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# Transport of Prostaglandins Through Normal and Diabetic Rat Hepatocytes

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Received May 15, 1980, from the *Department of Physiology, Faculty of Medicine, American University of Beirut, Beirut, Republic of Lebanon.* Accepted for publication April 14, 1982.

**Abstract** □ Transport of alprostadil (prostaglandin $E_1$) and dinoprost (prostaglandin $F_{2\alpha}$) was studied in enzymatically dispersed normal and streptozocin-treated rat hepatocytes prepared by collagenase perfusion. Cell suspensions incubated at 37° were sampled at time intervals for a period of 5 min and the supernatant analyzed for prostaglandins after centrifugation. The data analysis employed a theory and a model for solute transfer at the cell membrane–water interphase. Biophysical parameters such as the effective partition and the apparent permeability constants were used to define the transport mechanism. The apparent permeability coefficient of alprostadil and dinoprost transfer through normal hepatocytes was calculated to be $5 \times 10^{-3}$ and $3 \times 10^{-3}$ cm/sec with a mean partition coefficient of 1345 and 764 for both solutes, respectively. The permeability coefficient of alprostadil and dinoprost transfer through diabetic hepatocytes were $3 \times 10^{-3}$ and $2 \times 10^{-3}$ cm/sec with partition coefficient of 572 and 206, respectively. The results showed differences in prostaglandin transport between normal and diabetic hepatocytes, resulting from morphological and lipid alteration in the cytoplasmic membrane.

**Keyphrases** □ Prostaglandins—transport through normal and diabetic rat hepatocytes, alprostadil and dinoprost □ Alprostadil—transport through normal and diabetic rat hepatocytes □ Dinoprost—transport through normal and diabetic rat hepatocytes □ Hepatocytes, rat—normal and diabetic, transport of alprostadil and dinoprost □ Permeability—transport of alprostadil and dinoprost through normal and diabetic rat hepatocytes □ Partition coefficient—transport of alprostadil and dinoprost through normal and diabetic rat hepatocytes

Multicomponent and multicompartment diffusional models have been used to study transport of solutes across biological membranes. For example, erythrocyte permeability (1), lymphocyte permeability (2), Ehrlich ascites tumor cell permeability (3), and Burkitt lymphoma cell permeability (4) have been reported. The majority of the studies dealt with solute transfer through blood cell components and tissue culture cell suspensions. However, few mechanistic studies were attempted on cell suspensions obtained by enzymatic dispersion, *e.g.*, embryonic heart cells (5), adipocytes (6), and hepatocytes (7, 8).

Alprostadil (prostaglandin $E_1$) and dinoprost were chosen as solute models to mechanistically explain the transfer of acidic lipids through normal and diabetic rat hepatocytes. Furthermore, autoradiographic studies in mice have shown high concentrations of alprostadil and dinoprost in the liver 15 min after intravenous injection (9).

The present report describes the uptake mechanism of alprostadil and dinoprost through enzymatically dispersed normal and diabetic adult rat hepatocytes. The techniques, methodology, and the theoretical model employed are well suited to characterize interfacial barriers to interface transport in biological systems.

## EXPERIMENTAL

**Preparation of Rat Hepatocyte Suspensions**—Suspensions of isolated liver parenchymal cells were prepared by a modification of a previously described procedure (10). Male Sprague-Dawley rats weighing



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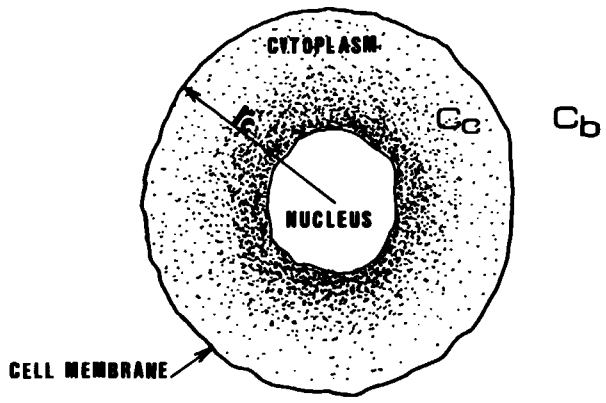


