Para-aminohippuric acid (PAH) accumulates against a concentration gradient in the ciliary body and independently in the iris of the rhesus monkey eye. This accumulation is inhibited by incubation at 0° C and shows saturation kinetics in both tissues. Cyanide, ouabain, dinitrophenol, iodopyracet, and probenecid effectively depress PAH uptake in both tissues, but anaerobic incubation conditions have little effect on uptake in either tissue. The washout of preaccumulated PAH occurs 2.5 times faster from the iris than from the ciliary body. The effects on washout of 10^-4 M PAH, 0° C, and 10^-4 M dinitrophenol are consistent with washout occurring by a diffusional mechanism in both tissues, with some reaccumulation occurring in the ciliary body only. In addition, nonsaturable uptake of PAH, studied in both tissues under high PAH concentrations, also occurs significantly faster in the iris than in the ciliary body. The kinetic analysis of active PAH uptake in both tissues is discussed in terms of initial uptake and in terms of a steady-state model. This steady-state model compensates for some technical problems in applying in vitro incubation techniques to primate tissues and also includes a correction for the additional exchange processes that affect the two tissues differently. Results of the kinetic analysis suggest that, at least to an order of magnitude, iris uptake is significant with respect to ciliary body uptake.

Key words: para-aminohippuric acid, active transport, washout, nonsaturable uptake, ciliary body, iris, blood-aqueous barrier, aqueous humor, rhesus monkey
not only that PAH accumulates actively in the primate ciliary body but also that it accumulates by an independent but parallel process in the primate iris. The present study also demonstrates the existence of a significant washout phenomenon in the iris that affects the kinetic analysis.

**Methods**

The eyes were obtained from rhesus monkeys used by the Bureau of Biologicals of the Food and Drug Administration as part of a polio vaccine testing program. The monkeys were deeply anesthetized with intramuscular phenobarbital and sacrificed by cardiac puncture and bleeding. The eyes were enucleated, transported to the National Eye Institute laboratories as quickly as possible, and used in the uptake studies within 1 hr of sacrifice.

The eyes were opened in the region posterior to the ora serrata, and the vitreous was cut free as atraumatically as possible. The lens zonules were then carefully cut, and the lens was discarded. The iris was disinserted or cut free from the ciliary body, and the ciliary body was then cut free from the scleral spur. In some experiments, the iris and ciliary body were removed and incubated as a complete structure, and the iris was disinserted after the incubation. In general, the ciliary body and iris were halved or quartered, with one segment serving as the control for other segments from the same eye. The use of alpha-chymotrypsin, trypsin, collagenase, and hyaluronidase to aid in the dissection of the lens zonules and adherent vitreous base in the monkey eye proved no more helpful than the mechanical dissection discussed above.

After dissection the tissue was incubated in 4 ml of Tyrode's solution which was buffered to pH 7.4 with 95% O₂ and 5% CO₂ and contained 20 mCi/mmole ¹⁴C-PAH (ICN Pharmaceuticals, Inc., Irvine, Calif.). For uptake studies, the incubation solution contained a PAH concentration of approximately 4.8 × 10⁻⁶M. In uptake experiments involving variation of the PAH concentration, unlabelled sodium p-aminophenol (Sigma Chemical Co., St. Louis, Mo.) was added to the incubation medium. The tissue was incubated with gentle shaking in a water bath at 37° C, generally for 60 min.

At the conclusion of the incubation, the tissue was gently blotted on filter paper, weighed, and then ground in a known volume of Tyrode's solution. The ground tissue was then spun at 3000 rpm for 20 min, and the supernatant was counted. The washed pellet from the centrifugation, either resuspended in Tyrode's solution or digested in NCS Solubilizer (Amer sham/Searle Corp.), contained less than 6% of the ¹⁴C counts for both the ciliary body and iris specimens. Ciliary body and iris specimens dried to constant weight indicated that the ciliary body specimens contained approximately 86% water and the iris specimens approximately 90% water. It was assumed that the tissue water spaces contained the counts measured, and counts per minute per volume of tissue water were calculated on the basis of the tissue water content. These corrected tissue counts were then compared to the medium counts at the conclusion of the incubation. Tissue-medium (T/M) ratios are reported. All specimens were counted in Aquasol scintillation counting solution (New England Nuclear) on a Beckman LS-355 scintillation counter. All specimens were tested with an external-standard channels ratio, and no quenching correction proved necessary.

