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Microcalorimetric measurements carried out on isolated tumorous and nontumorous tissue samples from organs in the urogenital tract in comparison to histological and impulse-cytophotometric investigations

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Abstract In this comparative study, microcalorimetric measurements were carried out on a total of 96 tumorous and nontumorous tissue samples taken from organs of the urogenital tract using a thermal activity monitor (TAM). Changes in the heat emission of the tissue samples were measured at 1-min intervals and graphically displayed as a function of time. The aim of the study was to compare the microcalorimetric results with impulse-cytophotometric and histological findings and provide evidence for the metabolic activity of tumorous and nontumorous tissue. In order to obtain the variation in metabolic activity, the maxima (P_{max}) of the curves were determined as a value of the maximum thermal power of a tissue sample, the mean values (P) were determined by the mean thermal power and the contour integrals (W) were defined by the behavior of the energy reserves and their mobilization. The first part of the study was carried out to investigate whether tumorous and nontumorous tissue samples differ in general according to their metabolic activity. We discovered, using the parameters described above, that in general tumorous tissue exhibited a higher metabolic activity than nontumorous tissue samples. For example, both W and P in tumorous prostate tissue samples were eightfold higher and the (P_{max}) value was 8.4-fold higher than in normal tissue. Additional investigations on testicle and kidney tissues were performed to find a possible correlation between microcalorimetric results and histological grading. We found that an increasing malignancy correlated with a higher metabolic activity of the tissue. Based upon these results we were able to differentiate the various histological gradings of these tumorous tissues by microcalorimetric measurements. The results show it is possible to differentiate between normal and tumorous tissue samples by microcalorimetric measurement based on the distinctly higher metabolic activity of malignant tissue. Furthermore, microcalorimetry allows a differentiation and classification of tissue samples into their histological grading. With the help of microcalorimetry, it might be possible in future to detect and record the metabolic processes of isolated tissue structures and changes in these activities as a result of medical intervention such as cytostatic treatment.

Key words Microcalorimetry · Isolated tissue samples · Metabolic activities

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The standard and reference method for determination of the malignancy grade of a tumor is the histological examination. Furthermore, the automatic measurement of the DNA content of the cell nuclei by impulse cytophotometry (ICP) [6, 22] gives additional information. The results from these procedures determine the degree of tissue differentiation under investigation and describe the level of malignancy of the tumor, which is a decisive factor in the prognosis and therapy [11]. The disadvantage of these procedures is the lack of information they said about the dynamic behavior of the tumor cells.

One method of determining the biological activity of a living system, e.g., a tissue sample, is by direct calorimetry. It provides a continuous measurement of the heat production and thereby gives information about the total metabolism of the tissue sample under examination. The first instrument to measure the heat

Fig. 1 Thermal activity monitor (TAM) with an IBM-compatible PC, keyboard and monitor

production of a biological system was developed by Lavoisier and coworkers in 1780. The so-called ice calorimeter detected the absolute heat emission in the metabolisms of small animals. Taking into consideration respiration rate, body weight and the specific melting heat of ice, Lavoisier determined the absolute body heat emission of a small animal metabolism during a designated period of time based on the quantity of melting water [16].

The principle of direct calorimetry has been further refined and developed and has led to the creation of modern microcalorimeters (Fig. 1). Its high degree of sensitivity has made it possible to measure even the slightest change in temperature – one millionth of a degree Celsius. Due to the precision of these instruments it was possible to create a procedure which allows rapid, sensitive and continuous monitoring of growth and metabolism in various biological systems. In a study on needle biopsies from human skeletal muscles at rest, it was possible, for example, to differentiate between tissue samples from the musculus rectus abdominis, the m. obliquus internus and the m. vastus lateralis [9].

Monti et al. [24] described the first calorimetric measurement of the metabolic activity of tumor cells in 1986. In his microcalorimetric study of the metabolism of tumor cells in patients with non-Hodgkin's lymphoma (NHL), the prognostic value of the measured total metabolic rates was examined. Monti detected an increased heat emission rate in the cells of highly malignant lymphomas and a reduced heat emission rate in cells with less malignancy. The results revealed a correlation between the degree of heat production of a tumor cell and the survival rate of the patient [23, 24]. It had been established previously that the determination of the proliferation kinetics of NHL cells provided more accurate information for prognosis than the morphological grading of the lymphoma [5, 7].

In another study cell lines from patients with renal cell carcinomas were studied to investigate the metabolic activity and responsiveness to cytostatic treatment [3, 10]. Tumor cell lines were incubated without and with a cytostatic drug (5-fluoruracil) and with two "biological response modifiers" (α-interferon-2a and interleukin-2). The microcalorimetric measurements revealed that untreated cell lines with a high increase in heat production were more susceptible to the antimetabolic agent and that the combination of 5-fluoruracil and α-interferon-2a provided an improved cytostatic effect.

