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Identification of Riboflavin Transport by MDCK Cells Using Quantitative Fluorescence Video Microscopy

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Summary. MDCK cells, when examined by low-light level video microscopy displayed an endogenous fluorescence with two differing patterns. A low intensity emission which was punctate and associated with cell organelles was observed with emission and excitation conditions generally used to observe either fluorescein (450–500 nm excitation/>510 nm emission) or rhodamine (514 nm excitation/>530 emission) type dyes. A second 5- to 10-fold brighter emission for 450–500 nm excitation was observed, which was unusual in that each cell appeared to be outlined. Evidence obtained from spectroscopy and from using culture media of altered composition supported the conclusion that the water-soluble vitamin riboflavin accumulated in the basolateral spaces and fluid-filled "domes" and was the source of this fluorescent emission. Quantitative measurements showed that exposure to cultures to 10 μM riboflavin resulted in accumulation in domes of 565 ± 80 μM. The transport rate was calculated to be 189 ± 30 pmol/min-cm^2. One mM probenecid, a known inhibitor of riboflavin transport in vivo, reduced transport to 54% of control, while 10 mM nearly abolished the uptake. The results demonstrate that removal of riboflavin reduces MDCK cell fluorescence to levels compatable with low-light level imaging. Furthermore, these cells actively transport riboflavin and provide a new in vitro model for this process.

Key Words vitamins · flavin transport · organic anion transport · probenecid

Introduction

Quantitative low-light level fluorescence microscopy combined with digital image processing is becoming an increasingly valuable method to study a wide variety of cell biological processes (Arndt-Jovin et al., 1985; Wang & Taylor, 1989). The present study employs quantitative microscopy for an analysis of riboflavin transport by MDCK cells. The study was prompted by the initial observation that MDCK cells displayed a very high endogenous fluorescence when examined by a low-light level imaging system. This intrinsic fluorescence interferred with the ability to quantitate other low-intensity signals from experimentally added fluorophores. Such "autofluorescence" has previously been attributed to a variety of sources including NADH, flavin proteins, riboflavin and phenol red, the culture media pH indicator (Aubin, 1979; Benson et al., 1979; Murphy, 1986). In the present study the identification of the vitamin riboflavin, present in the culture media, as the material primarily responsible for the high background fluorescence came in part from measurements with a microscope-based scanning spectrofluorimeter (Balaban et al., 1986). In addition, quantitative measurements using video microscopy demonstrated that riboflavin was accumulated in the lateral intercellular spaces between MDCK cells and within the fluid-filled "domes" at concentrations well above that in the culture media. Using these methods it has been possible to obtain kinetic data on this transport process and also show that, as seen in vivo (Jusko & Levy, 1975), the organic anion transport inhibitor probenecid blocks riboflavin transport. Finally, based on our findings, MDCK cells can be used as a new in vitro model to study transport of this anionic vitamin.

Materials and Methods

Cell Culture

MDCK cells were started from stocks obtained from American Type Culture Collection (Rockville, MD). Cells were grown in T-25 flasks and fed three times a week on media prepared from Dulbecco's minimal essential media (DME) containing 10% fetal bovine serum (Gibco, Grand Island, NY) and penicillin-strepto-

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myin (100 U/ml each), which was filtered before use. Cells were also grown in the same fashion, but in base DME media containing neither riboflavin nor phenol red. No apparent change in cells grown without these media supplements, including morphology, growth, ability to form domes or passage ability, has been observed to date (approximately 12 months and 20 passages).

Solutions

DME was obtained from the NIH Media Services Division. Riboflavin was added to the DME media in a concentration of 10 μM. Phenol red was added at a concentration of 10 mg/liter. In the chamber experiments utilizing quantitative video microscopy the perfusate was Earle's basic saline solution (EBSS) containing (in mM) 142 Na, 5.4 K, 1.8 Ca, 0.8 Mg, 127 Cl, 5 glucose, 4 $NaHCO_3$, buffered with 10 mM NaHEPES at pH 7.4. This formulation was chosen as there is likely to be sufficient bicarbonate for exchange mechanisms requiring this anion and pH is accurately maintained in the absence of CO_2 gassing (Freshney, 1983).

