Table 2. Observed and Calculated Mean Membrane Potentials of Frog Sartorii before and after Extrusion of Sodium into Chloride-free Recovery Fluids

	Sucrose–Ringer		Sulphate–Ringer	
	Observed	Calculated	Observed	Calculated
Before extrusion	75·5 ± 0·7	60·1 ± 1·1	68·9 ± 0·6	57·5 ± 0·6
After extrusion	—	—	70·0 ± 0·5	67·1 ± 0·5

The marked difference between the observed potential and the potassium-equilibrium potential here, even in the absence of external chloride, suggests that the sodium-pump is not neutral, but that its operation, particularly during the considerable extrusion of sodium taking place here, makes the interior of the fibre more negative with respect to the exterior, thereby causing potassium to move into the fibres to restore conditions of electrical neutrality within the fibres. Even when chloride should be moving out of the fibres along with sodium, as in the chloride-free recovery fluids, the exit of sodium can still result in a marked increase in membrane potential, suggesting that chloride does not contribute much to the observed potential under these conditions, but moves passively like the potassium to equilibrium at the end of the period of extrusion of sodium.

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PHARMACOLOGY

Fusidic Acid: a New Antibiotic

From the fermentation broth of a strain of *Fusidium* a hitherto unrecorded antibiotic, for which the name 'fusidic acid' is proposed, has been isolated.

The organism was cultured in deep culture fermentors at 24° C. and *p*H 6·5–7·5 in a complete medium containing sucrose and corn steep liquor, maximum production being achieved in about 120 hr. The activity was determined by the agar cup-plate method using *Staphylococcus aureus* as test organism.

The antibiotic was extracted from the clarified broth at *p*H 5 with methyl isobutyl ketone and concentrated further on extraction of the organic phase with aqueous sodium hydroxide at *p*H 11. From the concentrated aqueous solution thus obtained, fusidic acid was precipitated as a crystalline benzene solvate on acidification in the presence of benzene. Pure solvent-free fusidic acid (m.p. 192°–93° C., $[\alpha]_D^{20} - 9°$ (all rotations in chloroform)) separated from an ethereal solution of the recrystallized benzene solvate on standing. The acid is sparingly soluble in water, ether, and hexane, but soluble in alcohols, acetone, chloroform, and pyridine. The soldium salt is readily soluble in water.

Fusidic acid (I) is a carboxylic acid, which contains carbon, hydrogen, and oxygen only. The elementary analysis and the equivalent weight, obtained by electrometric titration in 50 per cent (v/v) ethanol, agree well with the formula $C_{31}H_{48}O_6$ (found: C, 72·03; H, 9·36 per cent; equiv. weight 518 ± 5. $C_{31}H_{48}O_6$ requires: C, 72·06; H, 9·36 per cent; M = 516·7). The *p*K-value found by titration is 6·35, corresponding to a *p*K of approximately 5·35 in water. A non-acidic monomethyl ester (II), $C_{32}H_{50}O_6$ (m.p. 153°–54° C., $[\alpha]_D^{20} - 14°$), is obtained on methylation with diazomethane. Fusidic acid contains one acetoxy group, and two hydroxy groups, one of which is readily acetylated. It can therefore be formulated:

$$C_{28}H_{42}\begin{cases} COOH \\ OCOCH_3 \\ OH \\ OH \end{cases}$$

Catalytical hydrogenation of (I) over palladium-on-charcoal in ethanol yielded dihydrofusidic acid (III), $C_{31}H_{50}O_6$, H_2O (m.p. 182°–83° C., $[\alpha]_D^{20}$ 0°), while hydrogenation over platinum oxide in glacial acetic acid afforded tetrahydrofusidic acid (IV), $C_{31}H_{52}O_6$ (m.p. 172°–73° C., $[\alpha]_D^{20} - 64°$). The difference between the ultra-violet spectra of (I) and (III) gives λ(EtOH, max.) 204 mμ (ε 4,200) for the chromophore first hydrogenated and shows that this is a trisubstituted isolated double bond. The second chromophore, lost in going from (III) to (IV) λ(EtOH, max.) 220 mμ (ε 8,300)), is characteristic of an α,β-unsaturated acid having not more than one hydrogen atom at the double bond. Since (IV) gave no colour with tetranitromethane, (I) must contain a tetracyclic ring-system. Further structural work, the results of which will be reported elsewhere, is in progress.

Table 1. Antimicrobial Spectrum of Fusidic Acid

Organism	Concentration required for 50 per cent inhibition (μgm./ml.)
Staph. aureus, penicillin-sensitive (6 strains)	0·04–0·1
Staph. aureus, penicillin-resistant (15 strains)	0·05–0·2
Str. pyogenes (2 strains)	4–20
D. pneumoniae (6 strains)	5–20
N. gonorrhoeae (3 strains)	0·4–0·8
N. meningitidis	0·6
C. diphtheriae (2 strains)	0·01–0·02
B. subtilis	0·6
Clostridium tetani	0·02
E. coli	> 100
K. pneumoniae	100
Sal. typhimurium	> 100
Sh. dysenteriae	> 100
P. vulgaris	> 100
Ps. aeruginosa	> 100
Myco. tuberculosis v. *hum.*	0·8
C. albicans	> 100
Asp. fumigatus	> 100
T. mentagrophytes	> 100

The sodium salt of fusidic acid was tested against a number of micro organisms by a serial dilution method. The concentrations which cause 50 per cent inhibition are given in Table 1.

Fusidic acid is non-toxic. The subcutaneous and oral LD_{50} in mice were found to be 1·2 gm. and 1·5 gm. per kgm. body-weight, respectively. The intravenous LD_{50} of the sodium salt was 0·2 gm. per kgm. body-weight.

Daily oral administration of fusidic acid to rats in doses of 0·4 gm. per kgm. body-weight over a period of 6 months was well tolerated. Post-mortem examination revealed no pathological changes.

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