Effect of sulpiride on cumulative volume of urine excreted in control and hypophysectomized rats

Treatment	n	Hours after dosing (ml urine/kg b.wt) 1	2	3	4	5	6	7
Normal control	5	1.3±2.0	5.5±3.8	6.2±4.2	8.6±3.1	9.6±1.6	11.4±3.0	14.1± 6.0
Normal+sulpiride	4	0	0	0	0	2.6±5.3	11.7±9.3	17.5±10.9
Hypophysectomized control	5	0	4.0±5.6	13.2±5.5	15.7±4.5*	19.0±6.0**	21.3±6.7*	22.5± 5.2
Hypophysectomized sulpiride	5	0	0	0	0	0	0	0

Data means± SD. * $p<0.02$ compared to normal control. ** $p<0.01$ compared to normal control.

Urine volume was expressed in ml/kg. Significance of the differences between means was determined by Student's t-test.

Results and discussion. Initial studies in rats which were not saline-loaded showed that either the oral or parenteral administration of sulpiride caused a reduction in urine volume for up to 6 h depending upon the dose. However, the urine volumes in the untreated nonhydrated rat were too low to permit any quantitative evaluation of a drug effect. Thus, in subsequent studies we used the saline-loaded rat. The inhibitory effect of sulpiride in this model was observed with doses greater than 120 mg/kg orally and at 40 mg/kg i.m. or i.p., doses which are considerably below the acute LD_{50} of sulpiride in the rat[9]. The pharmacologic effect observed with sulpiride is referred to herein as an antidiuretic effect since the measured effect was antagonism of saline-induced diuresis. Moreover, rats which failed to excrete urine during the test period were found to have empty urinary bladders at necropsy indicating that sulpiride inhibited urine formation and not micturition.

An i.p. dose of 120 mg sulpiride/kg inhibited urine excretion completely for up to 5 h in normal rats (table). The period during which there was essentially complete absence of urine excretion was followed by a period of marked diuresis so that by the 7th h the cumulative urine output by the sulpiride-treated rats was similar to that of the controls. Urine excretion was inhibited for at least 7 h in hypophysectomized rats. The seemingly greater inhibitory effect in the hypophysectomized rats occurred despite the fact that the untreated hypophysectomized rats excreted significantly more urine than did the untreated normal rats the first 6 h of the test.

In order to determine whether the antidiuretic effect observed with sulpiride was a nonspecific effect of high doses of antipsychotic drugs, haloperidol, clozapine, pimozide and chlorpromazine were tested at doses of 120 mg/kg i.p. None of these antipsychotic drugs inhibited the saline-induced diuresis.

Although it is not possible to conclude on the basis of the present studies that the mechanism of the sulpiride-induced antidiuretic response observed in humans and rats is the same, demonstration of the antidiuretic effect in the hypophysectomized rat indicates that the effect is not prolactin-mediated. Kohli et al.[3] have shown recently that sulpiride is a potent antagonist of dopamine stimulated renal vasodilation in the dog. Thus, the antidiuretic effect of sulpiride may be due to a direct effect on the renal vasculature.

Although we are not aware of any toxic manifestations attributable to the transient antidiuretic effect of sulpiride in the rat, the fact that this response has been observed in healthy women[8] and the report of hypertensive attacks occurring in some hypertensive patients receiving sulpiride[10], suggest that additional studies on the renal effects of sulpiride are warranted.

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Penetration barrier to sodium fluorescein and fluorescein-labelled dextrans of various molecular sizes in brain capillaries[1]

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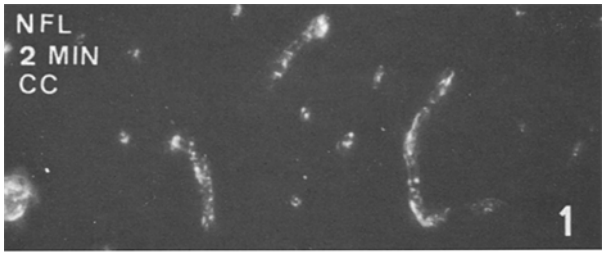
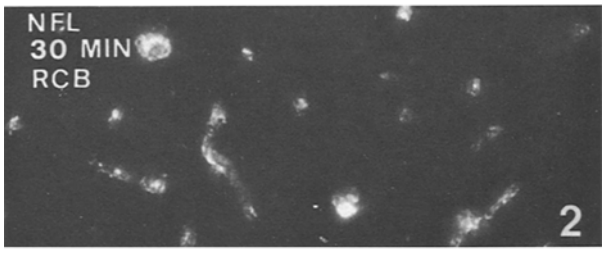
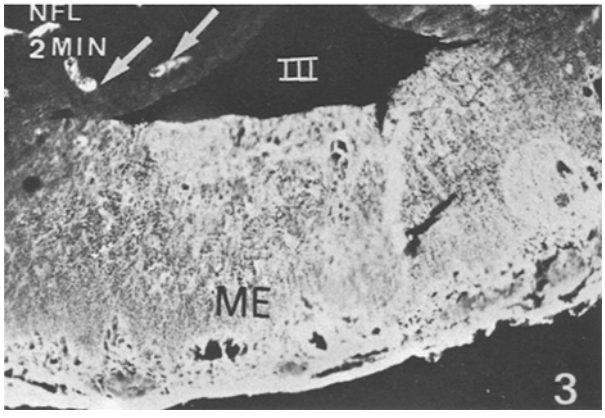
Summary. The permeability of the blood-brain barrier to sodium fluorescein, or fluorescein-labelled dextrans of various molecular weights, was investigated. Unlike the capillaries in both the area postrema and the eminentia mediana, the capillaries of the cerebral cortex were impermeable to all the intravenous tracer substances used.