The zero degree incubations were performed by placing the reaction vials in an insulated ice bucket attached to the moving platform in the water bath. The reaction vials were allowed to equilibrate to the temperature of the ice bath prior to inserting the tissue.

For testing the effects of anoxia, pure nitrogen was bubbled through the Tyrode's solution for approximately 45 min. The Tyrode's solution was then buffered to pH 7.4 with pure CO₂ and maintained in closed vials under a nitrogen atmosphere prior to and during the incubation.

For the washout studies, half-ciliary body and disinserted half-iris specimens were preincubated for approximately 45 min at 37° with 10⁻⁴M ¹⁴C-PAH. At the conclusion of the preincubation, each tissue segment was washed quickly in plain Tyrode's solution and then was placed in its own 10 cc reservoir of Tyrode's solution, pH 7.4, which initially contained no radiolabeled compounds.

The washout reservoirs were gently shaken in a constant temperature water bath, and small aliquots were sampled and counted at regular time intervals. At the conclusion of each washout study, the tissue was blotted, weighed, ground, spun, and counted as described above. From the residual tissue counts and the washout data, the total uptake at the conclusion of the preincubation period was calculated. For the washout part of the study, graphs were then constructed relating the percent of preaccumulated PAH remaining in the tissue as a function of washout time. Zero time was defined as the time when the preincubated tissue was transferred to the washout reservoir. Separate
washout experiments were conducted at 0°C, with 10⁻⁴M unlabeled PAH and with 10⁻⁵M dinitrophenol in the washout reservoir. In all cases, one half of the ciliary body or iris was incubated under the experimental conditions, and the other half of the tissue from the same eye was run as a control in plain Tyrode's solution at 37°C with no added chemicals.

The sources of additional chemicals were sodium cyanide (Fisher Scientific Co., Fairlawn, N. J.), ouabain (Sigma), 2,4-dinitrophenol (Fisher), probenecid (Dr. Clement A. Stone, Merck, Sharp & Dohme Research Laboratories, West Point, Pa.), iodopyracet (Dr. F. C. Nachod, Sterling Winthrop Research Institute, Rensselaer, N. Y.).

Results

Accumulation. After 1 hr incubation at 37°C, the half-ciliary-body specimens had a T/M ratio of 5.35 ± 0.22, and the half-iris specimens, a T/M ratio of 2.67 ± 0.13 (mean ± S.E. for 52 eyes). Incubation at 0°C significantly depressed the ¹⁴C-PAH accumulation in both tissues, yielding a T/M ratio of 0.63 ± 0.01 for the half-ciliary body specimens and 0.78 ± 0.03 for the half-iris specimens (mean ± S.E. for four eyes). The iris accumulation was of the same magnitude whether the iris was incubated after disinsertion from the ciliary body or incubated attached to the ciliary body and then disinserted prior to grinding.

As an additional control study, iris specimens were carefully cut free from the ciliary body, with a thin rim of iris root left on the ciliary body. Histologic studies of these dissections indicated a significant amount of iris root on the ciliary bodies and no evidence of any ciliary epithelial contamination on the iris specimens. The dissected iris specimens were cut in half. One half, incubated at 37°C for 1 hr, had a T/M ratio of 2.20 ± 0.27, and the other half, incubated at 0°C for 1 hr, had a T/M ratio of 0.90 ± 0.06 (mean ± S.E. for 8 eyes).

Diffusion of PAH across the iris root therefore appeared to be relatively insignificant under these in vitro conditions. Although iris disinsertion prior to incubation completely eliminates the possibility of diffusional exchange across the iris root, iris disinsertion also causes additional tissue trauma and creates a damaged tissue surface from which increased washout (see below) can occur. Individual studies therefore were designed in such a fashion to make the most effective use of the limited tissue available. Uptake studies were performed either before iris disinsertion or after iris disinsertion, but washout studies required prior iris disinsertion.

