Exit of Sulfanilic Acid and pAmino Hippuric Acid from Ventricular Fluid of the Dogfish

David P. Rall
National Cancer Institute

Studies reported last year showed that Sulfanilic Acid (SA), given by repeated intramuscular injections for 100 hours, failed to enter Ventricular cerebrospinal Fluid (VF) to an extent greater than 20% of the plasma concentration. The half-time to achieve this 20% ratio was about 10 hours. ParaAminoHippurate, (PAH) on the other hand, approached a VF plasma ratio of 100% with a t1/2 of about 50 hours. The relative exclusion of a compound indicates that some sort of active process is occurring to prevent the attainment of diffusion equilibrium. This year the exit rates of different concentrations of SA and PAH from the VF of the isolated perfused dogfish were determined. The dogfish was anesthetized with barbital. Fresh sea water ran into each spiracle via plastic tubing, perfused the gills and drained out the gill slits. The cranium was opened and 50-150 micro liters of drug solution at pH 7.4-7.8 were injected into the ventricular cavity with gentle barbotage. VF was then sampled at 30 sec. 15, 45, and 105 minutes and the concentration of SA or PAH was determined. At least 3 animals were used for each point in time with each drug concentration.

After 2.5 mg. of PAH, the exit half time was 110 min., after 2.5 mg. SA it was 85 min. When 0.2 mg. of drug was injected the half time's were 90 and 70 min. respectively. In a few experiments 3.0 mg Penicillin plus either 0.1 mg. PAH or SA was injected. Penicillin might be expected to compete with SA or PAH for a stereospecific transport system operating so as to remove drug from VF. Preliminary results indicated that the rate of disappearance of PAH and SA decreased by the penicillin.

These results do not exclude the possibility that SA and PAH might be removed from ventricular CSF by some stereospecific transport mechanism. Some mechanism, active in the broadest sense, must be operative since the exit rates are so much more rapid than the net entry rates.

(The excellent assistance of John Cohen and Richard Krugman is gratefully acknowledged.)

Permeability of the Blood-Cerebrospinal Fluid Barrier in the Dogfish to Sulfanilic Acid and pAminoHippurate

David P. Rall and Charles G. Zubrod
National Cancer Institute

Basic to the current hypotheses describing the blood-cerebrospinal fluid barrier is the supposition that this barrier is lipoidal in nature and is permeable to undissociated drug, but is impermeable to dissociated drug. This notion would be strengthened if it could be shown that a totally dis-

sociated compound were essentially excluded from CSF while a compound only slightly undissociated approached diffusion equilibrium. Previous studies have shown that both types of drugs are virtually excluded from CSF at 24 hours. Since 24 hours may be inadequate for equilibration the present studies have been extended to 96 hours. Sulfanilic acid (SA) and pAminohippurate (PAH) were compared. SA is often given a dissociation constant of 3.2, but is a strong acid, which is totally dissociated at body pH. PAH is a weak organic acid (pK=3.9); one part in 6,000 is undissociated at body pH. Plasma drug concentrations constant to ± 25% were maintained in dogfish by the subcutaneous injection of PAH every 8 hours, and SA every 12 hours. Three fish were studied with each drug at each time. Plasma, ventricular fluid (CSF), extradural fluid (EDF), bile, muscle and brain were sampled and analyzed by methods previously reported. The ratios of drug in fluid or tissue divided by mean drug plasma concentrations were calculated. At 96 hours the values with SA were CSF, 0.19; EDF, 0.85; bile, 0.54; brain, 0.18; muscle, 0.15. For PAH: CSF, 0.69; EDF, 0.83; bile, 1.74; brain, 0.20; muscle, 0.23. Ratios for SA at 24 and 48 hours were the same as at 96 hours. CSF and bile ratios for PAH were successively higher at 20, 40, 60 and 96 hours, while the ratios for EDF, muscle and brain were constant after 20 hours. These data clearly support the current hypotheses that the blood-CSF barrier may be lipoidal in nature, and is impermeable to dissociated drug, but is permeable to undissociated drug.

Transport of Organic Acid Dyes by the Isolated Choroid Plexus of the Spiny Dogfish, (S. Acanthias)

David P. Rall, Wendy Sheldon, and John M. Cohen
National Cancer Institute

Recent studies have indicated that organic acids are actively transported from the cerebrospinal fluid to the blood in the intact goat and in embryonal choroid plexus grown in tissue culture. It seemed of interest to determine if active transport of organic acid dyes could be demonstrated to occur *in vitro* in isolated choroid plexus tissue of a mature animal. This report presents evidence that the choroid plexus of the spiny dogfish can transport chlorphenol red across the choroidal epithelium. In this process the dye is concentrated in the lumen of the capillaries of the choroid plexus.

Fragments of dogfish choroid plexus about 1 mm. square were then obtained from all the ventricles and were placed in a dogfish Ringers solution. The usual concentration of the drugs was 3×10^{-5} molar. When the tissue was incubated in Ringers containing chlorphenol red, the lumen of the capillaries became bluish-red within 10-15 minutes. The color inside the capillaries unquestionably was more intense than the color of the surrounding solution. The cuboidal cells of the choroid plexus remained uncolored.

Incubation of the tissue at 2°C prevented the uptake of the dye and