# Histochemical and Lipid Studies on Human Choroid Plexus.* (28677)

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Our recent studies(1) on the lipids of human cerebrospinal fluid (CSF) suggested to us that a histochemical and lipid analysis of human choroid plexus might provide some data to aid in explaining a) its function and b) the origin of the CSF.

*Materials and methods.* Thirteen choroid plexuses (ages 35 to 78) were obtained at autopsy through the courtesy of Dr. Charles Dunlap. A representative sample of each was promptly frozen for the preparation of 10 $\mu$ cryostat sections; the remainder was freeze-dried and extracted with chloroform:methanol 2:1 v/v 20 ml per gram dry weight of tissue. The cryostat sections were examined by histochemical techniques for a) plasmalogen, b) succinic dehydrogenase, c) carbohydrate, and d) nucleic acids using the Feulgen, Nitro blue tetrazolium, PAS and methyl green pyronin techniques respectively (cf 2).

The lipids in the chloroform:methanol extract were identified by the same paper chromatographic methods employed in the analysis of CSF(1).

*Results.* Histochemical: The epithelial cells were uniformly and intensely stained for plasmalogen and by NBT for succinic dehydrogenase (Fig. 1). The PAS reaction for carbohydrate stained the basement membrane and the internal layer of the blood vessels but not the elastic fibers. The epithelial cells were moderately stained with pyronin and

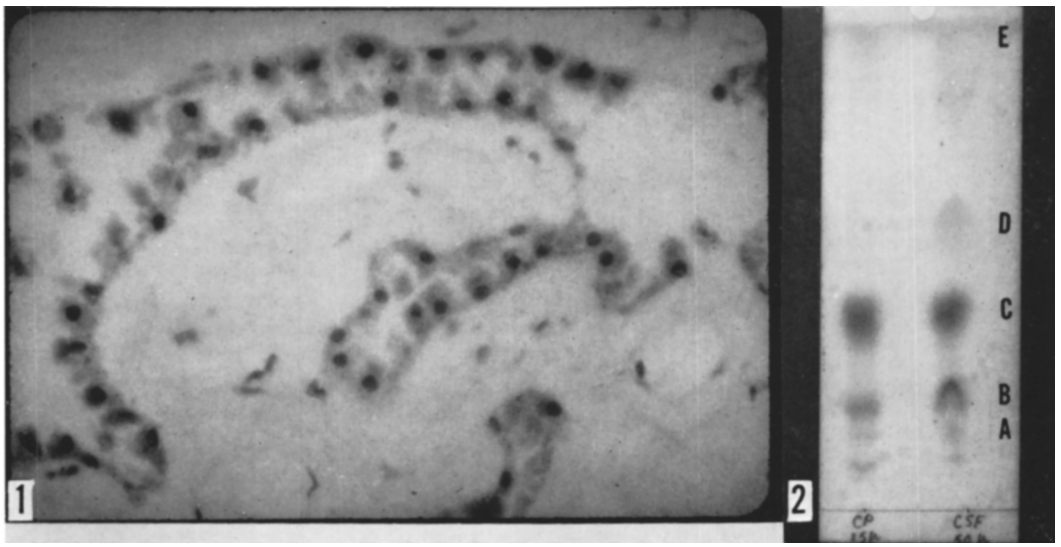


FIG. 1 (left). Human choroid plexus, fresh frozen cryostat section stained for succinic dehydrogenase by the NBT reaction. The cytoplasm of the epithelium shows a uniform finely granular lavender staining contrasting well with the dark nuclei which are stained by aqueous methyl green.

FIG. 2 (right). Chromatogram of choroid plexus (CP) and cerebrospinal fluid (CSF) which was run in the methanol system (cf 1) and stained for carbohydrate by the periodic acid Schiff (PAS) reaction. A) is monophosphoinositide (and sphingomyelin); B) is lecithin and choline plasmalogen; C) is phosphatidyl ethanolamine and ethanolamine plasmalogen; D) is cardiolipin. Neutral lipid is at the solvent front E and is more noticeable in the choroid plexus sample. The spot below A in both samples is presumably diphosphoinositide (see text). Cardiolipin is not seen in CP in this chromatogram, but would be revealed by Rhodamin 6G as expected from the strong mitochondrial demonstration by NBT in the cryostat sections.

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the elastic fibers of the connective tissue and blood vessels were very well stained.

Chromatography: Perhaps the most interesting observation was the presence of two phosphoinositides which were in greater relative concentration than in the CSF (Fig. 2). The lower inositide appeared to be diphosphoinositide on the basis of a direct chromatographic comparison with guinea pig brain extract and with fraction I isolated from guinea pig brain by the Folch technique(3). Although the choroid plexus contained cardiolipin (Fig. 2) as expected from the strong reaction for succinic dehydrogenase indicating the presence of mitochondria, unlike CSF it was negative for plasmalogen.

*Discussion.* We suggested earlier that lipids of the CSF may be contributed by the choroid plexus. The similarities of the respective lipid patterns, especially the 2 inositides (not examined for in the earlier CSF work) may strengthen this suggestion. The plasmalogen positive cardiolipin of CSF remains unexplained.

*Summary.* Some histochemical features of human choroid plexus are described and the lipid pattern of chloroform:methanol extracts of choroid plexus are compared with CSF. Two phosphoinositides were demonstrated in both specimens, and although cardiolipin is present in choroid plexus it is plasmalogen negative.

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## Measurement of Gamma Globulin in Germfree Guinea Pigs by Hemagglutination Inhibition. (28678)

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During experiments with germfree guinea pigs it became necessary to measure the gamma globulin of large numbers of animals with low serum gamma globulin concentrations. Because electrophoretic methods are not accurate for estimation of low levels of gamma globulin(1), a search was made for an immunochemical method which would be accurate at low levels, require only small amounts of serum, and could be carried out with minimum effort. Inhibition of the Coomb's reaction of human erythrocytes coated with specific anti-Rh antibodies, has been reported to be an accurate method(2-4) for measurement of low levels of gamma globulin in human sera. Latex particles coated with specific anti-human gamma globulin have been used to estimate serum gamma globulin concentrations, but this technique requires a specific anti-gamma globulin antibody, a complicated standardization procedure and has a graded endpoint(5). This report describes a hemagglutination inhibition procedure which is sensitive at low gamma globulin concentrations, can be applied to various species, has a sharp endpoint and, when combined with the microtiter dilution technique of Takatsy(6, 7), permits a rapid measurement of gamma globulin levels in large numbers of animals.

*Materials and methods. Preparation of gamma globulin.* Guinea pig gamma globulin was obtained by fractionation of pooled normal guinea pig serum by diethylaminoethyl cellulose chromatography(8). The first peak of protein was concentrated by ultra-filtration, dialyzed against 0.85% NaCl and tested for protein content by double diffusion in agar(9) and immunoelectrophoresis(10) against potent rabbit anti-whole guinea pig serum. Only a single gamma globulin precipitation line was obtained.

*Rabbit anti-guinea pig gamma globulin serum.* Antiserum was produced in rabbits by immunizing them with an aliquot of the