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Methodic problems in microcalorimetric measurements of human colonic mucosa

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Abstract

152 biopsies from the colon of healthy persons were incubated with several nutrition mediums in a microcalorimeter. The course of the heat-flow rate was measured at 1 min interval and graphically displayed as a function of time. For evaluation of the results, the maximum (P_{max}), the mean values (P), and the area under the curve as a measure of the total released energy (E_{tot}) of the power–time curves were determined.

The mean enthalpy change of all samples over time was 3.9 μW per sample. Depending on incubation conditions, mean values ranged between 2.5 and 7.1 μW, maximal values between 3.5 and 13.3 μW. The areas under the curve varied between 6.2 and 18.3 mJ. Our results also showed that bacterial growth can influence measured enthalpy changes if antibiotics are not present. Under identical incubation conditions, steel ampoules were better than glass ampoules with respect to methodic artifacts.

We conclude that microcalorimetric investigations on bioptic material from colonic epithelium are possible. Interestingly, our measured enthalpy changes were lower than those expected from the oxygen consumption rates and other metabolic processes. The reason is yet to be explored. © 1997 Elsevier Science B.V.

Keywords: Gentamicin; Gut; Microcalorimetry; Mucosa

1. Introduction

By improvement of microcalorimetric monitoring, recording of total enthalpy changes of chemical and physical reactions in biological systems has become possible in the last two decades. The microcalorimetric method has been established in the investigations of "living cells" [1–4]. In this way, the registration of heat-flow rates of very small tissue samples or of cell cultures has become possible [5–9]. The heat-flow rates are not only influenced by species differences but also by inflammatory and other pathologic processes. Factors like these could have effects on metabolism and therefore on heat-flow rates. Investigations on organ protection have shown that there are interrelations between the illness state of organs and energy metabolism [10]. In inflammatory situations, the oxygen consumption probably increases because of altered proliferation as well as by reason of immigration of leucocytes and increased phagocytosis [11,12].

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Inflammatory bowel diseases are characterized by diffuse or segmental inflammatory processes of the gastrointestinal tract. The etiology is unknown. Rectoscopy and coloscopy are established methods for the diagnosis and control of the illness activity. By these means it is possible to get information about severity and extension and to take mucosal biopsies for histological investigations [13]. Using appropriate methods, functional data can be gained from such tissue specimens. Functional deterioration is likely to precede morphological alterations.

At present, only few reports on the effect of inflammation on the overall metabolism of the gastrointestinal tract are available. A raised enthalpy change may be expected because of increased proliferation of mucosa, immigration of leucocytes and other inflammation cells. These effects make microcalorimetry a promising tool for detecting early mucosal alterations.

The main goal of this paper is to answer the question whether heat-flow rates produced by biopsies of the human bowel can be detected by the means of microcalorimetry and the problems expected thereof.

Specifically the questions are:

1. Which heat-flow rates of biopsies of colonic mucosa can be expected? Are the measured curves similar to those found with larger samples of biological tissues?
2. How important is the influence of the incubation medium on measured enthalpy changes?
3. Are there reproducible results with the method used?

2. Material and methods

At necessary endoscopic examinations, 152 mucosal biopsies were taken from the rectum at a height of 10 cm with bioptic forceps (opening width 1 cm). In accordance with the dependence on planned investigation, we took 2 to 8 samples of macroscopically and histologically normal colonic mucosa per patient. After rinsing with the incubation medium the samples were put in the measuring ampoule (operating volume 2.5 ml for glass ampoules, 4.5 ml for steel ampoules) with 2 ml incubation medium. The thermal equilibration took place in the pre-thermostating position for 20 min.

The measurements were carried out at 37°C corresponding to the physiological body temperature. Corresponding to the respective experimental protocol we used different clinical infusion solutions as incubation medium.