The time course of the accumulation of PAH in both tissues appears in Fig. 1. In these studies, quartered ciliary body and iris specimens from seven eyes were incubated from 15 to 90 min. For the ciliary body, an

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**Fig. 1.** Time course of accumulation of PAH in ciliary body and iris specimens at 37°C. See text.
Table I. Effect of inhibitors on PAH accumulation in 1 hr incubations at 37° C for the ciliary body and the iris

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>PAH uptake (% of control)</th>
<th>Ciliary body*</th>
<th>P value</th>
<th>Iris*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanide (5 x 10^-4M)</td>
<td></td>
<td>50 ± 7</td>
<td>&lt;0.001</td>
<td>59 ± 7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Ouabain (10^-5M)</td>
<td></td>
<td>34 ± 5</td>
<td>&lt;0.001</td>
<td>35 ± 7</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Dinitrophenol (10^-3M)</td>
<td></td>
<td>3 ± 1</td>
<td>&lt;0.001</td>
<td>18 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dinitrophenol (10^-5M)</td>
<td></td>
<td>46 ± 14</td>
<td>&lt;0.005</td>
<td>51 ± 18</td>
<td>&lt;0.0025</td>
</tr>
<tr>
<td>Iodopyraacet (5 x 10^-5M)</td>
<td></td>
<td>46 ± 10</td>
<td>&lt;0.005</td>
<td>41 ± 10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Probenecid (10^-5M)</td>
<td></td>
<td>63 ± 12</td>
<td>&lt;0.05</td>
<td>52 ± 11</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.E. from 8 eyes.

Inhibitors. Table I shows the effects of a variety of metabolic and competitive inhibitors on the accumulation of PAH during 1 hr incubations at 37° C. In these experiments one half of a ciliary body and one half of an iris were incubated in the presence of an inhibitor, and the other halves of the tissues from the same eye were incubated under identical conditions in the absence of inhibitor. The zero degree uptake for each tissue was used as an estimate of “nonactive” uptake. Inhibited and uninhibited T/M values for both tissues were corrected by subtracting the zero degree T/M value for the appropriate tissue, resulting in a T/M ratio that approximated the “active” component of uptake. The corrected T/M ratio from each inhibited tissue was compared to the corrected uninhibited control from the same eye, and the data were then averaged. As shown in Table I, all inhibitors tested depressed uptake in both tissues and had parallel effects on both tissues.

In testing for the effects of anoxia, one half of a ciliary body and one half of an iris were incubated under the usual aerobic conditions, and the other halves of the tissues from the same eye were incubated under the anaerobic conditions described above. After 1 hr incubation at 37° under aerobic conditions, the ciliary bodies developed a T/M ratio of 4.03 ± 0.45; the irises, a T/M ratio of 2.60 ± 0.25. Under anaerobic conditions, the ciliary bodies developed a T/M ratio of 4.78 ± 0.55; the irises, a T/M ratio of 2.43 ± 0.26. These results represent means ± S.E. from eight eyes. The difference between the aerobic and anaerobic values is not statistically significant by t statistics on the paired differences for either the ciliary body or the iris.

Washout studies. Fig. 2 represents typical findings from a washout study and shows that PAH washout from the iris was significantly more rapid than PAH washout from the ciliary body. These studies employed a large washout reservoir; the reservoir PAH concentration was negligible in comparison to the tissue PAH concentration in the initial portions of the washout and, in most cases, throughout the entire washout. In addition, the washout curves were exponential in character. No previous studies exist of PAH washout from anterior uvea. In other systems, however, like the well-studied renal tubule system, the washout rate of PAH and
PAH transport by ciliary body and iris

Fig. 2. Washout of PAH from ciliary body and iris. The study illustrated represents four half-ciliary-body and four half-iris specimens in which washout was studied at 37° C with a washout reservoir of plain Tyrode's solution. Each point represents the mean ± S.E. For some values, the symbol covers the error bars. The inset shows the semilog plots of the same data, as described in the text. The values for kw represent the means ± S.E. from a total of 12 such ciliary body and iris specimens studied under identical conditions, except for differences in the time of reservoir sampling.

