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An anomalous effect of the intermediate products of riboflavine photolysis on the intestinal absorption of poorly absorbed water-soluble drugs in rats

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The influence of the intermediate products of riboflavine photolysis on the absorption of poorly absorbed water-soluble drugs from the rat small intestine has been examined using an *in situ* recirculation technique. The absorption of phenol red, bromphenol blue (BPB) and their biliary excretion, and lactose isonicotinoyl-hydrazone (lactose-INH) and its plasma concentration were increased in experiments made in the presence of light in contrast to others made in the dark. The absorption of both phenol red and lactose-INH were concentration-dependent. On the other hand, a saturation phenomenon was demonstrated when the concentration of phenol red was kept constant while that of the water-soluble compound flavine mononucleotide (FMN) was changed. The results which were obtained from pretreatment experiments suggest an alteration in the permeability of the intestinal membrane. No enhancement effect could be demonstrated for lumichrome in the presence or absence of light.

Most investigations focused on studying the stability of riboflavine in pharmaceutical preparations (Uehara, Mizoguchi & Okada 1964; Uehara, Mizoguchi & others, 1966) have demonstrated a photochemical destruction of adenine and inactivation of NAD and NADP by irradiation with visible light in the presence of riboflavine. Recently *in vivo* experiments were made (Kostenbauder & Sanvordeker, 1973) that demonstrated an acceleration of bilirubin photodecomposition by riboflavine in the presence of light. No studies of the influence of riboflavine photolysates on biological membranes and the absorption processes of drugs have been reported. We therefore investigated whether they have an effect.

MATERIALS AND METHODS

Reagents. All the chemicals were reagent grade unless otherwise specified.

Source of light. This was two 60 W tungsten electric lamps placed 25 cm from perfusion glass tubes of 70 cm length and internal diameter of 2·0 mm and external diameter of 3·5 mm. During the absorption experiments the perfusion solutions were recirculated exposed to light. Protected experiments were made in the dark.

Perfusion solutions. The specified amounts of drugs were dissolved in 0·1 mM solution of riboflavine (unless otherwise specified) in pH 6·5 isotonic phosphate buffer contained the following concentrations of salts: 0·123 M Na_2HPO_4 and 0·163M NaH_2PO_4.

Intestinal absorption procedures. Male Wistar rats, 150–200 g, were prepared according to Koizumi, Arita & Kakemi (1964). The bile duct was ligated in all experiments except those in which biliary excretion was collected by cannulating the duct. The perfusion solution total volume 40 ml was perfused at a rate of 5 ml min^{-1}. After 1 h, or the specified time in time course experiments, the solution in the intestine was removed, the intestine washed with saline into the same perfusion flask and the final volume was adjusted to 100 ml. The pH of the final fluid was measured and it was found to be $6{\cdot}4 \pm 0{\cdot}1$. The decrease in the amount of drug in the perfusion solutions during the course of an experiment was determined and the amount absorbed was calculated.

Blood sampling. Blood was collected in a heparinized syringe through a heart puncture immediately at the end of the perfusion, centrifuged at 3000 rev min^{-1} for 20 min and the clear supernatant aspirated for assay of the drug.

Methods

Lactose isonicotinoyl-hydrazone was determined by the method of Kakemi Sezaki & others (1969) except that $NaNO_2$ was not added during its estimation in plasma.

Bromphenol blue in perfusion solution was measured by taking an aqueous sample from the perfused solution and diluting it with pH 6·5 phosphate buffer. Its extinction was then measured at 591 nm.

Bromphenol blue adsorbed on the intestinal membrane was measured at the end of the perfusion and after washing the gut with saline. The gut was removed, weighed, chopped and homogenized with an amount of pH 6·5 phosphate buffer equal in ml to double its weight in g. The final volume of the homogenate was recorded and then a 5 ml aliquot was mixed with 5 ml acetone. The mixture was shaken for 15 min then centrifuged at 3000 rev min^{-1} for 10 min and the extinction of the clear supernatant was measured at 596 nm. From a calibration curve for BPB the amount of dye adsorbed and that remaining after perfusion were determined, then the amount absorbed was calculated by difference.

Bromphenol blue in bile was measured by dilution of the bile with pH 6·5 phosphate buffer and measurement of the extinction at 600 nm. All the values were corrected for the control.

