It is generally believed that apolar substances, by their lipotropic virtue, penetrate through biological membranes and distribute widely in the body, whereas polar substances have limited access to the central nervous tissue. Thus, the term blood brain barrier is designated to describe a lipid-containing membrane or barrier which limits the accessibility of polar substances to the brain. This concept holds well in most cases. In a few instances, however, fairly liposoluble substances such as morphine (1, 2) demonstrate a low distribution to the brain. On the other hand, we have observed that chlorpromazine sulfoxide, a polar substance, was found in the brain in a substantial amount following an intraperitoneal injection to rats, even after a rigorous perfusion of the brain with hypertonic as well as isotonic NaCl or KCl solutions (unpublished observations). These facts raised the question whether liposolubility per se is the sole factor determining the permeability of a drug through a blood brain barrier.

In the present study, we examined the distribution of methylhydroxycoumarin (4-methyl-7-hydroxycoumarin) in the rat. This compound is an agent which has been claim-
ed to protect liver fibrosis due to carbon tetrachloride, to facilitate a bile secretion, and to have no anticoagulant activity (3-5). It has a hydroxyl group on the benzene ring and is intermediate in terms of liposolubility.

Male Sprague-Dawley JCL strain rats, weighing 200 to 250 g, were given 50 mg of methylhydroxycoumarin (1% suspension in 0.5% carboxymethylcellulose solution) per kg of body weight, by a stomach tube. Under ether anesthesia, blood was collected from abdominal aorta in a heparin-treated syringe. Brain, lung, heart, liver, kidney and skeletal muscle from a hindleg were excised, rinsed in a saline solution, blotted and weighed. Urine was collected from the individual rats using a metabolic cage. After the collection period, the cage was flushed with water and the total volume of the sample was adjusted to 30 ml. This was further diluted 30-fold before assay.

Extraction and fluorometric estimation of free and conjugated methylhydroxycoumarin were performed as described by Fontaine et al. (6). Free methylhydroxycoumarin was estimated using 0.5 g of the tissue or one-half of the heart. The tissue was homogenized in 5.0 ml of ethanol using a glass homogenizer. The homogenate was centrifuged at 950 xg for 5 minutes and the supernatant fluid was evaporated under nitrogen stream. The residue was dissolved in 2.5 ml of a chloroform-methylisobutylketone (4:1) mixture and filtered through a filter paper. The filtrate was shaken with 6.0 ml of 0.1 M glycocoll-NaOH buffer (pH 10.5) and the fluorescence of the buffer phase was assayed. Plasma was obtained by the centrifugation of heparinized blood and 2.0 ml were passed through a chromatographic column containing 3.6 grams of Sephadex G-25, and eluted with 0.1 M glycocoll-NaOH buffer (pH 10.5). After discarding the first 10 ml portion, 30 ml of eluate were collected and assayed for the fluorescence. A 2.5 ml aliquot of diluted urinary sample was adjusted to pH 3.0 with hydrochloric acid and extracted twice with 5.0 ml each of the chloroform-methylisobutylketone (4:1) mixture. The organic phases were combined and shaken with 10 ml of 0.1 M glycocoll-NaOH buffer (pH 10.5). Total methylhydroxycoumarin was estimated after a sulfatase hydrolysis of the conjugated form. Sulfatase (Sigma Chemical Company, St. Louis, Mo., Type H-2), 750 units in 0.2 ml, was added to a mixture containing 1.0 ml of 2.0 M acetate buffer (pH 5.0) and 1.0 ml of a sample, namely, plasma, diluted urine or 10% water homogenate of tissues. The mixture was incubated at 37°C for 24 hours and then added to 1.3 ml of 0.5 N NaOH, and centrifuged at 10,000 xg for 10 minutes. A 2.0 ml aliquot of the supernatant fluid was passed through a column of Sephadex G-25 as described above.

Fluorescence intensity was assayed using a Shimadzu Model GSS-16 spectrofluorometer, setting the activation and emission wavelength dials at 365 and 450 nm, respectively, and compared with those of known standards (authentic methylhydroxycoumarin dissolved in the glycocoll-NaOH buffer).

The cumulative amounts of free methylhydroxycoumarin recovered from urine after an oral administration of 50 mg/kg in rat were 2.96±0.50, 3.04±0.49 and 3.12±0.49% of the administered dose (mean±S.E.M. of 4 experiments) after 24, 48 and 72 hours, respectively. After the hydrolysis with sulfatase, however, these values were 85.7±3.9,
87.0±3.7 and 94.6±2.2%, respectively. Hence, the major metabolic pathway for this compound was a sulfate conjugation. Since almost complete recovery of methylhydroxy-coumarin should be attainable after a sulfatase hydrolysis, this value was referred as the total amount in the following text.

After an oral administration of methylhydroxy-coumarin, the plasma level of conjugated, as well as free, methylhydroxy-coumarin rose rapidly (Fig. 1). More than 97% of methylhydroxy-coumarin in plasma was in the conjugated form at 15 minutes indicating that the sulfate conjugation was unexpectedly rapid following this route of administration. Among the tissues examined, kidney had the highest concentration of the total and free methylhydroxycoumarin. Concentrations of total methylhydroxy-coumarin in liver, lung, cardiac muscle, skeletal muscle and brain were lower than that in plasma. There was a marked difference in the levels of total methylhydroxy-coumarin among these tissues (Fig. 1A). The brain level was 1 to 2 percent of the plasma level.

The distribution of free methylhydroxycoumarin was markedly different from that of the total methylhydroxycoumarin (Fig. 1B). In general, the level of the free form in the brain was not statistically different from those in cardiac muscle, lung and skeletal muscle, and was 20 to 30 percent of that in the plasma. Free form of the drug was predominant in the brain. Thus, only the free form of the drug penetrated into the brain and the penetration through the blood brain barrier was rather easy. Hence, apparent
impermeability of methylhydroxycoumarin through the blood brain barrier was due to
the predominance of impermeable metabolite, sulfate conjugated form, in plasma. Fur-
thermore, it should be noted that the brain level of free methylhydroxycoumarin decreased
parallel with the decrease in the plasma level, even though the plasma level was higher
than that in the brain. This indicates either one of the following two cases: 1. the presence
of a mechanism which extrudes methylhydroxycoumarin from the brain. If this were
the case, penetration of free methylhydroxycoumarin into the brain should be better than
the observed value, or, 2. in some compartment of the brain, which is accessible only by
the free form, the level of free methylhydroxycoumarin is very close to that in the plasma
and a rapid equilibrium exists between this compartment and the plasma.

In either case, free methylhydroxycoumarin, which is rather low in liposolubility,
penetrates through the blood brain barrier easily, although its lipid insoluble metabolite,
the conjugated form, does not gain access to the brain. Thus, the threshold of the blood
brain barrier, in terms of liposolubility, was lower than that generally believed. It may
be that the limited access to the brain of more liposoluble substances is due to the faster
metabolism of those compounds into polar forms. It may be postulated that the accessibility
of drugs to drug metabolizing enzymes in the liver increases with the increasing
liposolubility (7).

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ACTIONS OF CERTAIN POLYPEPTIDES
ON FROG SPINAL NEURONS

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Pharmacological actions of polypeptides have recently been extensively studied. How-
ever, relatively little is known about the effects of polypeptides on neurons of central nerv-
ous system (CNS). Several pharmacologically active polypeptides are known to exist
in mammalian CNS (1). Furthermore, Lembeck and Zetler suggested the possible trans-
mmitter role of Substance P which is known to be polypeptide(s) in nature (2). In the present