Such findings lead to the question of whether measurement of the biological activity of a tumor tissue sample could be used as a parameter of malignancy, which could perhaps give more accurate information on prognosis, dynamics and sensitivity to cytostatic treatment than purely morphological methods [8, 9, 14, 18, 19].

The aim of this study was to obtain thermographs and standard data from the heat-producing reactions of whole isolated tumorous and nontumorous tissue samples. The energy properties of the tissue samples were obtained and compared to histological and impulse-cytophotometric findings. The aim of the study was to examine whether it was possible to differentiate between normal and tumorous tissue by microcalorimetric measurements. A second part of the study tested whether microcalorimetry allows the differentiation of tumorous tissue samples with various morphological tumor gradings according to their biological activity.

Materials and methods

For the microcalorimetric measurements a thermal activity monitor (TAM) connected to an IBM-compatible computer was used. The data were processed by the ThermoMetric Digitam 3 software package (ThermoMetric, Järfälla, Sweden). A total of 96 tissue samples from urogenital tract organs were available. Thirteen measurements from prostate, 10 from bladder, 38 from kidney and 35 from testicular tissue samples were analyzed.

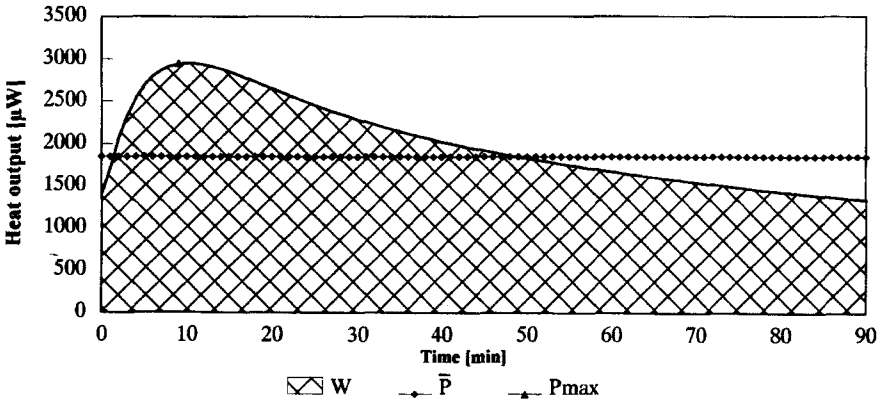

Fig. 2 Evaluation parameters for microcalorimetrically measured values: *shaded area* total energy released W, *dotted line* mean thermal power P, *point* maximum thermal power P_{max}

The samples originated from patients in the urological clinic at the University of Göttingen whose tumors had been resected. After the tissue samples had been transported to the calorimetric laboratory under hypothermic conditions, i.e., in Ringer's solution (B. Braun, Melsungen, Germany) at a temperature of 4 °C, they were divided into three parts, such that macroscopically identifiable tissue abnormalities were represented proportionately in every part, and then microcalorimetrically, histologically and impulse cytophotometrically examined. Hypothermia was maintained in order to reduce the metabolic activities of the tissue samples and to prolong ischemia tolerance during transport of the tissue from the operating theater to the laboratory. The samples, which had diameters ranging from 3 to 9 mm, consisted of histologically examined nontumorous and tumorous material from all phases of bladder, prostate, kidney and testis tissues. For the microcalorimetric measurements, the tissue samples were placed in a glass ampoule filled with 2.5 ml Ringer's solution and hermetically sealed. The measurements were carried out at a temperature of 37 °C. During each measurement, each taking 2 h, the heat emission of the sample was measured at 1-min intervals and continuously displayed on the monitor either numerically or graphically. At the conclusion of the measurement, the tissue sample was weighed to obtain the wet weight and then placed in a heating cupboard at a temperature of 100 °C for 12 h. Then the dry weight (DW) of the tissue was determined. After the sample-specific conversion factor was determined, the heat emission of the tissue samples was converted into microwatts per gram DW. For the evaluation of the measurement results, which were available in the form of heat flow-time curves, the following parameters were used to interpret and analyze the measured metabolic activities (Fig. 2):

A. The apex of the curve, representing the *maximum thermal power* (P_{max} in µW/g DW) of the tissue sample within the measurement period

B. The integral of the curve, serving as the *measure for the entire energy* (*W in mJ/g DW*) *released* in the period of observation

C. The calculated *mean value of a measurement*, representing the *mean thermal power* (P in µW/g DW) within one measurement period

To analyze the ICP results we calculated the DNA index (DI) of the histogram as described in detail by Zimmermann [32] and Zimmermann et al. [33]. Rainwater et al. [26] found that a DNA index of greater than 1.15 indicated an abnormal cell line.

