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Human Tumour pH and its Variation*

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Abstract—*The variation in human tumour pH values is large. The aim of this study was to analyse the reasons for these large variations and to determine whether tumour pH can be predicted on the basis of any easily measured parameter. One hundred and five determinations of tumour pH were performed in various human tumours, using the Philips C 902S tissue pH electrode. No correlations were found between the tumour pH and the tumour histology, degree of differentiation, tumour size, patient age or treatment history, and whether or not the tumour was ulcerated. However, tumour pH was significantly lower in primary tumours than in lymph node metastases. Tumours at their primary site (primary, recurrent or residual) were also more acid than distant metastases. The vascular disruption caused by the measuring technique was found to be acceptable.*

INTRODUCTION

INVESTIGATION of human tumour pH values is of great interest to the oncologist in view of the importance of environmental pH to the effectiveness of various cancer treatment modalities (for review see [1]). At our institute we have been collecting human tumour pH data for some years. That the pH of human mammary carcinoma is lower than that of normal tissue has already been confirmed [2]. However, a wide variation in values was found. The present study was directed towards analysing the reasons for such large variations, and investigating whether, on the basis of any easily measurable parameter, e.g. tumour location or histology, it might be possible to predict the acidity of a tumour. This paper presents the results of more than a hundred determinations in various human tumours.

What is actually measured by an invasive probe, such as was used in this study, is generally accepted to be largely dependent upon the pH of the interstitial fluid with an unknown component from damaged cells and blood released from ruptured capillaries. The vascular damage caused by the introduction of a (relatively) large probe into the tissue was investigated in experimental tumours.

MATERIALS AND METHODS

pH determination

Tissue pH determinations were performed using the Philips C 902S tissue pH electrode that has previously been described [2]. All patients taking part in this study gave consent after having been informed of its experimental nature. The technique was the same as that used by van den Berg *et al.* [2]. Briefly, following sterilization in Cidex solution (Johnson and Johnson, Benelux B.V.) the electrode was calibrated in sterile NBS buffers at pHs 6.841 and 7.385. As this type of electrode has a relatively fragile glass tip it cannot be used to puncture the skin. It was therefore necessary to make a small incision in the skin into which the electrode could be carefully inserted. This procedure was not always necessary in ulcerating tumours. In some cases the skin was numbed prior to incision using a chloroethylene spray, which we have found does not affect the tissue pH. The electrode was then secured to the skin using adhesive tape. In a number of patients it was possible to insert two electrodes into the tumour simultaneously, which enabled a comparison to be made of the variations in pH values found within tumours relative to those found between tumours. In many patients simultaneous

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determinations of normal (subcutaneous) tissue were also performed.

The ages and (local) treatment histories of all patients were recorded as well as a number of tumour characteristics such as histology, size, degree of differentiation, primary/metastatic and whether or not the tumour was ulcerated.

Assessment of vascular damage

In ten lightly anaesthetized WAG/Rij rats bearing the BA 1112 rhabdomyosarcoma (Radiobiological Institute TNO, Rijswijk) dummy electrodes made of Delrin (Dupont) were introduced in the same manner as when tissue pH determinations were performed. The animals were then given an intravenous injection of luconyl blue, a dye which remains in the circulation as long as this is intact. The animals were killed after 5 min and the tumours removed with the dummy electrodes still in place. The tumours were then subjected to a procedure that renders the blood vessels visible [3], enabling the vasculature to be examined under a stereo microscope.

RESULTS

In total 105 determinations of tumour pH were performed in 77 patients, giving a mean tumour pH of 7.25 ± 0.29 S.D. (n = 77). The normal subcutaneous tissue pH was measured on 60 occasions, the mean value being 7.54 ± 0.09 S.D. These values were significantly different ($2P <$ 0.0001, Student's t test). Tumour pH was also significantly lower than normal blood pH (7.40; $2P < 0.0001$) and subcutis pH was significantly higher ($2P < 0.0001$).

