onset of arrhythmia, ventricular fibrillation and cardiac arrest provoked by a slow infusion of ouabain (8 μg/kg every 90 sec).

In rabbits, the compound reduces the tachycardic effect of prolonged thyroxine treatment (0.2 mg/day/rabbit for 10 days).

The pressor activity of adrenalin and of occlusion of the carotids in anaesthetized rabbits is not modified by the compound just as the hypotensive action of acetylcholine remains unchanged. By contrast, the hypotensive effect of the stimulation of the peripheral stump of the vagus is reduced.

In anaesthetized rabbits, DA 1686 provokes bradycardia, widening of the QRS and mild hypotension. The effect on the cardiac frequency is greater than that exerted by quinidine and procainamide, while the effect on the QRS and pressure is minor.

The compound is well absorbed after both oral and intramuscular administration in rats and rabbits, producing higher blood levels than those of quinidine and procainamide administered at equal doses. In rats and rabbits, the compound is eliminated in the non-metabolized state to the extent of 50–60% during the first 24 h after treatment and 15–20% in the following 24 h.

Riassunto. Il DA 1686, corrispondente al 1,5-dimorfolino-3-(1-naftil) pentano, è un derivato della naftalina ad attività antiaritmica come risulta dalle prove in vitro (orecchiette isolate di cavia) e in vivo (cani, conigli, cavie e ratti). Il composto è ben assorbito dopo somministrazione orale e viene escreto nelle urine non metabolizzato.

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Novobiocin and the Binding of Magnesium Ions

It has been shown by WEINBERG[1] and BROCK[2] that Mg^{++} ions overcome the antibacterial activity of novobiocin against Gram-negative, but not Gram-positive, bacteria. In addition, evidence has been presented[2] to demonstrate that the antibiotic and cation form a complex, although more recent studies[3] may suggest otherwise. This problem has also been under study in our laboratories.

Novobiocin monosodium was a gift from Merck, Sharpe and Dohme Ltd., Hoddesdon (Herts, England). Magnesium chloride, $MgCl_2 \cdot 6H_2O$, and sodium chloride were of 'Analar' grade. The water used was obtained from an ion-exchange column, and had a specific conductance of 10^{-6} ohm^{-1} cm^{-1}. The spectra of novobiocin alone and in the presence of magnesium chloride were recorded over the range 200–450 nm in a Unicam SP 800 spectrophotometer, using 1 cm cells (Table I). It can be seen from the results that there is no evidence for the formation of a complex between novobiocin and Mg^{++}.

BROCK[2] carried out difference spectra with strong ($10^{-2}M$) solutions of novobiocin sodium and magnesium chloride, and obtained a peak at 360 nm; he therefore concluded that a complex was formed at high concentrations. Our findings with difference spectra (Table II), which are quite different in shape from the ordinary spectrum of novobiocin, suggest that the peak obtained by BROCK[2] was only an apparent, and not a true one. The results presented in Table II show that when solutions of novobiocin sodium of different concentrations are placed in the sample and reference cells, the peak obtained for the difference spectrum is displaced to longer wavelengths; the greater the concentrations, the greater is the shift. This shift is an apparent one, because when the spectrum of an $8 \cdot 10^{-4}M$ solution of novobiocin sodium (which is higher than the concentrations used in some of the difference spectra) was recorded against water, using 1 mm cells, the λ_{max} was still at 308 nm. Thus, the peaks obtained by difference spectra are false, since the spectrophotometer is insensitive to absorbtion differences between the sample and reference solutions when both are absorbing very strongly. In the experiments carried out by BROCK[2], a mixture of $10^{-2}M$ novobiocin sodium and $10^{-2}M$ magnesium chloride was placed in the sample cell and a $10^{-2}M$ solution of novobiocin sodium in the

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Table I. Absorption spectra of novobiocin sodium in presence and absence of magnesium chloride

Sample solution		Reference solution	λ^1_{max}	Absorbance at λ^1_{max}	λ^2_{max}	Absorbance at λ^2_{max}
Concentration of						
Novobiocin sodium	$MgCl_2$					
$8 \cdot 10^{-4}M$	absent	water	308	1.60	240	1.81
$8 \cdot 10^{-4}M$	$8 \cdot 10^{-4}M$	$8 \cdot 10^{-4}M$ $MgCl_2$	308	1.59	240	1.78
$8 \cdot 10^{-4}M$	$8 \cdot 10^{-2}M$	$8 \cdot 10^{-2}M$ $MgCl_2$	308	1.61	240	1.80