of similarly transported anions acts as a first-order process, proportional to intraluminal anion concentration. In view of the exponential character of the washout curves obtained for the ciliary body and iris preparations, it appears that a first-order decay was occurring here as well and that an equation of the following form applies:

\[ \frac{T'}{T^0} = e^{-k_w t} \]

where \( T' \) = tissue PAH concentration after washout for time \( t \), \( T^0 \) = tissue PAH concentration at the start of the washout study, and \( k_w \) = a washout constant. The slope of the semilog plot, \( \ln(\frac{T'}{T^0}) \) vs. \( t \), then gives the value for \( -k_w \), and the inset in Fig. 2 shows these semilog plots. The initial portions of these semilog plots were linear, but both plots deviated from linearity as washout progressed. For the iris, this deviation occurred at about 10% to 12% of preaccumulated PAH, probably because of tissue binding of PAH. As illustrated in Fig. 2, iris washout occurred so rapidly that this point was quickly approached. Ciliary body wash-

out occurred more slowly, but at 30 min this plot also began to deviate significantly from linearity. As will be discussed, active reaccumulation of PAH appeared to have occurred during the initial washout period for the ciliary body but not for the iris. Reaccumulation, as well as tissue binding, could have contributed to the deviation from linearity of the ciliary body plot. In addition, postmortem changes could have affected washout in both tissues since, because of the preincubation, the washout studies often did not start until at least 2 hr after enucleation. The present studies, however, are insuf-

Table II. Effects on \( k_w \) of altering washout conditions

<table>
<thead>
<tr>
<th>Washout conditions</th>
<th>( k_w ) (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ciliary body*</td>
</tr>
<tr>
<td>( 10^{-4} )M PAH</td>
<td>106 ± 7</td>
</tr>
<tr>
<td>( 0^\circ C )</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>( 10^{-2} )M DNP</td>
<td>124 ± 12</td>
</tr>
</tbody>
</table>

DNP = dinitrophenol. *Each value represents the mean ± S.E. from four eyes.
Table III. Values of $K_A$ and $V_{\text{max}}$ as determined from initial uptake and steady-state studies

<table>
<thead>
<tr>
<th>Experimental design</th>
<th>Ciliary body</th>
<th>Iris</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_A$ (mM/L)</td>
<td>$V_{\text{max}}$ (mM/L/hr)</td>
</tr>
<tr>
<td>Initial uptake*</td>
<td>0.32 ± 0.37</td>
<td>1.8 ± 2.1</td>
</tr>
<tr>
<td>Steady-state†</td>
<td>0.108 ± 0.078</td>
<td>1.7 ± 1.3</td>
</tr>
</tbody>
</table>

*Initial uptake data were calculated as described in the text, using 10 min incubations and correcting for nonsaturable uptake. Each value represents the mean ± S.E. for five eyes.
†Steady-state data were calculated as described in the text, using 1 hr incubations and applying equation 3. In the calculations, it was assumed that $k = 0.060 \text{ min}^{-1}$ for the ciliary body and 0.17 min$^{-1}$ for the iris. Each value represents the mean ± S.E. for six eyes.

Sufficient to characterize the effects of tissue binding, persistent uptake, and postmortem changes on washout during the latter parts of the washout studies in either tissue. Therefore it is assumed that the values of $k_w$ calculated from the initial linear portions of these semilog plots, where postmortem changes are least, reasonably reflect the true washout rates for both tissues. The values for $k_w$ shown in Fig. 2 represent the means ± S.E. for $k_w$ for the 12 half-ciliary body specimens and for the 12 half-iris specimens that served as control tissues in the washout studies. With this exponential formulation, it thus appears that iris washout occurs some 2.5 times faster than ciliary body washout.

Table II indicates the effects on $k_w$ of altering the washout conditions. The $10^{-4}$M PAH concentration in the medium represents the concentration of labeled PAH during the preincubation, and $10^{-3}$M dinitrophenol depressed uptake approximately 50% in each tissue. In each study, the value of $k_w$ under experimental conditions was compared to the control value of $k_w$ from the same eye, and then the data were averaged.

**Substrate effects.** The effect of high substrate concentrations on the initial velocity of uptake in both ciliary body and iris appears in Fig. 3.

The curves for each tissue represent measured initial uptake velocities for each tissue. These curves were linear, with slopes of 0.45 T/M units for the ciliary body and 0.83 T/M units for the iris. These values represent the nonsaturable component of the T/M ratio for each tissue during a 10 min incubation.