Spectrofluorimetry

Fluorimetric analysis of culture medium was performed using a scanning spectrofluorimeter (SLM 8000, SLM-Aminco, Urbana, IL). Excitation was at 360 nm and the emission was scanned from 500 to 700 nm with a 1-sec integration time. The combination of excitation and emission wavelengths was used to allow direct comparison of spectra to those obtained using the microspectrofluorimeter. Emission spectra obtained using 488 nm excitation were essentially identical (*data not shown*). Solutions analyzed were DME without riboflavin or phenol red and DME to which either riboflavin (10 μM) or phenol red (10 mg/liter) or both had been added. The concentrations of riboflavin and phenol red were equivalent to those in standard DME culture media. Microscopic fluorometric analysis of the MDCK domes was performed using a rapid scanning system attached to an inverted microscope (Balaban et al., 1986). Briefly, light from a 50-W mercury arc lamp was filtered using a 50% neutral density filter and passed through an excitation filter and dichroic mirror (model BV, Nikon) to produce 360 nm excitation). The unfiltered emission light was directed into a monochrometer and the spectrum obtained was projected onto the face of a silicon intensified target video camera (Princeton Applied Research, Princeton, NJ). The signal from the camera was gain corrected in software, and an emission spectrum from 380 to 580 nm obtained every 33 msec. Spectra were obtained from MDCK domes from cultures grown in either normal DME culture media or DME free of riboflavin and phenol red.

Video Microscopy

MDCK cells grown for 4 days after passage were mounted in a temperature-controlled perfusion chamber and maintained at 37°C. The cells were visualized with a quantitative video microscope (Ortholux II, E. Leitz, Rockleigh, NJ) equipped with a 63×/1.25 NA objective lens (Neofluar, Zeiss, Thornwood, NY) and a laser illumination system (Spring & Smith, 1987). Low-light level video imaging was achieved with a microchannel plate image intensifier (Model KS-1380, Videoscope, Washington, DC) connected to a television camera (model 65 MKII, Dage-MTI, Michigan City, IN). For each dome a bright-field image, using the Schlieren modification of the optics described by Axelrod (1981), was obtained of the focal plane at the base of the dome. The height of the dome was determined from the number of 0.5-μm steps needed to bring the apex into focus. These values were used to calculate the volume and surface area of the dome, assuming it was a symmetrical right hemisphere. It has been reported (Tanner, Framach & Misfeldt, 1983) and confirmed by our own preliminary results that this is an accurate method for the measurement of dome volume. The focal plane was adjusted to the base of the dome, and an initial fluorescent background image was recorded. In all cases this value did not differ from zero and was not needed for background subtraction. The perfusate was then switched to one of EBSS containing 10 μM riboflavin or 10 μM riboflavin plus the inhibitor probenecid at 1, 5 or 10 mM concentration. Images, of an 8-frame (0.25 sec) summation for noise reduction, were recorded on video disk (model VDR-1R, Arvin Echo, Mountain View, CA). The time interval between images was 15 sec to 1 min, with more frequent recordings at the start of the experiment. Fluorescent excitation was the 488-nm line of a 200-mW argon laser (Omnichrome, San Diego, CA), and the emission was filtered using a 520-nm dichroic mirror and 530-nm long-pass filter. The fluorescent excitation light was on only during the 250-msec data acquisition period. The gain setting of the image intensifier was adjusted periodically to prevent the fluorescence signal from overranging the video camera and digitizer. Image analysis was performed on a Trapix 5500 (Recognition Concepts, Incline Village, NV) image processor using user-written software based on a library of routines (TAU, Los Gatos, CA). The host computer for the Trapix was a Digital Equipment 11/73. Images (512 × 512 × 8 bits) were transferred from video disk to the image processor hard disk without any further signal averaging. Software routines allowed the observer to place a 100 × 100 pixel (0.18 μm/pixel) cursor box centered on the location corresponding to the dome apex. The mean grey level for this region of interest was determined for all time points, and the values were then corrected for the intensifier gain setting and the dome height (pathlength). The riboflavin concentration was then calculated from the detected fluorescence and a standard curve obtained from solutions of known riboflavin concentration in a well of known pathlength.

