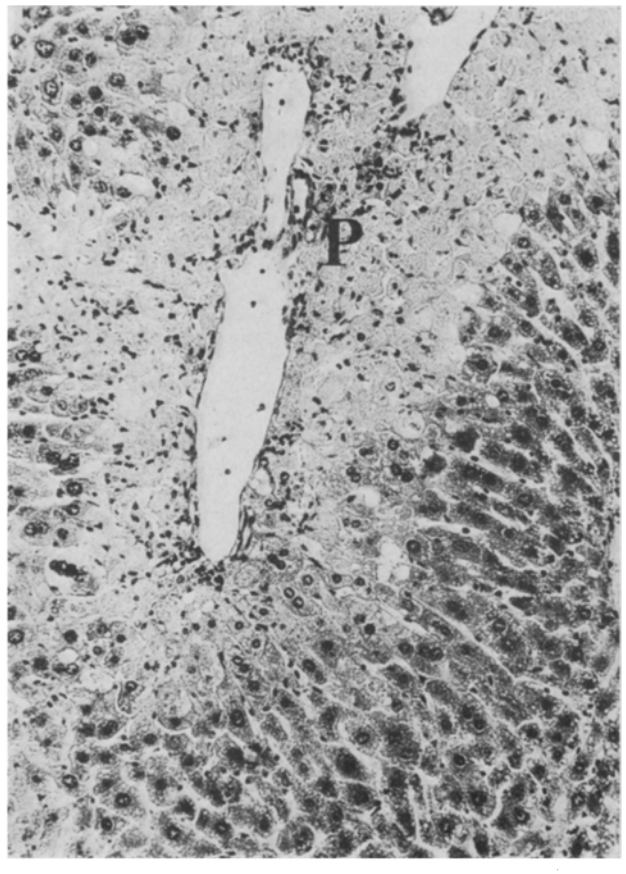


Fig. 3. Periportal necrosis of the liver (P) of a mouse caused by a single i.p. dose of 3-hydroxymethylfuran N,N-diethyl carbamate in arachis oil at 60 mg/kg after pretreatment with sodium phenobarbitone.

which together with ipomeamarone are believed to be responsible for disease in cattle characterised by pulmonary lesions following consumption of spoiled sweet potato tubers[18, 19].

Another synthetic analogue, 3-hydroxymethylfuran N,N-diethyl carbamate (VII) was found to be acutely hepatotoxic for mice with an LD_{50} of 60 mg/kg, causing centrolobular necrosis (Figure 2). Higher doses (about 100 mg/kg) killed more rapidly with acute severe pulmonary oedema as for (IV). Pretreatment of mice with phenobarbitone does not reduce the toxicity of (VII) for the animals but as in the case of the furanosesquiterpenes, causes the zone of injury in the liver to be transferred to the periportal hepatocytes (Figure 3).

Carbon disulphide (1 ml/kg orally) inhibits microsomal mixed function oxidation in the liver of the fasted normal rat for up to 3 days by destruction of the terminal oxidase of the enzyme system, cytochrome P-450[20]. We have shown that carbon disulphide has a similar effect on the liver of the mouse[21]. Pretreatment of animals with carbon disulphide reduces the hepatotoxic action of carbon tetrachloride given 6 h later[22], presumably through this inhibition of the microsomal oxidative metabolism of the latter compound[23, 24]. When compounds (IV) and (VII) are given to mice pretreated with carbon disulphide to inhibit the hepatic microsomal mixed function oxidases, toxic liver injury after (VII), but not (IV) is markedly reduced, although lung and renal lesions occur at higher doses.

