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# Role of Human Skin in the Photodecomposition of Bilirubin

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1. Human skin epithelium and human skin were found to absorb both free bilirubin and serum-bound bilirubin from an aqueous buffered medium. The serum-bound bilirubin thus absorbed was readily released when human skin epithelium or human skin were transferred to media containing no bilirubin. 2. The $K_m$ values for serum-bound bilirubin were $1.8\times10^{-3}$M and $2.2\times10^{-3}$M respectively for human skin epithelium and human skin; corresponding $K_m$ values for free bilirubin were $3.0\times10^{-4}$M and $5\times10^{-4}$M. The $V_{max.}$ for bound and free bilirubin was of the same magnitude, the apparent $V_{max.}$ being 1.0 and 1.66$\mu$mol/g of tissue for human skin epithelium and human skin respectively. 3. When human skin that had acquired a yellow tinge by absorbing bilirubin was incubated in a buffered medium and exposed to a mercury-vapour light, the yellow colour disappeared and decomposition products of bilirubin accumulated in the medium. 4. Experiments with [$^3$H]bilirubin indicated that the pigment absorbed by skin was photo-oxidized to products that were soluble in water and the quantity and number of such products increased with the time of exposure of human skin to the light-source. Under similar conditions [$^3$H]bilirubin alone in buffered medium was also oxidized and gave products which by paper chromatography appeared to be different from those released by human skin that had absorbed bilirubin. 5. The results suggest that by virtue of its large surface area human skin can act as a matrix for the degradative action of light on bilirubin.

Newborn babies suffering from jaundice have a highly yellow-tinted skin, and phototherapy with visible light not only bleaches the skin area exposed to light but also lowers serum bilirubin concentrations (Cremer *et al.*, 1958 Bergsma *et al.*, 1970; Anon., 1970*a*,*b*, 1972; Tabbe *et al.*, 1972; Bajpai *et al.*, 1973). Studies by Behrman & Hsia (1969) and Schmid (1971, 1972) suggest indirectly that skin can provide an alternative medium for the catabolism of bilirubin. However, detailed studies of the ability of skin to concentrate and degrade bilirubin have not been conducted. Previous studies from this laboratory have shown that strips of skin from albino rats, mice and guinea pigs can take up and release bilirubin (Kapoor *et al.*, 1973*a*,*b*). The present paper reports the role of human skin in the uptake and photodecomposition of bilirubin. A brief report of this work has appeared (Kapoor & Krishna Murti, 1973).

## Experimental

### *Materials*

*Chemicals.* Chemicals used were as specified by Kapoor *et al.* (1973*b*). The purity of bilirubin (E. Merck, Darmstadt, W. Germany) was confirmed by the procedure of McDonagh & Assisi (1972) and had an $\varepsilon$ value of 59000–61000 litre·mol$^{-1}$·cm$^{-1}$ at 450nm in chloroform.

### *Methods*

*Preparation of material.* Human skin epithelial strips were obtained from the dorsal surface of forearm and thighs of human adults (male) undergoing plastic surgery. Human skin strips were taken from the abdominal skin before suturing of the operated area. The samples were transferred immediately to chilled sterile 150mM-KCl. The adherent tissue was scraped off with a sharp blade and the piece was cut into smaller segments and used with minimum delay in uptake experiments.

*Uptake of bilirubin.* Human skin epithelium or human skin (500mg) was incubated in 5ml of Krebs–Ringer buffer, pH7.5 (122mM-NaCl, 5.0mM-KCl, 1.2mM-$MgSO_4$, 1.0mM-$CaCl_2$, 16mM-$Na_2HPO_4$), containing either serum-bound or free bilirubin (0.427mM) at 37°C. At various times the samples were washed free of medium, homogenized and used for the determination of bilirubin.

