the delayed effects of Sr were mediated by an increase in the slow inward current carried by Sr. These results were quite consistent with the properties of nonhibernating animals during the nonhibernating season. Thus, the myocardium of nonhibernating animals obtained during the hibernating season has a dual nature, one observed in hibernating animals and another observed in nonhibernating animals obtained during the nonhibernating season. This suggests that the cardiac function is changed during the hibernating season whether the animals hibernate or not. Paradoxically, some of the preparations obtained from nonhibernating animals during the hibernating season exhibited APps with relatively high amplitudes. The electromechanical characteristics of these preparations were similar to those seen in nonhibernating animals during the nonhibernating season (fig. 2a).

Nifedipine-sensitive electromechanical responses were induced by prolonged exposure to Sr in nonhibernating animals during the hibernating season, but not in the hibernating animals. A previous study[3] indicates that the lack of effect of Sr in hibernating animals is due to the inhibition of the slow inward current by a large transient outward current which has been shown to be independent on intracellular Ca. In nonhibernating animals during the hibernating season, however, blockade of Ca-activated potassium outward current[7,8] by Sr cannot be eliminated as a possible mechanism. Although the explanation for this difference between these two preparations is not clear, it is interesting that the electrophysiological changes in the myocardium during the hibernating season may be closely correlated to an outward current which is less sensitive to Sr.

In conclusion, the electrical and mechanical characteristics of cardiac muscle seen in hibernating animals occurs, at least in part, before the animals begin hibernating; the changes in cardiac function are not simply the result of hibernation. This suggests the possibility that hibernation is induced or regulated by as yet unknown factors[9–13]. If this is so, some responsible substance(s) may be present in animals during the hibernating season. Cardiac muscle may be one of the target organs affected by such substance(s). The present findings could provide a useful model for studying the mechanism of hibernation and the existence of some hibernation trigger substance(s).

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Renal handling of bilirubin photoderivatives[1]

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Summary. The renal handling of unconjugated bilirubin in the dark and during light exposure was analyzed using an isolated rat kidney preparation. The parameters tested were pigment disappearance from the perfusion medium, pigment uptake by tissue, and its renal clearance. The results indicated that despite the fact that pigment disappearance from the medium was similar for both forms of pigment, the extraction ratio was higher for irradiated pigment than for pigment in the dark. When renal clearance of pigment was plotted vs pigment uptake of tissue, the results indicated that irradiated pigment may be more efficiently removed by the kidney. In addition, data on the rate of secretion of p-aminohippurate suggested that both pigment forms shared a common site for secretion.

Key words. Bilirubin; phototherapy; organic anion transport; renal clearance.

Since 1958, phototherapy treatment of jaundiced infants has been widely employed both to prevent and to control neonatal hyperbilirubinemia[2]. Despite its widespread use, debate continues about several important points such as the most effective light source[3,4], sites of light action[5,6], intermediates, and final products of the photochemical reaction in vivo[7–10] and excretion fate of these products[7,8,10–15].

Experiments on Gunn rats provided the first key to the chemistry of phototherapy. First, wavelength-dependence studies indicated that the photoreceptor is unconjugated bilirubin (UB) itself[10,16]. Second, excretion studies showed that the slow decline in serum UB during phototherapy is preceded by a much faster, almost instantaneous excretion of yellow pigment in bile[8,11,12]. Moreover, despite some uncertainties, most data indicate that the photoisomerization pathway is far more important quantitatively than photooxidation in human infants and rats[15,17–19].

The role of the kidney in the excretion of UB is not clear enough[20–25], and data on the participation of that organ during phototherapy are scarce[12,14,15,26–29]. The main point analyzed was the chemical structure of the yellow pigment which appeared in the urine shortly after the phototherapy[14,15,26]. However, the mechanisms involved in the renal excretion of UB photoderivatives are poorly known. Therefore, in this study, the urinary excretion rate of UB photoderivatives was analyzed using an isolated rat kidney preparation, in comparison with the excretory rate of UB not exposed to light.

Materials and methods. Animals. Male Wistar rats weighing 300–350 g were used as kidney donors for all studies. Animals were allowed free access to a standard diet and tap water until used.