Results

In the first part of our study we investigated 23 benign and malign tissue samples derived from prostate and bladder, 13 samples from prostate and 10 from bladder. All samples were examined histologically, impulse cytophotometrically and microcalorimetrically. Marked differences in activity between tumorous and nontumorous tissue were found in prostate tissue samples. From 13 microcalorimetric measurements carried out [11 on nontumorous hyperplastic prostate tissue samples and 2 on moderately differentiated invasively growing prostate cancer (grade II)] from different patients, we discovered that tumorous tissue samples, in general, exhibited a higher metabolic activity seen in all calorimetric parameters described above, than nontumorous tissue samples. Figure 3 shows the histological findings and Figs. 4 and 5 the ICP results. The microcalorimetry curves shown in Fig. 6 revealed a clearly higher metabolic activity of tumorous prostate than of nontumorous prostate tissue samples. As also shown in Table 1, the values calculated from the tumorous prostate samples, were 8.4 times higher for P_{max} and were increased by a factor of 8 for W and P compared to normal tissue. Ten additional microcalorimetric measurements on tumorous (histological grades II, III) and nontumorous bladder tissue samples (Figs. 7–10) derived from eight patients were carried out. Here we detected an increase in metabolic activity in the tumorous tissue of 2.5 times for W and P, whereas the P_{max} value was 3.1 times higher (Table 1).

The second part of the study was designed to test whether it was possible to differentiate between histologically defined tumorous tissue samples of various malignancy levels by microcalorimetry. We examined 38 tumorous and nontumorous specimens from kidney tumors of different grades of malignancy. We divided the tissue samples into four different groups on the basis of histological examination (Table 2). As shown in Table 2 and Fig. 11, the metabolic activity increased as malignancy level increased. Normal kidney tissue showed the lowest activity, with a P_{max} of 1000.1 µW/g DW, a P of 450 µW/g DW and a W of 2460.8 µW/g DW. The DNA index was 1. Tumorous kidney tissue classified as grade I showed a significantly higher activity with a P_{max} of 1.75 and a P 3.1 times higher. The total energy W was increased by a factor of 2.5. An even higher metabolic activity was

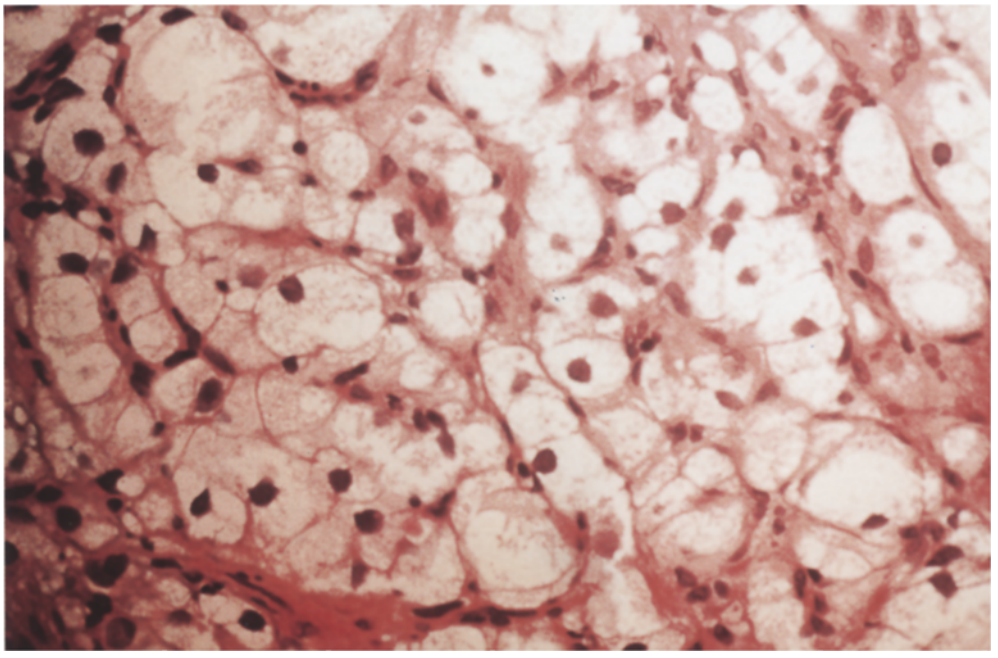

Fig. 3 Moderately differentiated invasively growing prostate cancer (82-year-old male patient). H & E