Results

MDCK Cell "Autofluorescence"

Figures 1 and 2*C* illustrate the results of our initial low-light level observations of MDCK cells. As shown in Figs. 1*A* and 2*C*, excitation at 488 nm and emission of >510 nm resulted in a bright fluorescent signal which appeared to outline the individual cell borders. This fluorescence was often easily observed with the intensified camera system at a relatively low gain setting. Comparing this emission image (Fig. 1*A*) with bright-field images (Fig. 1*C*) confirmed that image fluorescence coincided nearly perfectly with the lateral intercellular spaces when the plane of focus was at the basal surface of the cells. The other relatively short excitation wavelengths available from the laser source (458 nm, 476

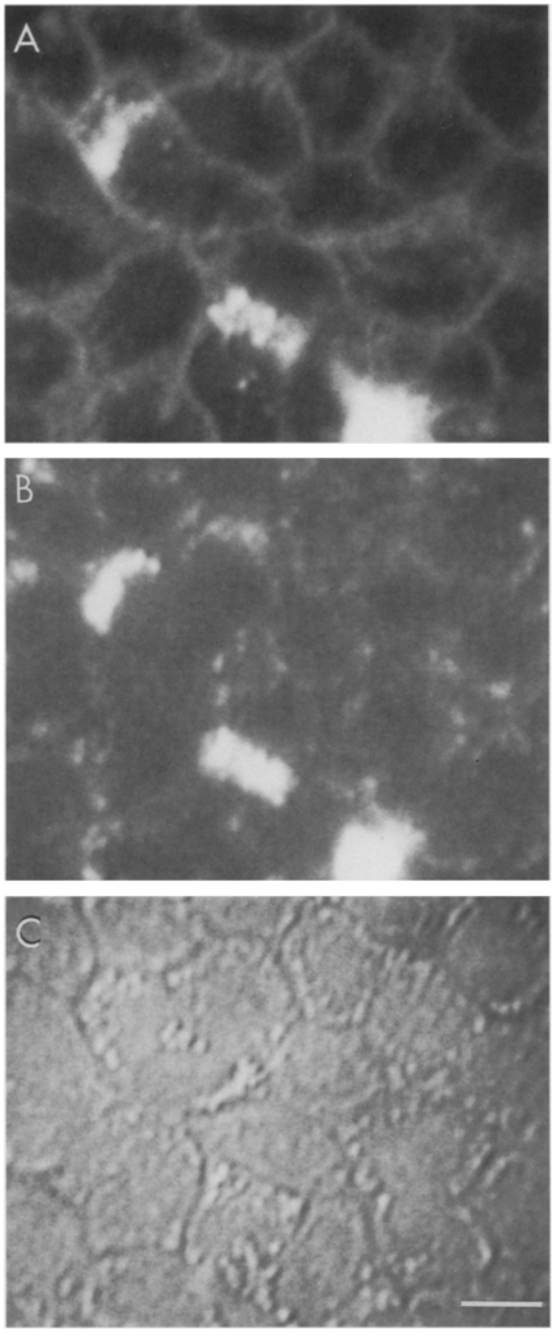

Fig. 1. Video microscopic images of MDCK cells. Cultures are all approximately 5 days old, completely confluent and grown in DME with 10% FBS (*see* Materials and Methods). (*A*) Fluorescent pattern typically observed from cells grown in DME using 488 nm excitation and 510 nm emission. Note that fluorescence outlines each cell and scattered light obscures cellular details. Panels *B* and *C* are observations of the same region of a single culture under the following optical conditions: (*B*) 514 nm excitation/530 nm emission. (*C*) Bright field (Schlieren optics). Image intensifier and video camera settings were identical for images *A* and *B*. Punctate fluorescence observed at 514 nm excitation (*B*) in many instances corresponds to intracellular organelles (*C*). The major fluorescence seen at shorter wavelengths (*A*) corresponds well to the cell borders (*C*). In this particular field the 488-nm sensitive emissions were less intense than usual, while punctate emissions were more intense, fortuitously allowing simultaneous observation of their relative positions. Scale bar = 10 μm

nm) resulted in the same pattern of fluorescence but with a reduction in intensity of approximately two-to-fourfold. The pattern of fluorescence for 514 excitation (emission > 530 nm) was quite different from that at shorter wavelengths, being primarily associated with intracellular organelles (Fig. 1*B* and *C*). Many of these fluorescent intracellular objects showed a perinuclear distribution, suggestive of being mitochondrial, Golgi and/or lysosomal-endosomal vesicles. In some cells polarized groups of vesicle-like structures could be observed, which showed intense fluorescence under both excitation conditions (Fig. 1*A* and *B*).

