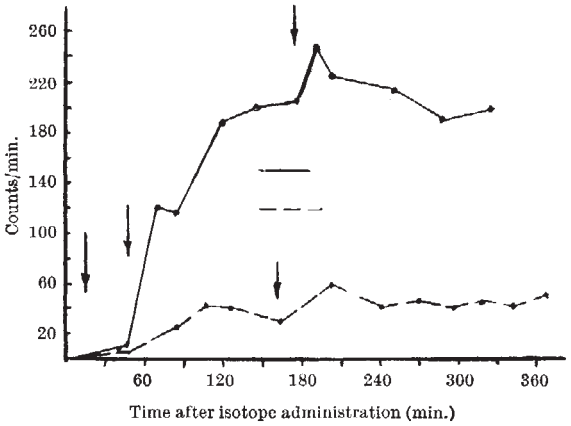

Fig. 2. Amphetamine and amylobarbitone sodium experiment on goat No. 18. 900 μc. phosphorus-32 administered into the teat. ——, Re-absorption after treatment with amphetamine; – – –, re-absorption after treatment with amylobarbitone sodium. The first arrows indicate the moment of isotope administration; the second arrows, massage of the udder

Adrenocorticotrophin is known to inhibit milk synthesis in the udder[5]. It was found that when injected intravenously into blood or directly into the udder adrenocorticotrophin also inhibits the re-absorption from the mammary gland of labelled sulphur and phosphorus.

It is also of interest to note that 10 mgm. ergotoxin may suffice to inhibit the re-absorption processes in the udder irrespective of the mode of its administration.

(4) Further experiments showed that not only does re-absorption accompany milk secretion but also the secretory process itself requires the re-absorption of milk constituents from the udder into the blood. The secretory process in the gland cannot proceed if the alveoli are completely freed of milk. If indeed the isotope is administered into the udder under conditions of maximum milk evacuation obtained by careful milking and injection of posterior-pituitary extract, re-absorption of the isotope from the udder is greatly retarded and so is milk secretion. In other words, a decrease in the re-absorption-rate is accompanied by a disturbance of the secretory process.

The residual milk in the udder is indispensable for the synthesis of additional milk in the intervals between milkings.

A complete analysis of the secretory process in the mammary gland should obviously take into account the re-absorption phenomena which are in some way necessary for the normal functioning of this organ. The mammary gland seems to require continuous stimulation, and it is possible that the continual transfer of milk constituents from the udder into the blood plays the part of such a stimulus.

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Membrane Potential Changes during Sodium Transport in Frog Sartorius Muscle

The concept of a sodium–potassium linked electrically neutral pump was first put forward by Hodgkin[1] for muscle and nerve. He suggested that a potassium ion might be transported into the cell for each sodium ion actively extruded, leading to an electrical neutrality with respect to the operation of the sodium-pump. This idea has been tested in the following experiments.

The membrane potentials of frog sartorii made rich in sodium by overnight soaking in cold potassium-free Ringer–Conway fluid (with 120 m.equiv. sodium/l.) were measured by the micro-electrode technique of Graham and Gerard[2] immediately on re-immersion, and with companion muscles similarly treated after 3 hr. re-immersion in recovery fluids containing 10 m.equiv. potassium/l.

In the first series of experiments the re-immersion fluid contained choline chloride replacing all the sodium salt of the Ringer fluid, and the 10 m.equiv. potassium was in the form of bicarbonate and phosphate buffer. In this immersion fluid loss of sodium from the fibres by diffusion or by the sodium-pump is greatly facilitated. In another series of experiments the sodium-rich companion muscles were re-immersed in Ringer–Conway fluid containing 104 m.equiv. sodium and 10 m.equiv. potassium per litre, under which conditions active extrusion of sodium has already been demonstrated[3–5].

After the potential measurements the muscles were analysed for potassium by the flame photometer following incineration in boiling concentrated nitric acid, and the potassium concentration in the muscle fibre water calculated. From this value the potassium equilibrium potential was calculated from the Nernst equation. The mean observed and calculated potentials are shown in Table 1.

Table 1. Observed and Calculated Membrane Potentials of Sodium-rich Frog Sartorii before and after Extrusion of Sodium into Recovery Fluids

	Mean membrane potentials (mV.)			
	In choline chloride		120/104 sodium procedure	
	Observed	Calculated	Observed	Calculated
Before extrusion	83·8 ± 0·6	50·1 ± 2·6	63·4 ± 0·7	52·3 ± 0·9
After extrusion	60·5 ± 0·6	58·0 ± 2·1	56·0 ± 1·1	58·0 ± 0·6

It is evident from Table 1 that the observed potential is greater than the potassium equilibrium potential while the marked extrusion of sodium is taking place, and that both potentials are in good agreement within the range of experimental error when extrusion declines after 3 hr. That this increased potential is associated with sodium movement rather than with chloride movement was shown by re-immersing the sodium-rich muscle in chloride-free recovery fluid, and measuring the membrane potentials under these conditions. In one case the sodium and chloride of the Ringer fluid was replaced by sucrose, providing good conditions for loss of sodium from the fibres. In another case where sodium extrusion against a concentration gradient was to be examined the chloride of the Ringer–Conway fluid was replaced by sulphate and the sodium concentration was reduced to 90 m.equiv./l. to give isotonicity and the same ionic strength[6]. Both recovery fluids contained 10 m.equiv. potassium as bicarbonate and phosphate. The observed and calculated mean membrane potentials in chloride-free recovery fluid are shown in Table 2.

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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