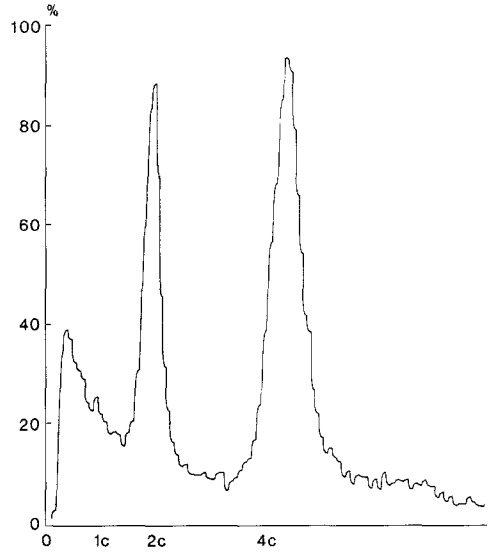

Fig. 4 Impulse cytophotometric results of tumorous prostate tissue from the same patient as in Fig. 3 with a high peak at about 4c and a DNA index of 1.53

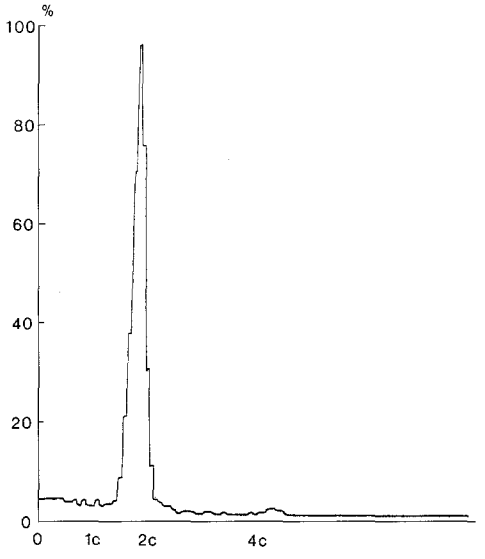

Fig. 5 Impulse cytophotometric results of periurethral nontumorous prostate tissue with slight chronic urocystitis (87-year-old male patient) and a DNA index of 1.05

measured from grade II tumorous tissue samples. The P_{max} value, for example, showed a threefold higher activity in tumorous kidney tissues than in normal kidney samples. The DNA index increased to 1.3 and confirmed the presence of abnormal cell lines. The metabolic activity in chronic inflammatory tissue showed similar values to those from normal kidney tissues and grade I tumorous material, whereas material from nephroblastoma showed metabolic activity values between those of chronic inflammatory and tumorous grade I tissue (Table 2).

The correlation between malignancy grade and metabolic activity determined by microcalorimetry was also confirmed from 35 testicular tissue samples. Table 3 shows that normal testicular tissue has a significantly lower metabolic activity than seminoma tissue at different levels of malignancy. Therefore, for example, the P_{max} measured from normal tissue was 1.2 times lower

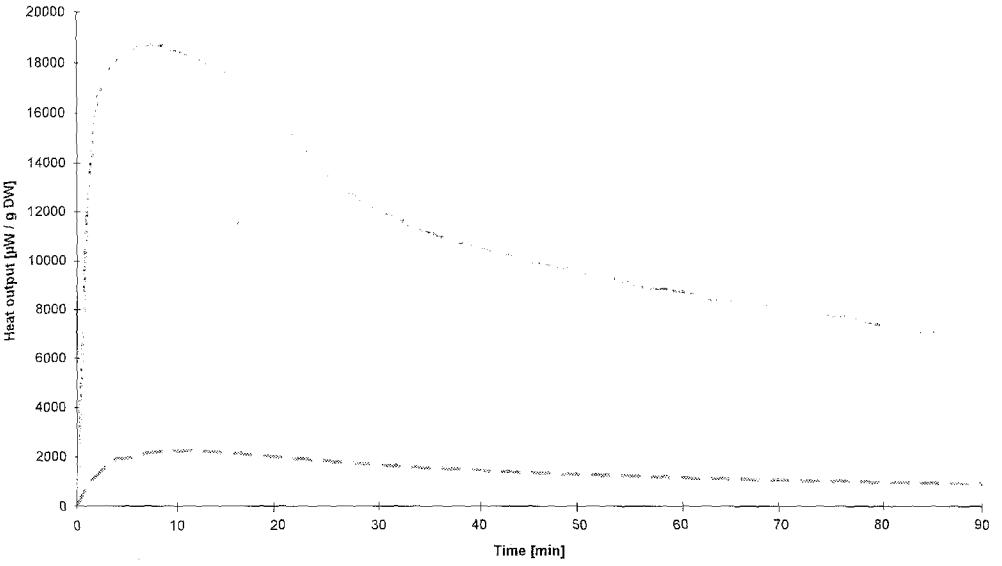

Fig. 6 Microcalorimetric evaluations of tumorous and nontumorous prostate samples [the values were calculated as a mean of 11 nontumorous tissue samples (*dashed line*) and 2 tumorous prostate samples (*solid line*)]

