Studies of the behaviour of isolated cells in an open system[1]

B. Paletta, R. Möller, W. Mlekusch, G. Meier, K. Hagmüller, M. Hanfstingl and M. Pabst

Medizinisch-Chemisches Institut und Pregl-Labor and Zoologisches Institut, Universität Graz, A-8010 Graz (Austria), 19 September 1979

Summary. A computer-aided arrangement was used to study the time function of lactate output of isolated intestinal epithelial cells in an open system. The results indicate better viability of the cells than in a closed system.

An attempt is made to build together individual processes into an overall mathematical process model[2,3] in such a way that the best possible agreement of mathematical model and biological object with respect to the response to exogenous influences is achieved[4]. The complexity of metabolism requires the limitation of mathematical modelling to subsystems of organisms. The behavior of the modelled subsystems must then be tested for agreement with the behaviour of the corresponding real biological subsystems (i.e. cells, cell organelles and enzymes) under the most natural conditions, especially under the conditions of an open system.

The correlation of different experimental cells and their subsystems also requires the multifactorial and timefunctional registration of inputs into the biological system, as well as a time-functional registration of the response reactions. This paper describes an arrangement which fulfills these experimental requirements.

Methods. The principle of the construction corresponds to a modification of an arrangement previously described[5], which allows studies of the time functional behaviour of enzymes in an open system (figure 1). The computer is programmed with the time functions of the modulator inputs and the molecular environment of the biological system. The substances are dissolved in buffer solutions and stored in stock containers (FS) or in dosing pumps (DP), from which they are fed to the thermostated (TH) cell chamber (CC) by dosing pumps and peristaltic pumps (P) respectively. The low molecular weight reaction products of the cells or their subunits are extracted by means of a microfilter (hollow fiber type) (MF). The outputs are collected in a fraction collector (FC) and are measured individually. The loss of volume in the cell chamber is restored by another peristaltic pump, which is controlled by a level indicator (LI). Isolated intestinal epithelial cells were used. For the preparation of the cell suspension adult male Sprague-Dawley rats were killed by a blow on the neck, the small intestine quickly removed, rinsed with physiological saline, ligated at 1 end, filled with incubation medium and ligated at the other end. The oxygenated medium (9 parts Tris buffer[6] 1 part 0.05 M succinic acid and 0.05 M α-ketoglutaric acid, pH 7.4) contained 0.1% papain and 0.25% bovine serum albumin (BSA). After shaking for 20 min at 37 °C the medium was discarded and the intestine rinsed and cut open lengthwise; the detached epithelial layer was removed and suspended in papain-free incubation medium containing 1% BSA. The cell suspension was filtered through nylon gauze, centrifuged at $56 \times g$ and washed twice with ice cold incubation medium. The method is described in detail elsewhere[7,8]. The pellet was then suspended in BSA-free medium containing 1% (v/v) penicillin/streptomycin (10.000 U/10.000 µg per ml) and the suspension transferred into the cell cage and into plastic vials for incubation. Lactate determinations were carried out by an enzymatic method[9].

Results and discussion. The time function of lactate production of isolated intestinal epithelial cells was determined in the open and closed system under comparable conditions. From a series of experiments giving principally the same results 1 is shown in figure 2, a, b, demonstrating that lactate production ceases after 3 h in a closed system, but not before 5 to 6 h in the open system. The much greater viability of the cells in the open system shows the importance of maintaining steady state conditions with continous removal of reaction products, especially when cells or their subunits are studied under stress conditions. Although lactate production is high enough to give a useful parameter of cell function it seems to be much lower than in other in vitro preparations of the intestine, even when those are vascularly perfused[10]. But we cannot at present compare these results on an exact quantitative basis, because of

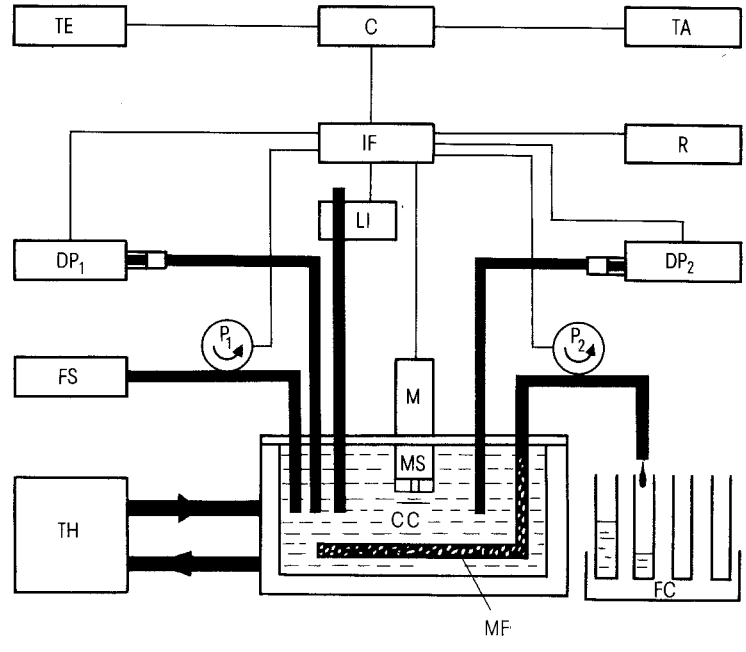

Fig. 1. The experimental apparatus: TE, terminal; C, computer; TA, tape; IF, interface; LI, level indicator; DP, dosing pumps (modulators); P, peristaltic pumps; FS, feeding substrate; MF, microfilter; CC, cell chamber; M, motor; MS, magnetic stirrer; TH, thermostat; FC, fraction collector.