The above findings suggest that hepatic microsomal oxidative metabolism has a role to play in the mechanism of the hepatotoxicity and possibly extrahepatic lesions in the animal caused by (VII) and other similarly toxic furans. The common structural feature present in this group of toxic furans is the furan ring substituted in the 3 position. The pathological changes produced in animals dosed with the compounds depend on the structure of the side chain. The 2 synthetic compounds described, (IV) and (VII), are simply prepared compounds which

[16] B. J. Wilson, M. R. Boyd, T. M. Harris and D. T. C. Yang, Nature, Lond. *231*, 52 (1971).
[17] M. R. Boyd, B. J. Wilson and T. M. Harris, Nature, Lond. *236*, 158 (1972).
[18] W. Monlux, J. Fitte, G. Kendrick and H. Dubuisson, SWest. Vet. *6*, 267 (1953).
[19] J. C. Peckham, F. E. Mitchell, O. H. Jones and B. Doupink, J. Am. vet. med. Ass. *160*, 169 (1972).
[20] E. J. Bond and F. De Matteis, Biochem. Pharmac. *18*, 2531 (1969).
[21] A. A. Seawright and M. D. Stonard, unpublished observations.
[22] A. A. Seawright, L. J. Filippich and D. P. Steele, Aust. vet. J. *48*, 38 (1972).
[23] A. A. Seawright, L. J. Filippich and D. P. Steele, Res. vet. Sci. in press (1973).
[24] P. Lange, D. Kästner and F. Jung, Acta biol. med. germ. *24*, K29 (1970).

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

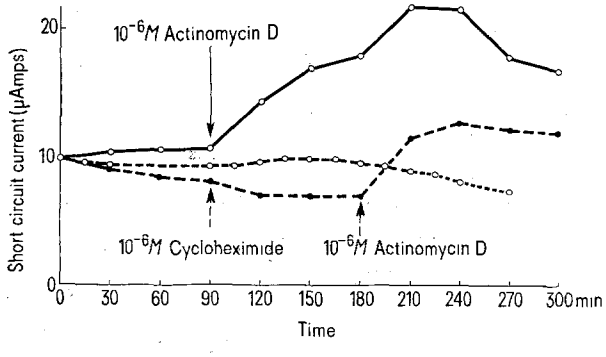


Fig. 1. Increased short circuit current across the isolated toad bladder stimulated by 10^{-6} M actinomycin D, additions as indicated, in the presence of 10^{-9} M aldosterone added at time zero to the serosal surface of both sections of bladder. The results represent the mean of 2 experiments.

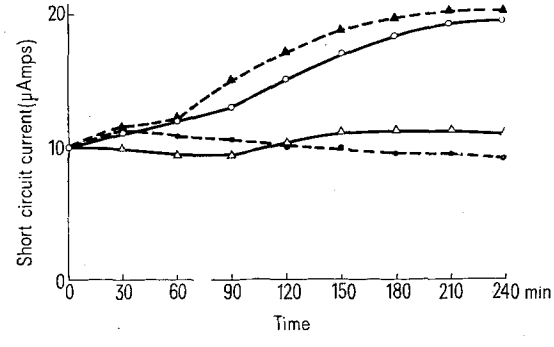


Fig. 2. Short circuit current across the isolated toad bladder stimulated by 10^{-7} M aldosterone in the absence (–○—○–) and presence of 10^{-6} M actinomycin D added 10 min after (--▲--▲--), 5 min after (–△—△–), and 10 min before (--●--●--) the hormone. The results represent the mean of 8, 4, 2 and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

1 R. S. SNART, J. Steroid Biochem. *3*, 129 (1972).
2 E. KIRSTEN, R. KIRSTEN, A. LEAF and G. W. G. SHARP, Pflügers Arch. ges. Physiol. *300*, 213 (1968).
3 T. DALTON and R. S. SNART, J. Endocr. *47*, 159 (1970).
4 R. S. SNART, Biochim. biophys. Acta *135*, 1056 (1967).
5 R. S. SNART, R. E. SHEPHERD and M. K. AGARWAL, Hormones *3*, 293 (1972).
6 G. W. G. SHARP and A. LEAF, Proc. natn. Acad. Sci. *52*, 1114 (1964).