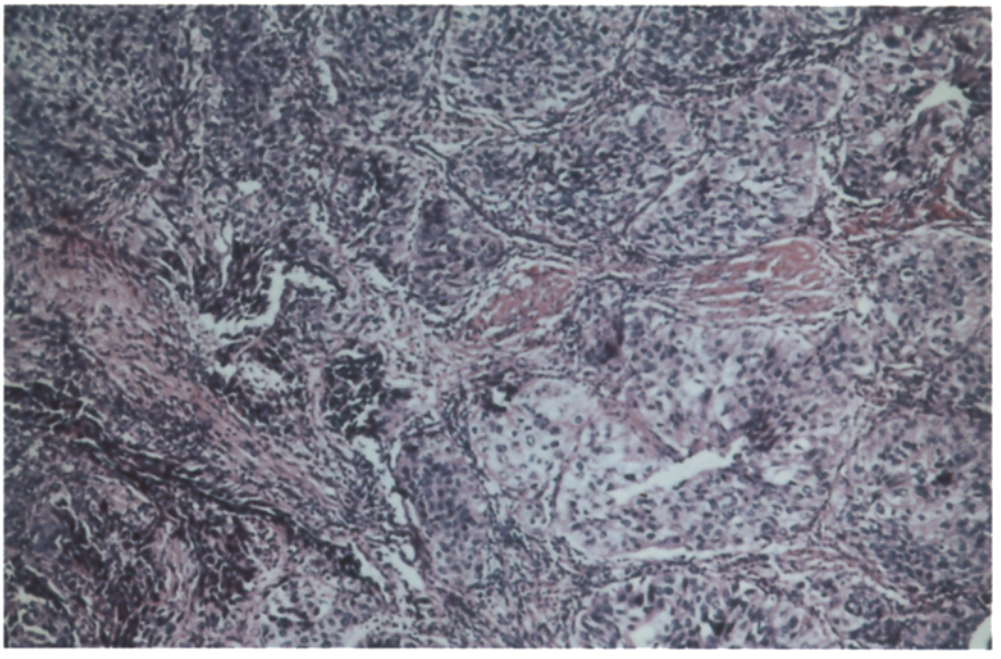

Fig. 7 Low differentiated invasively growing bladder cancer (grades II, III) in an 85-year-old female patient. H & E

than in tissue with moderate atypical nuclei and 2.5 times lower than in tissue with enlarged anisomorphic nuclei. High-grade atrophic seminoma tissue showed a reduced P_{max} in comparison to normal testicular tissue. This might be due to a high rate of fibrotic transformed cells. The coefficient of correlation between ICP, determined by the DNA index, and microcalorimetry was 0.98 (Table 3).

Discussion

One of the characteristics of neoplastic cells is the high aerobic and anaerobic glycolysis rate as described by Warburg and Minami [30]. In later studies, Macbeth and Bekesi [21] found that tumorous tissue consumed more oxygen than nontumorous tissue. Moreover, a preponderance of anaerobic glycolysis compared to the level of oxygen consumption has been determined in tumorous tissue [1, 2, 27, 31].

Comparative studies of enzyme activities in normal, benign and malignant human breast tissues revealed a correlation between degree of malignancy and specific enzyme activity [8, 13]. Pyruvate kinase activity was 45 times higher in tumorous tissue samples than in normal breast tissue. Benign breast tumors exhibited levels of enzyme activity between those of the tumorous and the nontumorous specimens.

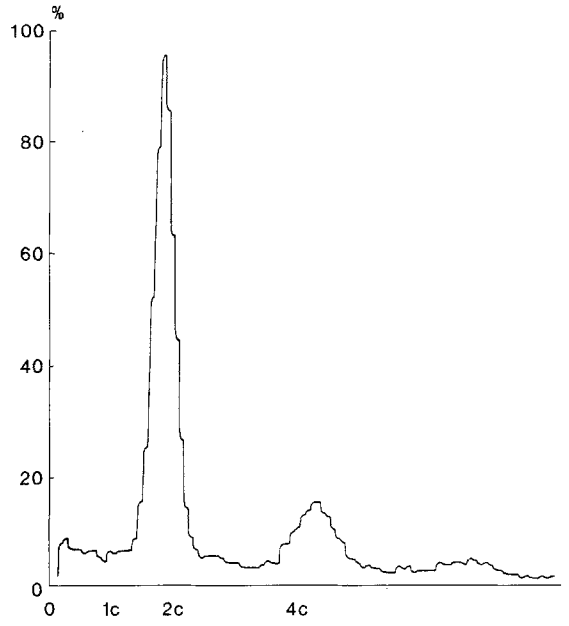

Fig. 8 Impulse cytophotometric results of tumorous bladder tissue from the same patient as in Fig. 7 with a peak > 12% at about 4c and a DNA index of 1.45