Perfusion procedure and apparatus. The animals were anesthetized with sodium pentobarbital (40 mg/kg b.wt, i.p.). The right kidney was prepared as previously described[24,25]. Arterial samples were collected from a catheter inserted in the mesenteric artery, and urine samples from an ureteral catheter. Venous effluent drained into a reservoir and recirculated. The perfusion medium (pH 7.5) consisted of Krebs-

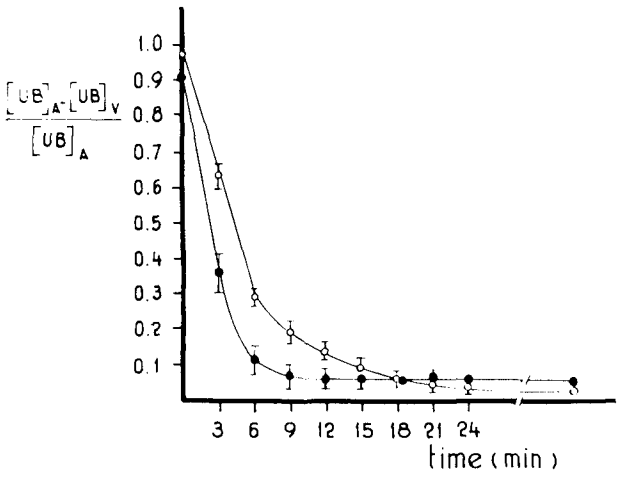

Figure 1. Kidney uptake vs time plots for UB irradiated, and not exposed to light. UB in the dark (○——○): A = 0.029 ± 0.007; B = 0.984 ± 0.011; K = 0.193 ± 0.006 min^{-1}; r = 0.997.
UB exposed to light (●——●): A = 0.050 ± 0.003 (*); B = 0.935 ± 0.020; K = 0.393 ± 0.007 min^{-1} (*); r = 0.976.
Data are mean values ± SEM. The asterisks indicate statistically significant differences ($p < 0.05$).

Ringer solution enriched with glucose (10 mM), sodium pyruvate (5 mM) and sodium lactate (5 mM), and contained creatinine (Cr) (400 mg/l) for measurement of glomerular filtration rate (GFR), and p-aminohippuric acid (PAH) (10 mM). The medium also contained 0.5 mM cysteine, 0.5 mM glutamic acid and 2.3 mM glycine in order to prevent the loss of glutathione from specific regions of the kidney and to improve the viability of the preparation[29]. The medium was constantly bubbled with O_2–CO_2 (19:1, v/v). The whole system operated thermostatically controlled at 37 °C. Perfusion flow through the isolated kidney in situ was performed with the use of a peristaltic pump (American Instrument Co. USA, cat. 5-8954) at a constant pressure of 100–110 mm Hg measured at the tip of the arterial cannula by means of a mercury manometer. The perfusion rate, measured with a flowmeter (Gilmont Instruments Inc. USA) inserted in the arterial line, ranged from 19–25 ml/min.

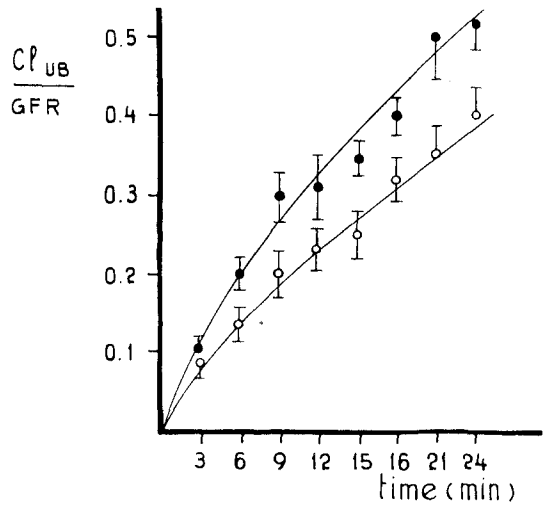

Figure 2. Renal clearance of UB irradiated, and not exposed to light. ○——○ UB in the dark; ●——● UB exposed to light. Data are mean values ± SEM.

