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Microcalorimetry for assessment of energy turnover of human gastrointestinal mucosa in vivo[1]

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Abstract

The objective of the present microcalorimetric investigation on mucosal biopsies from human gastric body (B) and descending duodenum (D) was to test whether a steady state of metabolism does persist and, if not, to find out how to assess the preexisting in vivo conditions. The measurements were performed at various incubation temperatures and partial pressures of oxygen. The measured curves fell exponentially after reaching an initial maximum without an initial steady state. Back-extrapolations to the time of taking the biopsies on the basis of a two-phase exponential decay curve gave baseline thermal power values at 37°C of 41 (D) and 15 (B) $\mu W/mg_{dw}$, respectively, which roughly correspond to known values of basal oxygen consumption of heart and kidney. The higher values of the duodenum may be founded on a higher transport activity in vivo. Altogether, microcalorimetry seems to be a method for comparative evaluation of metabolic activity of human mucosa samples. © 1998 Elsevier Science B.V.

Keywords: Energy turnover; Gastrointestinal tract; Microcalorimetry; Medicine; Mucosa

1. Introduction

In order to establish microcalorimetry as a routine method of diagnosis and treatment control of diseases of the gastrointestinal tract there are several prerequisites. First, tissue must be obtainable by simple and easily tolerable means, preferably at repeated times. Taking biopsies during endoscopy is such a possibility. Moreover, the thermal power must be great enough for detection. Only recently this has been shown to be the case [1]. More decisive are the questions if ex vivo measurements of unperfused mucosal biopsies are reflecting in vivo conditions. This may be assumed either when the measured curve shows a steady state during the first hours of measurement or when by extrapolation back to the time of the specimen removal from the gut, biologically reasonable values are obtained. The present paper considers this latter problem area. Moreover, by comparing results of biopsies from different regions of the gastrointestinal tract, evidence is accrued that diagnostically relevant differences of mucosal metabolism may be expected and detected.

2. Experimental

Patients undergoing endoscopic examinations were informed about the planned removal of additional

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Table 1
Incubation solution (mM)

NaCl	140
KCl	4
$CaCl_2$	1.2
$MgCl_2$	1
Phosphate buffer	10
Butyrate	2
L-Glutamine	2
D-Glucose	5
Gentamycin, mg/l	40
pH	7.4

mucosal biopsies for microcalorimetry and agreed by informal consent. During endoscopy 2 biopsies per sample region were taken from the body of the stomach (B) and from the descending duodenum (D), respectively. Within 10 min the specimens were brought to the microcalorimeter room and put in pairs into 4 ml steel ampoules, half filled with the nutrient solution specified in Table 1. During the transport to the microcalorimeter the biopsies were incubated in the same nutritional solution. The temperature during transport was 10°C when the temperature of the microcalorimeter during the later measurement was 27°C or it was room temperature when the measuring temperature was 37°C.

The ampoules were filled, closed and brought into the preheating position of the microcalorimeter for 20 min before measurements were started by sinking the ampoules to the base of the measuring channel. The preheating procedure was identical when ampoules were removed shortly from the microcalorimeter during the measurements, for experimental reasons. The medium of the reference ampoules was physiologic sodium chloride solution. The microcalorimeter used was the four-channel Thermal Activity Monitor (TAM) 2277-201 of Thermometric AB, S-17561 Järfälla, Sweden. Heat production was followed over at least 8 h in 2 min intervals.

After the measurements, the biopsies were removed from the ampoules and dried to weight constancy for dry weight determination. In some cases, lactate, partial pressures of oxygen and carbon dioxide, and pH were determined in the nutrient solution at the end of the measurements on a random basis (established routine methods of the clinical central laboratory). For conversion of the measured heat production values to dry weight related data and for plotting of the curves, the program Microsoft Excel was used. A two-phase exponential decay curve was fitted on the basis of the data from the descending side of each curve, using the program GraphPad Prism. With the calculated formula, the thermal power at the time of the removal of the specimens from the patient was extrapolated.

The measuring temperatures were 27 and 37°C, respectively. At 27°C, 10 pairs of specimens were obtained from the duodenum and 11 from the body of the stomach. The gas phase above the nutrient solution was always air at this temperature. At 37°C, five measurements with material from the stomach were carried out with air in the gaseous supernatant. In addition, at 9 B- and 10 D-measurements oxygen was blown into the ampoules before closing the top. On a total of 8 D-measurements either nitrogen or air were in the supernatant, or the partial pressure of oxygen in the ampoules was manipulated by opening the ampoules and short stirring of the nutrient solution.

Data are given as mean values and standard errors of the mean. For testing levels of significance, the Wilcoxon-U-test was applied [2].

