calf serum the drugs would be protein-bound to the extent 42–44% for TMP (Schwartz & Zeigler, 1969) and 64% for SMX (Hall, 1961).

Why these results differ from those of other workers using an apparently similar system is not clear. Con A and phytohaemagglutinin are both T-lymphocyte mitogens although in mice at least it is known that distinct subsets of cells respond differently to these two substances (Stobo & Paul, 1973). If TMP and SMX are acting through some interference with lymphocyte folate metabolism it may be significant that Medium TC 199 contains folic acid 0·01 mg litre^{-1} whereas the Eagle's minimal medium used by Gaylarde & Sarkany (1972) contains one hundred times this amount. The observations of Bain (1975) suggest that low folate media increase thymidine uptake without affecting the degree of morphological transformation of cultures. It is possible that our population as defined above in some way differs from the 'normal subjects' of Gaylarde & Sarkany (1972). Although SMX at high concentrations produces a small inhibition we believe the major inhibitory effect of co-trimoxazole on [^{3}H]thymidine incorporation to be due to TMP at the higher concentration and that any synergistic effect must be less common than hitherto suggested.

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LETTERS TO THE EDITOR

Contraversive circling behaviour produced by unilateral electrolytic lesions of the ventral noradrenergic bundle mimicking the changes seen with unilateral electrolytic lesions of the locus coeruleus

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In investigating the possible role of central nervous system noradrenergic neurons in motor activity we have found that unilateral electrolytic lesions in the region of the noradrenaline containing locus coeruleus in rats result in circling behaviour contraversive to the side of the lesion when the animals are systemically administered either apomorphine or (+)-amphetamine (Pycock, Donaldson & Marsden, 1975). This behaviour is transient and disappears after one to two months. It is accompanied by a similar transient rise in the ipsilateral striatal dopamine content by approximately 50%, which is present at five days post-operatively, but has disappeared by one to two months. There is a persistant fall in the ipsilateral cerebral cortical noradrenaline. It was postulated that a possible explanation of these results was that a pathway between the locus coeruleus region and the nigrostriatal system existed, and that this was facilitative serving to enhance nerve impulse flow between the substantia nigra and the striatum. The interruption of the proposed link was envisaged as causing a decrease in ipsilateral nigrostriatal activity with consequent decentralization supersensitivity (Ungerstedt, 1975) in the striatal dopamine receptors and a build up of dopamine. This would explain the contraversive rotation to both apomorphine and (+)-amphetamine.

If the observed changes were due to damage to noradrenaline-containing cells of the locus coeruleus and not to adjacent structures then these results might be expected to be reproduced by lesions of the ascending noradrenergic bundle systems. Damage restricted to either the dorsal or the ventral bundle would establish which of these two pathways was involved. The locus coeruleus is known to provide noradrenergic fibres to both of these bundles (Lindvall & Bjorkland, 1974).

Unilateral electrolytic lesions were made in the rostral pons and caudal midbrain of Wistar rats, of 150 ± 10 g weight at the time of operation. The level of the lesions was chosen so as to be caudal to the level of the substantia nigra at a point where the dorsal and ventral bundle were separate from each other. The lambda-bregma line was horizontal and the skull was held in a 'Stoelting' stereotaxic frame. The anode, a stainless steel electrode with a diameter of 0·65 mm, which was

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varnished except for the tip, was held vertical. A current of 2·5 mA was passed for 7 s. The co-ordinates are given in mm in relation to the interaural line (A), midline (L) and exposed surface of the cortex (V). Those used were A 1·0, L 1·2 and V 5·2 for the dorsal bundle and A 1·0, L 1·6, and V 6·2 for the ventral bundle. A group of rats had control electrolytic lesions placed in the tectum overlying, but not injuring the noradrenergic bundles. Post-operatively animals were given systemic apomorphine (1 mg kg^{-1}, i.p.) or (+)-amphetamine (5 mg kg^{-1},i.p.), and rotational activity was visually counted.