*Efflux of bilirubin.* Human skin epithelium or human skin from the uptake experiments was washed free of medium, suspended in fresh Krebs–Ringer buffer of the same composition as given above and shaken at 37°C in a metabolic bath (60

strokes/min, 2cm amplitude). After the desired period the tissue was removed and the medium assayed for bilirubin content.

Uptake and efflux studies were carried out in the dark, and skin segments and aqueous solutions in which bilirubin was estimated were protected from strong light.

*Photodecomposition of bilirubin.* Human skin (500mg) which had previously absorbed bilirubin was washed free from medium and suspended in 5ml of Krebs–Ringer buffer containing 50$\mu$g of streptomycin and 50units of penicillin G (Glaxo Laboratories of India, Bombay, India). Beakers containing the suspensions were exposed to an 80W shaded mercury-vapour lamp (Phillips, Calcutta, India) from a height of 20cm. Sterile water (0.25ml/h) was added to replace water lost by evaporation. The medium was centrifuged at 3000*g* at 4°C and used for study of decomposition products. Control experiments were run where the aqueous buffer without human skin contained bilirubin equivalent to the amount absorbed by 500mg of human skin.

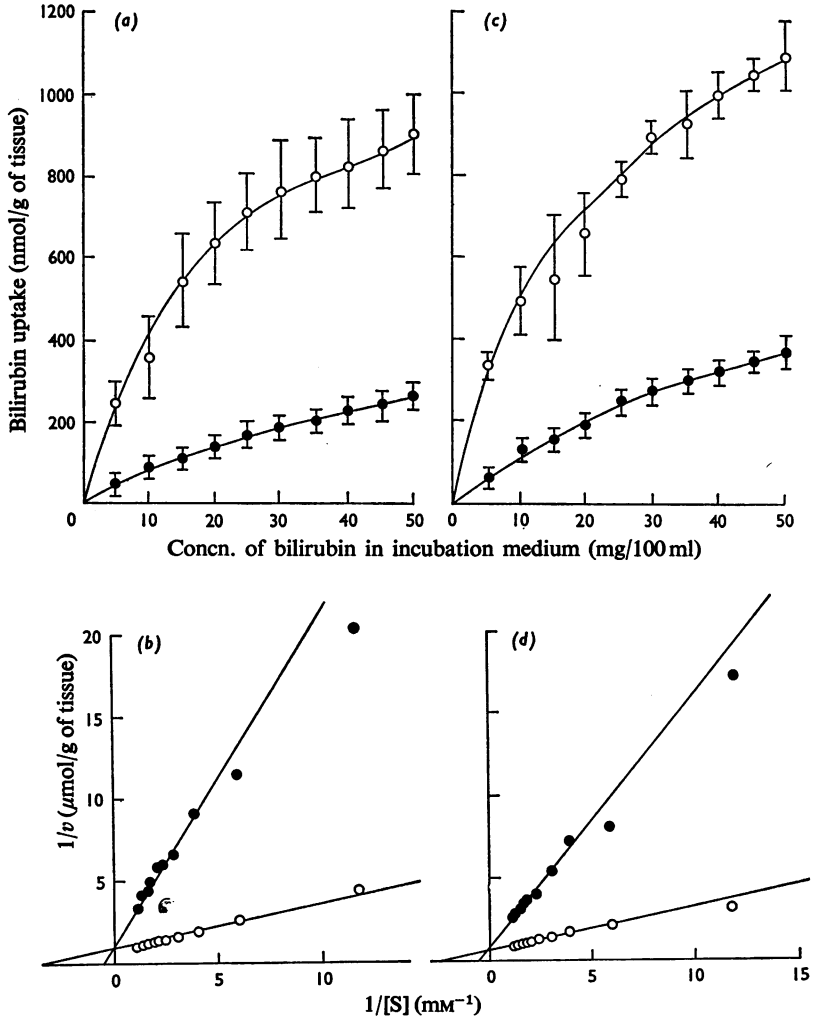


Fig. 1. *Uptake of bilirubin by human skin epithelium and human skin*

(*a*) Human skin epithelium; (*b*) graphical calculation of $K_m$ for human skin epithelium; (*c*) human skin; (*d*) graphical calculation of $K_m$ for human skin. Skin epithelium or skin (500mg) was incubated in the presence of 0.0854–0.854mM free or bound bilirubin in Krebs–Ringer buffer, pH7.5, at 37°C for 2h. The vertical bars represent ±S.D. of the mean value of three experiments with duplicates. ○, Free bilirubin; ●, serum-bound bilirubin.

