LETTERS TO THE EDITORS

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Mucolytic Activity of Diffusing Factor Preparations

Chain and Duthie[1] have observed that aqueous testicular extracts contain a mucinase which rapidly reduces the viscosity of synovial fluid and causes an increase in the reducing power of this material. They suggested that the diffusing factor in testicular extracts might be identical with this mucinase and that the spreading effect in the skin might be due to rapid hydrolysis of interfibrillar mucin. This at first sight attractive hypothesis has been accepted by several workers[2,3,4], although no evidence has been advanced of the identity of the two effects, other than the known fact that snake venoms and bacterial filtrates, both well-known sources of diffusing factors, also show mucolytic activity.

Recent improvements in the biological test for diffusing factors[5] and an accurate viscosimetric method for the assay of mucolytic activity[6] have made it possible to compare the degree of activity shown by the various diffusing factor preparations when assayed by both methods. Dry preparations from bull testicle and rattlesnake venom were used and some results are given in the accompanying table.

Preparation	Diffusing potency (% standard)	Mucolytic activity (% standard)
Glaxo Laboratories standard (testicle)[5]	100	100
Madinaveitia standard (testicle)[7] ..	68	24·6
Dialysed extract (testicle)	48	80
$(NH_4)_2SO_4$ purified extract (testicle)	58	149
Crude *Crotalus atrox* venom ..	5·35	18·4
Purified *Crotalus atrox* mucinase ..	4·9	61·1

While these results are only of a preliminary nature we feel that they are sufficiently clear to warrant publication at this stage. It seems that there is no parallel between diffusing factor potency and mucolytic activity in the materials examined. Moreover, Meyer and his colleagues have recently encountered a similar absence of correlation[8]. While it cannot be said that diffusing factors are therefore not in some way related to mucinases, it seems certain that the method of assaying diffusing factors by merely measuring their mucolytic activity is open to grave objection. The possibility of a complete separation of diffusing factor from mucinase is the subject of investigations now in progress.

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[1] Chain and Duthie, NATURE, **144**, 977 (1939).
[2] Meyer and Chaffee, *Proc. Soc. Exp. Biol. and Med.*, **43**, 487 (1940).
[3] Favilli, NATURE, **145**, 866 (1940).
[4] McClean and Hale, NATURE, **145**, 866 (1940); *Chem. and Ind.*, **59**, 347 (1940).
[5] Bacharach, Chance and Middleton, *Chem. and Ind.*, **59**, 348 (1940).
[6] Madinaveitia and Quibell. *Biochem. J.*, **34**, 625 (1940).
[7] Madinaveitia, *Biochem. J.*, **32**, 1806 (1938).
[8] Meyer, Hobby, Chaffee and Dawson, *Proc. Soc. Exp. Biol. and Med.* **44**, 294 (1940).

Concentration of Cations at Negatively Charged Surfaces

When ions of one sign are fixed at a surface, the concentration of ions of the opposite sign in the surface phase may be calculated from the Gibbs-Donnan equilibrium between the surface and bulk phases[1], or from the electrokinetic potential. By both methods it is predicted that the pH at a surface containing fixed anions will be less than in the bulk phase, and Hartley and Roe have shown[2] that the pH at the surface of cetyl sulphate micelles, as calculated from the ζ potential (circles of accompanying figure) is in reasonable agreement with the values observed using a surface colorimetric indicator (crosses of accompanying figure). We have now calculated the pH at the same surface from the Gibbs-Donnan equilibrium (continuous line of accompanying figure) and find values in reasonable agreement with those obtained by the other two methods. The thickness of the surface phase was taken as that given by the theory of Debye and Hückel.
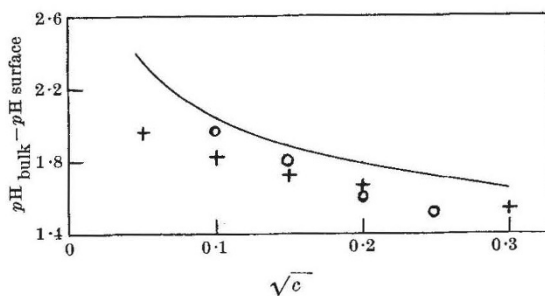
Wilbrandt has suggested[3] that the Gibbs-Donnan equilibrium can also be used to calculate the distribution of Na^+ and Ca^{++} between a surface and a bulk phase. We have studied this by chemical analysis of palmitate monolayers spread on solutions containing varying concentrations of Na^+ and Ca^{++}. As predicted by the Gibbs-Donnan relationship, there is a lower $Na : Ca$ ratio in the surface than in bulk, but the ratio found is much less than the predicted value (see accompanying table), which suggests that Ca^{++} may be bound at the interface by other forces in addition to that of simple electrostatic attraction.

Molar ratio $Na : Ca$ in monolayers of palmitate spread on 0·1 N saline.

Ratio in saline	100 : 1	200 : 1	400 : 1	2000 : 1
Ratio found in monolayer	0·13 : 1	0·18 : 1	0·27 : 1	0·31 : 1
Ratio calculated from Gibbs-Donnan equation	4·5 : 1	8·2 : 1	15·5 : 1	109 : 1

The negatively charged surfaces act as reservoirs of divalent ions, and the discharge or dissolution of these surfaces will release a disproportionate amount