macroneurones. Hassler[4] judged that his "thalamic internuncial cells" in the human dorsomedial nucleus, indistinguishable in his Fig. 14 from my "microneurones", suffer equal retrograde degeneration with the "specific" neurones after substantial cortical removals, but relatively less after very restricted lesions; and surmised that they project 'unspecifically' to the cortex. It may be that the tempo of degeneration is higher after wide cortical damage than after standard leucotomy; but in my judgment at least the majority of microneurones are wholly intrathalamic 'interneurones', susceptible to disuse atrophy rather than to retrograde degeneration, and I would still predict that they will be found to constitute structural essentials to the brain's attention-focusing mechanism, as well as to its electroencephalographic alpha-rhythm[1].

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[1] Andersen, P., and Eccles, Sir John, *Nature*, **196**, 645 (1962).
[2] von Monakow, C., *Arch. Psychiat.*, **27**, 1, 386 (1895).
[3] McLardy, T., *J. Neurol. Neurosurg. Psychiat.*, **13**, 198 (1950).
[4] Hassler, R., *Cong. Latinoamer. Neurocir.*, Montevideo, 754 (1955).
[5] Namba, M., *J. Hirnforsch.*, **4**, 1 (1958).
[6] McLardy, T., *Nature*, **194**, 300 (1962).
[7] Lemieux, L. H., *J. Neuropath. Exp. Neurol.*, **13**, 343 (1954).
[8] Turner, J., *Brain*, **26**, 400 (1903).
[9] Waddy, F. F., and McLardy, T., *Confin. Neurol.*, **16**, 65 (1956).

Reversal of Solubility Characteristics of 'Luxol' Dye-phospholipid Complexes

'LUXOL' fast blue *G* (Du Pont), the diarylguanidine salt of a sulphonated azo dye, forms complexes stoichiometrically with phospholipids[1]. These complexes have been found to be insoluble in a varying pattern in the lower alcohols. For this study phospholipid-dye complexes were prepared with the same dye and phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositol, or sphingomyelin. The complexes were produced by bringing together the dye and phospholipid in pyridine solution. After evaporation of the solvent, excess uncomplexed dye or phospholipid could be removed by the alcohol in which the complex was insoluble. The solubility of the complexes in methanol, ethanol, isopropanol and isobutanol was determined. The results are summarized in Table 1. It was found that the complexes were soluble to the extent of at least 0·1 per cent or else were completely insoluble in the alcohols used. When alcoholic solutions of dye and phospholipid were mixed, precipitates were formed in those alcohols in which the particular complex had been found to be insoluble. The absorption maximum for the dye alone in the alcohols used is 597 mμ. When alcoholic solutions of the dye and phospholipid were mixed, the absorption maximum dropped to 570 mμ before precipitation took place. If the precipitate was then centrifuged and the deposit redissolved in methanol or an alcohol in which it is soluble the absorption maximum was found to return to 597 mμ. This can be taken as evidence of dissociation where complexes are marked soluble in Table 1.

With the foregoing *in vitro* data as a guide, we tried to devise a method for the differentiation of phospholipids in tissue sections. Only partial success was achieved. In sections of brain tissue some differential staining of the phospholipids present was accomplished when solutions of the dye in the various alcohols were used. However, in other tissues some non-phospholipid material was stained. Tryptophan, present in the necrotic areas in livers of experimental animals, stained intensely with the dye in isopropanol solution. In methanol the dye stained neither phospholipid nor tryptophan but collagen and elastin were stained selectively. Frozen unfixed tissues were employed with similar results.

The dye 'Luxol' fast blue *G* is not a specific histochemical reagent for phospholipids, but its staining affinities for certain tissue components vary with the solvent used. This may explain some of the anomalous staining of phospholipids found in the literature. Recently, Wolman[2] found that the affected neurones in three cases of Neimann–Pick's disease were not stained by 'Luxol' blue; whereas Baker's procedure for phospholipids was positive. Current 'Luxol' blue techniques utilize ethanol as the dye solvent, and from reference to Table 1, it will be seen that sphingomyelin does not form insoluble complexes with 'Luxol' fast blue *G* in this alcohol. As an increase in sphingomyelin storage can be expected in Neimann–Pick's disease, the *in vitro* data furnish a possible explanation for the non-staining of the affected neurones by 'Luxol' blue dissolved in ethanol.

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[1] Salthouse, T. N., *Nature*, **195**, 187 (1962).
[2] Wolman, M., *J. Clin. Path.*, **15**, 324 (1962).

RADIOBIOLOGY

Effect of Multiple Injections of Calcium Compounds on the Survival of X-irradiated Rats

A MODERATE increase in the survival of X-irradiated rats has been observed when either parathyroid extract or ethylenediamine tetraacetate (EDTA) was injected before or after mid-lethal doses of X-rays[1–3]. The action of these substances has been attributed to bone resorption, which was thought to protect the adjacent radiosensitive bone marrow by exposing it to an increased concentration of newly-released calcium. In the work recorded here, we have tested this view directly by the injection of calcium compounds.

Adult males of a hooded strain of laboratory rat, weighing between 270–340 g, were used. Calcium was administered intraperitoneally as the acetate (0·12 M), lactate (0·12 M), or gluconate (0·067 M). The sodium salt of each of these compounds was also tested and administered in twice the molar concentration so that an equivalent amount of anion could be given in the same volume of fluid. All compounds were dissolved in a Krebs–Ringer salt solution[4] without phosphate buffer. The radiation source was a Philips constant-potential X-ray machine operated at 300 kV and 10 m.amp (half-value layer, 1·14 mm copper), with a target-object distance of 80 cm and a dose-rate in air of approximately 34 r./min. All rats received a whole body dose of 740 r. The animals were exposed 12 at a time in a revolving, partitioned, 'Lucite' cage. Each group of 12 consisted of 4 control animals (injected with Krebs–Ringer solution only), 4 animals injected with the sodium salt, and 4 animals with the calcium salt of the compound under investigation.

Table 1. SOLUBILITY OF 'LUXOL' DYE-PHOSPHOLIPID COMPLEXES

Dye or dye complex	Water*	Methanol	Ethanol	Isopropanol	Isobutanol	Pyridine
'Luxol' fast blue G	Insoluble	Soluble	Soluble	Soluble	Soluble	Soluble
Phosphatidyl choline—dye complex	Soluble	Soluble	Insoluble	Insoluble	Insoluble	Soluble
Phosphatidyl serine—dye complex						
Phosphatidyl inositol—dye complex						
Phosphatidyl ethanolamine—dye complex	Soluble	Soluble	Soluble	Insoluble	Insoluble	Soluble
Sphingomyelin—dye complex	Soluble	Soluble	Soluble	Insoluble	Soluble	Soluble

* *p*H 7·0