In *vivo*, the transport of \[^{14}C\]riboflavin into and from the isolated choroid plexus, the anatomical locus of the blood-cerebrospinal fluid barrier, was studied. With concentrations of \[^{14}C\]riboflavin of 0.7 \(\mu\)M (or greater) in the incubation medium, the choroid plexus accumulated \[^{14}C\]riboflavin against a large concentration gradient by a process that did not depend on binding or intracellular metabolism of the \[^{14}C\]riboflavin. The \[^{14}C\]riboflavin accumulation process in isolated choroid plexus could be described by Michaelis-Menten transport kinetics \((k_t = 78 \mu\text{M} \text{min}^{-1}, Y_{\text{max}} = 1.65 \text{nmol} \text{mg}^{-1} \text{hr}^{-1})\) and was inhibited by other flavins and probenecid but not by ribose, weak bases, or other B vitamins. The accumulation process was markedly depressed by iodoacetate and low temperatures. With a concentration of 0.08 \(\mu\)M \[^{14}C\]riboflavin in the incubation medium, 28% of the \[^{14}C\]riboflavin within the choroid plexus was converted to \[^{14}C\]FAD or \[^{14}C\]FMN intracellularly. Unlike the active transport of \[^{14}C\]riboflavin into choroid plexus, accumulated \[^{14}C\]riboflavin was metabolized by choroid plexus by a process independent of intracellular concentration or temperature. The efflux of \[^{14}C\]riboflavin from choroid plexus could be described by first order kinetics with a rate constant of \(-0.08 \text{ min}^{-1}\).

Riboflavin, an essential component of brain, is not synthesized in mammalian tissues and must enter brain and CSF from the blood (1, 2). In mammals, the concentrations of total riboflavin in brain, CSF, and plasma are approximately 8.8, 0.1, and 0.2 \(\mu\)mol/liter, respectively (1, 2). In rat brain, greater than 90% of the total riboflavin is present as FAD (Table I). The concentration of total riboflavin in brain (unlike liver and kidney) is maintained relatively constant (3). For example, the induction of severe riboflavin deficiency in rats resulted in marked depletion of total riboflavin in liver and kidney but not in brain (3). The constancy of the concentration of total riboflavin in brain in severe riboflavin deficiency is not due to slow turnover of total riboflavin since about 10% of the total riboflavin in brain of normal rats turns over/h (1). On the other hand, the injection of massive amounts of riboflavin (133 \(\mu\)mol/kg) into rats resulted in a small increase in the total riboflavin concentration in brain (1). These data suggest that a potent homeostatic mechanism(s) regulates the concentration of total riboflavin in mammalian brain.

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\(^{1}\) The abbreviations used are: CSF, cerebrospinal fluid; total riboflavin, sum of riboflavin, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD); T/M, tissue/medium ratios.

The overall purpose of the present studies was to investigate whether regulation of entry (and exit) or riboflavin into (and from) the central nervous system could be a part of the mechanism that controls total riboflavin levels in brain. As a first step, we investigated whether the choroid plexus, an anatomical locus of the blood-CSF barrier (4), might be a locus of riboflavin transport from blood to CSF. The choroid plexus is known to be involved in the transfer of several water-soluble vitamins from blood into CSF (e.g. ascorbic acid and folates) by specific, saturable, carrier-mediated processes (5-7). Frequently, substances that are concentrated by the isolated choroid plexus in *vivo* are transported between blood and CSF in *vivo* (5). Specifically, in the present study, we investigated whether the isolated rabbit choroid plexus could accumulate and release \[^{14}C\]riboflavin in *vivo*. Our experiments showed that the isolated choroid plexus contains a potent active transport system for riboflavin. In the present studies, the word "transport" refers to the substrate (vitamin) crossing the cell boundary, whereas "accumulation" and "uptake" refer to the observed level of intracellular vitamin.

**Experimental Procedures**

The concentrations of total riboflavin and the riboflavin vitamers in plasma, CSF, and choroid plexus are shown in Table I. In plasma and CSF, approximately 25 to 30% of the total riboflavin was present as riboflavin; in choroid plexus, as in most solid tissues including brain (1), little (6%) of the total riboflavin was present as riboflavin, whereas 4% was present as FAD (Table I).

The ability of the isolated choroid plexus to accumulate \[^{14}C\]riboflavin with either 0.7 \(\mu\)M or 0.1 \(\mu\)M riboflavin in the medium is shown in Fig. 1. The rate of accumulation was not linear and gradually declined over time at both concentrations. The accumulation of \[^{14}C\]riboflavin by choroid plexus was not due to the ability of the isolated choroid plexus to metabolize \[^{14}C\]riboflavin. When choroid plexuses were incubated for 45 min to 0.7 \(\mu\)M \[^{14}C\]riboflavin, 98 \pm 1% (S.E.; \(N = 6\)) of the \(^{14}C\) within the choroid plexus emerged with the riboflavin fraction on column chromatography. The results of the column chromatography were confirmed by thin layer chromatography since no \[^{14}C\]FMN or \[^{14}C\]FAD was detected in choroid plexuses incubated for 45 min in 0.7 \(\mu\)M \[^{14}C\]riboflavin. No [\(^{14}C\)]lumiflavin or [\(^{14}C\)]lumichrome was detected either. However, when choroid plexuses were incubated in 0.08 \(\mu\)M \[^{14}C\]riboflavin for 30 min, T/M ratios of 27.5