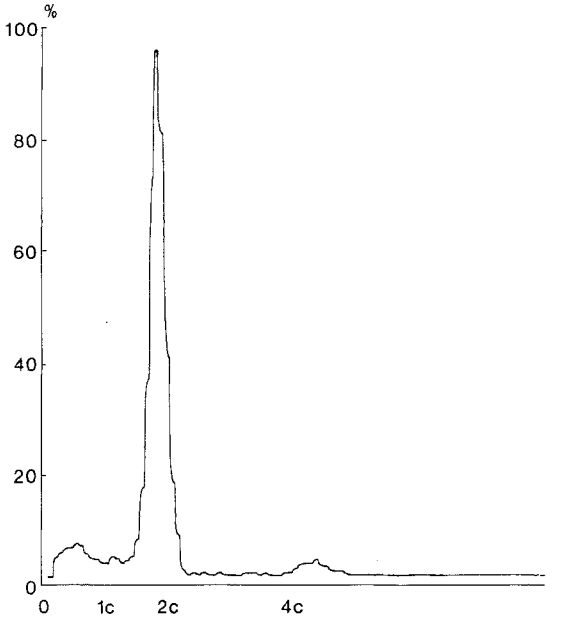

Fig. 9 Impulse cytophotometric results of hyperplastic transitional epithelial tissue without malignancy from an 83-year-old male patient and a DNA index of 1

More recent studies on enzyme activity in normal and neoplastic cells have once again shown that the pyruvate kinase activity in tumor cells is considerably higher than in normal cells. Moreover, it has been proved that the pyruvate kinase activity of metastatic cells is higher than that of neoplastic cells [4]. Furthermore, an increased glutaminase activity in neoplastic cells seems to correlate with the degree of malignancy [17, 20]. Thus, glucose and glutamine seem to be the most important energy and nitrogen sources for rapidly dividing cells.

In our study, tumorous and nontumorous tissue samples were investigated under ischemic conditions. A comparison of the heat flow-time curves resulting from the microcalorimetric measurements must, of course, take into consideration both the metabolic behavior of ischemic tissue and the special characteristics of tumor metabolism [25, 28]. The metabolic difference between tumorous tissue and nontumorous tissue is illustrated with particular clarity by the late measurement time point dictated by the methodology. In some cases, the tissue samples had undergone as much as 60 min of ischemia time before the measurement commenced. The curves from our measurements (Figs. 6, 10–12) show that tumorous tissue exhibited distinctly higher metabolic activity at the beginning of the measurement than nontumorous tissue from the same organ. The higher ischemia tolerance and higher glycolysis rate of tumorous tissue under anaerobic conditions, combined with the considerably higher metabolic activities of this tissue, are the reasons for the variation in metabolic activity. In addition it must be

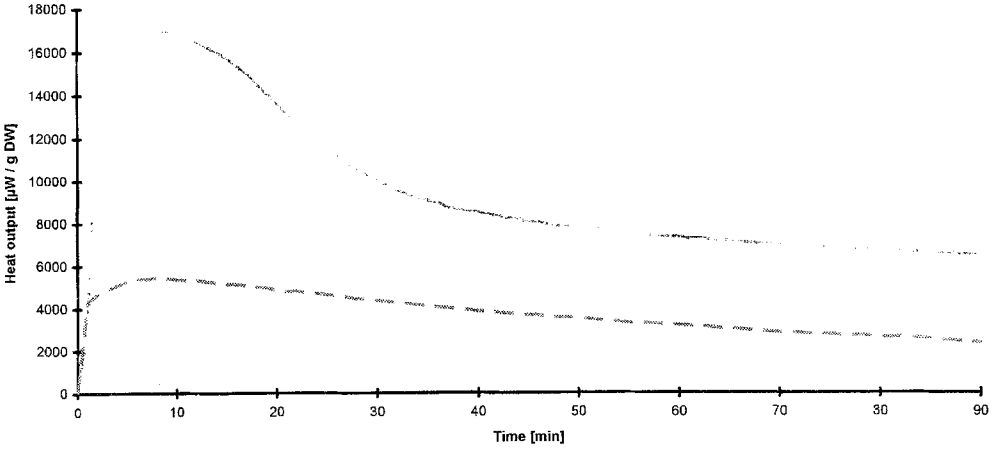

Fig. 10 Microcalorimetric investigations on tumorous and nontumorous bladder tissue [the values were calculated as means of eight tumorous tissue samples (*solid line*) and two nontumorous bladder samples (*dashed line*)]

Table 1 Histological, impulse cytophotometric and microcalorimetric results from tumorous and nontumorous tissue samples from prostate and bladder

