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Received November 29th, 1962

SC 2234

The isolation of squalene from human brain

In 1959 NICHOLAS AND THOMAS[1] gave evidence for the presence of squalene in rat brain after injecting intracerebrally sodium [2-^{14}C]mevalonate into the animal. More recently, HONEGGER[2] indicated its presence in brain tissue of multiple-sclerosis patients and normals by means of thin-layer chromatography. However, additional proof of identity remained to be done. JATZKEWITZ AND MEHL[3] mention also this hydrocarbon as a brain substance.

In this paper we give a report on the isolation and identification of squalene from human brain. Adult human brains were obtained *post-mortem* as soon as possible after death from cases in which there had been no pathological changes.

Two whole brains (about 2.5 kg) were freed from blood and meninges under running tap-water, minced in a Waring blendor and exhaustively extracted with cold acetone. The combined extracts were evaporated *in vacuo* to dryness and the residue crystallized from methanol. In this manner a great part of cholesterol was removed from a mixture. The filtrate was freed of solvent *in vacuo*, the semicrystalline residue dissolved in light petroleum (30–50°) and chromatographed on activated alumina. Each fraction was tested for squalene by paper chromatography. The light petroleum eluates (Fractions 1–6) showed a negative reaction and were discarded. The first benzene eluates (Fractions 7–12) were squalene positive and gave a viscous oil (880 mg) which still contained a considerable quantity of cholesterol. Therefore, it was dissolved in hexane and rechromatographed on Florisil (60/100 mesh, Floridin Company, Tallahassee, Florida) using the technique described recently by CARROLL[4]. A typical experiment is presented in Fig. 1. A sample of 92 mg of the oily product was put on 12-g columns of Florisil. An unidentified substance or mixture of substances (Area 1), squalene (Area 2) and cholesterol (Area 3) were successively eluted with hexane (30 ml), 5% diethyl ether in hexane (30 ml), 15% diethyl ether in hexane (25 ml) and 25% diethyl ether in hexane (25 ml) respectively. Only Fractions 8–13 were squalene positive. Approx. 13 mg of the highly purified hydrocarbon were isolated from this run. Thus, the clean-cut separation of squalene from other lipide classes could be effected.

The proof of identity for squalene was unambiguously presented on basis of following data: (a) A known mixture of both authentic squalene and cholesterol behaved similarly on Florisil columns as a mixture from brain; (b) Chromatograms on Whatman No. 1 filter paper impregnated with silicic acid, solvent system[5,6] diisobutyl ketone – acetic acid – water (40:25:5, v/v) showed a single spot (R_F 0.93)

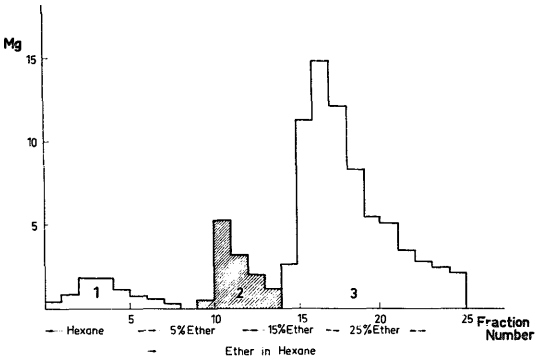

Fig. 1. Separation of a mixture of acetone-soluble brain lipids (92 mg) on a 12-g column of Florisil. 5-ml fractions were collected.

after exposure to iodine vapors[7,8] corresponding to that of the authentic squalene which was run side by side. (c) A specimen of the brain hydrocarbon was converted to the hexahydrochloride[9], which was identical with the authentic squalene derivative with regard to the melting point (120° with softening at 108°), crystal shape and infrared spectrum.

Although the procedure described is not entirely suitable for quantitative work, the amounts of squalene present in adult human brain would appear to correspond to 50 μg/g of fresh wet tissue. NICHOLAS AND THOMAS[1] gave a value of 29 μg squalene/g of fresh adult rat brain, as determined colorimetrically by a modified SOBEL method[10].

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Received November 12th, 1962