1. Electrolytic infusion solution – Elektrolytlösung E 153 (Serumwerk, Bernburg, Germany) with the following compounds: KCl 4.9 mmol l^{-1}; $CaCl_2$ 2.5 mmol l^{-1}; $MgCl_2$ 1.47 mmol l^{-1}; Na-acetate 50 mmol l^{-1}; NaCl 90 mmol l^{-1}; Cl^- 103 mmol l^{-1}; acetate-ions 50 mmol l^{-1}; pH 5.0–6.0; osmolarity 303 mosm l^{-1}. In case of a glucose addition (Serumwerk, Bernburg, Germany) the concentration was 10 g l^{-1}.
2. AKE 1100 with Glucose (Fresenius, Oberursel, Germany) with the following compounds: amino acids 30 g l^{-1}; Na^+ 50 mmol l^{-1}; K^+ 25 mmol l^{-1}; Mg^{2+} 3 mmol l^{-1}; Zn^{2+} 0.022 mmol l^{-1}; Ca^{2+} 3 mmol l^{-1}; Cl^- 80 mmol l^{-1}; glycerophosphate 10 mmol l^{-1}; glucose 60 g l^{-1}; pH 4.5–5.5; osmolarity 788 mosm l^{-1}.
3. Isotonic NaCl solution (Serumwerk, Bernburg, Germany) with NaCl 9 g l^{-1} in water; pH 5.0–7.0; osmolarity 303 mosm l^{-1}.
4. Gentamicin (Medphano, Rüdersdorf, Germany) was used (40 mg ml^{-1}) as gentamicin sulfate in water for injection.

The experiments can be split into the following groups:

Group A	incubation in glass ampoules, medium E 153, $n = 24$
Group B	incubation in glass ampoules, medium E 153 + 1% glucose, $n = 8$
Group C	incubation in glass ampoules, medium AKE, $n = 32$
Group D	incubation in glass ampoules, medium AKE + 50 mg l^{-1} gentamicin, $n = 12$
Group E	medium E 153+1% glucose +50 mg l^{-1} gentamicin, $n = 76$ glass ampoules EG, $n = 38$; steel ampoules ES, $n = 38$

After a preparation time of 10 min and an equilibration time of 20 min, the heat-flow rates were measured for over 12 h with a Thermal Activity Monitor (TAM), Thermometric, Sweden. The initial heat-flow rate was determined with back extrapolation

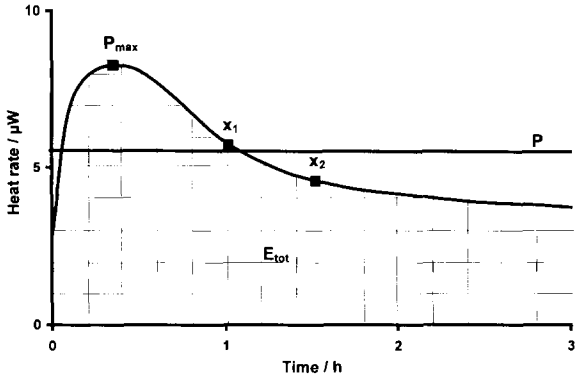

Fig. 1. Parameters used to analyse microcalorimetric records obtained from colonic tissue samples in medium A during the first 3 h after the experimental onset. An example of a single curve explaining the parameters of heat-flow rate in µW in dependence of the time in h. x_1: $T(60)$ i.e. heat-flow rate at 60 min, x_2: $T(90)$ i.e. heat-flow rate at 90 min; P_{max}: maximal power, shaded area: E_{tot} i.e. integral under the curve as a measure of the total energy released during the measurement; horizontal line: P i.e. mean power.

of the measured exponential curves. The heat-flow rates after 60 (T60) and after 90 (T90) min were compared. We also compared the course of power–time curves over the complete registration time (0 to 12 h). In addition, the maximal thermal power (P_{max}), the mean thermal power (P) and the integral under the curve as a measure of the total energy (E_{tot}) released during measurements were determined (see Fig. 1).

In a previous set of experiments the weight of the mucosal biopsies was determined. After measurements the samples were rinsed with isotonic sodium chloride and dried at 100°C until weight constancy was achieved. In this study only spot checks were made.

For all results mean values and standard deviations were calculated.