Histological results	ICP results	n	Number of patients	P_{max} (μW/g DW)	W (mJ/g DW)	P (μW/g DW)
Benign hyperplastic prostates	Nontumorous tissue	11	8	2239	7506	1422
Moderately differentiated invasively growing prostate cancer (grade II)	Tumorous tissue	2	1	18734	59839	11333
Hyperplastic transitional epithelium	Nontumorous tissue	2	2	5433	19289	3920
Low differentiated invasively growing bladder cancer (grades II, III)	Tumorous tissue	8	6	17059	48679	9894

Table 2 Histological, impulse cytophotometric and microcalorimetric results from nontumorous and tumorous kidney tissue with different levels of malignancy and from chronic inflammatory material, nephroblastoma and cystic kidney tissue

Histological results	n	Number of patients	P_{max} (μW/g DW)	W (mJ/g DW)	P (μW/g DW)	ICP (DNA index)
Normal kidney tissue	7	5	1000.1 ± 471	2460.8 ± 881	450 ± 161	1
Tumorous tissue grade I	19	10	1745.1 ± 348	6311.5 ± 1500	1395.1 ± 274	1.01
Tumorous tissue grades I, II	2	2	2759.4 ± 188	8903.9 ± 104	1630.7 ± 92	1.2
Tumorous tissue grade II	4	3	2983.3 ± 300	8305.5 ± 1728	1525.7 ± 319	1.3
Chronic inflammatory tissue	1	1	1282.3	3941.8	721.9	1
Nephroblastoma	2	1	1552.3 ± 162	4689.6 ± 1158	858.9 ± 212	0.9
Cystic-kidney tissue	3	1	2105.9 ± 719	4173.5 ± 63	762.1 ± 328	1

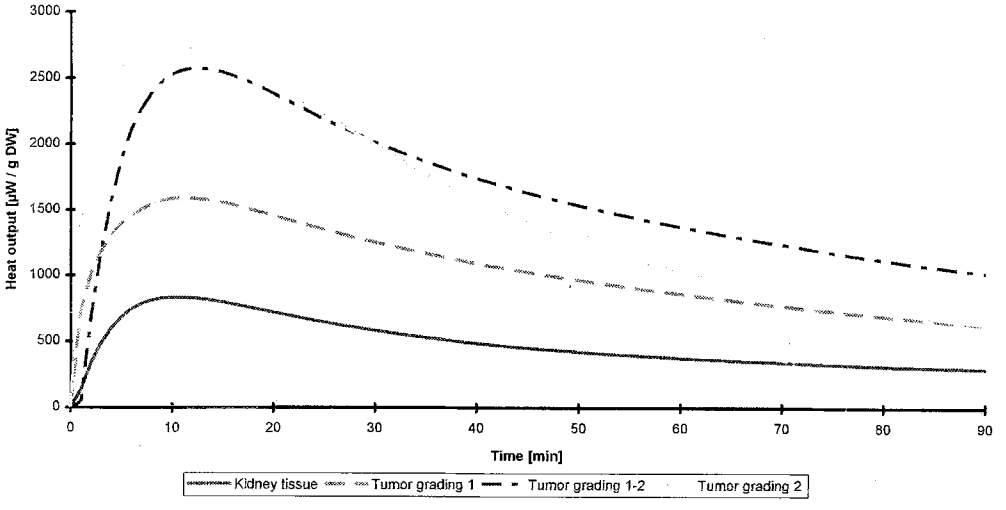

Fig. 11 Microcalorimetric results from nontumorous and tumorous kidney tissue of different levels of malignancy [the values were calculated as means of 7 normal kidney tissue samples, 19 samples from tumorous samples grade I, 2 samples grades I, II and 4 tumorous kidney samples grade II (Table 2)]

considered that the higher cell decay rate leads to an increased heat emission of the tumorous tissue [29]. In the course of the measurement a considerably delayed metabolic reduction of tumorous samples was also observed. This observation also shows the higher ischemia tolerance of tumorous tissue than of nontumorous tissue. Based on the fact that glycolysis is the essential metabolic function of tumorous tissue, the measurements confirm the increased anaerobiosis capacity of the tumor tissue under ischemic conditions.

The lowest metabolic activity was recorded in predominantly necrotic parts of carcinoma. With this result, a methodological problem in tissue calorimetry becomes evident. Despite the fact that it is tumorous material, the measured metabolic activity is distinctly lower than in the nontumorous tissue. A reason for this low activity is that a large number of nonvital cells are associated with a considerably reduced metabolism. Such results show that a very accurate and careful histological examination of the samples cannot be dispensed with.