The pattern seen with 488 nm excitation was reminiscent of immunocytochemical labeling of plasma membrane cell surface antigens. Therefore, our initial concern was that this was a previously undescribed cell membrane-associated autofluorescence, which would render MDCK cells unsuitable for low-light level quantitative microscopy. However, subsequent observations suggested that the fluorescence was due to freely mobile material in the interspaces. Introduction of polarizing filters into either the emission light path or into both the excitation and emission pathways only reduced the intensity by a factor as great as would be expected for the transmittance of the filters, with no effect of polarizer orientation. This result was consistent with a fluorochrome which was freely mobile in solution because fixed molecules would show fluorescence anisotropy and therefore an orientation-dependent decrease in intensity (Axelrod, 1989).

Observation of the fluid-filled hemicysts or "domes," which commonly occur in MDCK cell cultures, also provided evidence for a fluid phase distribution. In Fig. 2 the bright-field (Fig. 2*A*) and the fluorescent image (Fig. 2*B*) and fluorescent intensity profile (Fig. 2*D*) for a single dome are shown. The profile shows the intensity is greatest at the center. The greatest pathlength for a fluid marker is the center of the dome, whereas the edge of the dome is the greatest pathlength for a cell-associated fluorescence. As described above, Fig. 2*C* shows the fluorescence pattern of cells adjacent to the dome, illustrating the pericellular distribution of the fluorescence in flat regions of the culture. These results suggested that a soluble substance in the culture media or some cellular metabolite excreted by the cells was responsible for this fluorescence.

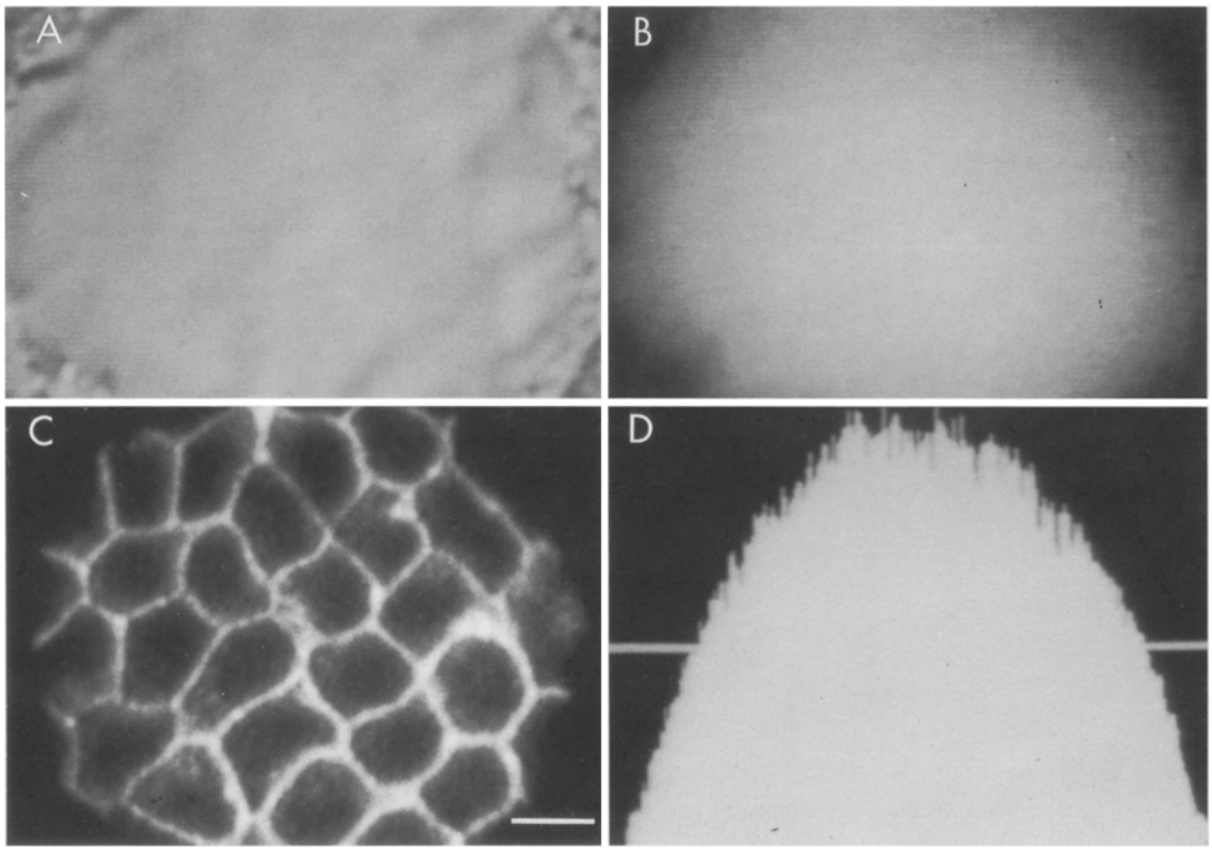

Fig. 2. Fluid-filled dome observed under (*A*) bright field and (*B*) fluorescence microscopy (488 nm excitation/510 nm emission). Panel *C* shows the fluorescence pattern of the cells adjacent to the dome at an approximately fourfold higher image intensifier gain setting. Panel *D* shows the intensity profile across the center of the image in panel *B* as indicated by a horizontal line. The intensity is greatest at the dome center and decreases towards both edges. Scale bar = 10 μm