Experimental procedure. The isolated kidney was perfused for about 20 min (equilibration time) until perfusion pressure and flow remained constant. The viability of the preparation was assessed in every experiment. For this purpose, clearance studies were performed at 3-min intervals for 24 min with arterial and venous sampling at the midpoint. Clearance of Cr, tubular reabsoption of glucose and sodium, and tubular secretion of PAH were systematically determined.
The filtration fraction and the water excretion fraction were also calculated. Three experimental groups were studied. 1) Preparations perfused with UB which was added to the perfusate solutioin as a single dose immediately after the equilibration time; UB was previously dissolved in 0.1 M NaOH and Krebs-Ringer solution containing an amount of albumin enough to give a molar ratio of bilirubin to albumin of 20:1[30]. Samples for clearance studies were collected as stated above. UB concentrations in arterial, venous and urine samples were also measured in addition to the viability parameters. All the procedures were carried out under a red photographic safety light. 2) Preparations perfused with UB exposed to light irradiation as soon as it was added to the perfusate solution; irradiation was maintained throughout the experiment. UB solution was prepared as described above. Light irradiation was provided by two circular daylight fluorescent lamps (Phillips, TLE 32W/54) placed at a distance of 15 cm around the reservoir. They provided 3.1 (± 0.4). 10^6 cuantas/s per cm^2 of radiant flux at the reservoir surface. Samples were collected as described above. 3) Control preparations to assess kidney viability throughout the experiment in the absence of pigment.
At the end of the experiment the kidney was removed, gently blotted on filter paper, and weighed.
Analytical methods. Cr was determined by Jaffe's reaction, PAH by Waugh and Beall's procedure, and sodium and potassium by flame photometry as described previously[24,25]. Urine volume was estimated gravimetrically, and pigment concentration was determined by direct spectrophotometry at 450 nm assuming the same molar absorption coefficient as described by Lamola et al.[31]. UB and its photoderivatives were not separated owing to the unavailability of methods. The absorption spectra of samples collected from artery, vein and ureter catheters were recorded from 350 to 500 nm against Krebs-Ringer solution. In order to identify spectral changes throughout the experiment, the differential spectrum was determined for each sample at various times of perfusion, using the sample from the first 3-min period as a blank (Varian 634, Varian 9176 Recorder, Australia).
Calculations. All the parameters of kidney viability were calculated conventionally. The uptake rate of pigment by renal tissue was calculated for each clearance period applying the arterious-venous extraction ratio. The relationship between the pigment uptake rate during the first 3-min period and the pigment concentration in the perfusate solution was also evaluated. Pigment clearances were calculated for experimental groups 1 and 2.
Statistical analyses. The results are presented as mean ± SEM. Differences between groups were assessed by the t-test for unpaired data. The first-order decline in perfusate pigment concentration was calculated by the method of least squares.
Chemicals. All chemicals were of the highest grade commercially available. UB was from Koch-Light.
Results. Functional criteria of the preparation. Data showing functional characteristics of control preparations during the first 3-min clearance period are presented in the table and they were similar to those reported previously[24,25]. The perfusion flow (ml/min), the GFR (ml/min/g) and the glucose reabsorption rate referred to GFR (μmol/ml), remained constant throughout the experiment. The percentage of sodium reabsorption and the water excretion were unchanged

Functional parameters obtained for control isolated rat kidney preparations during the first 3-min clearance period

Kidney weight (g)	1.78 ± 0.05
Perfusion flow (ml/min)	18.0 ± 0.7
Urine volume (ml/min/g)	0.18 ± 0.02
GFR (ml/min/g)	0.31 ± 0.03
Glucose reabsorption rate/GFR* (μmol/ml)	7.14 ± 0.38
PAH secretion rate (μmol/min/g)**	0.072 ± 0.010
Sodium reabsorption rate/GFR (μmol/ml)	73.85 ± 2.72

Values are mean ± SEM (n = 20). The asterisks refer to concentrations in the perfusate solution: *glucose, 10.0 ± 0.5 mM; **PAH, 25.0 ± 2.0 μM.

throughout the perfusion, except for a 15–20% decrease during the last clearance period. Therefore, functional data on the isolated kidney indicated that they were in a constant state for at least seven 3-min periods after equilibration. In addition, pigment incorporation in the perfusion medium did not affect the viability of the preparation.