3. Results

The mean dry weight of the 2 biopsies measured together in one ampoule was 1.49±0.55 mg (mean±standard error of the mean). There was no significant difference between the dry weights of biopsies from the gastric body and the duodenum. At a temperature of 27°C with 2 ml air in the supernatant above the nutrient solution, the measured curves from the duodenum and the gastric body had a similar shape. After an initial rise from negative values the curves reached a maximum and then fell exponentially. However, the D-curves reached a distinctly higher maximum and the measured values were significantly higher with biopsies from the duodenum, compared to the values from gastric body specimens (at 1 h and at 6 h: $p<0.001$) during the following exponential decay (Fig. 1).

At 37°C, the B-curves were about 4 $\mu W/mg_{dw}$ higher than corresponding curves at 27°C. Measuring duodenal biopsies of the maximum curve was not only higher at the upper temperature but the exponential decay was also distinctly steeper at 37°C, so that after 4 h the measured values fell below the B-curve values

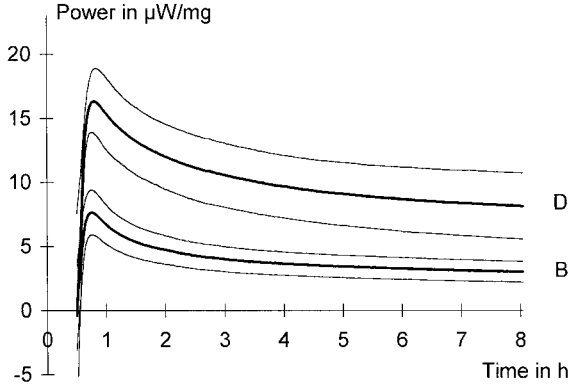

Fig. 1. Thermal power curves of biopsies from human duodenum (D, n=10) and gastric body (B, n=11) at 27°C with air in the supernatant above the nutrient solution. Thick curves show mean values, thin curves show the standard error of the mean.

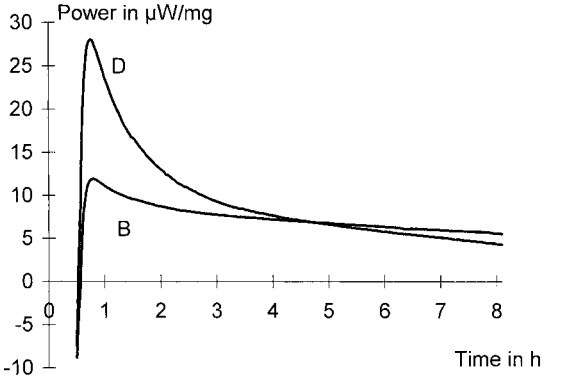

Fig. 2. Thermal power curves of biopsies from human duodenum (D, n=1) and gastric body (B, n=5) at 37°C with air in the supernatant above the nutrient solution.

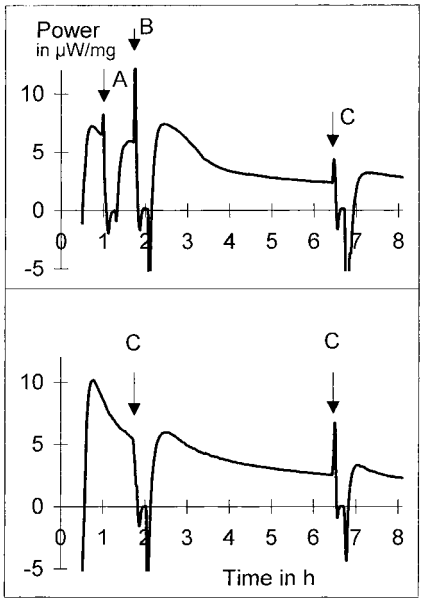

Fig. 3. Thermal power curves of two respresentative experiments with biopsies from human gastric body with air in the supernatant above the nutrient solution (above: Exp. 114, below: Exp. 113). A: During the ongoing measurement the ampoule with the biopsies was taken out of the microcalorimeter and shortly opened, before it was reinserted into the preheating and then into the measuring position. B: The ampoule was opened and gastrin (2.5 µg/ml) and histamine (10 µg/ml) were added. Before closing the top, the nutrient solution was stirred. C: The ampoule was taken out of the microcalorimeter, a short stirring was performed before the ampoule was brought back to measurement.

of the same temperature, especially when air was in the gaseous supernatant of the ampoules (Fig. 2).

Fig. 3 shows what happened when the ampoules were manipulated. When ampoules with body biopsies were only removed for a short period and afterwards reimmersed in the microcalorimeter (procedure A, Fig. 3), the measured values after temperature adjustment were as high as they would have been without the short removal. When ampoules were not only opened, but also when gastrin (2.5 µg/ml) plus histamine (10 µg/ml) as stimulators of gastric body acid secretion were added, followed by a short stirring, the measured values afterwards were distinctly higher than they would have been without such a manipulation (procedure B, Fig. 3). Post-reimmersion values were also higher when ampoules were only opened for a brief stirring (procedure C, Fig. 3).