Rats with lesions aimed at the dorsal noradrenergic bundle area showed tight ipsiversive circling in response to either systemic apomorphine or (+)-amphetamine. This behaviour was persistent in rats kept up to three months. These animals had lesions involving their dorsal and not their ventral noradrenaline bundles, as shown by a fall in ipsilateral cortical noradrenaline content by 27% without any change in the ipsilateral hypothalamic noradrenaline. Routine light microscopy confirmed that the dorsal bundle area was damaged, while the ventral bundle area was spared. In the brains examined by histochemistry the dorsal noradrenergic bundle showed an accumulation of fluorescent material caudal to the lesion, but the ventral bundle was not seen. In such rats killed at five days, there was no difference in the dopamine content of the two striata.

In a group of rats with electrolytic lesions aimed at the ventral noradrenergic bundle region, some showed the same type of ipsiversive circling in response to apomorphine and (+)-amphetamine as has been seen in the rats with lesions of the dorsal bundle area, while others showed contraversive circling when given either of these two drugs. The ipsiversive rotation persisted in a group of rats tested for three months, but the contraversive circling disappeared between two and three weeks post-operatively. In the rats showing ipsiversive circling the ventral bundle area was histologically involved in all, but in the majority the damage was only partial. In addition, the damage had extended into the dorsal bundle region in all animals. This was confirmed by a fall in ipsilateral cerebral cortical noradrenaline by 20%, and in ipsilateral hypothalamic noradrenaline by 13% in this group. In the rats which circled contraversively, the histological damage was restricted to the ventral bundle region, which was totally destroyed in all animals. Confirmation that the damage involved the ventral and not the dorsal bundle regions was shown by a 24% fall in ipsilateral hypothalamic noradrenaline content without any difference in the cortical noradrenaline concentrations between the two sides. In brains from these contraversively circling rats that were examined by fluorescent histochemistry the ventral bundle was visible caudal to the lesion, but the dorsal was not seen. It has been shown above that damage to the dorsal noradrenergic bundle area does not alter the ipsilateral striatal dopamine content. However, when the lesion involves the ventral noradrenergic bundle area there is a considerable rise in the ipsilateral striatal dopamine content by 60% at five days, although this had fallen to normal by one to two months. Involvement of the dorsal noradrenergic bundle area seemed to prevent the contraversive circling that was seen when the ventral bundle area alone was damaged, although it did not prevent the increase in dopamine.

Although unilateral lesions involving the median raphé nuclei have been reported to produce contraversive circling in rats to apomorphine and (+)-amphetamine (Costall & Naylor, 1974), this cannot be the mechanism in these animals as the raphé nuclei were not involved histologically, and there was no fall in ipsilateral cerebral cortical 5-HT which accompanies such median raphé lesions (Marsden & Guldberg, 1973). Damage to the adjacent substantia nigra would not seem to be the cause of the rise in striatal dopamine, as no elevation was seen at five days in another group of rats with electrolytic lesions of this structure.

A group of rats with control electrolytic lesions in the tectum dorsal to the noradrenergic bundles showed no circling in response to apomorphine or (+)-amphetamine, and no change in cerebral cortical noradrenaline, cerebral cortical 5-HT, hypothalamic noradrenaline or striatal dopamine.

These results, which will be described in full elsewhere, show that unilateral electrolytic lesions of the ventral noradrenergic bundle region mimic unilateral electrolytic lesions of the locus coeruleus and produce transient contraversive circling in response to systemic apomorphine and (+)-amphetamine plus a transient rise in ipsilateral striatal dopamine. It would seem likely that this is due to interruption of the noradrenergic fibres from the locus coeruleus which ascend in the ventral bundle and that this is the route of the proposed facilitative noradrenergic pathway which is envisaged as enhancing impulse flow in the nigrostriatal system. Involvement of the dorsal noradrenergic bundle area results in ipsiversive apomorphine and (+)-amphetamine-induced circling. When this region is damaged in addition to the ventral bundle area, this ipsiversive rotation seems to replace the contraversive turning seen with lesions of the ventral bundle area alone. The structures and mechanisms involved in this ipsiversive response following dorsal bundle lesions have not been definitely identified, but may not be due to damage to ascending noradrenergic fibres, for such ipsiversive turning does not occur with lesions of the locus coeruleus, which is the major source of noradrenergic fibres ascending in the dorsal bundle.

