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Lipid Composition of Human Cerebrospinal Fluid.* (27811)

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It has recently been reported(1) that human cerebrospinal fluid (CSF) contains cerebroside, sphingomyelin, cholesterol, cholesterol esters and triglycerides as well as a number of unidentified lipids. The present paper will present data confirming and extending some of these observations.

Materials and methods. The 14 samples of CSF examined in this study were obtained through the cooperation of Dr. R. C. Llewellyn during the course of pneumoencephalographic examination. The freshly collected samples (20-80 ml) were either centrifuged at 25,000 *g* for 20 minutes or they were filtered through coarse sintered glass filters, only water-clear samples were examined. The supernates or filtrates were promptly shell frozen in a dry-ice ethanol bath and freeze dried; the dry residue was extracted with 0.5 ml chloroform-methanol (2:1) per ml of CSF. Extracts obtained by both methods seemed to be identical and there were no obvious correlations to be made relating the resulting lipid pattern to the neurological complaint. Because of the large proportion of water soluble extractives, the chloroform : methanol extract, after filtering through a sintered glass filter (Corning porosity C), was fractionated according to Folch's technic(2) by addition of 0.73% NaCl. The resulting chloroform extract was dried over anhydrous Na_2SO_4, filtered again through sintered glass, and its volume reduced to 1/10th by means of a stream of nitrogen with the sample in a water bath at 40°C. Twenty-five to 50 μl of this concentrate was generally adequate for analysis by the paper chromatographic technic described elsewhere(3). Our experience has shown that when multiple spot-test analysis is applied to paper chromatograms their usefulness is greatly extended and serves to quite adequately identify various phosphatides.

Results and discussion. Fig. 1 clearly demonstrates the presence of both ethanolamine and choline plasmalogens; on hydrolysis with $HgCl_2$ (*cf.* 4) it was possible to show that both diester analogs were also present. As can be seen from the figure, ethanolamine plasmalogen was proportionately greater, whereas in plasma the reverse was true; this would suggest that CSF is not a simple plasma transudate. Choline spot-tests verified the presence of both lecithin (and choline plasmalogen) and sphingomyelin. When compared with authentic brain cerebroside and ganglioside (through the generosity of E. Klenk), no corresponding components, characterizable by the periodic acid-Schiff reaction, were observed in the CSF using the ketone, pyridine, water chromatographic system(5). There was, however, detectable by this system, a trace amount of a plasmalogen component which occupied a position on the chromatogram just above phosphatidyl ethanolamine. Due to insufficient material this component has not been characterized further, although it closely resembled cardiolipin when compared with a sample of this phosphatide (isolated from dog heart by column chromatography on silicic acid).

A second uncharacterized material could be seen between the phosphatidyl choline and phosphatidyl ethanolamine, stainable only by Rhodamin 6G and fairly rapidly leached by the subsequent SO_2 washings. This material did not give a positive phosphorus reaction.

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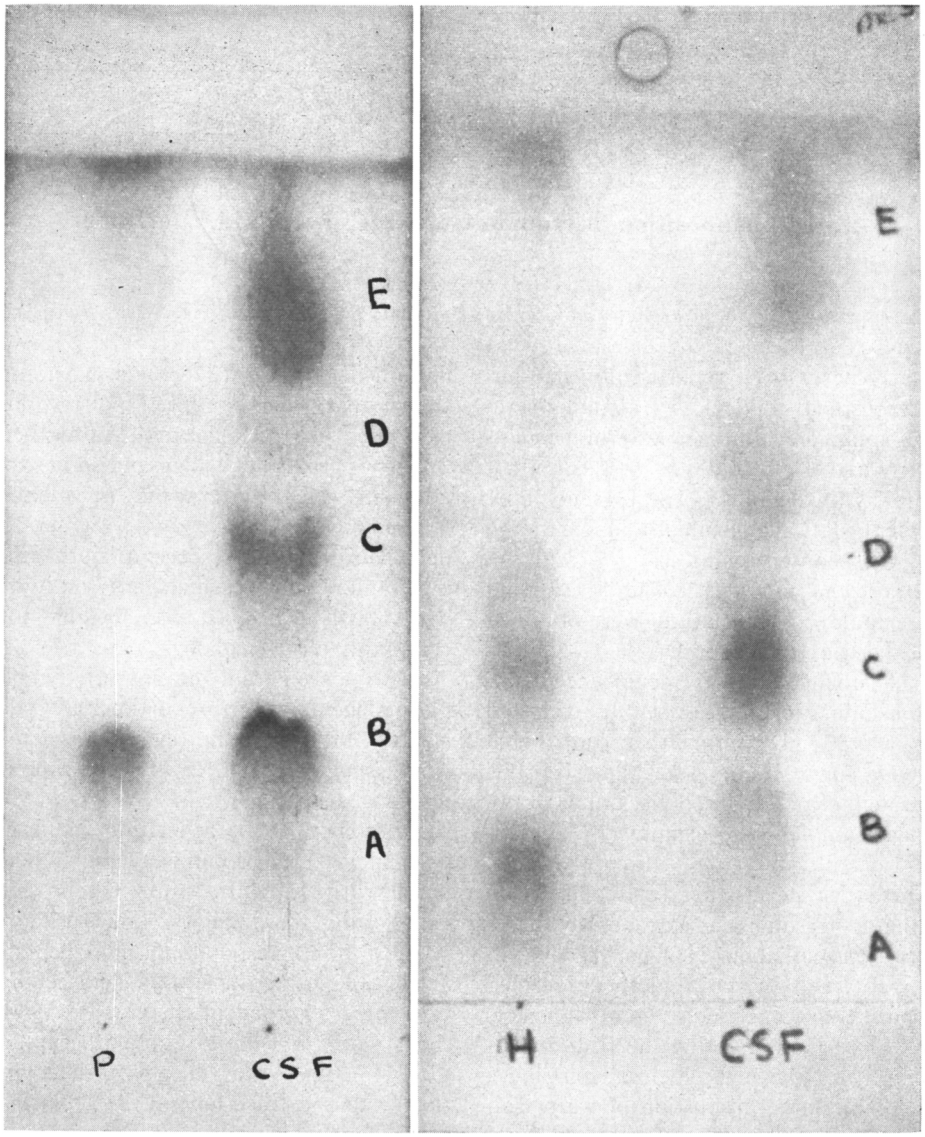

