had the opposite effect on its sensitivity to BuCh. It is also possible that the guinea-pig ileum preparation was not set up as we have done; the authors' reference to Chang and Gaddum[5] for information on this point is unhelpful because these authors did not use the guinea-pig ileum.

Our present results do not stand in isolation. They agree with our own earlier experience that ACh can be distinguished quite readily from either propionyl- or butyryl-choline and are in line with what Chang and Gaddum reported. Moreover, contrary to what Hosein and Koh have claimed, there is no discrepancy between the results of Dale and Dudley[6] and the later observations of Banister *et al.* Dale and Dudley only examined the identity of the ester ACh in horse spleen; although they go so far as to say that there was a "similarly acting unstable principle in the spleen of the ox" they did not attempt to characterize it further. This is very important because horse spleen, unlike ox spleen, only contains ACh (ref. 7); so the fact that their parallel assay only revealed ACh does not constitute disagreement with the findings of Banister *et al.*[4], who detected both acetyl- and propionyl-choline in the ox spleen.

We conclude that parallel assay still offers a valid first approach to the identification of cholinomimetic substances.

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Freeze Dried Cellulose Acetate Membranes with 1000 Å Pores

CELLULOSE acetate has been found to be the most suitable material for casting porous membranes from solvent mixtures for use in reverse osmosis[1]. The aim of the present investigation was to examine a simple alternative method of producing micropores, which might be expected to traverse thin sections of cellulose acetate. Freeze drying its solutions offers such an opportunity, because removal of the solvent will not cause the polymer to collapse. The porosity of the product decreases as the concentration of the solution increases, and the strongest possible solutions were therefore investigated.

Most solvents with freezing points in a region potentially useful for freeze drying have a vapour pressure well below 1 mm at this temperature, and therefore sublime only slowly. Apart from water, benzene is the only other solvent that has been used for freeze drying; Lewis and Mayo[2] found that polymers dissolved in benzene could be rapidly freeze dried to constant weight, and therefore considerably improved the analytical separation of these polymers from solvents and monomers. Vinogradov and Titkova[3] obtained polymers with very large specific surfaces by freeze drying from benzene.

p-Dioxane has a vapour pressure of 21 mm of mercury at its freezing point of 12° C. Because it dissolves large quantities of cellulose acetate, solutions were prepared by leaving mixtures of cellulose acetate and *p*-dioxane (dried by passing through a molecular sieve) in sealed test-tubes, with occasional shaking, at 60° C until uniform clear solutions were obtained. (For preparing solutions containing more than 25 per cent cellulose acetate by weight, it was found quicker to use highly porous cellulose acetate that had previously been freeze dried from a 10 per cent solution in dioxane.) The solutions were then frozen at −10° C before opening the tubes in a cold room to avoid the formation of surface films. Solid pieces were put inside a glass ring on a glass plate and resealed in an atmosphere saturated with dioxane. On warming to 60° C the solution spread evenly and adhered to the inside of the ring; after refreezing and opening, the glass ring was turned over, so that both surfaces could be simultaneously freeze dried in a vessel which was evacuated to 10^{-2} mm, while collecting the dioxane in a trap at −80° C.

Solutions containing up to 27 per cent cellulose acetate by weight could be rapidly freeze dried at 0° C. Below 10 per cent, many pores could be seen in the product, while 20 per cent cellulose acetate solutions gave visually uniform white products. 27 per cent solutions freeze-dried to thin, strong, white membranes with a specific gravity of 0·91. Because cellulose acetate has a specific gravity of 1·33, 32 per cent of the volume consisted of pores. After several hours in benzene, the membranes became transparent and then had a density corresponding with that of solid cellulose acetate, suggesting that continuous pores traverse the polymer. Electron micrographs of cuts along the direction of freeze drying confirmed that pores predominated in one direction, while cuts at right angles to the direction of freeze drying showed fewer randomly oriented pores. The diameter of these pores was approximately 1000 Å, which is too large to make them useful in reverse osmosis.

More concentrated solutions of cellulose acetate in dioxane were too viscous to produce thin membranes by the technique described, and were therefore freeze dried directly in the tube in which they were made. Softening temperatures of cellulose acetate in dioxane of 12, 9, 5, −1 and −15° C were obtained for weight per cent mixtures of 0, 10, 20, 30 and 40 per cent respectively.

A 31 per cent solution could not therefore be freeze dried at 0° C, but even fairly thick sections were satisfactorily prepared by freeze drying at −20° C. The product had a specific gravity of 1·01, corresponding to a porosity of 24 per cent. Approximately a day was needed before benzene completely penetrated the pores, the specific gravity then being 1·33, equal to that of solid cellulose acetate. Electron micrographs did not distinguish between the directions of cutting and showed randomly orientated pores of diameter about 1000 Å. This suggests that with highly concentrated solutions of cellulose acetate, the present freeze drying technique decreases the number of pores, but not their diameter, at increased specific gravities.

35 per cent solutions of cellulose acetate in dioxane could not be satisfactorily freeze dried in several hours at −20° C. The rate of freeze drying is greatly dependent on the procedure used for cooling the solutions. Rapid cooling in liquid nitrogen produces glasses, which even at 20 per cent cellulose acetate did not dry satisfactorily. It is possible that thin sections of highly concentrated frozen solutions, made in a microtome cryostat under optimum cooling conditions, may dry to give smaller pores than found here.

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