

taminants. They were rich in nitrogen (5.4–12.5 per cent). The hexose content ranged from 1.0 to 8.1 per cent; the neuraminic acid from 1.2 to 3.1 per cent; and the higher concentrations of both carbohydrates occurred in the slowest moving substances. The glycamino substances have now been extracted from human grey matter with chloroform:methanol (2:1) and under these circumstances also show the same properties as those extracted with water, with the exception that the nitrogen content is lower. They have also been shown to be present in crude mitochondrial and microsomal subcellular fractions of grey matter.

These substances are not phospholipids. This is clear from the absence or occasional presence of trace amounts of phosphorus and by their infra-red spectra. They are not proteolipids since they are extracted by water, while proteolipids are not. They are neither gangliosides, nor cerebroside, nor aminoglycolipids⁹, since they are extractable by aqueous solvent, and do not have the composition of these glycolipids. Further studies on the structure and function of these newly isolated substances from brain are in progress.

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Fluorescent Lipid from Brain

A SUBSTANCE has been obtained from the brain of rabbits killed with diisopropyl fluorophosphate that appears to have interesting properties and chemical structure. The substance can be obtained in amounts of 1.0–1.3 mg/g of brain by extraction with cold aqueous 5 per cent perchloric acid, followed by precipitation with phosphotungstic acid, and chromatography of the portion of the precipitate soluble in chloroform on silica with chloroform-methanol mixtures as eluant. The substance, obtained as a wax, gave a single fluorescent spot of R_F 0.95 by thin layer chromatography on alumina with a pyridine-amylic alcohol-water (3:3:2) solvent system. The same areas also gave positive reactions with acidified permanganate, diazotized *p*-nitroaniline, Folin's phenol reagent, Schiff's fuchsin reagent, 2,4-dinitrophenylhydrazine, and with alkaline hydroxylamine followed by acidified ferric chloride solution. Elementary analysis showed some variation from lot to lot, with an average of 75.5 per cent carbon and 12.0 per cent hydrogen, and a range of ± 1 per cent for carbon and ± 0.5 per cent for hydrogen.

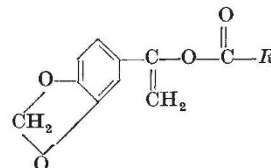
Reduction of the lipid with zinc dust and glacial acetic acid, followed by alkaline hydrolysis, yielded a product which, after crystallization from cyclohexane and from methanol, formed white crystals that had a blue-white fluorescence under ultra-violet light and melted at 111°C. Analysis showed the presence of 70.01 per cent carbon and 5.06 per cent hydrogen, corresponding with the formula $C_{18}H_{16}O_5$, for which formula the calculated composition is carbon, 69.22 per cent; hydrogen, 5.13 per cent. This product is apparently identical with a substance obtained by

Mameli¹, namely 2,3-dimethyl-2,3-bis-3,4-methylenedioxyphenylethylene oxide, and results from a dimerization during the reduction process^{2,3}. A sample prepared by Mameli's method melted at 111°C, contained 70.62 per cent carbon and 5.97 per cent hydrogen, and showed similar fluorescence. The natural and synthetic material had identical R_F values of 0.89 on thin layer chromatography on silica using a benzene-dioxane-acetic acid (90:25:4) system. The presence of the methylenedioxy ring in the original lipid was confirmed by acid hydrolysis followed by steam distillation, and demonstration of the presence of formaldehyde in the distillate by the dimedon and chromotropic acid tests. It was therefore concluded that the structure of the substance included a 1-ethyl-3,4-methylenedioxyphenyl skeleton.

Hydrolysis of the lipid with 10 per cent phosphoric acid followed by steam distillation and extraction of the distillate with ether gave a product with the general appearance, melting point (24°C), and composition (71.33 per cent carbon, 12.14 per cent hydrogen) of a mixture of fatty acids. Methylation with diazomethane yielded methyl esters which were shown by gas chromatography to consist of about 80 per cent methyl stearate and 20 per cent methyl palmitate.

Two kinds of product were obtained from the reaction of the lipid with 2,4-dinitrophenylhydrazine. The first of these, obtained in the cold, gave a high R_F with the thin layer chromatographic system of Dhont and de Rooy⁴, and a composition suggesting that it was an addition product of the lipid and the reagent. Heating with acid yielded a product that gave an R_F of 0.77, identical with that of acetopiperone-2,4-dinitrophenylhydrazone, and contained carbon, 50.64 per cent; hydrogen, 4.95 per cent; and nitrogen, 16.97 per cent. The calculated values for the acetopiperone derivative are carbon, 52.35 per cent; hydrogen, 3.49 per cent; and nitrogen, 16.28 per cent. The melting points of both products showed broad ranges.

The data suggest to us that the lipid is a fatty-acid ester of the *enol* form of acetopiperone, that is



where *R* represents the hydrocarbon portion of the fatty acid chain. This is consistent with its ability to react with reagents for carbonyl groups (2,4-dinitrophenylhydrazine and Schiff's reagent) and ester groups (alkaline hydroxylamine and ferric chloride). Similar compounds are known to react with diazonium compounds⁵. The structure shown appears to be the only structure consistent with the principal derivatives obtained.

The methylenedioxyphenyl group occurs naturally in a considerable number of alkaloids and of essential oils. So far as we know, it has not previously been found in a constituent of animal tissue.

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