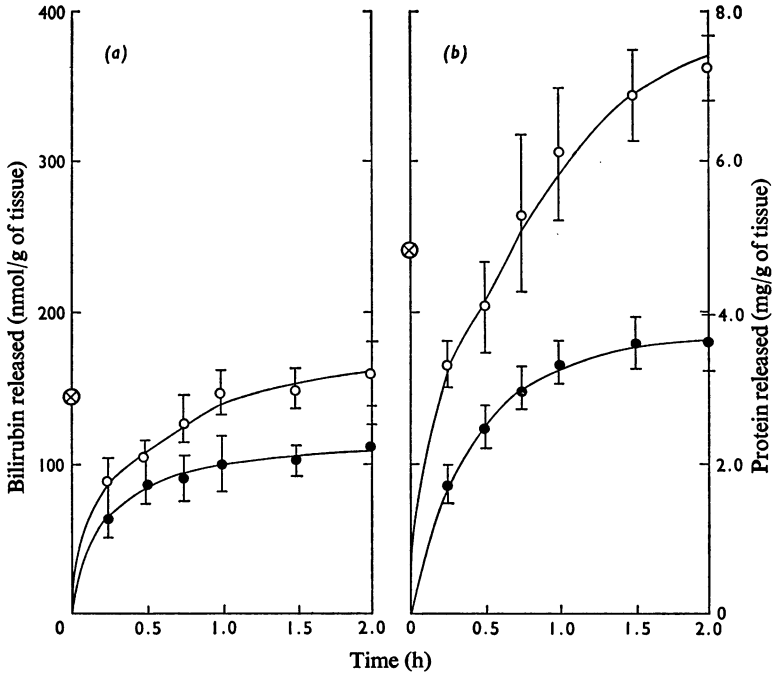


Fig. 2. *Efflux of bilirubin from human skin epithelium and human skin*

(*a*) Human skin epithelium and (*b*) human skin (500mg) were incubated in 5ml of Krebs–Ringer buffer, pH7.5, containing 0.427 mM-serum-bound bilirubin at 37°C for 2h, washed free from medium and then reincubated in 5ml of fresh Krebs–Ringer buffer, pH7.5. Vertical bars represent ±S.D. of the mean value of three experiments with duplicates. ●, Bilirubin released into medium in 2h; ⊗, protein released into medium in 2h. ⊗, Initial concentration of bilirubin in skin epithelium or skin when added to fresh Krebs–Ringer buffer.

*Preparation of skin homogenates.* Homogenates (5%, w/v) were prepared by grinding the segments to a paste first with the aid of a pestle and mortar and subsequently in a power-driven glass homogenizer fitted with a Teflon pestle (3000rev./min for 2–3min).

*Purification of [$^3$H]bilirubin.* [$^3$H]Bilirubin prepared by the Wilzbach procedure as described by Grodsky *et al.* (1962) was supplied by the Isotope Division of Bhabha Atomic Research Centre, Bombay, India. After two crystallizations from chloroform and methanol, the material was diluted with carrier bilirubin (0.25mg/ml of chloroform) and crystallized five times from chloroform and methanol until the final product showed a constant specific radioactivity (Ostrow *et al.*, 1961). Purified [$^3$H]bilirubin was dissolved in 0.1 M-$Na_2CO_3$, equilibrated with aqueous bovine serum albumin and extracted with acetone to constant specific radioactivity. Before dilution the $\varepsilon$ value in chloroform at 450nm was 56000 litre·mol$^{-1}$·cm$^{-1}$. The specific radioactivity was 7$\mu$Ci/$\mu$mol, measured against an internal reference standard, *n*-[1,2-$^3$H]hexadecane (The Radiochemical Centre, Amersham, Bucks., U.K.).

