In vitro Studies on Bilirubin Transport by the Small Intestine of the Rat[1]

In vivo experiments have demonstrated that unconjugated bilirubin is absorbed from the gut[2,3] as well as transfered from the plasma across the intestinal wall[4,5]. However, there is no clear indication of the phenomena involved exclusively at the intestinal wall level. In this investigation we studied the transport of unconjugated ^{14}C-bilirubin across the wall of isolated rat intestinal segments.

Methods. Adult Wistar rats of both sexes were killed by cervical dislocation. A lenght of 15–18 cm of the proximal jejunum was resected and, in the non-everted or everted position, mounted in the apparatus shown in Figure 1 which was placed in a water bath at 37 °C. Oxygen consumption by rings of jejunum was determined with Warburg's method[6].

^{14}C-bilirubin was crystallized[7] from the bile of rats injected with ^{14}C-ALA[8] (δ-aminolevulinic acid-4-^{14}C hydrochloride, CEA, France) and recrystallized until it reached a constant specific activity (2000–3200 dpm/μg). Radioactive pigment was dissolved in 0.2 ml 0.1 N NaOH and bovine albumin (80 mg/mg bilirubin) in a total volume of 12–14 ml of Ringer Krebs solution. This solution (internal bathing solution), (IS) perfused the lumen of the intestinal segment (non-everted or everted). Sampling of the opposite or external solutions (ES) (12–14 ml of R. KREBS) was performed at intervals of 30 min for 120 min.

Transmural fluxes of ^{14}C-bilirubin from mucosa to serosa in non-everted segments (J_{ms}^{bil}) and from serosa to mucosa in everted segments (J_{sm}^{bil}) were estimated by dividing radioactivity (disintegrations per minute, dpm) in ES by the specific activity of ^{14}C-bilirubin in IS (at the beginning of the experiment). Fluxes were expressed as μmoles/h/cm.

The integrity of the intestinal segment was tested by perfusing a Methylene Blue solution at the end of the experiment.

ES were treated in 2 ways. In some experiments the pigment was diazotized, concentrated[9] and chromatographed on paper[10]. In others they were pooled and, in these cases, the samples were acidified with acetic acid and extracted with 2:1 mixture of methanol and chloroform[4]. Unlabelled unconjugated carrier bilirubin was added and the combined pigment crystallized for radioassay[7].

Bilirubin concentration was estimated by the diazo reaction in a DU Beckman spectrophotometer. Radioassay of samples and pigment crystals was performed in a Packard Tri-Carb Liquid Scintillation Spectrometer.

Scanning of the paper strips was carried out by a previous method[11] and densitometry in a Densicord 542 A (Photovolt, N.Y., USA) with a 545 nm filter.

Results. Oxygen consumption by intestinal rings was shown to be constant for at least 160 min of perfusion with values of 9–9.8 μl/mg wet tissue/h. The passage of ^{14}C-bilirubin to ES was proved: a) by crystallization of bilirubin from pooled samples of ES where more than 50% of their total counts could be recovered as crystalline ^{14}C-bilirubin in both types of intestinal segments, b) by ascending paper chromatography which showed that in non-everted as well as in everted segments, ES contained an azo pigment identified as azo pigment A. Scanning of paper strips showed that nearly 40% of total radioactivity was present in this area coincident with the peak registered by densitometry (Figure 2). These experiments

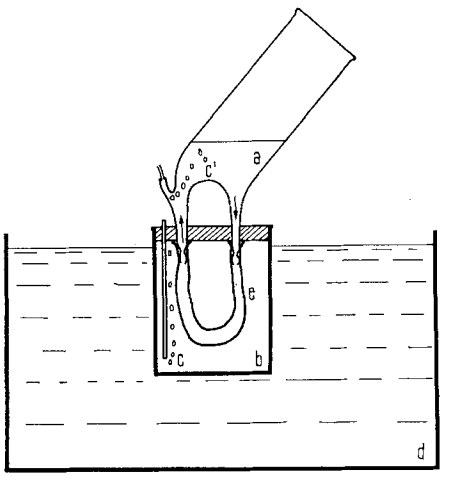

Fig. 1. Diagrammatic representation of the perfusion apparatus. a) Internal bathing solution. b) External bathing solution. c) and c') 95% O_2 5% CO_2 (c', bubble pump). d) Constantly stirred water bath. e) Intestinal segment.

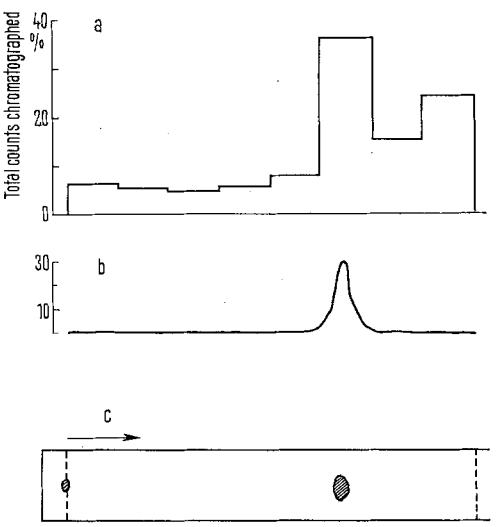

Fig. 2. Chromatography of pools of external solutions. a) Distribution of radioactivity. b) Recording of color spots. c) Chromatogram showing a spot corresponding to azopigment A.