FIG. 1. This figure illustrates the 3 principal phosphatides found in CSF compared here with plasma (P), and with cardiac muscle (H). A) Sphingomyelin. B) Lecithin and choline plasmalogen. C) Phosphatidyl ethanolamine and ethanolamine plasmalogen. D) Cardiolipin (and cardiolipin plasmalogen in CSF). E) Neutral lipid. Chromatogrammed in 2, 6-dimethyl-4-heptanone, methanol, water mixture (*cf.* 3) at room temperature for 2 hr; plasmalogen-Rhodamin 6G stain.

The presence of cholesterol, cholesterol ester and triglyceride was verified by chromatography on S & S 2043b silicic acid impregnated paper in n-heptane : 2,6-dimethyl-4-heptanone (100:1); the chromatographic run was one hour. Exposure of these chromatograms to OsO_4 vapors resulted in staining (brown-black spots) of all the lipids due to their unsaturated fatty acid content. The cholesterol and cholesterol ester were detected by means of the Liebermann-Burchard reaction by immersing the paper in cold acetic anhydride : sulfuric acid (20:1) for 10 minutes. This treatment destroyed the chromatogram due to hydrolysis of the paper so that photography of the green cholesterol spots was impractical.

Discussion. The low lipid content of CSF

makes its analysis difficult; appropriately prepared extracts of freeze-dried material, however, can be examined accurately by paper chromatography as here illustrated. The lipid pattern disclosed indicates that the CSF cannot be a simple plasma transudate; the disproportionately high content of ethanolamine phosphatide might imply contributions by the choroid plexuses or ependymal cells. The origin of the cardiolipin-like plasmalogen is similarly unknown and especially puzzling because cardiolipin is generally believed to be exclusively a mitochondrial lipid.

Summary. Freeze-dried chloroform-methanol extracted samples of human cerebrospinal fluid (CSF) were examined for various lipids by means of paper chromatography. Phosphatidyl ethanolamine and ethanolamine plasmalogen were characteristically high as compared with blood plasma. Sphingomyelin, phosphatidyl choline and a small amount of choline plasmalogen were also present. The neutral lipids were composed of triglycerides, cholesterol and cholesterol ester. A plasmalogen resembling cardiolipin was present in low concentration. The cerebrosides were not detected.

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Effects of Vasoactive Drugs on Serum Electrolytes in Hypertensive and Normotensive Humans.* (27812)

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Previous studies have suggested that extracellular sodium decreases during acute rises in blood pressure and increases as pressure falls acutely(1-4). Inverse movements of potassium of lesser amounts usually accompany these sodium movements. It was concluded that sodium transfer systems are the primary determinants of smooth muscle tone(3,4). However, experimental data have been neither consistent nor conclusive. In addition, drugs, such as epinephrine and epinephrine-like substances, which were used in some studies, have extravascular metabolic effects as well as effects on vasomotor tone(5,6).

The present study reviews the acute effects of drugs, which primarily affect vasomotor tone, on the distribution of electrolytes in man. Patients with "essential" hypertension and normotensive subjects were studied to determine whether the effects of vasoactive drugs upon electrolyte distributions are influenced by initial blood pressure levels.

Materials and methods. The subjects of this study were divided into 4 groups (Tables I-IV). Groups A and C include 10 and 7 patients, respectively, with chronic arterial (essential) hypertension (blood pressures, 140/90 mm Hg or higher), and Groups B and D include 7 and 4 subjects, respectively, with normal blood pressures. Antihypertensive therapy was avoided for 2 weeks prior to these studies. The patients with normal blood pressures were convalescent hospital subjects believed to have normal hearts, lungs, and kidneys.

The patients were in the postabsorptive state and were not given premedication. A cardiac catheter was passed into the right atrium from a peripheral vein. Control data were obtained during an initial period of 60

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