Identification of the Fluorophore

The classes of compounds in DME most likely to be fluorescent were the amino acids, the vitamins and phenol red, the pH indicator added to most culture media. The amino acids seemed a less likely source because their absorption maxima are in the UV. Measurement of the fluorescence of solutions of phenol red and each of the vitamins showed that phenol red was weakly fluorescent and riboflavin was strongly fluorescent when excited at 488 nm. A culture medium was prepared in which both of these compounds were eliminated and MDCK cells were grown for several passages. These cultures no longer showed the strong fluorescence seen at 488 nm excitation but retained the weaker punctate emission when excited at 488 or 514 nm. Reintroduction of media containing phenol red for 15–120 min failed to change this pattern. Exposure of cultures to riboflavin-containing media for as little as 5 min resulted in the bright cell outlines and domes seen in unaltered DME. The effect of longer term reintroduction of each of these compounds was also examined to determine the effect of accumulation over time and/or cell replication. Data from one representative group of sister cultures is shown in Table 1. The MDCK cells grown in the absence of riboflavin and phenol red were subcultured into each of the media indicated and grown to confluency over 8 days. Multiple regions were examined, and the mean fluorescence intensity was determined. Only cultures containing riboflavin showed high levels of fluorescence. Although the combination of phenol red and riboflavin appears to be elevated versus riboflavin alone, culture variability can result in differences of similar magnitude. Importantly, removal of riboflavin for as little as three days resulted in cultures whose fluorescence was indistinguishable from those passaged multiple times in riboflavin-free DME.

Table 1.

Culture conditions	Fluorescence intensity
DME − phenol red − riboflavin	87 ± 4
DME + phenol red − riboflavin	90 ± 14
DME − phenol red + riboflavin	245 ± 32
DME + phenol red + riboflavin	368 ± 84
DME + phenol red + riboflavin 8 days + phenol red − riboflavin 3 days	79 ± 8

Fluorescent data are means ± SE for 4–9 regions of a single culture for each treatment. Multiple qualitative observations established that the major source of intensity variations occurred between regions of a culture. MDCK cells grown for 6 months in DME − phenol red and − riboflavin were subcultured (1 : 5) into the media indicated and grown to confluence. For the last treatment the cultures were fed with the two different media for the period indicated. The fluorescence intensity is the mean grey level for a 200 × 200 pixel region enclosing 10–15 cells using 488 excitation/510 emission.

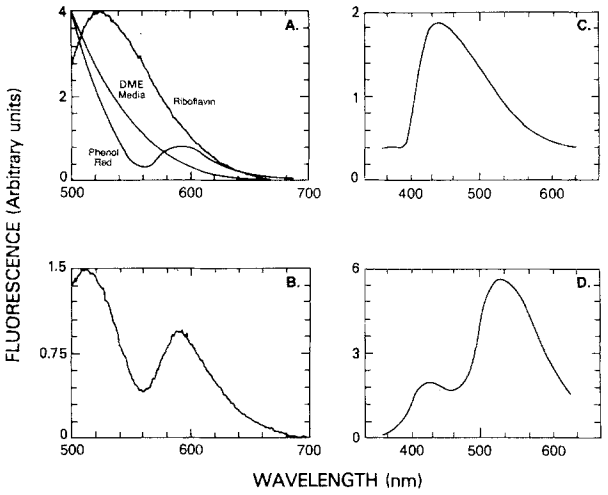

Fig. 3. Fluorescence emission spectra of culture media (*A* and *B*) or MDCK cell dome contents (*C* and *D*). Spectra were obtained with 360 nm excitation. Panel *A* shows the emission for DME culture media without riboflavin or phenol red, with phenol red only or with riboflavin only at their normal media concentrations. *B* is culture media containing both phenol red and riboflavin and illustrates the approximately 3-nm blue shift in the peak maximum from 520 to 517 nm seen in the riboflavin spectrum. *C* is the spectrum from a MDCK cell dome grown without riboflavin or phenol red. The major peak is at 448 nm, and none is seen above 500 nm. *D* is the emission spectra for a MDCK dome grown in standard culture media. The major peak is at 517 nm, the minor peak at 448 nm, and a small shoulder is at 529 nm. The major peak corresponds well to the blue-shifted riboflavin peak seen in *B*. Note changes in fluorescent intensity scale between spectra, especially *A* and *B* and *C* and *D*