Pigment perfusate disappearance. The decay with time of medium pigment concentration was found to obey first order kinetics in both groups, at least over the time of the experiment. In spite of using different initial pigment concentrations (6–10 μg/ml), there were no differences in decay constants (k, min^{-1}) between groups (UB: −0.028±0.004, n = 12; UB+Hv: −0.020±0.003, n = 6).

Kinetics of pigment extraction from perfusion medium. Pigment removal from the perfusate by the kidney estimated by the extraction ratio (fig. 1) could be described as a monoexponential phenomenon in both experimental groups. Data were adjusted to a monoexponential uptake model ($y = A+B\ e^{-kt}$), by a standard non-linear least squares technique (BMDP P3R).

The actual physiological significance of the parameters was as follows: A was the steady-state value of pigment extraction from the medium by kidney tissue; this value, although small (in accordance with a medium being almost a reservoir) was significantly higher when UB was illuminated than when UB was in its native form. A+B was the value expected to represent $(\{UB\}_A-\{UB\}_V)/\{UB\}_A$ at zero time and, accordingly, it tended to be equal to 1 for each situation. The time constant κ were statistically different in the two groups.

Renal clearance of pigments. The time course of the renal clearance of pigments is presented in figure 2. A correlation

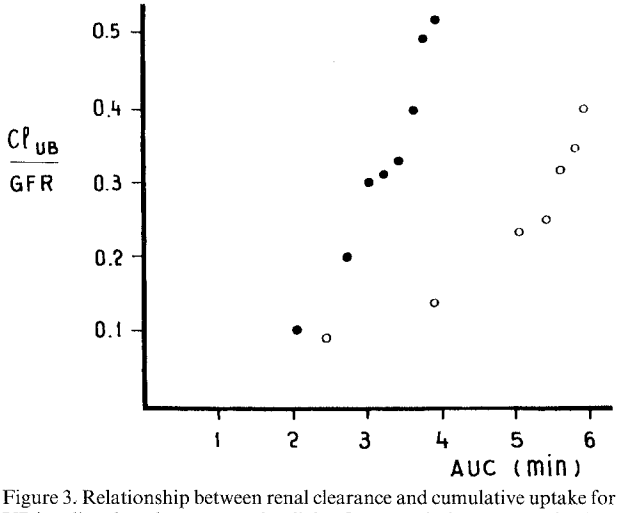

Figure 3. Relationship between renal clearance and cumulative uptake for UB irradiated, and not exposed to light. Open symbols correspond to UB in the dark (n = 12) and closed symbols for UB exposed to light (n = 6). Data are mean values; n: number of experiments.

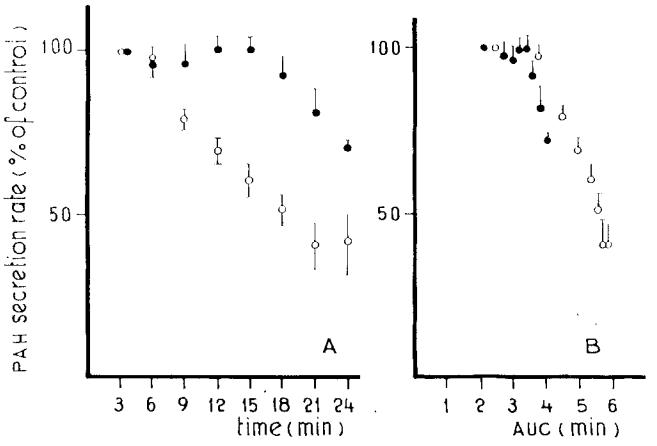

Figure 4. Relationship between PAH secretion rate vs time (A), and between PAH secretion rate vs cumulative uptake (B) for irradiated UB, and UB not exposed to light. Open symbols correspond to UB in the dark and closed symbols to irradiated UB. Data are mean values ± SEM.

between renal clearance and medium pigment disappearance could not be demonstrated.

Relationship between renal pigment cumulative uptake and pigment clearance. Figure 3 shows the time course of renal clearance of pigment as it was taken up by renal tissue estimated from the cumulative extraction ratio. This latter was calculated from the area under the extraction ratio-time curve for each experimental clearance period using the trapezoidal method. It could be seen that a good correlation (not linear) existed between the two variables. Although a higher value for uptake (25% of the total theoretical value) was obtained for UB in the dark than for irradiated UB (17%) the respective clearance value was higher for UB in the presence of light than in the dark.

