A Simple Method for the Study of Tryptophan Binding to Serum Albumin by Small-Scale Equilibrium Dialysis: Application to Animal and Human Studies

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The equilibrium between albumin-bound and freely diffusible tryptophan in blood is considered to be one of the major factors affecting the rate of tryptophan access into the brain, and hence the rate of synthesis of the neurotransmitter 5-hydroxytryptamine.

The large-scale equilibrium dialysis method used by McMenamy & Oncley (1958) in their original investigations of the albumin binding of tryptophan is unsuitable for either repeated investigations in human subjects, or investigations on small animals, because of the relatively large volumes of serum required. Curzon et al. (1973) and others have used ultrafiltration to estimate the proportion of tryptophan bound to albumin. Although this method uses only small amounts of serum, it is open to the criticism that it is a dynamic non-equilibrium method, and therefore may bear little resemblance to the equilibrium established in vivo. Further, it is not easy to estimate the binding parameters $K_d$ (the dissociation constant of the tryptophan–albumin complex) and $P_0$ (the concentration of binding sites available to tryptophan) by using such a method.

An equilibrium-dialysis method has been developed in this laboratory which allows determination of the proportions of tryptophan bound to albumin and freely diffusible, by using samples of serum as small as 60 µl. It is therefore appropriate for studies on mice and rats. Further, the apparatus is readily available, inexpensive, and can be treated as disposable; the plastic cups used are in fact the caps supplied with disposable plastic tubes and sample vials.

For studies on human serum, 300 µl of serum is pipetted into a plastic cup of 250 µl capacity, thus forming a convex meniscus. The cup is covered with a square of cellophane dialysis membrane, soaked in distilled water for several hours, and blotted dry immediately before use. This expels excess of serum leaving 250 µl inside. The cellophane membrane is secured in place by an open-ended plastic or glass tube, to create a second chamber, above the membrane. Then 100 µl of a solution of [14C]tryptophan (50 µCi/µmol, 0.1 µmol/ml in 0.15 M NaCl) is pipetted into the upper chamber covering the membrane. A rack of such dialyses is placed in an air-tight container, and left overnight at 4°C to allow equilibration. As there is a relatively large surface area (5 mm²), complete equilibration can be achieved within 8 h, without the need for agitation.

After equilibration, replicate 20 µl samples are withdrawn from the upper chamber, and, after removal of the upper tube and membrane, also from the lower chamber, for counting of radioactivity. A water-miscible scintillator is used (PPO (2,5-diphenyloxazole) 3 g, POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] 0.3 g, in 400 ml of 2-ethoxyethanol+600 ml of toluene); in the Packard Tri-Carb liquid-scintillation spectrometer used counting efficiencies in excess of 89% are usually observed.

Radioactivity in the upper chamber corresponds to diffusible tryptophan alone, and that in the lower chamber to diffusible+protein-bound tryptophan. It is thus possible to calculate the proportion of tryptophan bound to albumin, and, knowing the total concentration of tryptophan in the sample (determined fluorimetrically by the method of Denckla & Dewey (1967)), the concentration of tryptophan which is freely diffusible in the serum sample can be estimated.
For studies with mice, a smaller size of plastic cup has been used, and the volumes have been decreased to: lower chamber (serum) 55 µl; upper chamber ([14C]tryptophan) 20 µl; 10 µl samples are removed for radioactive counting.

By varying the amount of tryptophan in the upper chamber (0.2–1.0 mM, at a specific radioactivity of approx. 1.5 µCi/µmol), it is possible to estimate the dissociation constant of the tryptophan–albumin complex (K_{diss}) and the concentration of binding sites available to tryptophan in the serum sample (P₀), by Scatchard-plot analysis (Scatchard, 1949). In this way it has been shown that in 16 normal subjects the mean value of K_{diss} was 32 ± 1 µM⁻¹, and P₀ was 0.42 ± 0.09 mM. In ten chlorpromazine-treated chronic schizophrenics the mean value of K_{diss} was the same, but P₀ was decreased to 0.22 ± 0.03 mM, a highly significant difference (P < 0.001), indicating a non-competitive inhibition of tryptophan binding in these patients compared with normal subjects. Chlorpromazine added in vitro had no effect on the percentage of tryptophan bound to albumin, or on the estimated values of P₀ and K_{diss}. (Bender & Bamji, 1974).

Non-esterified fatty acids are known to displace tryptophan from albumin binding; increasing concentrations of potassium palmitate from 0.3 to 1.2 mM (within the normal physiological range of variation) increased the value of K_{diss} from 42 to 53 µM⁻¹, without affecting the value of P₀. This confirms the observation of McMenamy & Oncley (1958) that fatty acids are competitive with respect to tryptophan for albumin binding.

Studies on the effects of Δ⁹-tetrahydrocannabinol administered intraperitoneally to mice have shown that the drug has no effect on the percentage of serum tryptophan bound to albumin (A. P. Boulton, unpublished work).


Variations of Certain Lipids in the White Matter of Developing Brain

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There exists some evidence that the composition of plasmalogens and glycerol ether phospholipids varies with age in a way that does not parallel changes in total lipid (Svennerholm & Thorin, 1960). On the other hand, in certain parts of the white matter alterations in the number and composition of cells are taking place to a certain extent during brain development (Friede, 1961). The main purpose of this study was to investigate possible quantitative variations in total lipid and lipids containing ether in relation to age during the development of the brain.

Materials and methods

Various parts of the brain, namely the parietal and temporal lobes, corpus callosum and internal capsule, were obtained by anatomical preparation. All brains belonged to individuals free of clinical history of central or peripheral neurological disorders.

The method of Folch et al. (1957) was used for total lipid extraction and that of Thompson & Kapoulas (1970) for the determination of glycerophosphatides containing ether.

Results and discussion

In this study, white matter from the parietal and temporal lobes, the internal capsule and the corpus callosum of 55 human brains, belonging to individuals of different ages, was examined in order to determine possible quantitative changes in total lipid and ether-