Assuming first-order kinetics for this non-saturable uptake process, one can write

$$\frac{dT}{dt} = k_u (M - T)$$

where $k_u =$ nonsaturable uptake constant, $T =$ tissue concentration of PAH after incubation for time $t$, and $M =$ medium PAH concentration. Integrating, with $T = 0$ at $t = 0$, gives

$$\frac{T}{M} = 1 - e^{-k_u t}$$

From this equation, $k_u$ can be calculated from the nonsaturable component of the T/M ratio. The results are 0.060 min$^{-1}$ for the ciliary body and 0.18 min$^{-1}$ for the iris. These results are in close agreement with the values for $k_w$ determined from the washout data.

The effect of lower substrate concentrations on initial velocity of uptake in the ciliary body and in the iris appears in Fig. 4. In these studies, the experimental T/M values are corrected to reflect saturable uptake by subtracting the nonsaturable component of the T/M ratio from the experimental T/M values for each tissue. From the corrected T/M ratios, the initial PAH uptake was calculated for each tissue. The results are data which estimate the accumulation from saturable uptake during a 10 min incubation. For both the ciliary body and the iris, a number of extraneous data points were eliminated in the analysis. Lineweaver-Burk plots were then constructed for both tissues, and linear regression analysis was used to calculate values for the apparent Michaelis-Menten constants, $K_A$ (half-saturation concentration) and $V_{\text{max}}$ (maximum accumulation rate). These data appear on the top line of Table III. The
Fig. 4. Effect of varying PAH concentration on the saturable uptake of PAH. Experimental conditions were identical to those in Fig. 3 except for lower PAH concentrations. Initial uptake here represents saturable uptake only and was calculated as described in the text. Each value represents the mean ± S.E. from five eyes, unless the symbol covers the error bars.

Fig. 5. Lineweaver-Burk plots from the data used in Fig. 4, corrected for nonsaturable uptake. Each point represents the mean from five eyes. Error bars show ± S.E., unless hidden by the symbol.

Lineweaver-Burk plots from these studies, using the corrected data, appear in Fig. 5. As shown in Figs. 4 and 5, the data showed considerable scatter, even with the elimination of the extraneous data, and as a result, there was considerable error in the values for $K_A$ and $V_{max}$ calculated from these initial uptake data. Reasons for this experimental scatter are discussed below.

Fig. 6. PAH accumulation in quartered ciliary body and iris preparations, incubated at 37°C for 1 hr in varying PAH concentrations. PAH uptake = ($T/M$ ratio at 1 hr) × (medium PAH concentration). Each value represents the mean ± S.E. for six or eight specimens. For some values, the symbol covers the error bars.

To overcome some of the limitations in using initial uptake data, steady-state data were used as an alternative method of estimating $V_{max}$ and $K_A$. This type of approach to kinetic data has a precedent in nonocular systems, but it has been used primarily to calculate diffusion constants in situations in which reliable values for $K_A$ and $V_{max}$ exist from initial uptake data and not as a means to calculate $K_A$ and $V_{max}$.

Assume that $k_w = k_u$, as shown above to be experimentally valid both in ciliary body and in iris, and let $k = k_w = k_u$. Also assume that the active component of uptake is given by

$$\frac{V_{max} \cdot M}{K_A + M}$$

Further assume no active outwardly directed transport. Then

$$\frac{dT}{dt} = \frac{V_{max} \cdot M}{K_A + M} + k(M - T)$$

Assume that $M$ is approximately constant,
which can easily be achieved experimentally with a sufficient volume of incubation medium. Integrating this last equation and setting \( T = 0 \) at \( t = 0 \) gives

\[
\frac{T}{M} = \frac{V_{\text{max}}}{k(K_A + M) + 1} (1 - e^{-kt})
\]

Equation 1 gives the \( T/M \) ratio as a function of time and substrate concentration. It is equivalent to previous theoretical formulations that have incorporated diffusional exchange. At steady state, \( t \to \infty \), and equation 1 reduces to

\[
\frac{T}{M} = \frac{V_{\text{max}}}{k(K_A + M) + 1}
\]

which can be rearranged to

\[
\frac{1}{T} = \frac{K_A}{M(K_A + \frac{V_{\text{max}}}{k} + M)} + \frac{1}{K_A + \frac{V_{\text{max}}}{k} + M}
\]