Following introduction of the electrode typically an 'acid shift' followed by a 'recovery phase' was observed. The greater the pH drop, the longer the recovery phase tended to be until stabilization was achieved. The acid shifts were noticeably smaller, and stabilization times hence considerably shorter, during normal tissue determinations. Normally only 20-30 min was required for subcutaneous pH determination, compared to 50-90 min for tumour. A typical trace is shown in Fig. 1.

Tumour characteristics and pH variation

The tumour pH data is shown in relation to the type of lesion (Fig. 2) and the histopathology (Fig. 3). All 105 determinations are shown. Calculation of the means plus standard deviations and the statistical evaluations were, however, performed using the mean value *per tumour* in cases where more than one determination per tumour was performed. These values are given in Table 1.

It can be seen in Fig. 2 that non-ulcerated

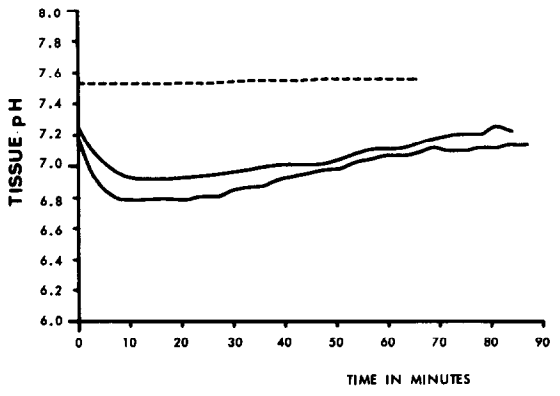

Fig. 1. A typical pH recording obtained from a patient with a poorly differentiated mammary carcinoma. The solid lines show the determinations performed at two sites in the tumour. The dashed line shows the normal (subcutaneous) values.

tumours are somewhat less acid than ulcerated tumours, although this difference is not significant. However, when metastatic lesions only are divided into these two categories the difference is significant ($2P$ = 0.047). Furthermore, it can be seen that lymph nodes were generally less acid than the other groups, in particular when compared to primary tumours ($2P = 0.035$). There were no differences between the other four groups individually. However, when the data were divided into tumours at their primary site (primary, locally invasive and residual lesions) and distant lesions (distant metastases and lymph node lesions), once more a significant difference was found ($2P$ = 0.008).

Comparison of the different tumour pathologies revealed no apparent differences between the groups, with the single exception of lung tumours (Fig. 3). It should be noted, however, that this group of determinations was performed in lymph nodes, as the primary lung tumours were unfortunately inaccessible to the measurement probe. As has already been discussed, lymph node metastases were less acid than the other types of lesions. (Lymph node metastases of lung carcinoma were not significantly different from the other lymph node metastases.)

A large variation in values can be seen in all of the groups. Comparison of the tumour pH values with other characteristics — patient age, local treatment history, tumour size and degree of differentiation — revealed no correlations. In 25 patients, however, more than one pH determination per tumour was performed. From these paired determinations it was possible to calculate the inter- and intratumour variance. The variance *within* tumours was found to be considerably less than that *between* tumours: 0.016 and 0.037 respectively (one-way analysis of variance, random effects model).

Fig. 4. A dummy pH electrode in situ *in an experimental tumour (rhabdomyosarcoma BA* 1112*). The tissue has been treated such that only the blood vessels are visible.*