**Figure 1**—*Physical model describing uptake across hepatocyte–water interface.*

200–250 g and fed *ad libitum* were anesthetized by injection of 80 mg/kg ip of the sodium salt of 5-*sec*-butyl-5-ethyl-2-thiobarbituric acid[1]. The liver was initially perfused through the portal vein at a flow rate of 30–40 ml/min with 200-ml heparinized $Ca^{2+}$-free Krebs–Henseleit (I) solution to clear it of its blood supply. Perfusion was continued with 250 ml of I containing 0.4 mg/ml of collagenase[2] at a flow rate of 100 ml/min using a peristaltic pump[3]. All perfusion media were aereated with 95% oxygen–5% carbon dioxide throughout the experiment. After 30 min of perfusion, the fibrous tunic was scraped and the hepatocytes dispersed by gentle motion. The cells were filtered through a course 250-μm nylon-mesh filter, washed and suspended into 30 ml of $Ca^{2+}$- and $Mg^{2+}$-free phosphate buffered saline (II) (pH 7.4). Hepatocyte viability was then estimated by the trypan blue exclusion test.

**Preparation of Diabetic Rats**—Male rats were injected with 65 mg/kg iv streptozocin[4] in saline acidified to pH 4.5 with 0.05 *M* solution of sodium citrate. They were fed *ad libitum* and checked daily for diabetes by urine sugar analysis paper[5] and a blood glucose test (11). Animals whose blood glucose levels were >250 mg/100 ml after day 7 were used in the experiments; their livers were perfused by the same procedure mentioned above to obtain cells from diabetic rats.

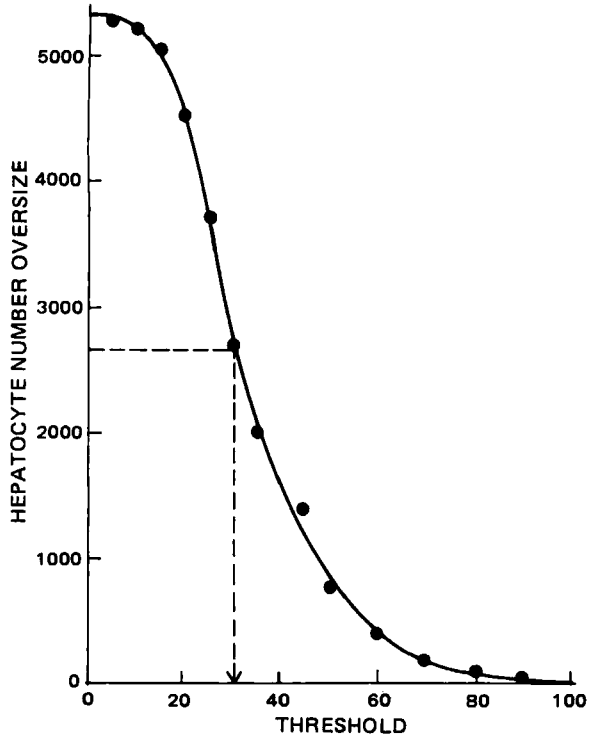


**Figure 2**—*Cumulative size distribution for rat hepatocytes.*

[1] Inaction, Byk Gulden, Konstanz, West Germany.
[2] Type CLS II, 140-175 U/mg, Worthington Biochemical Corporation, Freehold, N.J.
[3] Ministaltic pump, Manostat, New York, N.Y.
[4] U-9889, lot 60,273-1, Courtesy of The Upjohn Co., Kalamazoo, Mich.
[5] Eli Lilly Co., Indianapolis, Ind.

