Biliary excretion of [^{14}C]edrophonium and its glucuronide conjugate

Although edrophonium chloride (ethyldimethyl(3-hydroxyphenyl)ammonium chloride) is occasionally used for diagnostic purposes in man, little is known of its metabolism and excretion. In general, chemically reversible anticholinesterase drugs have rapid and evanescent effects (Randall, 1950); in these conditions, detoxication may have little or no influence on pharmacological action. Indeed, it is commonly assumed that most quaternary amines are not metabolized, since the lipophilic endoplasmic reticulum may restrict the penetration and limit the metabolism of polar compounds (Gaudette & Brodie, 1959). These limitations do not necessarily apply to all polar drugs (Mazel & Henderson, 1965) or low molecular weight quaternary compounds (Somani, Wright & Calvey, 1970). For these reasons, and since no previous studies of the metabolism of edrophonium have been reported, we have studied the excretion of this drug and its metabolic products in bile.

The common bile duct of Wistar rats of either sex was cannulated under urethane anaesthesia, and bile was collected at hourly intervals after intravenous injection of [^{14}C]edrophonium chloride (2·0 μmol/kg) in saline. Specimens were assayed for radioactivity by liquid scintillation spectrometry, and [^{14}C]edrophonium and its metabolites were detected by paper and thin-layer chromatography in at least five different solvent systems. Conjugates of edrophonium in bile were identified by incubation with β-glucuronidase; control specimens were incubated with the enzyme in the presence of the specific inhibitor glucaro-(1→4)-lactone.

In 24 experiments, 4·7 $\pm$ 2·3% (mean $\pm$ s.d.) of the dose of [^{14}C]edrophonium was excreted in bile in 6 h. The only significant metabolite identified was a 3-oxyglucuronide conjugate of edrophonium {[^{14}C]ethyldimethyl(3-oxyphenyl)ammonium glucuronide}. Approximately 89% of the radioactivity in bile was present as this metabolite 1 h after administration of the drug (Table 1); some 10% was excreted as [^{14}C]-edrophonium. Only small amounts of the unchanged drug were identified 2–6 h after injection. Thus, the presence of unchanged edrophonium in bile may represent diffusion of the drug from periductular plasma downstream from the biliary canaliculus.

The trimethyl analogue of edrophonium (trimophonium) is partly demethylated before biliary excretion (Somani & others, 1970). In the present experiments, approximately 1% of the total radioactivity in bile was eliminated as an unidentified metabolite; it is possible that this substance represents a dealkylated conjugate of edrophonium.

Table 1. *Excretion of* [^{14}C]*edrophonium and its glucuronide conjugate in normal rat bile.* Values (based on mean $\pm$ standard deviation of at least five experiments) represent the proportion of total radioactivity present as each compound.

Time (h)	Proportion of total radioactivity (%) [^{14}C]Edrophonium	[^{14}C]Edrophonium glucuronide
1	10·4 $\pm$ 3·8	88·9 $\pm$ 3·8
2	1·3 $\pm$ 0·5	97·9 $\pm$ 0·8
3	0·9 $\pm$ 0·3	98·2 $\pm$ 0·4
4	1·0 $\pm$ 0·3	98·2 $\pm$ 0·3
5	0·7 $\pm$ 0·1	98·2 $\pm$ 0·4
6	0·7 $\pm$ 0·3	97·8 $\pm$ 0·6

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A common error in assessing the significance of percentage change in neuropharmacology

It has become common practice to express values for drug-treated animals as a percentage of (or as a percentage change from) values observed in saline controls. This procedure would be straightforward and easily understood if one could measure the effect of drugs in each experimental animal both after a saline control treatment and after a drug treatment, or if there existed some rational justification for pairing individual saline and drug-treated animals and if the numbers of animals employed in each treatment were the same. However, the calculation and interpretation of percentages is not so simple when these requirements are not met.

We have noted that the standard errors of mean percentages published in many experiments of this kind are erroneously small. In these instances, the standard error is unjustifiably small because it reflects only the variation within the drug sample and does not reflect the variation inherent within the control sample.

The error arises in one of two ways. It is made in one way when the standard error of the mean percentage change is calculated from a series of percentages, each derived from an observation made on an individual drug-treated animal, by dividing the mean percentage change by the mean of the controls. It is made in another way when the standard error of the mean percentage change is obtained by dividing the standard error of the drug mean by the mean of the controls.

Let us consider a hypothetical experiment involving a certain drug and brain 5-hydroxytryptamine concentrations. We assume that this drug has no effect on the concentration of 5-HT and hence that the experimental animals should have the same average value for 5-HT as do the saline controls. Let us assume that the available animals are from a normal population having a 5-HT concentration with a mean and standard deviation of 1000 and 150 ng/g brain. Thus, if one randomly takes 25 animals for each of the two groups the standard deviation of the sample mean of the control as well as the treatment group would be $150/\sqrt{25} = 30$ ng/g. Thus, it would be quite reasonable to obtain sample means of 1045 and 965 ng/g 5-HT for the saline and drug group, respectively. If one ignores the variability of the control group we can estimate that the figure for the drug group is 92·3% of that of the control group with a standard error of $(30 \times 100)/1045$ or 2·87%. We then see that the drug appears to lower brain 5-HT by 7·7% which is 2·68 standard errors lower than the control and normally the conclusion would be made that the drug significantly lowers brain 5-HT. However, if the variability in the control group is considered, the standard error for the ratio of the two means, say, $\bar{X}/\bar{Y}$, must be