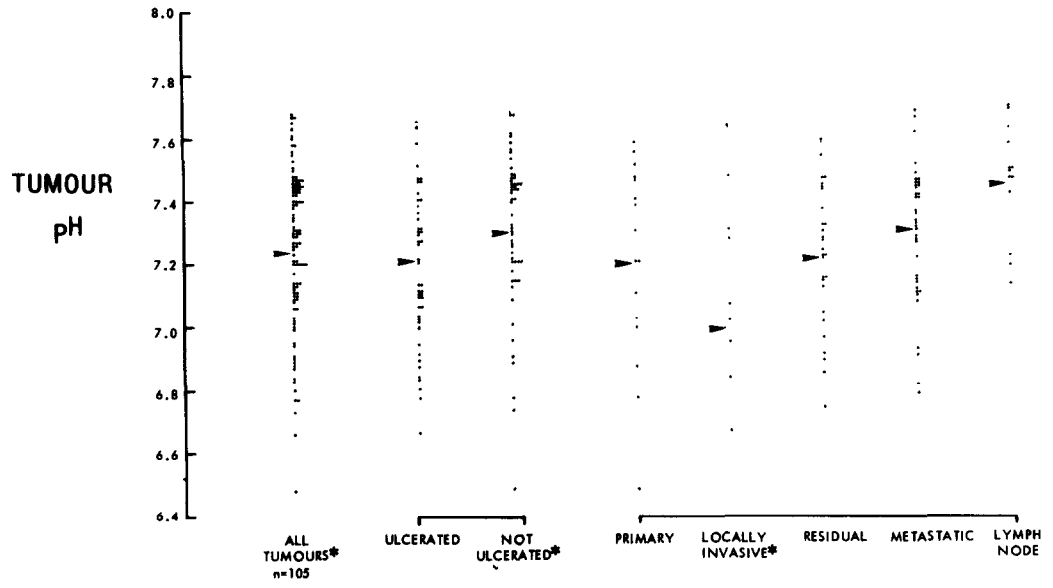

*Fig. 2. Human tumour pH determinations shown with respect to the type of lesion. The left-hand column represents the data from all determinations and the other columns the data split according to the type of lesion. *Not shown is a single determination in a locally invasive, non-ulcerated malignant melanoma; pH 5.98.*

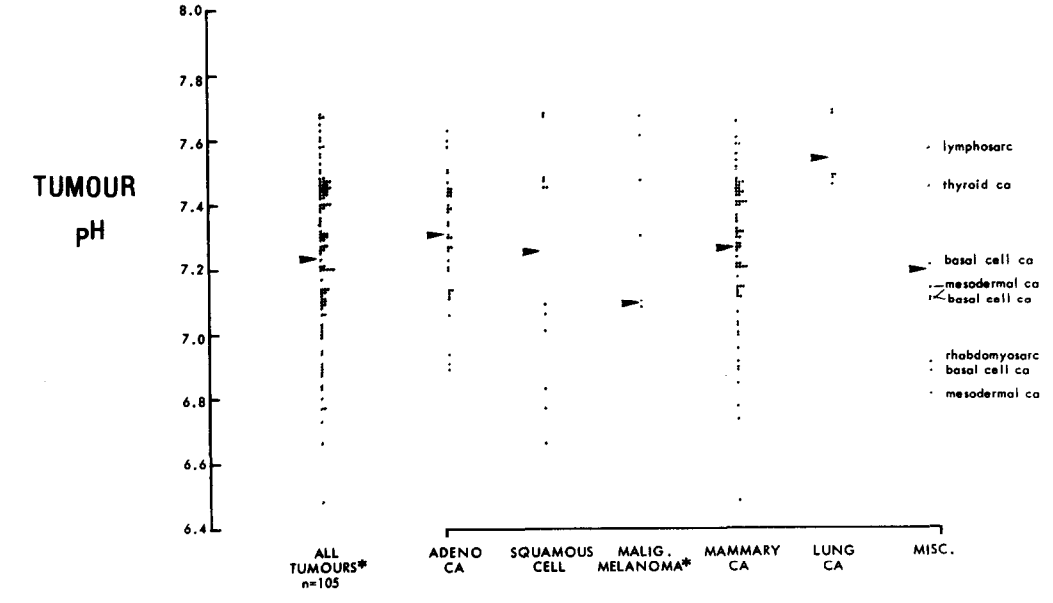

*Fig. 3. Human tumour pH determinations shown with respect to tumour histology. The left-hand column represents the data from all determinations and the other columns the data split according to the tumour histology. *Not shown is a single determination in a locally invasive, non-ulcerated malignant melanoma; pH 5.98.*

Vascular damage

In the experimental rat tumour, rhabdomyosarcoma BA 1112, a series of tumour blood vessel preparations was made into which a dummy electrode had been introduced. An example is shown in Fig. 4. It can be seen that disruption of the blood vessels is within acceptable limits and that little haemorrhaging has taken place around the tip of the electrode.