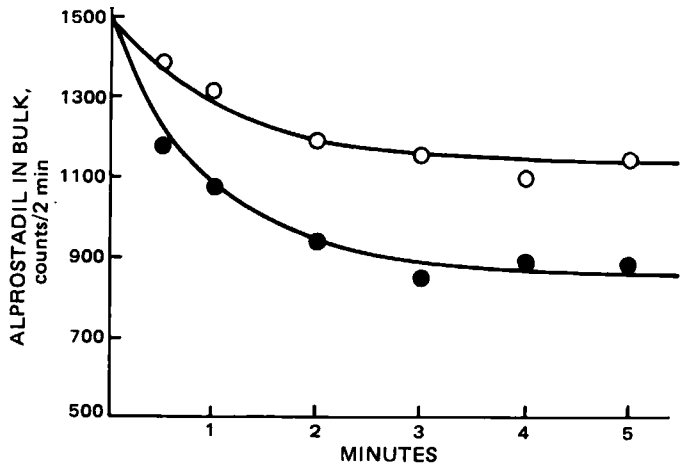


**Figure 3**—*Comparison of experimental data with theory for uptake of alprostadil by normal hepatocytes. Key: (○) 13,169,475 cells; (●) 27,362,137 cells;* P = 5 × $10^{-3}$ *cm/sec;* K = 1345.

**Determination of Mean Hepatocyte Count and Diameter**—An automated counter[6] was used to determine the mean hepatocyte count and diameter. The instrument was precalibrated with a suspension of polystyrene divinyl benzene latex (16.6-μm diameter).

The hepatocyte suspension (0.5 ml) was placed in a 200-ml solution of II, and cell counts were read at thresholds 2, 5, 10, 15, 20, 30, 35, 40, 60, 80, and 100, respectively.

**Uptake of Alprostadil and Dinoprost by Hepatocytes from Normal and Diabetic Rats**—One microcurie (~0.635 n*M*) of [5,6(*n*)-$^{3}$H]-alprostadil or 1 μCi (~3.81 n*M*) or [1-$^{14}$C]dinoprost[7] was added to 225 ml of a solution of II at 37° in a water-jacketed beaker. A 1-ml sample from time zero was withdrawn from the beaker into a liquid scintillation vial for radioactive assay in a liquid scintillation counter. Following this, a measured volume of hepatocyte suspension was added into the beaker to obtain ~1.3 × $10^{7}$ or 2.6 × $10^{7}$ cells/225 ml of a solution of II. The hepatocytes were kept in suspension throughout the experiment by stirring with a 120-rpm synchronous motor. At 0.5-, 1-, 2-, 3-, 4-, and 5-min intervals, 3-ml aliquots of the suspension were withdrawn and the cells immediately sedimented by centrifugation at 800×*g* for 15 sec. One-milliliter samples of the supernatant solutions were assayed for their radioactive content.

## THEORETICAL

The selected experimental method involves the dispersion of hepatocytes in an isotonic aqueous phase. The following discussion describes a physical model with relevant interrelationships between experimental

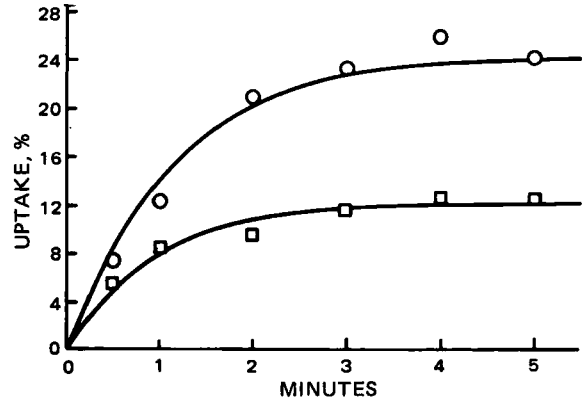


**Figure 4**—*Normalized plot, by percent uptake, of experimental data with theory of alprostadil by normal and diabetic hepatocytes. Key: (○) 13,169,475 normal cells;* P = 5 × $10^{-3}$ *cm/sec;* K = 1345; *(□) 13,450,000 diabetic cells;* P = 3 × $10^{-3}$ *cm/sec;* K = 572.

[6] Coulter Counter model A, Coulter Electronics, Hialeah, Fla.
[7] The Radiochemical Centre, Amersham, England.