*Determination of radioactivity.* At various times 0.2ml portions of medium were removed and spotted on 2cm×2cm squares cut out of Whatman 3MM paper. After drying the squares were placed in 15–20ml of scintillation fluid [0.4% 2,5-diphenyloxazole and 0.04% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in distilled toluene] and counted for radioactivity in a Packard Tri-Carb liquid-scintillation spectrophotometer (efficiency 40–43%). Counts registered by 0.1 and 0.2ml portions of [$^3$H]bilirubin in toluene were 10793 and 20865d.p.m. respectively. The results have been corrected for this quenching by Whatman 3MM paper.

*Chromatographic separation of photodecomposition products.* Media from light-exposure experiments were freeze-dried. From the residue, water-soluble decomposition products were extracted by the procedure of Ostrow & Branham (1970). The material thus extracted was applied to Whatman no. 1 sheets and developed in the descending direction in the solvent system of Gray *et al.* (1972). The paper was

then dried. The distance between origin and solvent front was divided into 2cm sections. Squares (2cm×2cm) were cut out of the strip, dropped into scintillation fluid and counted for radioactivity.

*Chemical determinations.* Bilirubin in skin homogenates was measured by the procedure of Van Roy *et al.* (1971) with a recovery of 94%. Bilirubin in aqueous solutions was determined by the procedure of Malloy & Evelyn (1937). Protein in serum or medium was determined by the colorimetric method of Lowry *et al.* (1951) with crystalline dry bovine serum albumin (Sigma Chemical Co., St. Louis, Mo., U.S.A.) as a standard.

*Absorption spectra.* Absorption was measured in a Beckman DU model spectrophotometer in 1cm light-path glass cuvettes.

## Results

### *Uptake and efflux of bilirubin by human skin segments*

The uptake of free bilirubin or serum-bound bilirubin by human skin epithelium and human skin was affected by the concentration of bilirubin as shown in Fig. 1. Both free bilirubin and serum-bound bilirubin were readily absorbed, the former 3.5-fold more than the latter. Double-reciprocal plots of the data were linear. The apparent $K_m$ values for serum-bound bilirubin were $1.8\times10^{-3}$M and $2.2\times10^{-3}$M respectively for human skin epithelium and human skin. Corresponding $K_m$ values for free bilirubin were $3.0\times10^{-4}$M and $5.0\times10^{-4}$M, whereas $V_{max.}$ values were 1.0 and 1.66$\mu$mol/g of tissue for human skin epithelium and human skin.

The serum-bound bilirubin absorbed by human skin epithelium or human skin was released into the medium when the skin segments which had absorbed the pigment were incubated in Krebs–Ringer buffer. The mean rates of efflux were 0.9nmol/h per g for human skin epithelium and 1.5nmol/h per g for human skin (Fig. 2). The efflux was relatively fast in the first 30min. The relatively large amount of free bilirubin absorbed by skin was, however, not released into the medium.

### *Photodecomposition of bilirubin*

[$^3$H]Bilirubin dissolved in buffer or absorbed by skin segments, suspended in buffer, was exposed to