Microspectrofluorimetry

Additional evidence for riboflavin being the source of the fluorescence was obtained by microspectrofluorimetry. Figure 3*A* and *B* shows emission scans for culture media minus phenol red and riboflavin, media with each compound, as well as media with both compounds. The emission maxima are 520 and 580 nm for riboflavin and phenol red, respectively. Riboflavin shows a slight blue shift to 515 nm in the presence of phenol red. Figure 3*C* and *D* shows spectra obtained from large domes using a microspectrofluorometer. In Fig. 3*C* the culture was grown in media free of phenol red and riboflavin, while the culture in Fig. 3*D* was exposed to normal DME for 30 min prior to the scan. A large emission at 420 nm is seen in both instances and most likely is from cytochromes. In the absence of phenol red and riboflavin no additional maximum is seen beyond 500 nm. In the presence of these two compounds a single additional maximum exists at 517 nm, but none exists in the region corresponding to phenol red. From these observations we concluded that the major source of fluorescence within the domes was riboflavin.

Transepithelial Riboflavin Transport

An important implication of the above finding was that riboflavin was transported from the apical bath to the basal surface of the MDCK cells. We performed experiments to examine this transport phenomenon using quantitative video microscopy of the fluorescence arising from the domes. Figure 4 shows a representative time course of riboflavin uptake into a single dome, grown in the absence of riboflavin and then exposed to 10 μM riboflavin in the perfusate. Maximum concentration is reached within 10 min, with the uptake rate being relatively linear for the first 5–7 min. Figure 5 shows mean values for the first 5 min of uptake measured at 30-sec intervals (control). Thirty seconds after riboflavin addition to the perfusate, the dome concentration has increased to 30 μM, significantly above the perfusate concentration of 10 μM. The mean concentration at 5 min was 565 ± 80 μM (n = 11; Table 1), or approximately a 60-fold increase above the perfusate concentration. Values for individual domes ranged from 100 to 1000 μM. The mean rate of riboflavin transport into the domes was 189 ± 30 pmol/min-cm^2 (Table 1), which was calculated from concentration values obtained between 1 and 3 min after riboflavin addition.

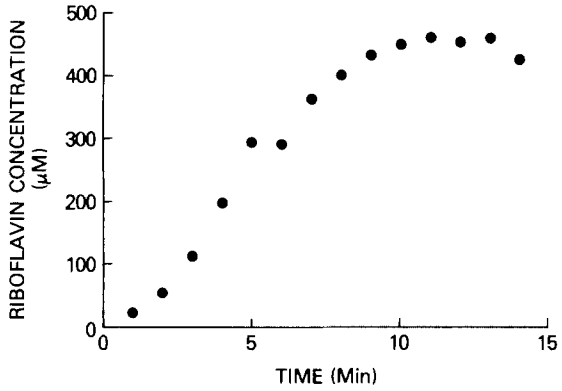

Fig. 4. Riboflavin concentration in MDCK cell domes *versus* time. Time course is for a single representative dome. Uptake rate appears to be linear for approximately 5 min

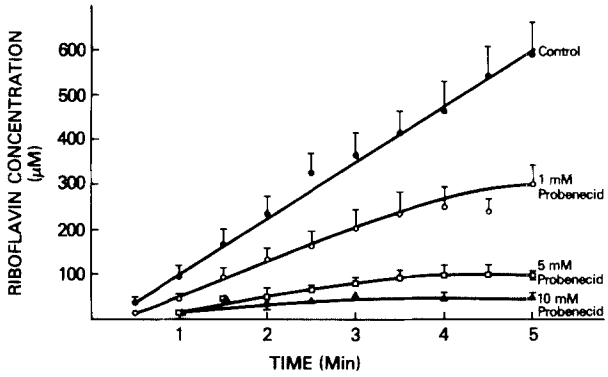

Fig. 5. Riboflavin concentration in MDCK cell domes *versus* time in the presence of varying concentrations of probenecid. Data are means ± SE (*see* Table 2 for respective *n* values)