3. Results

The mean dry weight of the mucosal biopsies was 0.92±0.28 mg. For all the results taken together, the mean heat-flow rate was 3.9±1.9 µW per biopsy, independent of incubation conditions over a registration period of 3 h. Table 1 shows the differences between the groups depending on the incubation

Table 1
Influence of the incubation medium on the measured heat-flow rates. Heat-flow rates in µW using different incubation media (mean values plus standard deviation). Registration during the first 3 h of the experiment. P: mean value in µW; P_{max} : maximal value in µW; E_{tot} : area under the curve as a measure of the total energy release in mJ

	P/µW	P_{max}/µW	E_{tot}/mJ
Group A (n = 24)	4.10±3.22	4.95±4.18	9.18±8.35
Group B (n = 8)	7.14±6.15	13.25±12.21	18.30±15.89
Group C (n = 32)	3.03±2.56	8.74±6.71	6.73±6.48
Group D (n = 12)	2.74±1.72	4.99±2.81	6.95±4.41
Group EG (n = 38)	2.49±2.27	3.51±2.53	6.23±5.75

medium. The enthalpy change in Group B with a glucose addition is twice or triple that in Group A. Also mean values, maximal values, and total energy over a period of 0–3 h after the onset of the experiment were higher when there was an addition of glucose in the incubation medium. The maximal heat-flow rate produced by specimens incubated in AKE is also higher than the respective values of specimens incubated in pure electrolytic solution. Mean and the total energy are nearly identical.

The enthalpy changes in Group B without an antibiotic addition are higher than in Group E with gentamicin over the complete registration time. Fig. 2 shows characteristic heat-output time-curves with and without additional gentamicin, respectively. Especially, the steep rise of curves without an antibiotic addition from the second hour after beginning of registration is remarkable. But already in the first 90 min of the experiment there are differences in the heat-flow rates (Table 2).

By using glass ampoules a part of 23% of the registered curves could not be evaluated. Despite the same incubation and registration conditions only 8% of the curves registered with steel ampoules had to be rejected. The criteria for rejection followed from the power–time curves running only in the negative enthalpy range. On the average, the measured enthalpy changes with glass ampoules (EG) are higher than with steel ampoules (ES). Table 3 shows the mean values and the standard deviations.

Typical power–time curves of colonic tissue biopsies incubated and measured in glass and steel

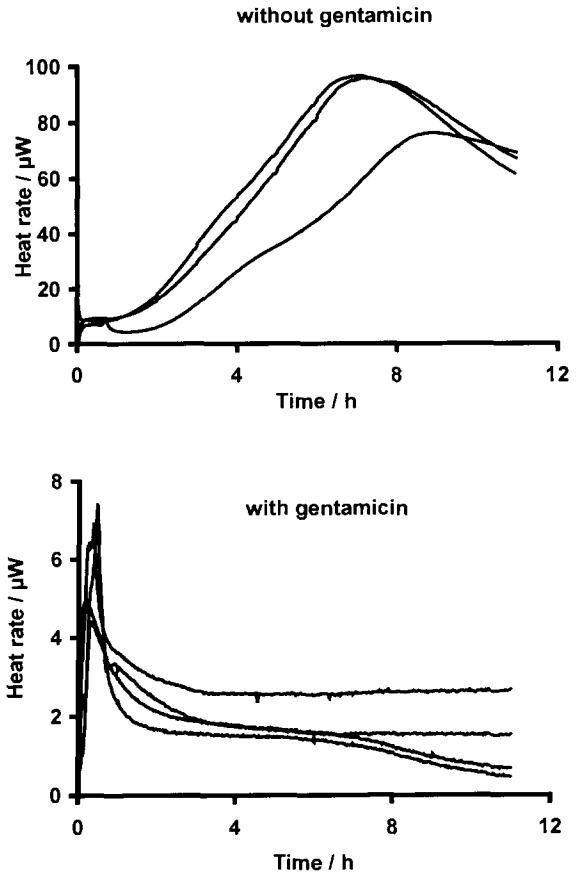

Fig. 2. Comparison between heat-flow rates in µW in 1% glucose medium during the time 0–12 h after experimental onset in dependence of an antibiotic addition of three and four independent experiments, respectively. – without gentamicin (above), medium B – with gentamicin (below), medium E.

ampoules under completely equal conditions are represented in Fig. 3.