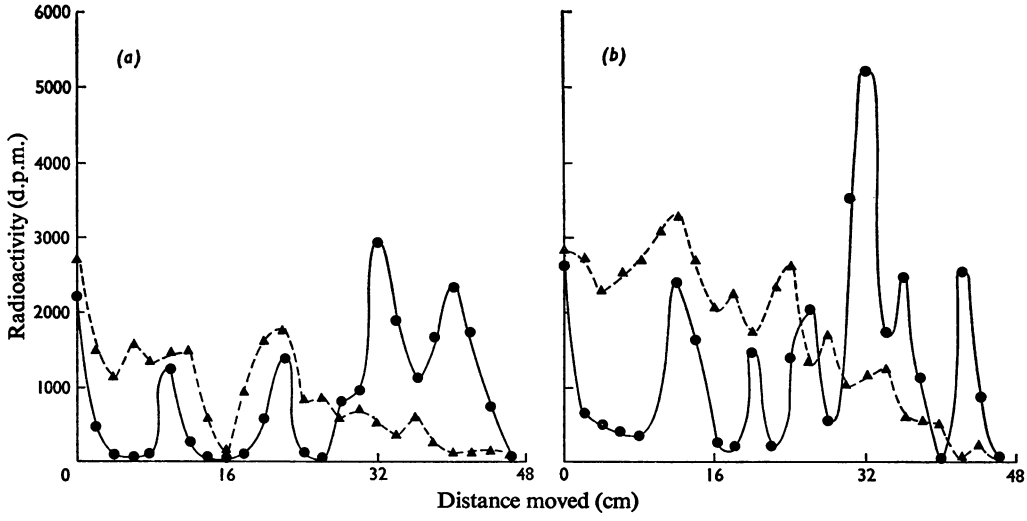


Fig. 3. *Photodecomposition of bilirubin in Krebs–Ringer buffer or of free bilirubin bound to skin and suspended in Krebs–Ringer buffer*

Human skin (500mg) was first allowed to equilibrate with 0.427mM-bilirubin (equivalent to 0.43$\mu$Ci/$\mu$mol of [$^3$H]bilirubin). The samples were then exposed to the light-source as described in the text. Another set of beakers holding the same volume of Krebs–Ringer buffer and containing 0.427mM-bilirubin with the same amount of [$^3$H]bilirubin as used for skin were also exposed to the light-source. The photodecomposition products obtained at 4h (*a*) and at 8h (*b*) were subjected to descending paper chromatography in the system ethanol-aq. $NH_3$ (sp.gr. 0.88)–water (16:1:3, by vol.). The paper chromatograms were scanned for radioactivity; the ordinate shows d.p.m. per unit length and the abscissa distance (cm) moved from origin of paper chromatogram. ●, [$^3$H]Bilirubin absorbed in skin; ▲, [$^3$H]bilirubin in Krebs–Ringer buffer. (*a*) 4h-irradiated samples; d.p.m. applied were 22050 (●) and 27550 (▲). (*b*) 8h irradiated samples; d.p.m. applied were 38200 (●) and 55945 (▲).