Fig. 4 presents data from an experiment in which three pairs of specimens from the duodenum of the same patient were measured with either oxygen (A), air (B) or nitrogen (C), in the gaseous supernatant above the incubation fluid. Whereas the maxima of the curves A and B were rather similar, the decay velocity was higher in measurement B. On the contrary, the maximum was markedly lower in curve C than in curve A or B. Calculating two-phase exponential decay curves on the basis of the data from the descending parts of the measured curves and extrapolat-

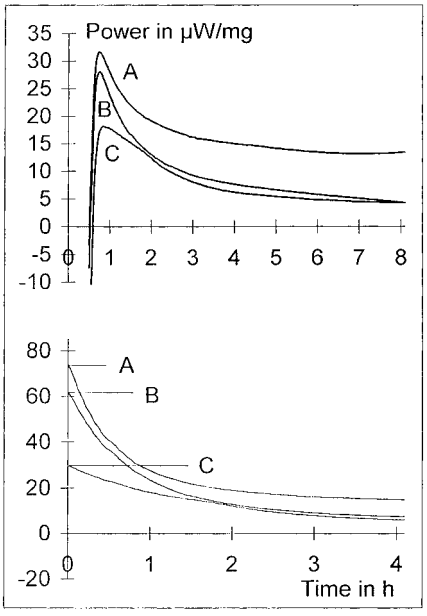

Fig. 4. Measured power curves curves of duodenal specimens (above) and two-phase exponential decay curves calculated from the values of the descending part of the respective measured (below), all samples from the same patient. In measurement A oxygen was blown into the ampoule before closing the top. In measurement B air was in the supernatant above the nutrient solution. In experiment C nitrogen was present in the supernatant. The horizontal lines in the lower part indicate the extrapolated values at the time of taking the biopsies (A: 74, B: 62, C: 30 µW/mg_{dw}).

ing back to the time of the sample removal from the duodenum, the values were 74 µW/mg_{dw} for measurement A, 62 µW/mg_{dw} for measurement B and 30 µW/mg_{dw} for measurement C.

In an oxygen atmosphere the decline of the measured values of duodenal biopsies at 37°C is less steep than with air. However, it is nevertheless steeper than with specimens from the body. The extrapolated values at the time of endoscopical removal are 41 and 15 µW/mg_{dw} for duodenum and body, respectively (p<0.001) (Fig. 5). With 29 µW/mg_{dw} for extrapolated starting values of duodenal biopsies at 27°C, though with air as supernant, a temperature coefficient of roughly 1.4 between 27 and 37°C for duodenal mucosa may be calculated.

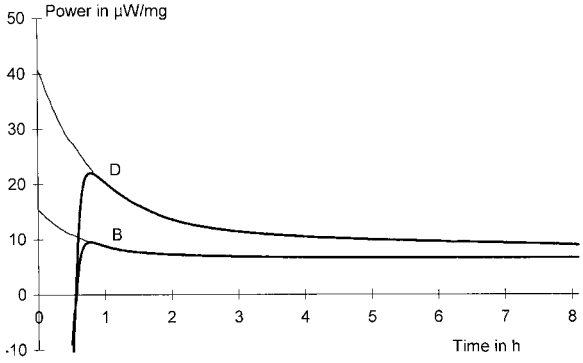

Fig. 5. Mean values of thermal power curves of duodenal (D, n=10) and gastric (B, n=9) biopsies at 37°C under oxygen atmosphere. The backextrapolated values are 41 and 15 µW/mg_{dw}, respectively.

4. Discussion

For answering the question whether in our experiments the biological material showed a steady state of metabolism, it is necessary to bring out which part of the measured curves is due to thermal equilibration and effects of friction or, more in general terms, to reasons of methodology and from which time only the metabolism of the examined samples determined the measured values. In Fig. 3, upper part, an experiment is shown where after reaching the curve maximum the ampoule with the specimens was removed, opened and immediately afterwards reinserted into the microcalorimeter. Just as the start of the experiment, the following curve signal came from deeply negative values because the ampoule had to take up heat again from the environs because it had lost temperature during the reexposal to the room milieu. However, following a rapid rise, the increase slowed down signifying the end of the equilibration process and the curve levelled off and just approached the level of the assumed course of the curve made previously. This type of experiment was reproduced several times and shows that from the curve maximum on the measured values reflect the metabolism of the biological specimens. Hence it follows from the curve characteristics, that in the first hours of measurement there is no steady state of metabolism, at least with duodenal biopsies at 37°C.