If one chooses low substrate concentration so that

\( M < K_A + \frac{V_{\text{max}}}{k} \)

equation 2 becomes

\[
\frac{1}{T} = \frac{K_Ak}{M(V_{\text{max}} + K_Ak)} + \frac{k}{(V_{\text{max}} + K_Ak)}
\]

Equation 3 is a double reciprocal plot of \( 1/T \) vs. \( 1/M \), except that the slope and the intercept have a more complex formulation than in the traditional Lineweaver-Burk approach. For equation 3, let \( S = \) slope and \( I = \) intercept. Solving the simultaneous equations yields

\[
K_A = \frac{S}{I}
\]

\[
V_{\text{max}} = \frac{k}{I} (1 - S)
\]

To generate data for this model, quartered ciliary body and iris specimens were incubated in varying PAH concentrations for 1 hr. Fig. 1 shows that at this time the iris appears to be in steady state and the ciliary body appears to be approaching steady state. One hour was used as an incubation time that gives values approximating steady-state values without allowing excessive tissue deterioration. Fig. 6 illustrates the PAH accumulation in both tissues under these incubation conditions.

In one series of experiments, quartered ciliary body and iris specimens were incubated for 1 hr in 0.048 to 0.60 \( \times 10^{-4} \)M PAH. The data were plotted according to equation 3, and these graphs appear in Fig. 7. The plots were linear for both tissues, and linear regression analysis was used to calculate val-
ues for $K_A$ and $V_{max}$ as described above. The values for $K_A$ and $V_{max}$, calculated from these steady-state experiments, appear on the bottom line of Table III.

In the derivation of equation 3, it is assumed that, in the experimental conditions

$$M + (K_A + \frac{V_{max}}{k}) < 1$$

At the highest substrate concentration used for the ciliary body experiments in Fig. 7, this fraction is 0.10. For the iris, this fraction at the highest substrate concentration is 0.38, somewhat higher. Elimination of the highest iris concentration data, gives $K_A = 0.048 \pm 0.028$ mM/L and $V_{max} = 0.57 \pm 0.33$ mM/L/hr (mean ± S.E.). These values do not differ significantly from the values reported in Table III, and the data in Table III are used directly in the discussion.

Discussion

The kidneylike mechanism by which the anterior uvea accumulates organic anions has been most extensively studied in the rabbit eye. Intravitreal injections of iodopyracet into monkey eyes in vivo have also demonstrated an outwardly directed system for transport of iodopyracet, but more extensive studies of this kidney-like transport have not been performed in primate eyes. Because the iris epithelium contains tight junctions, lateral interdigitations, and a reasonable number of mitochondria, it has been suggested, but not previously documented, that the iris also participates in transport phenomenon.

The present study not only further characterizes the kidneylike mechanism in the primate ciliary body but also demonstrates that a similar process occurs in the primate iris. In both tissues, PAH accumulates against a concentration gradient. This accumulation is inhibited by cold (0°C), ouabain, cyanide, and dinitrophenol, and it displays saturation kinetics. It appears, then, that an active uptake process, perhaps coupled to cation transport, is present in both tissues. Although the anatomic location of iris transport is unknown, it is assumed in the present discussion to occur at the posterior epithelium.

The primate ciliary body does not achieve as high T/M ratios as the rabbit ciliary body-iris preparation. Although this difference may reflect species variation, the following technical features of the present study may depress uptake: the unavoidable delay between sacrifice and incubation, the tissue trauma resulting from the extensive dissection necessary to free the lens and disinsert the ciliary body from the scleral spur in the primate eye, the residual adherent vitreous in the primate eye, and perhaps the use of phenobarbital anesthesia.

Cutting the iris anterior to the root slightly diminishes the amount of PAH accumulation seen. Since the iris stroma is thicker anteriorly than at the root, this dissection may increase washout through increased stromal injury and thus depress the T/M ratio. Alternatively, a gradient of transport activity may exist across the iris surface, becoming less active near the pupil. To test this latter hypothesis, three ring-shaped pieces of iris tissue were cut from each of several iris specimens at the pupillary, midstromal, and root areas. Each of these thin tissue segments accumulated significant 14C-PAH, but no gradient in activity was demonstrated with these crude dissections.

Incubation at zero degrees effectively inhibits uptake in both tissues. Ouabain, cyanide, and dinitrophenol have similar effects in both tissues.

