A further application of the open-ended capillary method for measuring diffusion coefficients

SIR,—At the 1966 British Pharmaceutical Conference we described two methods for studying the tracer diffusion of sodium-22 in protein solutions and phospholipid sols, which were based on the open-ended capillary method of Anderson and Saddington and on the continuous monitoring method of Mills (Castleden & Fleming, 1966). These techniques suffered from the limitation that it was necessary to use an isotope with radiation sufficiently strong to penetrate the walls of the capillary and the metal surrounding the sodium iodide crystal of the scintillation counter. Preliminary experiments, in which the fluid surrounding the capillary has been analysed for radioactivity, have shown that this modification makes the above methods more versatile; for instance, it is possible to use tritium or carbon-14 labelled substances (or a mixture of both).

Using the Anderson and Saddington technique, we set the apparatus up as before, except that only one capillary was immersed in the inactive bulk solution. At various times, t, the activity of the bulk solution, C, was estimated by withdrawing 0·5 ml samples and assaying them for radioactivity using a liquid scintillation counter (Packard 3015). At the end of the experiment the contents of the capillary were added to the bulk solution, and after thorough mixing, its activity was determined and used to calculate the initial activity, C_0, at time $t = 0$. The relation between C, C_0, and C_t, which is the activity remaining in the capillary at time, t, is given by the expression: $C_t = C_0 - C$. Log C_t is linearly related to t, and the diffusion coefficient, D, was calculated as described previously. It was found that the accuracy was improved by weighing the samples into the counting vials, and this was done with the alanine experiment.

Table 1 shows values obtained for the diffusion coefficients of sodium-22 in sodium chloride solution, and of carbon-14 labelled alanine in alanine solution. From the results we conclude that a suitable method based on the above technique can be developed for studying the diffusion of compounds labelled with weak β-emitter isotopes.

TABLE 1. DIFFUSION COEFFICIENTS OF SODIUM-22 IN SODIUM CHLORIDE AND OF ALANINE IN ALANINE SOLUTIONS

Experiment	Compound	Concentration	$Dx10^5$ cm^2 sec^{-1}
1	NaCl	0·1M	$1{\cdot}27_0$
2	NaCl	0·1M	$1{\cdot}31_8$
Mills & Godbole (1958)	NaCl	0·1M	$1{\cdot}277_9$
3	Alanine	1%	$0{\cdot}476_5$
Longsworth (1952)*	DL-β-alanine	0·618%	0·45
,, ,,	DL-α-alanine	0·624%	0·4317

* As there are no values for the self-diffusion coefficient of alanine in the literature, we quote the diffusion coefficient determined by Longsworth (1952) to indicate the order of magnitude to be expected.

The School of Pharmacy,
University of London,
29–39 Brunswick Square,
London, W.C.1, England.

JENNIFER A. CASTLEDEN
R. FLEMING

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Tolerance experiments with barbitone-dependent rats

SIR,—Recently Ungar & Cohen (1966) found that morphine tolerance could be transferred to mice by the injection of a brain extract prepared from morphine-tolerant rats or dogs. It has also been reported that the respiration of brain slices removed from morphine-tolerant rats is refractory to the depressant effect of morphine (Takemori, 1961; 1962). Adopting experimental procedures similar to those of the above authors we have looked at barbiturate tolerance in experiments with barbitone-dependent and barbitone-withdrawn rats.

Female Wistar rats, about 50 g at the beginning of the experiment, were made dependent on barbitone by the administration of up to 400 mg/kg/day barbitone sodium in the drinking water over a five week period. Withdrawal was effected by replacing barbitone solution by drinking water. Control animals received drinking water throughout. Animals were killed after five weeks of barbitone treatment or 48 hr after withdrawal.

Groups of mice were injected intraperitoneally with a whole brain homogenate (0·5 ml/25 g) prepared from barbitone-dependent, withdrawn or control rats. Each ml of homogenate contained 400 mg of brain in normal saline. Either 3 or 24 hr after administration of the homogenate, the sleeping time after intraperitoneal injection of 100 mg/kg hexobarbitone sodium was measured. At the same time hexobarbitone metabolism *in vitro* was assayed on liver microsomal preparations from other groups of similarly treated mice. The method used involved the determination of the metabolite formed from ^{3}H-labelled hexobarbitone.

Respiratory rate was measured by conventional manometric techniques. Two cortical slices, one from each hemisphere, were placed in each flask and after an initial equilibration period of 15 min at 37° respiration was measured over 60 min. The effect of barbitone (5×10^{-3}M) was measured on the respiration of both unstimulated and stimulated (0·1M KCl) cortical slices taken from barbitone-dependent, withdrawn and control rats.

Although Ungar & Cohen (1966) found that pentobarbitone tolerance was not transferred, their method of producing tolerance was such that it is unlikely that the tolerance observed was due to a central mechanism but rather that the drug stimulated the activity of enzymes concerned with its metabolism. Our method to produce physical dependence on barbiturates was that first described by Crossland & Leonard (1963) who showed that a characteristic withdrawal syndrome developed on cessation of barbitone administration.

TABLE 1. HEXOBARBITONE SLEEPING TIME AND RATE OF HEXOBARBITONE OXIDATION *in vitro* BY LIVER MICROSOMES OF MICE TREATED WITH RAT BRAIN HOMOGENATE

	Pretreatment (hr)	Type of brain homogenate		
		Control	Barbitone-dependent	Withdrawn
Sleeping time (min)	3	61 ± 20(8)	56 ± 14(8)	49 ± 13(8)
Sleeping time (min)	24	66 ± 20(8)	46 ± 15(8)*	55 ± 16(8)
Hexobarbitone metabolism[1]	24	2·8 ± 0·9(4)	3·8 ± 0·9(4)	—

* $P < 0.05$.
[1] μmoles hexobarbitone oxidized/9,000 g supernate from 1 g liver/30 min.