\(^2\) Portions of this paper, including "Experimental Procedures," Figs. 1 to 4, and Tables I and II, are presented in miniprint following the discussion. Miniprint can be easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20014. Request Document No. 79M-1143 cite author(s), and include a check or money order for $1.65 per set of photocopies.
The concentration of total riboflavin in CSF is approximately 50% of the concentration of total riboflavin in plasma (Table I). Our results of total riboflavin in plasma and the percentages as FAD and FMN are similar to those of other investigators (2, 11). Since approximately 50% of the total riboflavin in plasma is bound to serum proteins (mainly albumin) (14), the unbound plasma concentration of total riboflavin is approximately equal to the CSF concentration. Because CSF is constantly leaving the central nervous system (total turnover time, approximately 8 h) (4), riboflavin (or its vitamins) must continually be supplied to the newly formed CSF. In view of the large size and very poor lipid solubility of riboflavin, and, even more so, of FMN and FAD, the entry of riboflavin and its vitamins into CSF and brain via carrier-mediated or other specialized processes appeared reasonable.

The choroid plexus, a locus of blood-CSF barrier, was a possible site for the hypothesized riboflavin transport system. In fact, the isolated choroid plexus vigorously accumulated [14C]riboflavin in vitro (Table II). The accumulation process for [14C]riboflavin did not depend on metabolism or intracellular binding of the [14C]riboflavin. Moreover, the transport process in choroid plexus for riboflavin was almost totally inhibited by iodoacetate and cold temperature (Table II and Fig. 3). The transport process was specific in that other flavins and probenecid inhibited riboflavin transport into choroid plexus but weak bases and several other vitamins did not (Table II). Finally, after subtracting off the nonsaturable transport process (presumably simple diffusion), the saturable transport process could be described by a Michaelis-Menten transport model with a $K_T = 79 \mu M$ and a corrected $Y_{max} = 1.65 \text{ mmol kg}^{-1} (15 \text{ min})^{-1}$. Thus, the choroid plexus accumulation system for riboflavin satisfies all the criteria for active transport (15).

The efflux of [14C]riboflavin from choroid plexus could not be depressed by increasing the intracellular concentration of riboflavin from 13 to 964 \text{ pmol of riboflavin/kg of choroid plexus} or by lowering the temperature of the efflux medium to 7°C (Fig. 4). Thus, the efflux of riboflavin from choroid plexus followed first order kinetics with an efflux constant approximately equal to 0.08 min$^{-1}$ (Fig. 4) (15). These results, however, do not exclude a carrier-mediated efflux transport system with a $K_T$ (one-half saturation concentration) much greater than 1 mM or much less than 10 \text{ pmM}.

Thus, there are, at least, three processes that regulate the riboflavin concentration in the isolated choroid plexus in vitro if the concentration of riboflavin in the medium is greater than 0.7 \text{ \mu M} to prevent any metabolism; active transport into choroid plexus (Fig. 2), nonsaturable efflux from choroid plexus (probably simple diffusion) (Fig. 4) and simple diffusion into choroid plexus. At concentrations below 0.7 \text{ \mu M} riboflavin in the medium, metabolism of the [14C]riboflavin within choroid plexus to [14C]FMN and [14C]FAD and the endogenous flavin concentration would need to be taken into account.

The above described studies were performed at concentrations, at least, 5 to 10 times higher than the total riboflavin concentration in CSF and plasma (Table I). At lower (physiological) concentrations, some of the [14C]riboflavin accumulated by choroid plexus was metabolized to FAD and FMN; however, these results do not exclude FMN or even FAD entering choroid plexus cells directly. Similarly, our finding that lumiflavin, FAD, and probably FMN inhibit [14C]riboflavin uptake by choroid plexus (Table II) do not show that these substances actually enter choroid plexus by the active transport system for riboflavin. Further studies will be required to establish this point.

The above studies, although suggestive, do not establish
the role of the choroid plexus in transporting riboflavin or its vitamers between blood and CSF. Accumulation studies with the isolated choroid plexus as with kidney slices do not show sidedness, i.e. the direction of transport from blood to CSF or vice versa (4). Moreover, it is possible that the active transport system for riboflavin in the choroid plexus is present to provide riboflavin exclusively for the internal use of the choroid plexus. Further studies in vivo will be required to establish this point.

At a plasma concentration of riboflavin above 0.5 μM, the kidney secretes riboflavin from blood into urine (14). The secretion of riboflavin by the kidney is inhibited by probenecid but not weak bases (14). In the choroid plexus, probenecid but not weak bases also inhibit riboflavin transport (Table II). In a very elegant study, yeast, like choroid plexus, was shown to contain an active transport system for riboflavin similar in some respects to riboflavin transport systems in kidney and yeast. The physiological role of this system remains to be established.

REFERENCES

References are found listed below in the miniprint.
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