Potassium-free medium markedly decreases iodopyracet uptake in rabbit ciliary body-iris preparations, and other transport systems in the anterior uvea show a marked sensitivity to ouabain. The marked sensitivity to ouabain of PAH uptake in both the ciliary body and the iris of the primate is therefore not surprising and suggests that PAH accumulation is coupled to cation transport. PAH uptake in both tissues is less sensitive to cyanide than to dinitrophenol, but these results are similar to iodopyracet uptake in the rabbit.

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Competitive inhibitors also give similar results in the primate and the rabbit. Probencid (10^{-4}M) causes about 50% inhibition of iodopyracet uptake in the rabbit ciliary
body–iris preparation and causes about the same inhibition of PAH uptake in the primate ciliary body and iris. In the rabbit, $2 \times 10^{-5}$M PAH causes about 50% inhibition of iodopyracet uptake, and in the monkey tissues, $5 \times 10^{-5}$M iodopyracet causes about the same inhibition of PAH uptake.

Because the absence of oxygen does not affect PAH accumulation, it appears that glycolysis can support this uptake mechanism for 1 hr. Although oxygen lack eliminates iodopyracet uptake in the rabbit ciliary body–iris, other active transport systems in both the rabbit uvea and the guinea pig uvea are resistant to the effects of anoxia. It is also possible that, despite the precautions, trace amounts of oxygen remained in the incubation medium.

Both the washout and saturation studies provide evidence for a second exchange process occurring in the primate tissues that differs from the active uptake process.

The interpretation of the washout data, however, is not straightforward. The failure of $10^{-4}$M PAH in the washout medium to affect washout suggests that this washout is occurring by simple passive diffusion rather than by a carrier-mediated phenomenon like exchange diffusion or active secretion. However, PAH washout from renal tubules apparently occurs by a complex mechanism involving biphasic responses to various inhibitors. Iodopyracet, accumulated in the kidney tubules by the same uptake mechanism, similarily demonstrates a complex washout mechanism, and three phenomena have been implicated to explain iodopyracet washout from kidney tubules: simple passive diffusion, active uptake opposing washout, and a carrier-mediated mechanism displaying characteristics of both exchange diffusion and facilitated diffusion. The use of only a single PAH concentration in the present uveal washout studies is insufficient to demonstrate such complex behavior.

The inhibition of washout at zero degrees is similar to that in the renal system. However, low temperatures can decrease passive diffusion across cell membranes, and the zero degree temperature effects may represent an effect on diffusion and not the depression of an active component of washout.

In terms of the effect of dinitrophenol on washout, a relatively simple explanation can be offered for the results observed in the anterior uvea. The increase in washout observed by incubating ciliary bodies with dinitrophenol may occur because the uptake mechanism is inhibited, thus preventing reaccumulation. No effect on iris washout is seen, perhaps because anatomic differences between the iris and the ciliary body allow the PAH molecules to diffuse from the uptake sites in the iris before significant reaccumulation can occur. Only one dinitrophenol concentration, however, has been tested, and it is therefore impossible to exclude the possibility of the existence of complex biphasic responses to inhibitors as seen in the renal system.

The results of these washout studies in the ciliary body and iris are consistent with washout occurring by the following mechanism: simple passive diffusion in both tissues, modified by active reaccumulation in the ciliary body only. The experimental data, however, do not prove that washout in the ciliary body and iris occur by such a simple mechanism, and further work to characterize washout is necessary. The study of the washout of alpha-aminoisobutyric acid from rabbit ciliary body–iris preparations represents another uveal system in which washout has been studied, and some similar difficulties in interpretation are encountered here.

Incubation of both tissues with high PAH concentrations demonstrates the presence of a nonsaturable component of PAH uptake in both tissues. Assuming first-order kinetics, an uptake constant, $k_u$, was calculated above both for the ciliary body and for the iris. The agreement between $k_u$ and $k_w$, the washout constant, for both tissues suggests that the washout studies and the nonsaturable uptake studies are opposing experimental approaches to a reversible process.

Therefore there is an exchange process between the anterior uveal tissue and the medium, which differs from the active uptake...
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process. Both the washout phenomenon and the nonsaturable uptake are more active in the iris than in the ciliary body. The bulk of this exchange probably occurs by simple passive diffusion; however, as discussed above, the presence of the other mechanisms cannot be excluded on the basis of present data.