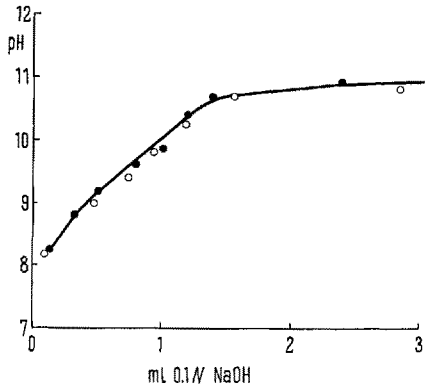
1 cm cells were used in all experiments.

reference cell; a peak was obtained having a λ_{max} at 360 nm. In similar experiments, with slightly less concentrated solutions, we have obtained a small peak with a λ_{max} at 346 nm (see Table II); however, when the experiments were repeated with an equal ionic strength solution of sodium chloride instead of magnesium chloride, the same small peak having a λ_{max} at 346 nm was obtained, which is further evidence that no complex formation is taking place between novobiocin and Mg^{++} ions.

Table II. Difference spectra

Sample solution Concentration of novobiocin sodium	Reference solution Concentration of novobiocin sodium	λ_{max}
$8 \cdot 10^{-4} M$ [a]	absent (water)	308
$5 \cdot 10^{-4} M$	$1 \cdot 10^{-4} M$	323
$1 \cdot 10^{-3} M$	$6 \cdot 10^{-4} M$	333
$1 \cdot 10^{-2} M$	$9.6 \cdot 10^{-3} M$	351
$5 \cdot 10^{-3} M + 5 \cdot 10^{-3} M$ $MgCl_2$	$5 \cdot 10^{-3} M$	346
$5 \cdot 10^{-3} M + 1.5 \cdot 10^{-2} M$ NaCl	$5 \cdot 10^{-3} M$	346

[a] 1 mm cells used here; 1 cm cells in the other experiments.



Potentiometric titration curves for 20 ml of a solution of a mixture $5 \cdot 10^{-3} M$ novobiocin sodium and $5 \cdot 10^{-3} M$ magnesium chloride (●); summation curve (see text) (o).

In addition, potentiometric titration curves have been recorded, using 0.1 N NaOH as titrant, for 20 ml solutions of (a) $5 \cdot 10^{-3} M$ novobiocin sodium, (b) $5 \cdot 10^{-3} M$ magnesium chloride, and (c) a mixture of $5 \cdot 10^{-3} M$ novobiocin sodium and $5 \cdot 10^{-3} M$ magnesium chloride. It was found that on summation of the titration curves (a) and (b), an identical curve was obtained to that obtained with the mixture (c) (Figure). This finding confirms the results recently reported by NIEBERGALL et al.[3].

Our results are not intended to throw light on the exact mode of action of novobiocin. Whether the primary effect of the antibiotic is on the bacterial cytoplasmic membrane[4] or on deoxyribonucleic acid synthesis[5] is not yet known. However, the results presented here demonstrate clearly that novobiocin and Mg^{++} do not form a complex; it is thus extremely unlikely that novobiocin acts by inducing an intracellular deficiency of Mg^{++}.

Résumé. La novobiocine et les ions de magnésium ne forment pas un «complexe». De ce fait, il est très peu probable qu'elle occasionne une déficience des ions de magnésium dans la cellule bactérienne.

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Fluorescence of Blood Cells of the Tunicates *Phallusia mamillata* and *Ciona intestinalis*[1]

The ascidiaceae *Phallusia mamillata* Cuvier (family Ascidiidae) and *Ciona intestinalis* L. (family Cionidae) possess different types of blood cells[2–6]. Most of these cells are vanadocytes, that contain in electronmicroscopically dense areas (vanadophores[5]) vanadium of low valency (reduction of osmic acid) and an acid (indicator reaction with methyl red), first observed with *Ph. mamillata*[7] and later with *C. intestinalis*[8]. The presence of disulfatovanadium(III) acid and of a yellow acid reductone-like substance, belonging to the hemovanadin system, has been proved in the hemolysate of the vanadocytes of *Ph. mamillata*[9]. A second cell type of both species are vacuolized compartment cells, that in the case of *Ph. mamillata* have been considered as direct precursors of the vanadocytes[4]. Further blood cells, which have also

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