4. Discussion

The goal of the present paper is to test whether the method of direct static microcalorimetry is suitable as an in vivo technique for investigations on biopsies of colonic mucosa with regard to clinical interests. In a first set of experiments mucosa samples of patients with macroscopically and histologically healthy rectum mucosa were examined with respect to their total metabolic activity.

Table 2
Influence of an antibiotic on heat-flow rates in µW at different times. Group B: without gentamicin; Group E: with gentamicin; $T(0)$: experimental onset; $T(60)$: after 60 min; $T(90)$ = after 90 min.

	Heat rate /µW		
	T (0)	T (60)	T(90)
Group B (n = 8)	5.10±3.12	5.58±3.19	6.46±4.55
Group EG (n = 38)	2.73±0.90	2.87±0.61	2.52±0.79

Table 3
Heat-flow rates in µW at different times (T) and various ampoule materials. Group EG: glass ampoules; Group ES: steel ampoules; $T(0)$:experimental onset; $T(60)$: after 60 min; $T(90)$: after 90 min

	Heat rate /µW		
	T (0)	T (60)	T (90)
Group EG (n = 38)	4.31±0.98	4.44±1.16	3.58±0.80
Group ES (n = 38)	4.01±1.29	3.79±1.20	3.42±1.12

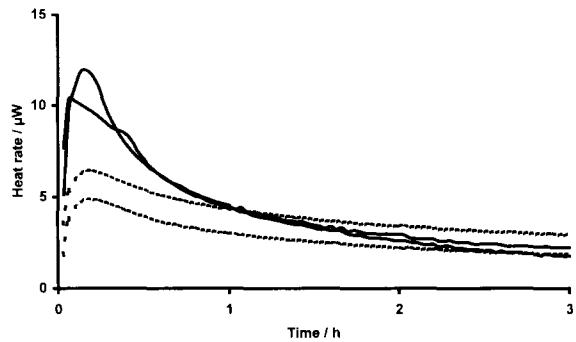

Fig. 3. Exemplary representation of the power–time curves using steel and glass ampoules simultaneously with identical incubation medium (E).– glass ampoules · · · steel ampoules.

The typical microcalorimetric curve pattern seen with biological tissues at anaerobic conditions was also found in our experiments. The decrease of the metabolic activity following an initial rise to a maximum may be explained by an exhaustion of usable cellular stores of energy-rich substrates.

In the low energy range the optimal standardization of the method is of utmost importance. Using commercial endoscopic forceps the sample size can be influenced only in a small range (set up angle, entering depth, anatomical conditions, manual skill of the investigator). By taking 2 biopsies per ampoule it is

possible, not only to increase the tissue amount, but also to equalize differences due to sample size. In this way, the amount of bacteria brought in together with the specimens should be comparable. All the biopsies were taken from the colon without special preparation in order to avoid side effects. Thus, a higher bacterial contamination is probable.

In the experiments we used a clinically applied infusion solution with a composition corresponding to the need of a human organism in homeostatic conditions with regard to electrolytes, pH and osmolarity. The AKE solution is used for substitution of electrolytes and fluids and for parenteral supply of essential amino acids and glucose. We also tested a simple combination of an electrolyte solution and a definite addition of glucose. With glucose as a substrate the measured enthalpy changes were twice to three times that without glucose. In the literature about human epidermal tissue a rise of metabolic activity from 2 mW to 11.6 mW g_{ww} due to the presence of substrate has been described. The authors, however, did not give information about the kind of tissue, sample quantity and a specification of the compounds of the incubation medium [14]. Nilson has also reached an increase of the heat output by a factor of 2 to 3 by an addition of glucose and insulin in investigations on adipocytes at in vitro conditions [6]. Nevertheless, a further improvement of the incubation medium seems to be necessary. In addition to substrate supply, pH, osmolarity, the amount of electrolytes and the kind of buffer systems, should also be respected. Wadsö points out that the metabolic rate of cells is affected by minimal pH changes [4]. For instance, a change of medium pH of 0.01 starting from a pH of 7.4 effects the enthalpy change by 1.2% in investigations on erythrocytes [15].