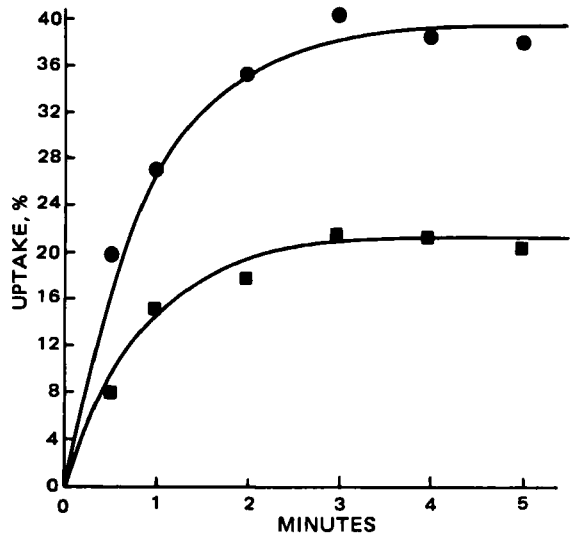


**Figure 5**—*Normalized plot, by percent uptake, of experimental data with theory of alprostadil by normal and diabetic hepatocytes. Key: (●) 27,362,137 normal cells;* P = 5 × $10^{-3}$ *cm/sec;* K = *1345; (■) 27,000,000 diabetic cells;* P = 3 × $10^{-3}$ *cm/sec;* K = *572.*

and theory which incorporates parameters physically describing the system.

The model shown in Fig. 1 is similar to the one proposed previously (12) for rapid equilibration in the heterogeneous cytosol with no binding of solute molecules to components outside the cell. The model describes a hepatocyte of radius $r_c$ suspended in buffer II with $C_c$ as the intracellular solute concentration and $C_b$ as the aqueous bulk solute concentration. The resistance to solute transport may be described as a barrier to transfer across the cytoplasmic membrane. It is best characterized by a transport coefficient (permeability coefficient, $P$) which can be a function of solute and membrane characteristics.

A quasi-steady-state rate of uptake of solute by a cell was described (13) by:

$$\frac{dC_c}{dt} = \frac{4\pi r_c^2 P}{V_c}\left(C_b - \frac{C_c}{K}\right) \qquad \text{(Eq. 1)}$$

where $P$ is the apparent permeability coefficient for the interfacial resistance, $K$ is the effective cell-water partition coefficient, and $V_c$ is the hepatocyte volume.

From mass balance:

$$T_{Ab} + T_{Ac} = \text{Constant} \qquad \text{(Eq. 2)}$$

can be written where $T_{Ab}$ and $T_{Ac}$ are the total amounts of solute in the bulk phase and in the cells at any time $t$. Therefore:

$$V_b C_b + 4/3\pi r_c^3 N C_c = \text{Constant} \qquad \text{(Eq. 3)}$$

where $V_b$ is the bulk volume, and $N$ is the number of hepatocytes in the suspension.

Differentiating $C_b$ and $C_c$ in Eq. 3 with respect to time and rearranging:

$$\frac{dC_b}{dt} = -\frac{4/3\pi r_c^3 N}{V_b}\frac{dC_c}{dt} \qquad \text{(Eq. 4)}$$

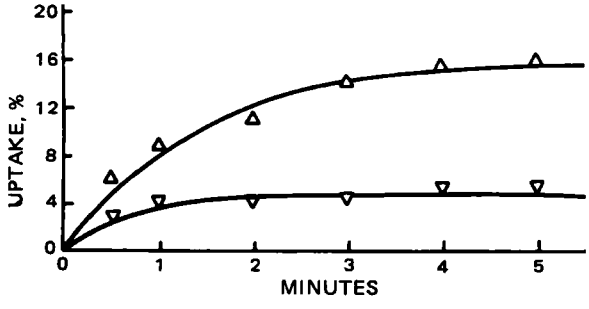


**Figure 6**—*Normalized plot, by percent uptake, of experimental data with theory of dinoprost by normal and diabetic hepatocytes. Key: (△) 11,948,182 normal cells;* P = 3 × $10^{-3}$ *cm/sec;* K = *860; (▽) 13,033,125 diabetic cells;* P = 2 × $10^{-3}$ *cm/sec;* K = *206.*