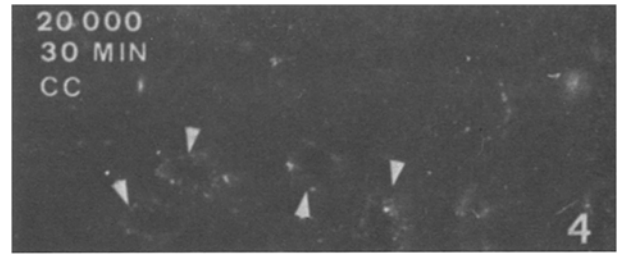
In most brain regions, including the cerebral cortex, the blood-brain barrier (BBB) prevents diffusion of lipid-insoluble particles from the blood stream into the brain parenchyma. This barrier has been localized histologically mainly in the capillary endothelium[2-4].

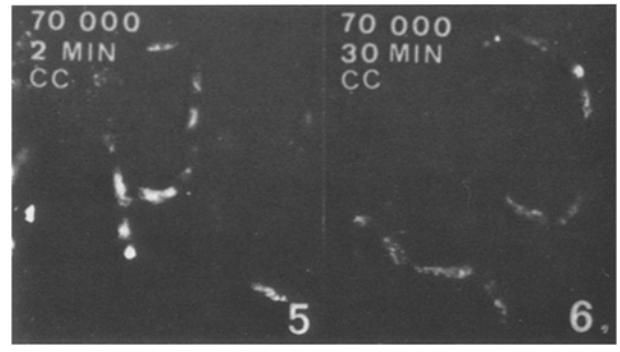
The transcapillary transport of lipid-insoluble particles takes place through small pores or channels of the capillary endothelium in most tissues of the body[5]. On the basis of theoretical calculations from physiological data, Fenstermacher and Johnson[6] proposed that pores with a diameter











Figures 1-6. Fluorescence due to Na-fluorescein (NFL) or FITC-Dextrans (mol. wt is indicated in appropiate figures) in the cerebral cortex (CC) or eminentia mediana (ME; figure 3) of the rat brain. Fig. 3. The non-permeable brain capillaries are marked with arrows. Fig. 4. The arrowheads point at capillaries with weakly fluorescent endothelium. The time (either 2 or 30 min) after an injection with the tracer is indicated in each figure. RCB: excretion of urine has been stopped by closing the renal vessels bilaterally. Magnifications. Figures 1, 2, 5 and 6: ×310; figure 3: ×155; figure 4: ×550.

of about 7 Å might exist in the cerebral capillaries. Morphological studies have not revealed such pores[3].

In order to study the correlation between mol. wt, anatomical location, and permeability of graded mol. wt substances through capillaries of the cerebral cortex, eminentia mediana and area postrema, sodium fluorescein and fluorescein-labelled dextrans of various molecular sizes were used as i.v. tracers.

Material and methods. 30 adult albino Sprague-Dawley rats of both sexes were injected either with sodium fluorescein diluted in 0.6 ml of saline (Merck, Germany) or with equal volume of fluorescein-labelled dextrans (FITC-Dextrans, Pharmasia Fine Chemicals, Sweden) of graded mol. wt. The animals were not exsanguinated before the tracer infusion. In preliminary experiments with sodium fluorescein, 0.6 ml of blood was aspirated before the injection with the tracer. However, omission of exsanguination changed neither the permeability of the blood-brain barrier nor the capillary fluorescence. FITC-Dextrans were prepared by the factory from very narrow fractions of dextran obtained by high resolution gel filtration. The tracers used in the present work, their mol. wts, dosage and Stokes' radius are shown in the table.

Injections were given into the femoral vein and the animals were killed by cutting the neck 2 or 30 min after the injection under ether anaesthesia. In some experiments, the renal was blocked by ligating the renal artery circulation and vein of both kidneys just before injection with a tracer. Subsequently pieces of cerebral cortex, area postrema and eminentia mediana were immediately excised and quickly frozen in liquid nitrogen. The specimens were then freeze-dried at $-45\,^{\circ}C$ in a vacuum of 10^{-4} mm Hg for 1 week. After embedding in paraffin wax, 5-µm thick sections were cut and mounted on glass slides and deparaffinized with xylene. The sections were viewed with a Leitz Ortholux fluorescence microscope equipped with an HBO 200 mercury lamp and a TK 150 dichroic mirror. The primary filters were BG 38, BG 12 and a KP 500 interference filter, while a Leitz K 510 served as a secondary filter.