Since, according to microbiological testing predominantly gram negative bacterial growth prevails in the incubation media, the use of the antibiotic gentamicin seems beneficial. Without an addition of gentamicin the enthalpy change at the starting point is higher by a factor of 1.8, the enthalpy change after 60 min by a factor 1.9, and the enthalpy change after 90 min by a factor 2.6. With gentamicin, especially, the delay of the later rise of the curves is obvious.

Because of the smallness of the samples obtained by biopsy, we suppose that they have a more or less totally aerobic metabolism. The anaerobic part can probably be neglected [9,16]. According to the examinations of Singer [16] with an average dry weight of 0.9 mg per sample, it is justified to suppose that the surface area to volume ratio is large enough and the diffusion distance for oxygen is not greater than 0.5 mm, allowing oxygen present in the measuring ampoules to be used optimally. Whether the "crowding effect", plays an important role in investigations of cell cultures, describing the decrease of the metabolic rate in terms of cell number [16], is relevant in our experiments is not clear.

Since with lower heat-flow rates the influence of methodic disturbances is growing a careful experimental operation is essential. A course of the enthalpy curves only in the negative range, as in some of our experiments, is partially explicable by leaking and relaxation effects of the gum gaskets. Altogether, we may conclude that the real heat-flow rate of the mucosal samples is overlaid by the heat effects of vaporization and friction. Since non-utilizable curves were found in all groups where glass ampoules were used, it seems justified to mutually compare the results of these groups. Measuring with steel ampoules in this low-energy range, methodic artifacts unequivocally are smaller. Steel ampoules appear better suited for measurement of small heat-flow rates.

In the literature, there are only a few results from investigations on intact human tissue. On the contrary, results on blood samples and cell cultures, especially tumor cell colonies, are found abundantly. In a paper from Karnebogen et al. [1] enthalpy changes of human bowel were measured. At sample weights in the gram range (samples extracted from surgical intervention without exact weight indications), a comparison of the findings is difficult. The average heat-flow rates measured in our investigations are in the range below 10 μW per sample with an average dry weight of 0.9 mg. Karnebogen et al. found a maximal power of about 2 $\mu W\ g^{-1}$ on normal colonic tissue and about 11.5 μW g on tumorous tissue. The mean enthalpy change was 1 and 7 $\mu W\ g^{-1}$, respectively, and the total energy was about 5 $mJ\ g^{-1}$ in the normal tissue and 35 $mJ\ g^{-1}$ in the tumors. Obviously, the absence of significant aerobic energy metabolism due to larger sample size had led to distinct weight-related lower heat-flow rates. The significant higher metabolic activity in tumorous tissue (bladder, prostate, kidney, colon) compared to the healthy tissue under ischemic

conditions is in accordance with the results of Warburg [17]. Already in 1930 Warburg described an increased anaerobic glycolysis in tumorous against non-tumorous tissue. This is partly due to an altered enzyme pattern in the neoplastic cells [17]. According to the results of Henninger [18] oxygen consumption rates of small bowel samples from the cat are about: 7–10 ml min^{-1} 100 g^{-1}. Reinhart [19] found an oxygen consumption rate of 3–5 ml min^{-1} 100 g^{-1} in colonic mucosa of the pig. Our results are lower by an order of magnitude. Besides the influences of the incubation medium, probably the lack of physiological transcellular transport conditions is the decisive reason for the differences.

In conclusion, microcalorimetric investigations on biopsies of human colonic mucosa are possible and promising despite some problems yet to be solved. Further investigations have to clarify whether some benefit for the diagnosis or treatment of inflammatory bowel diseases will be obtained by microcalorimetric examinations.

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