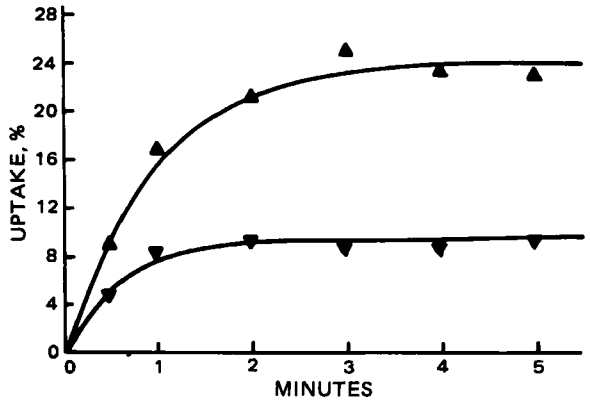


**Figure 7**—*Normalized plot, by percent uptake, of experimental data with theory of dinoprost by normal and diabetic hepatocytes. Key: (▲) 26,320,500 normal cells;* P = 3 × $10^{-3}$ *cm/sec;* K = *667; (▼), 27,169,687 diabetic cells;* P = 2 × $10^{-3}$ *cm/sec;* K = *206.*

Equations 1 and 4 are combined to give:

$$\frac{dC_b}{dt} = -\frac{4\pi r_c^2 NP}{V_b}\left(C_b - \frac{C_c}{K}\right) \qquad \text{(Eq. 5)}$$

Equations 1, 3, and 5 are used to solve for $C_b$ as a function of time when $V_b$, $K$ (which can be calculated from the steady-state data points and Eq. 3), $r_c$, $P$, and $C_b^0$ (concentration of solute in bulk phase at $t = 0$) are known. These equations can be solved analytically to yield a solution of the form:

$$C_b = Ae^{-\alpha t} + B \qquad \text{(Eq. 6)}$$

where $A$, $\alpha$, and $B$ are constants and are expressed as:

$$A = \frac{C_b^0 K V_c N}{(KV_c N + V_b)} \qquad \text{(Eq. 7)}$$

$$\alpha = \frac{3NP(KV_c N + V_b)}{r_c V_b K} \qquad \text{(Eq. 8)}$$

and:

$$B = \frac{C_b^0 V_b}{(KV_c N + V_b)} \qquad \text{(Eq. 9)}$$

However, to manipulate this solution is tedious and prone to errors considering the expressions for $A$, $\alpha$, and $B$, especially in situations of nonuniformity in size distribution of the cells (14). A more practical approach is to resort to numerical methods using the Hamming's predictor–corrector method. The computer program calls on a subroutine which employs the fourth-order Runge–Kutta method to yield the starting values for Hamming's algorithm (15). Comparing the numerical solution with that obtained analytically for some of the data yielded a very close fit, thus, supporting the results.

## RESULTS

**Determination of Mean Hepatocyte Count and Diameter**—The number of isolated cells per liver was ~1.2 × $10^8$ hepatocytes. Hepatocyte preparations showing ≥90% viable cells were used in the experiments.

The mean hepatocyte count corresponds to threshold 17 for the calibration material polystyrene divinyl benzene latex (16.6-μm diameter). Figure 2 represents a plot of the number of normal oversize cells *versus* threshold of the suspension with mean count at threshold 30. Identical size distribution curves were observed for hepatocytes from diabetic animals. A procedure similar to the one reported previously (16) was used to calculate the mean diameter, which was estimated to be 20 μm.

**Uptake Studies**—Uptake studies of alprostadil and dinoprost by 6 × $10^4$ and 12 × $10^4$ cells/ml from normal and diabetic rats were attempted to verify that the calculated physical constants remain the same, irrespective of cell number in the uptake medium. The theoretical calculations are presented as smooth curves in Figs. 3–7 with the apparent permeability coefficient ($P$) experimentally determined as the value which gives the best fit to the data points.