RESULTS AND DISCUSSION

The results (Table 1) show that the absorption of phenol red, BPB and lactose-INH was significantly increased in the experiments in which riboflavine had been exposed to light relative to those made in the dark. The enhancement was confirmed by biliary excretion of both phenol red and BPB, and plasma concentration of lactose-INH. As aqueous solutions of riboflavine are susceptible to light, this anomalous phenomenon can be attributed to an effect induced by photodegradation products of riboflavine.

We wished to examine further the role of these photolysates and their effect on absorption processes. Hence, time course experiments for the absorption of phenol red were made. From Fig. 1 it can be seen that the absorption of phenol red after 15 min exposure proceeded at a constant rate following apparent first order kinetics.

Its rapid disappearance in the early minutes can be ascribed to uptake by the intestinal mucosa.

Studies were made to determine the relation between the concentration of the substance and its rate of absorption. Fig. 2a shows that the absorption of phenol red and lactose-INH was concentration-dependent. This observation suggests that the process responsible for their absorption under the influence of the intermediate products of photolysis was saturable.

Table 1. *Effect of riboflavine photolysates on the intestinal absorption of drugs and their biliary excretion or plasma concentration.*

Drug*	Concn (mM)	% absorbed in 1 h† Protected		Exposed		Biliary excretion or plasma concentration Protected		Exposed	
Phenol red	0·14	7·3	s.d. 0·2	22·5	s.d. 0·2[a]	4·8	s.d. 1·2	8·1	s.d. 1·0[1,c]
Bromphenol blue	0·10	3·4	2·0	13·0	1·4[b]	1·6	0·9	3·2	0·3[1,c]
Lactose-INH	0·10	1·7	1·5	29·8	0·7[a]	2·7	1·0	9·4	1·1[2,a]
	2·00	6·2	0·8	17·3	0·4[b]	10·1	1·6	54·3	6·9[2,b]

* Each drug was dissolved in 0·1 mM riboflavine solution in pH 6·5 phosphate buffer.
† The % absorbed in 1 h expressed as the mean of at least three determinations with s.d.
[1] Biliary excretion in 90 min (μg). The mean of at least three determinations with s.d.
[2] Plasma concentration after 60 min (μg ml^{-1}). The mean of at least three determinations with s.d.
a, Significantly different from protected experiment, $P < 0{\cdot}001$; b, $P < 0{\cdot}01$; c, $P < 0{\cdot}05$.

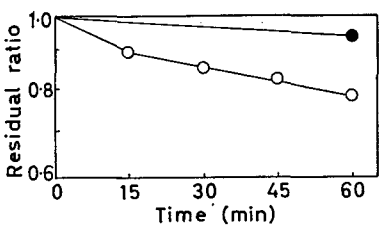

FIG. 1. Semilogarithmic plots of the disappearance of phenol red from the lumen of the rat small intestine *in situ*. Protected (solid circle), exposed (open circles). Phenol red (0·14 mM)dissolved in solution of riboflavine (0·1 mM) in pH 6·5 phosphate buffer.

The conditions were then reversed. The concentration of phenol red kept constant and the initial concentration of riboflavine was changed. Fig. 2b demonstrates a significant increase in the absorption of phenol red on increasing the concentration of riboflavine from 0·05 to 0·1 mM in exposed solutions. Examination of the effects of higher concentrations of riboflavine were precluded because of its limited solubility. In its place the water soluble compound flavine mononucleotide (FMN) was used. From Fig. 2b a saturation phenomenon can be observed. The inhibition on using 5 mM FMN was insignificant. A possible explanation for this is that the number of photons which attacked the drug molecules remained constant because the source of light was kept constant throughout the experimental work. Hence, it would appear that increasing the concentration of FMN beyond a certain limit has no effect on the absorption process.

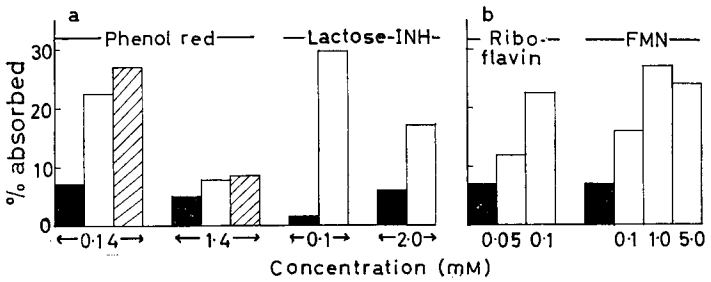

FIG. 2 a. The relation between the concentration of phenol red or lactose-INH and their intestinal absorption. Control, protected (solid columns); 0·1 mM riboflavine, exposed (open columns); 1·0 mM FMN, exposed (hatched columns). Drugs were dissolved in riboflavine (0·1 mM) or FMN (1·0 mM) solution in pH 6·5 phosphate buffer and perfused through the intestine for 1 h.