Results. Regardless of the mol. wt of the tracer used, the specific fluorescence was observed inside the capillaries of the cerebral cortex 2 min after the injection (figures 1 and 5). All the labels used penetrated through the capillaries of both the eminentia mediana and the area postrema, which was indicated by the fact that these brain regions fluoresced intensely (figure 3).

	Molecular weight	Dose (mg/kg in 0.6 ml of saline)	Stokes' radius (Å)	Number of animals examined
Sodium fluorescein	376	48	5.5	10
FITC-Dextran 3	3000	48	12	4
FITC-Dextran 20	20000	48	30	4
FITC-Dextran 40	40000	48	45	4
FITC-Dextran 70	70000	48	60	4
FITC-Dextran 150	150000	48	80	4

Sodium fluorescein and fluorescein-labelled dextrans with a mol. wt smaller than 70,000, fluoresced more weakly 30 min following the injection than 2 min after it (figure 4). Dextrans 70,000 and 150,000 also fluoresced intensively 30 min after the injection (figures 5 and 6). Specific fluorescence was never observed in the parenchyma of the cerebral cortex.

If both the artery and vein of both kidneys were ligated before injection of the tracer, even the smallest label, sodium fluorescein, fluoresced brightly in the cortical capillaries 30 min after the infusion but did not, however, leak into the brain tissues (figure 2). In all experiments, the parendryma of cerebral cortex showed only a weak background fluorescence.

Discussion. Fluorescein-labelled dextrans are very stable molecules[7,8] and suitable for microcirculation studies[7,9]. Several successful methods for localizing sodium fluorescein in freeze-dried specimens habe been reported[9-12]. In the present study, the freeze-drying technique of Rodriquez-Peralta[11] and Baurman[12] yielded a good localization of the label fluorescence and well reproducible results with both Na-fluorescein and FITC-Dextrans.

Neither Na-fluorescein nor any of the FITC-Dextrans used penetrated the BBB. Similar results, concerning the permeability of retinal vessels to Na-fluorescein[13] or to FITC-Dextrans[9], have been reported previously.

The fluorescence in the capillaries of the cerebral cortex was weaker 30 min rather than 2 min after an injection with a tracer smaller than FITC-Dextran 70,000. This seems to be explained both by re-distribution of the marker into other tissues of the body and by its elimination through renal excretion. In man, dextrans with a mol. wt smaller than 50,000 are rapidly excreted into urine[14]. In the present study the fluorescence due to the smaller tracers (mol. wt < 70,000) persisted for over 30 min in the cortical capillaries if both the renal artery and vein were closed bilaterally. This operation is very likely to be followed by disruption of the BBB. This, however, did not take place during the first 30 min.

Both the eminentia mediana and the area postrema were brightly fluorescent as early as 2 min after an injection with any of the tracers, which indicates that the fenestrated capillaries in these brain regions are permeable to all the labels used and that 2 min was not too short a time for demonstrating transcapillary leakage.

Both Na-fluorescein and fluorescein-labelled dextrans proved to be reliable and suitable for blood-tissue barrier research. It seems that the transcapillary vesicular transport[15] rather than the possible 7-Å pores proposed before[6], are involved in the exchange of water-soluble particles through continuous brain capillaries[16]. However, non-fenestrated capillaries and barrier systems with pore size inside the range of dextran molecules will undoubtedly be an even more suitable subject for studies with FITC-Dextrans of graded mol. wts.

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Hepatocellular iron-containing deposits in relatives of patients with latent idiopathic hemochromatosis: a qualitative and quantitative approach[1]

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Summary. The quantitative and qualitative study in electron microscopy of hepatic fragments taken in apparently healthy collaterals of idiopathic hemochromatosis, shows the presence of siderosis, undetectable by Perls reaction comparatively with normal liver samples.

Previous clinical[3,4] and histological[5,6] studies of hemochromatosis relatives have already been made. Hepatic biopsy, associated with ferritine determination[7], is one the most important criteria to appreciate the iron overload[8] in the liver and to detect the nonsymptomatic stades of the disease. This morphological investigation attempts to clarify the cellular mechanisms of tissular overload which remain obscure.

The purpose of the present study is to correlate an electron microscopy quantitative approach of the ferric inclusion in the hepatocytes[9], with a light microscopy evaluation of heterogenous lipofuscin[10] aggregates in hemochromatosis. This correlation is made between the liver of the relatives of patients with latent idiopathic hemochromatosis and patients with normal liver.

Material and method. The propositus (table 1, II A 1), 32 years old, was admitted to the hospital for hepatomegaly and diabetes. His seric iron level was 275 µg/ml and the needle hepatic biopsy showed, after Perls reaction, a massive ferric overload, associated with fibrosis.