**Uptake of Alprostadil and Dinoprost by Hepatocytes from Normal Rats**—Figure 3 compares the experimental data with theory for uptake of alprostadil by normal hepatocytes. A similar graph was obtained for dinoprost uptake. Steady state was observed in 5 min, and the effective cell-water partition coefficient was 1345 and 764 for alprostadil

and dinoprost, respectively. The apparent $P$-value for both solutes was in the range of $5 \times 10^{-3}$ and $3 \times 10^{-3}$ cm/sec, respectively.

**Uptake of Alprostadil and Dinoprost by Hepatocytes from Diabetic Rats**—Plots similar to Fig. 3 were observed with diabetic cells. The effective cell–water partition coefficient was 572 for alprostadil and 206 for dinoprost. The apparent $P$-value was estimated to be $3 \times 10^{-3}$ and $2 \times 10^{-3}$ cm/sec, respectively.

**Normalized Plots of the Data**—Figures 4–7 are normalized plots for alprostadil and dinoprost uptake by hepatocytes from normal and diabetic rats at almost identical cell counts. At steady state the percent uptake of alprostadil and dinoprost by normal cells was almost double that of cells from diabetic rats, because of differences in the $K$-values of both solutes between the two types of cells.

## DISCUSSION

**Diffusion- and Permeability-Controlled Transfer Through Cell Suspension Models**—Solute transfer through cell suspensions is generally controlled by two physically defined situations, namely the diffusion of the solute from bulk phase to the proximity of the cell membrane, and then the permeation of the solute through the membrane. In the present study, movement of solute from the bulk phase to the hepatocyte membrane interface will be contributing minimally, if any, to the rate of solute transport. Therefore, solute transfer through the cell membrane becomes rate limiting. Membrane permeation is then physically assessed by the isolation of the apparent permeability coefficient. Since this coefficient is in the range of $10^{-3}$ cm/sec, then the effective diffusivities ($D$) of both solutes in an ~100-Å thick ($d_c$) hepatocyte membrane may be estimated to be in the range of $10^{-9}$ cm$^2$/sec. Therefore, the lag time for solute transport may be expressed by:

$$\tau = \frac{d_c^2}{D} \qquad \text{(Eq. 10)}$$

where $\tau$ has a value of $\sim 10^{-3}$ sec, a short lag time when compared with 5 min, the time needed to reach steady state.

**Assessment of the Data**—It was reported (11) that in streptozocin-treated rats, cell membranes have shown significant changes in sialic acid and cholesterol content which support the findings on concanavalin A-induced agglutination[8] that cells from diabetic rats flocculate at a slower rate than those from controls. This suggests that diabetic cells, when compared with normal cells, are either less rich in glycoprotein receptors (sialic acid-rich receptors) or the receptors are arranged differently in the cell membrane. The data on the $K$-values presented here show that normal cells are more lipoidal than diabetic ones. For example, the ratio of the $K$-values of both prostaglandins in normal cells (1345 for alprostadil and 763 for dinoprost) is 1.76, which agrees fairly well with previous data (17) on the partition coefficient ratio of alprostadil and dinoprost between $n$-octane and water (ratio 1.72). Furthermore, the ratio of the $K$-values of the same solutes in diabetic cells is 2.78, which agrees with the ratio of their partition coefficients (ratio 2.11) between 1-octanol and water as well.

The initial rate of transport of alprostadil and the apparent permeability coefficients are larger in normal as compared with diabetic hepatocytes (Figs. 4 and 5). This is also observed in Figs. 6 and 7 for dinoprost. However, alprostadil is transported faster and to a greater extent in both normal and diabetic cells than dinoprost (Figs. 4 and 6, Figs. 5 and 7, respectively). Therefore, it can be concluded that solute partitioning plays a prominant role in the transport mechanism, since the apparent permeability coefficient is a function of the effective partition coefficient.

[8] Unpublished data.

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## ACKNOWLEDGMENTS


This work was supported in part by Grant 38-5766 from the Lebanese Council for Scientific Research and by Grant 18-5205 from the American University of Beirut Medical Research Fund.