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.

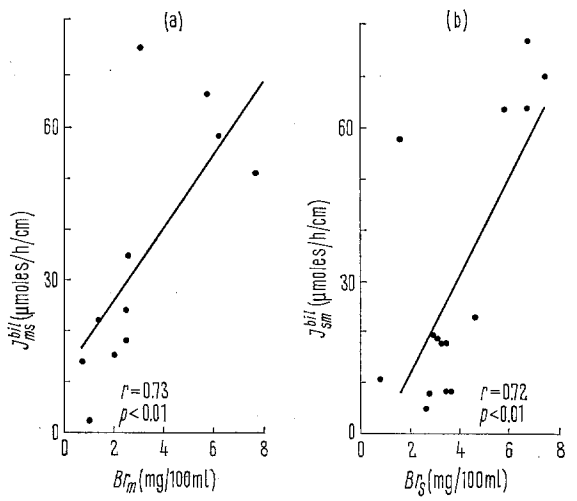

Fig. 3. Correlation between bilirubin *cis*-concentration and bilirubin flux. a) Noneverted segments; Br_m, bilirubin concentration at the mucous side. b) Everted segments; Br_s, bilirubin concentration at the serous side.

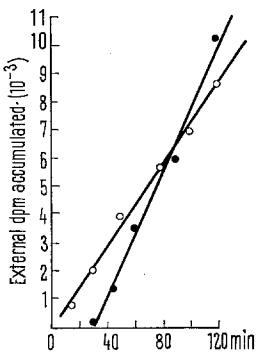

Fig. 4. Isotope incorporation at the external solution as a function of time. ●, noneverted segment; ○, everted segment.

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

water as the solvent: Diluent A 8.5 g NaCl, 26.7 g sucrose, 0.7 g glucose, 0.4 g fructose; Diluent B 8.5 g NaCl, 26.7 g sucrose, 0.4 g fructose; Diluent C 8.5 g NaCl, 26.7 g sucrose, 0.7 g glucose; Diluent D 8.5 g NaCl, 0.7 g glucose, 0.4 g fructose; Saline 8.5 g NaCl.

Five replicates of the following step-wise procedure were run for each of the saline and sugar diluents: A, B, C, and D. 4 test tubes, hereafter noted as 1, 2, 3, and 4, were used in each replicate. Tubes 1 and 2 each received 1 ml of saline diluent; tubes 3 and 4 each received 1 ml of the saline and sugar diluent being tested.

Step A: 1 µl of honey bee semen introduced into each tube and gently mixed with the diluent. Step B: 1 drop of the mixture from each tube placed on a separate microscope slide, covered with a coverslip, and spermatozoal motility and morphology appraised by phase contrast microscopy. Step C: Motility of spermatozoa in tube 1 rechecked, and then 3 drops (approx. 0.16 ml) of the test saline and sugar diluent added to it. Motility checked again after this addition. (Final approximate sugar concentrations here and at Step E below were 0.39% for diluents A, B, and C and 0.016% for diluent D.) Step D: Motility in tube 2 rechecked and then oxygen bubbled through the mixture for 1 min followed by another check for motility. Step E: 3 drops of the test saline and sugar diluent added to oxygenated tube 2 and again checked for motility. Step F: Final reexamination of tubes 3 and 4 to verify observation in Step B.

In addition, a series of on-slide motility examinations was made for each saline and sugar diluent, using saline as a control, as follows: 1 drop of saline was placed on each of 2 slides. 1 drop of saline and sugar diluent was placed on a third slide. Then 1 drop of semen was placed into the fluid on each slide, covered with a coverslip, and examined microscopically for spermatozoal motility. Next a drop of saline was placed adjoining the coverslip on one of the saline slides, and a drop of saline and sugar diluent was placed likewise on the other saline slide. All 3 slides were again examined microscopically.

Results and discussion. In both series of tests, differences in spermatozoal motility between saline-semen mixtures and saline and sugar-semen mixtures were so great that quantification in terms of percent motile spermatozoa was unnecessary. The response was nearly all-or-none.

Few spermatozoa (not more than 1–2%) were capable of movement in the saline-semen mixtures. Nearly all were folded at the center of their length. In comparison, nearly all spermatozoa in all of the saline and sugar-semen mixtures exhibited vigorous, normal-appearing motility and normal morphology. No differences were detected in relation to the omitted sugars in diluents B, C and D.

Oxygenation of the semen-saline mixtures did not induce motility and did not seem to alter the folded configuration of the spermatozoa. In contrast, adding 3 drops of any saline and sugar diluent to either oxygenated or unoxygenated saline-semen mixtures immediately produced vigorous movement of the spermatozoa although most remained folded.

The results of the on-slide motility tests were consistent with the foregoing. Semen placed in saline remained in a tight clump, and nearly all of the spermatozoa were immotile. Semen placed in any of the saline and sugar diluents immediately dispersed into swirling foci of motile spermatozoa. Additional saline allowed to diffuse into the original semen-saline preparations had no effect other than partial disruption of the clump by streaming diluent. In contrast, swirling foci of motile spermatozoa formed rapidly when any of the saline and sugar diluents were allowed to diffuse into the semen-saline preparations.

These results suggest that sugars in concentrations as low as 0.016% can induce spermatozoal motility in dilute mixtures of honey bee semen and 0.85% saline. They also indicate that no one of the sugars tested is indispensable for the induction and support of spermatozoal motility. We suppose that motility occurred in mixtures containing sugar because the sugar provided a metabolizable energy source not previously available. This conjecture is supported by similar findings in other species[1] and by observation of rapid fructolysis in honey bee semen[2].

The motility difference between saline and sugar-semen mixtures and saline-semen mixtures of equivalent dilution is evidence that sugar was more important to spermatozoal motility than the dilution itself. This is inconsistent with the conclusion of others[3,5] that motility is induced merely by dilution, probably with secretions of the spermathecal gland, when spermatozoa are released or removed from the spermatheca. Further work should be done on the composition of spermathecal gland secretion to determine whether it too contains significant sugar.

Zusammenfassung. Es wird gezeigt, dass frischgewonnenes Sperma der Honigbiene *Apis mellifera* L. in 0,85% NaCl-Lösungen mit Rohr-, Frucht- und Traubenzuckerzusatz bewegungsfähig bleibt, während in 0,85% NaCl nur wenige Spermien bewegungsfähig sind.

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Interrelationships Between the Contractile Effects of Metabolic Substrates, β-Adrenergic Blocking Agents and Endogenous Catecholamine Depletion on Isolated Rat Atria[1]

The effects of adrenergic influences on the contraction of the heart muscle have been extensively studied over the years. On the other hand we have explored[1–8] the importance of metabolic substrates and enzyme inhibitors to maintain and modify electrical and contractile characteristics of the isolated myocardium. However, little is known regarding the possible mutal influences between these two aspects.

In the present study we have attempted to explore whether the myocardial contractile effects elicited by