differences in measurement periods and in the preparations used and possibly because of differences in anaerobic and aerobic metabolism. By Laser-Raman spectroscopy[11] an improvement of the measurement technique can be expected in registering simultaneously the time function of multiple outputs. For the purpose of a 1st qualitative model-based explanation of the processes involved, we thus obtain for the time-course of the cell population the simple expressions:

1. $Z(t) = Z_0$ for $t \leq T_e$, and

2. $Z(t) = 0$ for $t > T_e$; where

$Z(t)$ means the number of cells at time t,
Z_0 represents the constant number of living cells,
T_e stands for the life-span of the cells.

With these assumptions the concentration-profile of lactate in the closed system is described by an expression representing the accumulation of lactate in the following way:

3. $c(t) = c_0 + \frac{\pi t}{V}$ for $0 \leq t \leq t_m$, and

4. $c(t) = c_0 + \frac{\pi t_m}{V}$ for $t > t_m$, where the symbols have the following meanings:

c_0, the concentration of lactate at the beginning of the registration ($t = 0$),
π, the cell productivity assumed as constant,
V, the volume of the reaction chamber,
t_m, the registration time point of cell death;
if we further define
T_r as the time which has passed since the formation of the cells until the onset of lactate registration, the relation

5. $T_e = T_r + t_m$ holds.

Figure 3a shows the theoretical time-course of lactate concentration computed on the basis of equations 1–5 with the following parameters:

$c_0 = 0.12$ mg/ml, $\pi/V = 0.10$ mg/ml,

$t_m = 2$ h; from the calculations the following estimates for T_r and T_e result:

$T_r = 1$ h, $T_e = 3$ h.

In the case of the open system we have to consider the combination of production and washing-out of lactate which is reflected in the dynamical relations:

6. $\dot{c} = \frac{\pi}{V} - \frac{D}{V} c$ for $0 \leq t \leq t_m$, and

7. $\dot{c} = -\frac{D}{V} c$ for $t > t_m$.

Integration of these differential equations give the time functions of lactate concentration:

8. $c(t) = \left\{c_0 - \frac{\pi}{D}\right\} e - \frac{D}{V} t + \frac{\pi}{D}$ for $0 \leq t \leq t_m$, and

9. $c(t) = \left\{\left(c_0 - \frac{\pi}{D}\right) e - \frac{D}{V} t_m + \frac{\pi}{D}\right\} e - \frac{D}{V}(t - t_m)$,

constant dilution-rate.

Figure 3b shows the theoretical time-course of the lactate concentration for the following parameter estimates put into 8. and 9.:

$c_0 = 0{,}12$ mg/ml, $V = 12$ ml,
$\pi = 0.40$ mg/h, $t_m = 3.5$ h;
$D = 3$ ml/h,

with $T_r = 1$ h inserted into 5. we get:
$T_e = 4.5$ h.

With results of further experiments and a more detailed mathematical model it should be possible to achieve a more precise agreement of the experimental biological system with the corresponding mathematical process model.

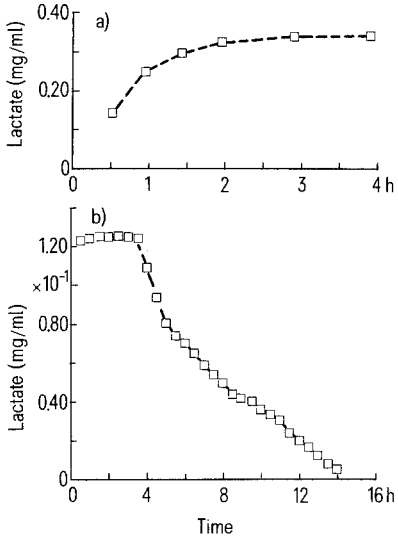

Fig. 2. Experimental time profile of lactate concentration produced by intestinal epithelial cells in a) a closed system, b) an open system.

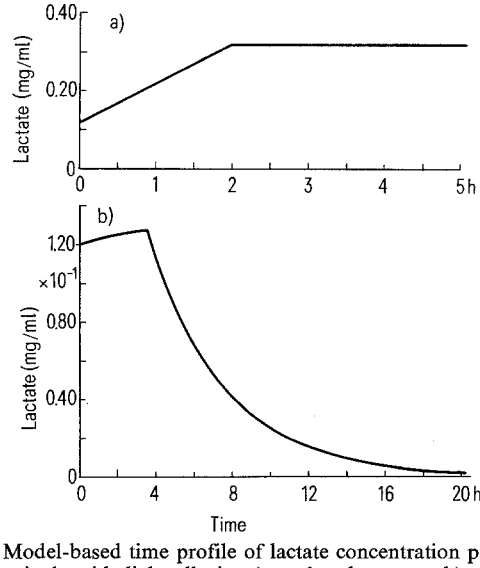

Fig. 3. Model-based time profile of lactate concentration produced by intestinal epithelial cells in a) a closed system, b) an open system.

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