Relationship between PAH secretion rate and pigment uptake by kidney. Figure 4 shows that the PAH secretion rate was progressively impaired as pigment was extracted from the perfusate solution and presumably accumulated in the kidney. Such an impairment was more pronounced in the preparations in the dark than in those illuminated (fig. 4A). There were no differences between groups in the impairment detected at similar values of pigment uptake (fig. 4B).

Spectral analysis of samples. Differential spectra from both experimental groups showed that the urinary pigment for both situations was spectrally similar to UB in water solution; pigment disappearance from the perfusate was mainly due to pigment which absorbed at 450 nm.

Discussion. Although the renal excretion of a brownish-green urine in newborns placed on phototherapy is familiar to clinicians, the renal excretion of bilirubin derivatives during such an exposure is not well documented. The urinary excretion of ^{14}C-bilirubin during and after phototherapy has been recorded in homozygous Gunn rats[13]. It was also reported that photobilirubin accumulates in the plasma of rats with interrupted bile flow[8] whereas others indicated that renal excretion participated during irradiation[28]. In this context, the rapid urinary excretion of different UB photoderivatives has been described[15]; photoderivateves were also found in the urine of premature infants during phototherapy[27]. Furthermore, although photooxidation is not the major photochemical event associated with phototherapy, its products may be detected in the urine of jaundiced neonates[11,16].

In this study we analyzed the role of the kidney in the excretion of UB photoderivatives using an isolated rat kidney preparation perfused with UB exposed to irradiation. Previous investigations suggested that UB renal excretion was

accomplished by filtration of the unbound pigment[24] plus a secretory step accompanied by a significant back-diffusion from the lumen into the cells[26]. In this study we found that pigment disappearance from the perfusate was similar in the preparations perfused with irradiated and non-irradiated UB. These data,and the linear relationship observed between pigment uptake by tissue and medium pigment concentration, indicated that the range of concentrations used were below saturation levels. The slight decay in pigment medium concentration seen in both experimental groups was in accordance with previous data[9,13–15]; such an effect might be due to the perfusion medium acting almost as a reservoir.

Data on pigment uptake by tissue indicated taht the process was less efficient in the presence of light, which suggested a higher affinity for UB in its (Z-Z) conformation. It is well known, that many anionic compounds with a high degree of hydrophobicity exhibit increased affinity for the transport system, but reduced transport rates[33]. Such differences in hydrophobicity exist between UB (Z-Z) and UB photoderivatives because, the photoisomerization enables the carboxylate groups to dissociate[7].

Although a marked difference between uptake and excretion existed at earlier times, the steady-state value for extraction ratio and clearance, and the rate of reaching the steady-state, were higher for irradiated UB; this suggested a fairly efficient mechanism of renal excretion for UB photoderivatives.

As reported previously[26], we found that the secretory component of UB transport shared a common site with PAH, inhibited by probenecid; this inhibitory effect of UB on PAH secretion was found to be more marked in this study than that seen for irradiated UB, though it was not distinguishable when it was analyzed as a function of pigment uptake. This allowed us to postulate that the two pigment structures shared a common organic anion uptake system but showed quantitative differences in affinity for binding sites.

Even though the data suggested a higher secretory capacity for UB photoderivatives, it was not possible to discard the possibility that they may be less reabsorbed by non ionic diffusion than UB (Z-Z) due to their more polar structure. It might also be possible that as we employed a pigment to albumin ratio less than one, structural isomerization occurred (10) and the lumirubin excreted (measured at 450 nm) could maintain its anionic charge and hence a diminished passive non-ionic diffusion capability. The spectral analysis assured us that we were analyzing pigments with the spectral characteristics of UB in water solution.

Although the identity of excreted yellow intermediates was not analyzed in this study, the results indicated the important role of the kidney in pigment excretion during phototherapy. Such a role may be particularly relevant in those clinical settings in which hepatic function is impaired. Moreover, the diminished pigment uptake by tissue in the presence of light demonstrated in this study might prevent the deleterious effects described for UB when it was bound to membrane structures.

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