Probenecid Inhibition of Riboflavin Uptake

Riboflavin transport studies in a variety of tissues and preparations, including in vivo renal clearance measurements, have demonstrated that an active transport process for this compound is sensitive to the competitive inhibitor of organic anion transport, probenecid (Jusko & Levy, 1975). Figure 5 and Table 2 also show the results of uptake studies in the presence of 1, 5, and 10 mM concentrations of probenecid. At all concentrations of probenecid, inhibition of uptake was observed as early as the 1-min time point (Fig. 5). Probenecid inhibited both the dome riboflavin concentration and uptake rate in a dose-dependent fashion. 10 mM probenecid almost completely inhibited uptake, the dome concentration and rate being reduced to 8 and 16% of control, respectively (Table 2). The inhibition of uptake by 1 mM probenecid was 56%, suggesting that this concentration is likely to be close to the true IC_{50} and apparent inhibitor constant (K_I) for this compound.

Discussion

Riboflavin Fluorescence and Autofluorescence in MDCK Cells

The finding that MDCK cell cultures transport riboflavin is a solution to an important methodological problem. The effort to discover the cause of what we initially believed to be autofluorescence was motivated by wanting to use these cells in quantitative low-light level fluorescent microscopy experiments. Previous characterization of the low-light level imaging system demonstrated that specimen emission levels of 5×10^{-7} to 5×10^{-6} foot candles were likely to give sufficient radiometric precision and temporal resolution for subsequent physiological studies (Spring & Lowy, 1989). Use of the lowest acceptable light levels is essential to reduce the time-intensity integral of specimen exposure to the excitation light, thus reducing the degree of photobleaching and photodamage, which can induce artifacts. Specimen autofluorescence is an important parameter in setting the practical limit of probe molecule concentration and excitation intensity necessary to obtain good signal-to-noise levels (Taylor & Salmon, 1989; Murphy, 1986). We had expected to find some level of autofluorescence, as emissions associated with cytochromes (NADH), protein-bound flavins and intracellular riboflavin have been observed in diverse cell types (Aubin, 1979; Benson et al., 1979). In kidney tubules autofluorescence has been used to distinguish between mitochondria-rich cells and other cell types (Kohler & Fromter, 1985). Similar to reports by these authors, we observed a punctate fluorescence with broad excitation between 360 and 514 nm and emission above 510 nm. We also identified cellular emissions which are likely due to NADH at 448 nm (Fig. 3*D*). Unlike previous reports, we observed a fluorescent emission above 500 nm that was not punctate and in most cases was considerably more intense than that associated with cell organelles (Fig. 1*A*). The fact that the intensity was approximately fivefold higher than the previously determined optimal operating limits for low-light level studies suggested that the utility of this cell line for imaging methodology was seriously compromised. Fortunately, the major source of this high-level background was identified as riboflavin. Removal of this one compound from the culturing media reduced the cell background emission for excitation wavelengths near 480 nm to levels well within acceptable limits for low-light

Table 2.

Condition	Dome riboflavin concentration		Riboflavin uptake rate	
	μmol at 5 min	% Control	pmol/min-cm²	% Control
Control	565 ± 80	100	189 ± 30	100
$n = 11$				
1 mM Probenecid	304 ± 47	54	83 ± 12	44
$n = 9$	$P < 0.02$		$P < 0.001$	
5 mM Probenecid	99 ± 7	18	35 ± 2	18
$n = 3$	$P < 0.02$		$P < 0.01$	
10 mM Probenecid	48 ± 20	8	31 ± 17	16
$n = 3$	$P < 0.01$		$P < 0.01$	

Data are means ± SE for indicated n. P values are unpaired t test of control *versus* probenecid treated cultures. The concentration 5 min after riboflavin addition was used for these quantitative comparisons. After 5 min a significant number of the domes examined appeared to collapse, but the time course of the shape change was variable. There was no readily observable difference in the initial appearance of domes which did or did not change shape.

level imaging. The fluorescence remaining in MDCK cells in the absence of riboflavin appeared to be largely from cytochrome or cytochrome-like sources. We have not attempted to grow MDCK cells in any of the completely defined culture media developed for MDCK cells, and it is likely some riboflavin is present in the fetal bovine serum (Aubin, 1979). Such an approach may or may not compromise these cell's nutritional requirements but probably would not reduce the fluorescence levels further as this approach was not successful in other cell lines (Aubin, 1979). We would caution that we have not attempted to carefully characterize the nutritional effects of eliminating exogenous riboflavin from the culture media formulation. Most media, including those such as MEM, also commonly used for MDCK culture, have considerably less riboflavin in the standard formulation. Also over one year of study we have noticed no gross changes in cell morphology, rate of growth or survival during passage, but these parameters have not been examined quantitatively.

