bution of the (^{3}H) corticosterone fraction in different tissues. Distribution within the liver was different from that in other tissues. This difference is of interest because of the wide range of effects that result after glucocorticoid treatment. The subcellular distribution was similar in extrahepatic tissues whether they are recognized target tissues of corticosterone or not.

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Binding of Sulfobromophthalein Sodium (BSP) and Other Organic Anions By Isolated Hepatic Cell Plasma Membranes *in vitro*.* (31819)

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The movement of organic anions from blood to bile involves their transfer across the sinusoidal and bile canalicular portions of the plasma membrane of the parenchymal liver cell. Plasma membranes may be isolated from rat liver by differential centrifugation(1), and their lipid, protein, and enzymatic compositions have been investigated (2-5). Because the liver selectively and rapidly removes BSP and other organic anions from blood, the ability of isolated hepatic plasma membranes to bind BSP *in vitro* was investigated. Since several organic anions compete with BSP for hepatic uptake *in vivo*(6), their effect on BSP binding by isolated hepatic plasma membranes *in vitro* was also investigated.

Materials and methods. Cell membranes were isolated from rat liver according to the method of Neville(1). The final sucrose gradient centrifugation was deleted which permitted a better yield of membrane fraction for studies *in vitro*. Final membrane preparations were essentially free of nuclei, mitochondria, and microsomes when examined by phase and electron microscopy and cytochrome oxidase(7), glucose-6-phosphatase(8) and glucuronyl transferase activities (9) were absent or negligible. BSP binding by plasma membranes *in vitro* was investigated in an aqueous solution of 2.1 ml containing 1 mg BSP in 0.05 M Na_3PO_4 buffer at pH 7.4 and 100 λ of the membrane preparation. The mixture was incubated with constant shaking at 37°C for 10 minutes, immediately chilled to 3°C, and centrifuged for 10 minutes at 2000 $\times$ g. The supernatant was discarded and the membrane resuspended in phosphate buffer and gently mixed for 20 seconds. Following recentrifugation, the membrane was immediately harvested free of its supernatant and suspended in 0.1 M NaOH. Bound BSP was determined colorimetrically at 565 mμ.

Binding was expressed both as μg BSP bound per mg of membrane protein(10) and per unit of Co^{++}-stimulated cytidine monophosphatase (Co^{++}-CMPase) activity.‡ Because Co^{++}-CMPase was observed by Novikoff(11) to be concentrated in hepatic cell plasma membranes, this enzyme was used to estimate the quantity of membrane present

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‡ 1 Co^{++}-CMPase Unit = 1 μg P liberated per 15 min in incubation system(11).

as well as its relative purity. Following estimation of Co^{++}-CMPase activity in liver homogenates, the theoretical total BSP binding capacity by hepatic plasma membranes was calculated.

In competitive binding studies *in vitro* between BSP and a series of organic anions (sodium fluorescein, indocyanine green, bilirubin, sodium taurocholate, flavaspidic acid glucaminate, and iodipamide methylglucamine), the incubation mixture contained 500 μg of BSP and equimolar amounts of the aforementional organic anions. Interference with BSP binding was expressed as the percent of BSP not bound to membrane as compared with control samples in each membrane trial with BSP alone.

Results and discussion. On the average, 160 $\pm$ 2 μg BSP was bound per mg of membrane protein and 1.4 $\pm$ 0.1 μg BSP bound per unit of Co^{++}-CMPase activity in 15 membrane isolates. BSP binding was constant between pH 7 to 8, whereas increased binding occurred at lower pH's. BSP binding was maximal at 500 μg BSP/ml in the incubation mix with proportionately less binding observed at lower concentrations. BSP concentrations above 500 μg/ml reduced BSP binding and produced dissolution of cell membranes. Maximum BSP binding was complete by 3 minutes and did not increase on incubation up to 120 minutes. BSP binding was not enhanced by addition or substitution of K^+, Ca^{++}, Mg^{++}, or Cl^- ions to the buffer system at 0.05 M; however, binding was greatly depressed by substitution of distilled water for the Na_3PO_4 buffer.