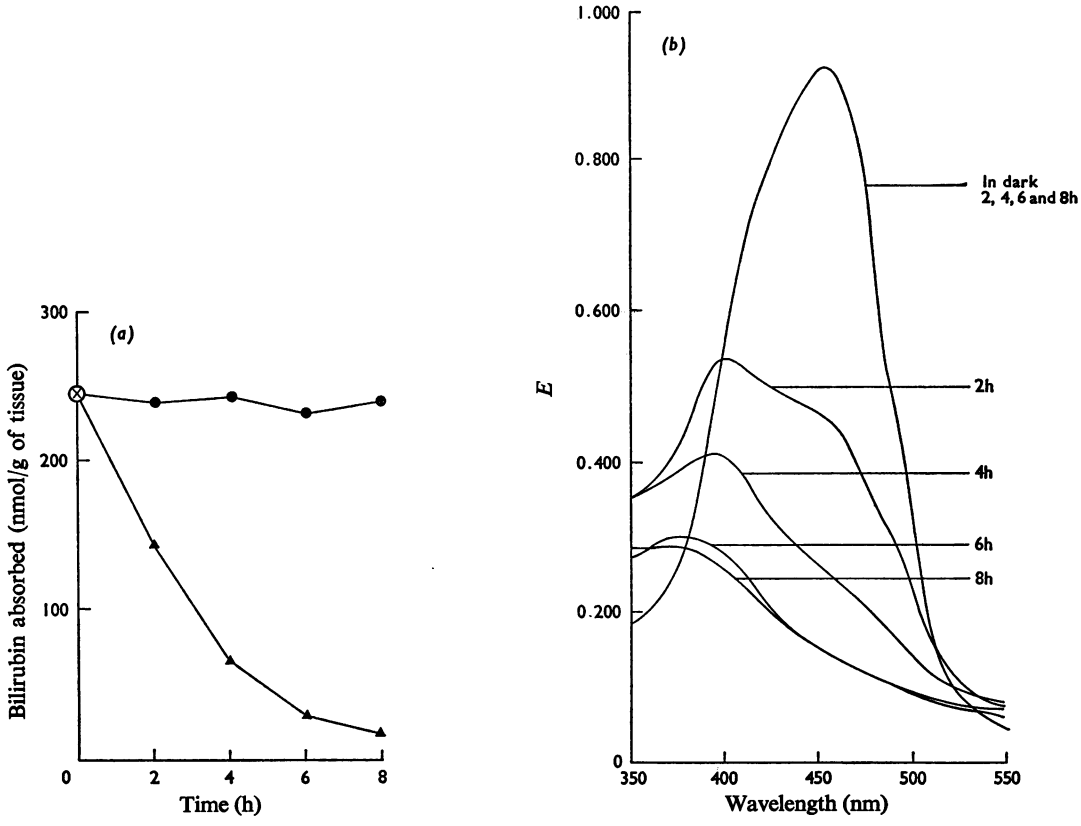


Fig. 4. *Photodecomposition of bilirubin absorbed from serum-bound bilirubin by skin*

Human skin (500mg) was incubated at 37°C for 2h in 5ml of Krebs–Ringer buffer containing 0.427mM-serum-bound bilirubin. It was washed free from medium, suspended in 5ml of fresh Krebs–Ringer buffer and incubated either in the dark or under exposure to the source of light. (*a*) Change in bilirubin content of skin incubated in the dark (●) and skin exposed to source of light (▲). (*b*) Visible absorption spectrum of medium after exposure to source of light.

the light-source and the products accumulating in the medium were resolved by paper chromatography. Fig. 3, shows that although [$^3$H]bilirubin undergoes photodecomposition even in aqueous solution the extent of photodecomposition is much greater when the pigment is absorbed by skin and exposed to the source of light. The products of decomposition also appear not to be the same as those obtained when bilirubin is retained by the skin.

The above experiments were repeated with human skin which had previously absorbed unlabelled free bilirubin or unlabelled serum-bound bilirubin. About 70–80% of the bilirubin was released into the medium intact when human skin segments which had absorbed serum-bound bilirubin were incubated in the dark for 2h (Fig. 4*a*). When such human skin segments were suspended in buffer and irradiated, not only were they bleached but products showing an absorption maximum in the range of 390–400nm were recovered in the medium. The absorption maximum of bilirubin is 460nm (Fig. 4*b*).

Free bilirubin absorbed by human skin segments showed a different response. There was no release of bilirubin into the medium when segments which had absorbed free bilirubin were incubated in the dark. However, when such segments suspended in buffer were irradiated, there was not only a gradual bleaching of the segments but also release of photodecomposition products into the medium (see Fig. 5).

## Discussion

The present study provides evidence to show that human skin is involved in the homeostasis of bilirubin. Efflux studies indicate that serum-bound bilirubin can exchange with serum proteins in the