The higher heat power after stirring in comparison to the level just before the removal of the ampoule

(procedure C in Fig. 3) suggests that the oxygen partial pressure of the nutrient solution is one of the factors determining the height of metabolism. The higher the metabolism with higher temperature, the more oxygen supply by diffusion is impaired which explains the correspondingly steeper decrease of the measured curves, especially for duodenal samples with their higher metabolism in comparison to gastric body specimens. In addition, the slower decrease of the heat power with oxygen in the supernatant and the then concomitantly higher oxygen partial pressure which was confirmed by corresponding measurements of the nutrient solution underline the limiting role of oxygen for the metabolism of the specimens in the ampoules (Fig. 4, upper part, Figs. 2 and 5). Nevertheless, also an insufficient supply of metabolic substrates and a hampered removal of metabolic waste products might be involved in the lack of a steady state. Again the higher the metabolic need the more distinctly metabolism is likely to be impaired. On the other hand, by reason of the superposing effects of altered oxygen partial pressures, it cannot be clearly stated to what extent the higher measured values after the addition of stimulating hormones of acid secretion (procedure B in Fig. 3) are due to secretion stimulation. For such questions perfusional microcalorimeter devices are more promising. Anyway, it may be emphasized that it is possible to increase metabolism of incubated specimens in the setup used in our experiments.

If it is true that the descending part of the measured curves is caused merely by the biological object under observation, the consideration regarding this part of the curve as an exponential function which may be extrapolated back to the time of taking the biopsies seems to be self implicit. Despite some limitations, a two-phase exponential decay curve is the best suited to describe this part of the curve. With such an extrapolation, energy turnover of duodenal mucosa at the time of taking the biopsies was calculated to be 41, the corresponding value of mucosa from the gastric body was 15 $\mu W/mg_{dw}$. On the basis of a dry-to-wet weight ratio of about 1 : 6 [3] and of an energy equivalent to 21 J/ml O_2, assuming virtually a mere aerobic metabolism at this time, oxygen consumption rates of 2.0 and 0.7 ml $O_2\ min^{-1}\ 100\ g^{-1}$ are calculated. These values are in agreement with basal oxygen consumption values of kidney and heart [4–6].

Under incubation conditions in a nutrient solution without a transepithelial concentration gradient, both passive and active transport activities should be greatly reduced and in our experiments by and large basal conditions of basal oxygen consumption should have prevailed. Although data from different species must be compared only with caution, this assumption is reinforced by data from the literature according to which normal oxygen consumption rates of the small intestine of the cat range between 7–10 and of the porcine colon between 3–5 ml $O_2\ min^{-1}\ 100\ g^{-1}$ [7,8] whereby energy turnover rates of mucosa from colon and gastric body are probably roughly comparable (unpublished preliminary results). On the other hand, a higher physiologic transport activity in vivo is likely to be associated with a higher basal oxygen consumption rate because the synthetic activity for transport proteins and the energetic demands for volume regulation should be higher in such circumstances [3]. Hence, the higher extrapolated energy turnover rates of duodenal tissue compared to mucosa from the gastric body (Fig. 1 and Fig. 5) seem to be plausible. The extrapolated values of the energy turnover under the condition of a N_2-atmosphere being lower but anyhow nearly half as high as values with oxygen or air in the supernatant (Fig. 4, below), should not be misinterpreted that anaerobic metabolism does contribute substantially to the energy turnover of mucosal tissue in vivo. Rather that oxygen reserves in the form of dissolved oxygen is insufficient right from the onset to maintain fullscale aerobic metabolism.

Although in Fig. 4 the curves A and B come close together at about half an hour after the onset of the experiments, the difference seems to increase again if one traces back both curves to their origin (Fig. 4, below). This implies that the method used for back-extrapolation is not an ideal one. Further error probability does arise from the fact that, with the formula used, a possible initial deviation from the characteristics of a two-phase exponential decay curve, e.g. by the presence of a small initial plateau, is not taken into account. In addition, the effects of the cooling of the biopsies during transport to the microcalorimeter is uncertain. On one hand, the oxygen consumption should have been reduced during this period leading to an overestimation of the in situ oxygen consumption. On the other hand, mucosal metabolism might have been disturbed in an unknown fashion by such a

change of temperature, though at least the gastric mucosa might be accustomed to temperature changes due to eating and drinking. Altogether, there are possible sources of error in the determination of the basal energy turnover in situ using the backextrapolation method. However, the possible error range is tolerable and the values obtained are plausible from a physiological point of view. In addition, methodological errors of the kind mentioned above are negligible to a comparative evaluation of biological differences of mucosal tissue when the only values compared are those obtained in the same standardized way.

In conclusion, microcalorimetry is suited for comparative examinations of mucosal biopsies of different origin or nature. According to results obtained by this method the energy turnover of duodenal specimens is nearly three times higher than that of biopsies taken from the human gastric body when an extrapolation back to the time of the removal from the human body during endoscopy is performed.

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