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indicate that at least about 50% of the measured radioactivity corresponded to bilirubin.

Unidirectional flux of ^{14}C-bilirubin from mucosa to serosa could be directly related to the bilirubin *cis*-concentration[12] ($r = 0.73$, $p < 0.01$) (Figure 3, a). A similar linear correlation was observed for the flux from serosa to mucosa ($r = 0.72$, $p < 0.01$) (Figure 3, b). Analysis of covariance[13] showed that both slopes did not differ significantly ($F = 0.715$; $p > 0.05$). Within the range of concentrations used (up to 13 mg/100 ml), no saturation tendency was observed.

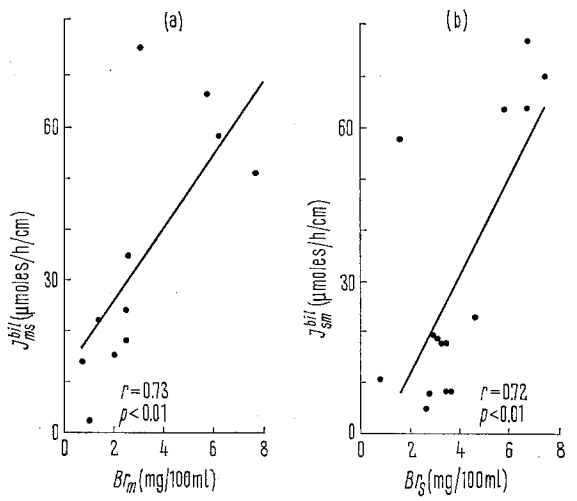

Fig. 3. Correlation between bilirubin *cis*-concentration and bilirubin flux. a) Noneverted segments; Br_m, bilirubin concentration at the mucous side. b) Everted segments; Br_s, bilirubin concentration at the serous side.

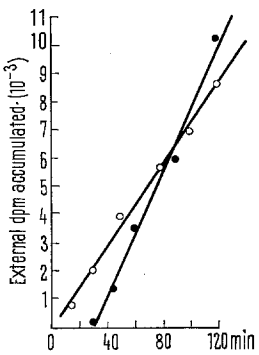

Fig. 4. Isotope incorporation at the external solution as a function of time. ●, noneverted segment; ○, everted segment.

Paired experiments of intestinal non-everted segments (in one of them unlabelled bilirubin was placed at the external side) were performed to detect an effect of bilirubin *trans*-concentration[14]. The analysis of data showed that the difference between both groups was not statistically significant ($P > 0.05$).

Discussion. Our results confirm the bidirectional passage of unconjugated bilirubin across the intestinal wall. The incorporation of radioactivity to the external side followed a linear function (Figure 4). This suggested that no significant retroflux of isotope occurred.

Among the forces that could be involved in bilirubin transport across the intestinal wall, we found that bilirubin *cis*-concentration at the mucosal compartment (in non-everted segments) and at the serosal compartment (in everted segments) was directly related to the unidirectional flux. Lack of tendency to saturation and of a *trans*-concentration effect suggests a mechanism of passive diffusion. This is in agreement with the findings reported by some authors that explained the in vivo absorption of bilirubin from the intestine[3] or the gall bladder[15] by passive diffusion. Since the molarity of the bathing solution was not significantly changed by bilirubin, the effect of solvent drag was assumed to be irrelevant.

Fluxes expressed per unit of length had similar values for both types of intestinal segments. It may be argued that when expressed per unit of area, J^{bil}_{sm} would be much greater than the opposite. However, the intestinal wall cannot be described as a homogeneous compartment[16] and the epithelial structure responsible for the diffusion barrier is as yet unknown.

Resumen. Se estudió el transporte de bilirrubina no conjugada en asas intestinales aisladas y perfundidas de rata. Los resultados indican un pasaje bidireccional con la probable participación de un mecanismo de difusión simple.

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Induction of Motility in Honey Bee (*Apis mellifera* L.) Spermatozoa by Sugars

Sugars, especially fructose, are an important exogenous energy source for spermatozoal motility in many species[1]. Glucose, fructose, trehalose, and sucrose are found in the reproductive tract, seminal plasma, and hemolymph of honey bees[2–4]. Fructose in seminal plasma is rapidly metabolized by honey bee spermatozoa in vitro[2]. Nevertheless, Lensky and Schindler[5], and Schindler and Volcani[3] have concluded that dilution is the principal factor initiating honey bee spermatozoal motility over a wide range of diluent pH and ionic composition.

We have begun to evaluate the influence of naturally occurring sugars on motility of honey bee spermatozoa.

Materials and methods. The following semen diluents were prepared (g/l of solution) with triple glass-distilled