b. Effect of the initial concentrations of riboflavine and FMN on the intestinal absorption of phenol red. Protected (solid columns); exposed (open columns). Phenol red (0·14 mM) was dissolved in various concentrations of either riboflavine or FMN solutions in pH 6·5 phosphate buffer and perfused through the rat small intestine for 1 h.

To clarify the mechanism of action of riboflavine photolysates on the absorption of drugs, pretreatment experiments were made. Table 2 shows that the percentage of phenol red absorbed from the solution immediately after pretreatment was much

Table 2. *Effect of pretreatment with riboflavine photolysates on the absorption of phenol red from the intestine.*

Experimental condition	% absorbed in 1 h*	
Control[1]	3·5 s.d. 0·9	
Pretreatment[2]	10·1	1·1[b]
Simultaneous recirculation[3]	22·5	0·2[a]

[1] 0·14 mM phenol red solution in pH 6·5 phosphate buffer was perfused through the intestine for 1 h.

[2] 0·1 mM riboflavine solution in pH 6·5 phosphate buffer was perfused through the intestine for 1 h in the presence of light, then it was washed out with saline and replaced by 0·14 mM phenol red solution in pH 6·5 phosphate buffer which was perfused for 1 h without light.

[3] 0·14 mM phenol red was dissolved in 0·1 mM riboflavine solution in pH 6·5 phosphate buffer and perfused through the intestine for 1 h exposed to light.

[a] Significantly different from control value, $P < 0{\cdot}001$.

[b] Significantly different from control value, $P < 0{\cdot}01$.

* % absorbed in 1 h expressed as the mean of at least three determinations with s.d.

greater than that absorbed from the control solution but less than that from its simultaneous recirculation. From these results the possibility of an interaction between the photolysates and phenol red and consequently its effect on the absorption process can be excluded. At the same time these results show a transient and reversible effect of the photolysates of riboflavine on the intestinal membrane which suggests an alteration in its permeability with a return to normal within a short period. This assumption is conceivable as there have been previous reports (Feldman & Gibaldi, 1969; Feldman, Salvino & Gibaldi, 1970; Kakemi, Sezaki & others, 1970; Feldman, Reinhard & Willson, 1973) demonstrating a change in the permeability of the intestinal membrane due to the effect of bile salts and thus enhancing the absorption of drugs.

We also investigated the influence of irradiation on lumichrome and other flavines and their effect on the absorption of phenol red from the intestine. It is clear from

Table 3 that with lumichrome, the percentage absorbed is unchanged in the presence or absence of light. As it has been reported previously (Oster, Bellin & Holmstrom, 1962) that lumichrome is one of the end products of aerobic photolytic decomposition of riboflavine, this result strongly supports the concept of the involvement of some intermediate products in such a process. The slight but significant effect of flavine-adenine dinucleotide (FAD) photolysates can be ascribed to the quenching effect of

Table 3. *Effect of lumichrome and photolysates of different flavines on the absorption of phenol red* from the intestine.*

Compound	% absorbed in 1 h† Protected	Exposed
Riboflavine	7·3 s.d. 0·2	22·5 s.d. 0·2[a]
Flavine mononucleotide	6·9 1·3	15·8 1·6[b]
Flavine-adenine dinucleotide	5·8 0·5	10·0 0·8[b]
Lumichrome	6·4 2·0	6·2 1·8

* 0·14 mM phenol red was dissolved in 0·1 mM solution of either riboflavine, FMN, FAD or lumichrome in pH 6·5 phosphate buffer.

† The % absorbed in 1 h expressed as the mean of at least three determinations with s.d.

a, Significantly different from protected experiment, $P < 0{\cdot}001$; b, $P < 0{\cdot}01$.

the adenine part of the molecule which acts as a stabilizer for the FAD molecule against photolytic decomposition (Weber, 1950). The difference between the percentage of phenol red absorbed in the presence of riboflavine and that in the presence of FMN can be attributed to a difference in the lipophilicity of two molecules. Riboflavine is much the more lipophilic and its photolysates might be expected to retain the same property.

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