On the average, a 69-fold increase in Co^{++}-CMPase activity was observed in 29 isolated membrane fractions as compared with the enzyme activity in the original liver homogenates. On the basis of total Co^{++}-CMPase activity in each of 15 rat livers, 3.3 $\pm$ 0.34 mg of BSP by calculation would have been bound to membranes derived from 10 g of rat liver. The rapid disappearance of 1.25 mg of BSP when injected intravenously into a 250 g rat with a 10 g liver (5 mg/kg) is not surprising.

Competitive inhibition between BSP and several organic anions for binding by hepatic plasma membranes *in vitro* is presented in Table I. Many organic anions interfere with either BSP uptake or excretion by the parenchymal liver cell(6). It was of interest that anions such as taurocholate(12,13), bilirubin (14) and fluorescein(15), which do not compete for hepatic uptake with BSP when simultaneously injected *in vivo,* exhibited little or no interference with BSP binding by hepatic cell plasma membranes *in vitro.* By contrast, other organic anions, such as indocyanine green(16), flavaspidic acid glucaminate (17), and iodipamide methylglucamine(18), which compete with BSP for hepatic uptake *in vivo,* significantly reduced BSP binding by isolated membranes *in vitro.* These observations suggest that studies of organic anion binding by isolated liver cell plasma membranes may be useful in studying the mechanism of transfer of organic anions from blood to bile.

TABLE I. Competition by Equimolar Amounts of Various Organic Anions with BSP for Binding by Rat Liver Plasma Membranes *in vitro.*

Organic anion	Exp No.	Average interference with BSP binding (%)
Sodium taurocholate	4	0
Bilirubin	4	0
Sodium fluorescein	3	5
Iodipamide methylglucamine	3	49
Indocyanine green	4	51
Flavaspidic acid glucaminate	4	64

Summary. Isolated, hepatic cell plasma membranes bound on average 160 $\pm$ 2 μg of sulfobromophthalein (BSP) per mg of protein. Taurocholate, bilirubin and fluorescein, which do not compete for hepatic uptake with BSP when simultaneously injected *in vivo,* exhibited little or no interference with BSP binding by hepatic cell plasma membranes *in vitro.* Other organic anions, such as indocyanine green, flavaspidic acid glucaminate, and iodipamide methylglucamine, which compete with BSP for hepatic uptake *in vivo,* significantly reduced BSP binding by isolated membranes *in vitro.*

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Suppressive Effect of Anti-Allergic Mediators on Experimental Encephalomyelitis.* (31820)

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Experimental allergic encephalomyelitis (EAE) is an autoimmune response induced in animals and man by the injection of central nerve tissue, or a basic protein extracted from central nerve myelin, most often in combination with Freund's adjuvant containing tubercle bacilli(1). After approximately 2 weeks, clinical symptoms including weight loss, paresis, incontinence and ascending paralysis follow the development of central nervous system lesions essentially marked by perivascular lymphocytic cuffing. The immune mechanism is a cellular response, passive transfers having been accomplished successfully only with viable lymphocytes in immunologically receptive animals(2-5).

However, the role of antibody, either produced or transported by infiltrating cells, has not been excluded from an inducing role or, at the least, as an enhancing factor(6). In the event that whole tissue is used to induce disease, antibodies to non-myelin brain tissue or vascular elements may possibly initiate a local inflammatory response. Increased vascular permeability, due to released mediators, may facilitate the infiltration of myelin-sensitized lymphocytes. Myelin antibody might also enhance tissue damage by infiltrating the parenchyma following the lymphocytic perivascular invasion. At any rate, if a local antigen-antibody reaction does influence the course of EAE, then pharmacologic mediators may also be involved. Histamine and serotonin(7,8), perhaps the best characterized mediators of hypersensitivity, have consequently been investigated for their effect on the occurrence and severity of EAE.

Although anti-histiminic agents, which compete with histamine for active cellular sites, have been found ineffective in EAE (9-11), it was of interest to study the effect of depletion of available histamine which has recently been shown to occur in guinea pigs treated with repeated doses of anaphylatoxin. This treatment renders these animals refractory to passive anaphylactic shock for about

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