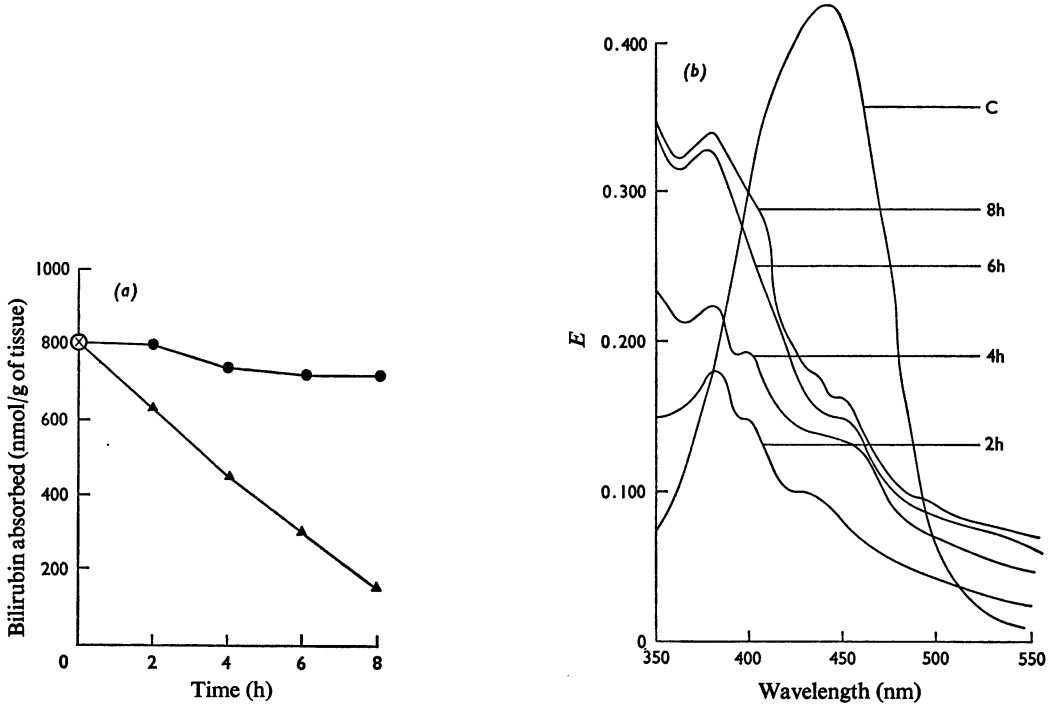


Fig. 5. *Photodecomposition of free bilirubin absorbed by human skin*

Human skin (500 mg) was incubated at 37°C for 2 h in 5 ml of Krebs–Ringer buffer containing 0.427 mM-bilirubin. It was washed free from medium, suspended in 5 ml of fresh Krebs–Ringer buffer and incubated either in the dark or under exposure to the source of light. (*a*) Bilirubin content of skin incubated in the dark (●) and skin incubated under exposure to source of light (▲). (*b*) Visible absorption spectra of bilirubin (curve C) and of decomposition products in medium.

intravascular compartment of the skin (Kapoor *et al.*, 1973*b*). When sufficient bilirubin-binding sites are available in serum proteins, bilirubin in circulation could be transported into liver for its normal disposal by conjugation with glucuronic acid. This mechanism of disposal would probably be over-strained in hyperbilirubinaemia, when free bilirubin in circulation would be far in excess of the quantity that normally saturates the available binding sites on serum albumin.

The results presented show that human skin has a greater affinity *in vitro* for free bilirubin than for serum-bound bilirubin. The differences in the relative rates of uptake and efflux of free or serum-bound bilirubin presumably arise from the differential rates at which the pigment can be absorbed by different components of the skin. Lipid components of skin and collagen can absorb bilirubin (Kapoor, 1973, 1974) and collagen appears to hold a relatively high concentration of bilirubin as compared with serum. The pigment bound to collagen is not readily released but is nonetheless sensitive to light. It seems likely, therefore, that the excess of free bilirubin in the serum in neonatal jaundice can be absorbed by skin in an irreversible manner. In contrast the serum-bound bilirubin apparently exchanges with the serum proteins in the intravascular space of skin. Although bilirubin even in aqueous solution is degraded by light, the absorption by skin facilitates the decomposition of water-soluble products. The chemical nature of these products will have to be established before a plausible pathway of photodecomposition can be postulated.

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