The existence of the diffusional exchange process greatly complicates the kinetic analysis of the active uptake in the ciliary body and the iris. This exchange enhances the experimental initial uptake T/M ratio and depresses the experimental steady-state T/M ratios, and these effects are more marked in the iris than in the ciliary body.

With the use of a more traditional approach to generating kinetic data, the experimental T/M ratios from 10 min incubations with varying PAH concentrations were corrected by subtracting the nonsaturable component of the T/M ratio generated from independent studies using 10 min incubations and high PAH concentrations.8-18 This approach assumes washout to be negligible during the initial 10 min of uptake. As discussed, however, there is considerable scatter in these corrected data points. The scatter arises because of several serious limitations in applying initial uptake studies to primate ciliary body and iris specimens.

First, the adherent vitreous base cannot be effectively removed from the primate ciliary body, either mechanically or enzymatically, without also significantly damaging the tissue. The vitreous thus represents a space adjacent to the ciliary epithelium that is poorly mixed and interferes with substrate access to the epithelium. In addition, because the amount of vitreous cannot be controlled from one tissue sample to the next, the vitreous induces a further source of error.

Another difficulty arises with iris specimens. In correcting the T/M ratios for “nonactive” uptake, the correction for the ciliary body is relatively small. For the iris, however, some 50% to 60% of the initial 10 min accumulation occurs by nonactive mechanisms. In addition, the T/M ratios at this time are relatively low, generally 1.2 to 1.5. Because of the large correction factor necessary, small experimental errors become magnified into large percentage errors in the corrected data, and considerable data spread can occur.

Because of these difficulties with initial uptake data, a steady-state formulation is applied to generate values for $K_A$ and $V_{\text{max}}$. The use of steady-state T/M values minimizes the vitreous problem and does not require the use of large correction factors. Table III shows that the steady-state values for $K_A$ and $V_{\text{max}}$ are in reasonable agreement with the values from initial uptake studies, considering the sources of experimental error discussed above. The steady-state values are more accurate. Significant spread in the data still occurs, however, because of variation from animal to animal and because of the small number of eyes studied.

The theoretical time predictions of equation 1 are a further test of the validity of the proposed steady-state model. According to equation 1, $(1 - e^{-kt})$ is 0.97 for the ciliary body at 60 min and 0.97 for the iris at 20 min, assuming $k$ to be 0.060 min$^{-1}$ for the ciliary body and 0.17 min$^{-1}$ for the iris. These predictions are in reasonable agreement with the experimental data in Fig. 1. Even at 10 min, $(1 - e^{-kt})$ is 0.82 for the iris, according to equation 1. This calculation shows that because of the rapid iris diffusional exchange, even the 10 min iris uptake is not an “initial” value, further illustrating the limitation of initial uptake studies with this tissue.

The anatomic differences between the ciliary body and the iris make direct kinetic comparisons between the two tissues difficult. It is hoped, however, that by the use of corrections for nonsaturable uptake and washout, some qualitative comparisons between the two tissues can be made.

Of interest, then, is the relative similarity of the $K_A$ values for the ciliary body and for the iris. This similarity may imply a parallel between the receptor characteristics of the two tissues.19 In addition, $V_{\text{max}}$ here is expressed as millimoles of PAH accumulated per hour per liter of tissue water. As discussed above, the ciliary body contains 86% water, and the iris

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contains 90% water. Since the ciliary body specimens weigh about seven times more than the iris specimens, since the projected area of monkey ciliary processes is about 0.94 times the iris projected area as determined by direct measurement of several eyes, and since the true epithelial surface area of the ciliary processes (in rabbit) is about five times the projected area,\(^{20}\) it appears that the transport capacity of the ciliary body is only

\[
\frac{1.7}{0.83} \times \frac{0.96}{0.90} \times \frac{7}{0.94 \times 5}
\]

or 2.9 times greater than the iris when corrected for epithelial surface area. The calculation assumes that iris uptake occurs at the epithelial surface and not, for instance, at the blood vessel walls, and it assumes no role for the pars plana. This finding suggests that, at least to an order of magnitude, the PAH transport capacity of the iris is significant with respect to the PAH transport capacity of the ciliary body in the primate eye.

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