Our study proved it is possible to differentiate between tumorous and nontumorous tissue from organs of the urogenital tract by microcalorimetry. Additional

Table 3 Histological, impulse cytophotometric and microcalorimetric results from seminoma tissue with different levels of malignancy and from embryonic and choriocarcinoma tissue

Corresponding graph	Histological results	*n*	Number of patients	P_{max} (μW/g DW)	*W* (mJ/g DW)	*P* (μW/g DW)	ICP (DNA index)
Normal tissue	Normal testicular tissue	2	2	1348.6 ± 181	3198.2 ± 321	585.7 ± 49	1
1	High-grade atrophic seminoma	6	5	827.8 ± 232	3054.3 ± 1087	559.4 ± 198	1.25
2	Moderate atypical nuclei, exuberant cytoplasm and fibrous stroma of seminoma tissue	4	2	1623.9 ± 18	6400.7 ± 381	1172.3 ± 70	1.56
3	Medium-sized and unrounded nuclei, prominent nucleoli and fine focus necrotic tumor tissue of seminoma	5	2	2104.3 ± 177	7168 ± 309	1312.8 ± 186	1.83
4	Medium-sized nuclei and coarse-grained chromatin of seminoma	3	2	2863.7 ± 167	9708.3 ± 554	1778.1 ± 101	2.11
5	Medium-sized to enlarged, anisomorphic nuclei of seminoma tissue	5	2	3386.2 ± 409	11 504.2 ± 1859	2107 ± 347	2.39
	Embryonic carcinoma tissue	6	3	2034 ± 790	8617 ± 2666	1601 ± 488	1.53
	Choriocarcinoma	4	1	2616 ± 893	7011 ± 1377	1284.1 ± 252	1.33

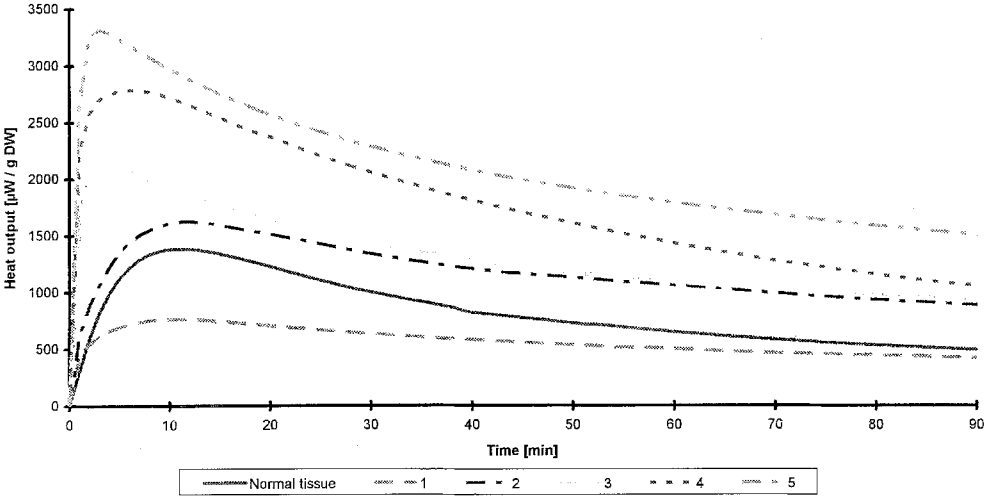

Fig. 12 Microcalorimetric results from seminoma tissue of different levels of malignancy [the values were calculated as from means of 23 seminoma tissue samples and 2 normal testicular tissue samples (Table 3)]. We calculated from all seminoma tissues a correlation coefficient between DNA index and microcalorimetry of 0.98

comparative, microcalorimetric measurements from malign and benign colon, cervix and liver tissues confirmed the higher metabolic rate in tumorous tissue samples [15]. With regard to the seminoma tissue samples of different malignancies, ICP and microcalorimetry gave equal results. However, embryonic and choriocarcinoma showed, despite their clinical malignancy and metastatic spread, low DNA indices and high standard deviations on measurement by microcalorimetry. An explanation for this phenomenon might be the high heterogeneity of the tissue samples [12].

The degree of metabolic variation between the samples depends on the organ of origin (Tables 1–3). For example, prostate and bladder tissues exhibited considerably higher metabolic variation between tumorous and nontumorous tissue than kidney tissue. With the present investigation it could not be distinguished whether a higher ischemia tolerance, an increased cell decay rate or a higher metabolic force was responsible for the variation in the degree of metabolic activity in the organ tissue samples. In order to draw any conclusions about the different metabolic behavior of various organ specimens additional measurements must be carried out.

In conclusion, microcalorimetry, as a means of measuring differences in metabolic activity between tissue samples, can be viewed as a supplementary procedure in tumor diagnostics. Its application may in future provide valuable information regarding prognosis of diseases. After standardization of the measurement procedure, which will be an essential factor for

obtaining reliable diagnostic information, microcalorimetry could become helpful in screening and preoperative staging.

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