Riboflavin Transport

The second major finding of this study is that MDCK cells are capable of the transepithelial transport of riboflavin. This report appears to be the first in vitro study of transepithelial riboflavin transport for any renal tissue or renal-derived cells. The concentration gradient measured across the dome is considerable, averaging approximately 60-fold. A passive process resulting in redistribution due to electrochemical gradients across the domes seems unlikely. The standard MDCK cell line, including the domes, is a leaky epithelium, and the magnitudes of transepithelial driving forces are modest. The reported transepithelial potential across the domes of +1.2 mV (Rabito et al., 1978) is considerably less than the approximately 100 mV Nernst potential that would be necessary to account for the observed riboflavin concentration. Similarly, if a weak anion transport mechanism is assumed, a pH gradient on the order of two units, e.g., an internal pH of 5.5, would be needed to maintain the anion redistribution. Measurements of cation gradients are in keeping with the modest transepithelial potential and low transepithelial resistance of this cell line. Sodium and potassium concentrations in the domes of MDCK cells were shown to be not significantly different from those of the bathing medium (Lifschitz, 1986; Rabito et al., 1978). Only chloride has been found to be reduced in the dome fluid by 22 mM, attributed to secondary active transport as identified in a wide variety of tissues (Lifschitz, 1986). The specific transport mechanism for riboflavin needs further characterization. However, while there is a considerable literature on the biochemical and nutritional role for this vitamin (Rivlin, 1975), studies on riboflavin transepithelial transport in general or in kidney tissues in particular are not extensive. In vivo clearance studies in humans, rats and dogs demonstrated that riboflavin was not only cleared by glomerular filtration but also secreted and reabsorbed by the renal tubules of humans and dogs (Jusko & Levy, 1975). The site of the secretory component was thought to be in the proximal tubule, but no suggestion has been made for the absorptive component (Jusko & Levy, 1970; Jusko, Rennick & Levy, 1970*b*). Probenecid was shown to inhibit renal excretion in a dose-dependent fashion

and evidence was obtained for an effect both on the secretory and reabsorptive processes (Jusko et al., 1970*a*,*b*). The only previous in vitro study to date examined the uptake of radiolabeled riboflavin into kidney slices (Spector, 1982). It was concluded that an active absorption process existed and that this process was inhibited 37% by 1 mM probenecid. Evidence was also presented that uptake was inhibited by weak acids, including PAH, but not the weak base tolazine. In rat small intestine (proximal jejunum) voltage-clamp studies have demonstrated sodium-dependent reabsorption of riboflavin (Daniel, Wille & Rehner, 1983). This process had an apparent K_m of 0.54 μM. At 37°C in the presence of 10 μM riboflavin, a transport rate of 0.8 pmol/min-cm^2 was observed. The transport rate of 189 pmol/min-cm^2 exhibited by MDCK cells is far more potent. At present the data concerning riboflavin transport do not exclude any of several mechanisms reported for organic ion transport. Transport of acidic vitamins in the intestine is thought to be primarily via a sodium-dependent cotransport process, although it has been suggested that nonionic diffusion coupled to Na-H exchange may also play a role in the transport of organic anions (Moller & Sheikh, 1983; Murer & Burckhardt, 1983). A broadly specific reabsorptive system for aliphatic and monocarboxycyclic acids that is sodium dependent has been characterized in proximal tubule (Ullrich et al., 1982). Organic anion exchange mechanisms, with or without coupling to Na-H exchange, could also participate in the movement of this compound (Guggino, Martin & Aronson, 1983). It also remains to be determined whether the direction of transport represents a process occurring in distal tubules, the possible origin of MDCK cells (Rindler et al., 1979) or is an anomalous process unique to MDCK cells. The finding of this previously undescribed property of MDCK cells provides a new opportunity to study transport of this and possible other organic species in renal tissue. MDCK cells are readily cultured, genetic variant subclonal lines already exist and there are well established methods for studying transepithelial transport. Therefore, they should provide an ideal preparation in which to apply both physiological and molecular biological methods to characterize this renal transport mechanism.

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