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A possible metabolic explanation for drug-induced phospholipidosis

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A large variety of amphiphilic cationic drugs which are in widespread clinical use produce a generalized phospholipidosis when administered for prolonged periods. These drugs, which vary widely in their potency in causing phospholipidosis, include chlorphentermine, fenfluramine, triparanol, *trans*-1,4-bis (2-chlorobenzylaminoethyl)-cyclohexane (A79944), azacosterol, 5,5'-diethylaminoethyoxyhexestrol, 1-chloroamitriptyline, iprindole, 2-*N*-methyl-piperazino-methyl-1,3-diazofluoroanthen 1-oxide (AC 3579), chlorcyclizine, chloroquine, chlorpromazine, thioridazine, imipramine, clomipramine, haloperidol and boxidine (Yamamoto, Adachi & others, 1971a, b; Shikata, Kanetaka & others, 1972; Hruban, Slesers & Ashenbrenner, 1973; Lüllman, Lüllman-Rauch & Wasserman, 1973; Wherrett & Huterer, 1973; De La Iglesia, Feuer & others, 1974; Kasama, Yoshida & others, 1974; Lüllman-Rauch, 1974a, b, 1975; Schmien, Seiler & Wasserman, 1974). Although these drugs have a variety of therapeutic effects they are physicochemically rather similar, in that they all possess both a hydrophobic region and a primary or substituted amine group which can bear a net positive charge. This amphiphilic nature enables the drugs to interact with phospholipids, particularly the anionic phospholipids which are quantitatively minor constituents of membranes (e.g. phosphatidate, phosphatidylinositol, phosphatidylserine, cardiolipin). Their capacity both to cause phospholipidosis and to interact with lipids depends largely on the size and hydrophobicity of the apolar portions of the molecule.

We recently suggested that interactions of these drugs with anionic phospholipids might cause some of the therapeutic actions or side-effects of these drugs (Brindley, Allan & Michell, 1975).

The lipids which accumulate in the lysosomes of a variety of tissues during drug treatment are mainly glycerophospholipids. There are clear indications that compared with normal tissue, these tend to include increased proportions of anionic phospholipids (phosphatidate, phosphatidylinositol, phosphatidylglycerol and lysobisphosphatidate) and decreased proportions of triglyceride and of the major zwitterionic glycerophospholipids (phosphatidylcholine and phosphatidylethanolamine) (Yamamoto & others, 1971a, b; Wherrett & Huterer, 1973; Kasama, Yoshida & others, 1974; Allan & Michell, 1975; Karabelnik & Zbinden, 1975). This pattern of lipid accumulation is in marked contrast to that seen in the classical hereditary lipidoses in which sphingolipids, particularly glycosphingolipids, are the main lipids which accumulate in lysosomes.

One proposed explanation of this effect is that phospholipids are normally degraded in lysosomes by phospholipases, but that when amphiphilic cationic drugs form complexes with the phospholipids this prevents phospholipase attack and the phospholipid-drug complexes therefore accumulate and engorge the lysosomes (Lüllman & others, 1973; Lüllman-Rauch, 1974a). Although this mechanism would explain many of the experimental findings it does not provide a complete explanation. For example, it does not explain

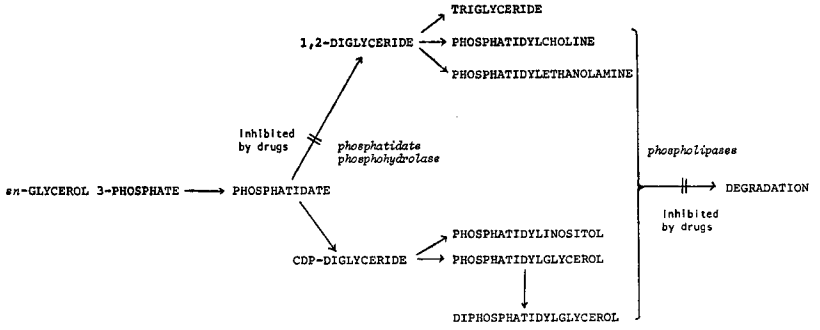

Fig. 1. Effect of amphiphilic cationic drugs on the metabolism of glycerolipids.

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