DISCUSSION

Data on human tumour pH values are scarce [2, 4–6]. Tumours are assumed to have a low pH compared to normal tissue, largely on the basis of the animal data that have accumulated over the years. These data have recently been reviewed extensively [1]. The occurrence of low pH values in tumours may modify the effectiveness of some forms of cancer therapy, favourably in the case of

Table 1. Human tumour pH values

	n	Mean pH	S.D.
All tumours	77	7.25	0.29
Primary	13	7.19	0.33
Locally invasive	6	6.98	0.57
Residual	18	7.20	0.23
Metastatic	29	7.29	0.22
Lymph node metastases	12	7.43	0.18
Ulcerated (all)	32	7.20	0.23
Non-ulcerated (all)	48	7.29	0.32
Ulcerated (metastases)	8	7.17	0.24
Non-ulcerated (metastases)	22	7.34	0.19
Tumours at primary site	37	7.16	0.34
Tumour at distant site	41	7.33	0.21
Adenocarcinoma	24	7.31	0.19
Squamous cell carcinoma	9	7.25	0.35
Malignant melanoma	5	7.09	0.66
Mammary carcinoma	52	7.26	0.24
Lung carcinoma	5	7.53	0.09
Others	6	7.18	0.27
Subcutaneous tissue	60	7.54	0.09

Apparent discrepancies in the numbers can be explained by the fact that three patients, in whom two simultaneous tumour pH determinations were performed, had two masses with differing characteristics.

hyperthermia treatment and unfavourably in the case of, for example, radiation therapy. Work is also in progress to develop methods of treatment that would exploit low tumour pH values [7].

It can now be taken as established beyond doubt that human tumour pH is, *on average*, lower than that of normal tissue. All studies to date find tumour values to be significantly lower than either normal tissue or blood pH [1]. In this study the mean tumour pH of 7.25 was significantly lower than either blood pH or the mean subcutaneous pH, 7.54. The latter value may seem to be somewhat high when compared to blood pH, but this is unlikely to be due to an artefact in the determination. In a pilot study the Philips C 902S electrode was compared to a different tissue electrode (Micro electrodes Inc., Londonderry, NH, U.S.A., diameter 800 μm) used in another laboratory.* Both types of electrodes were found to give comparable pH values for rat tissues. These determinations are summarized in Table 2. Relatively high human subcutis pH values have also been found by other investigators [1]. Whether there is a difference in pH values between human and rodent subcutis is presently being investigated.

Table 2. Comparison of two tissue pH electrodes in rat tissues

	Philips electrode*	Microelectrode†
Rat subcutis, mean	7.28 ($n = 9$)	7.35 ($n = 10$)
DS carcinosarcoma:		
before glucose administration	6.83 ($n = 1$)	6.81 ($n = 1$)
after 4 ml 10% glucose i.v.	6.21 ($n = 1$)	6.40 ($n = 1$)

*Electrode used in this study.
†Electrode used in the Physiologisches Institut, University of Mainz.
Arterial blood pH values were monitored during the experiments and these never fell below pH 7.40. The experiments were performed at the Physiologisches Institut in Mainz (F.R.G.).

The only other studies in which both normal and tumour tissues were examined showed slightly larger differences of 0.62 and 0.71 pH units, although only very small numbers of patients were used in these two studies [4, 5]. In general it can be stated that, although the mean tumour pH is low, a large variation in values exists. Some tumours are clearly not acid. This means that no assumptions can be made concerning the acidity of individual tumours. Nor does it appear possible to predict the acidity of a tumour on the basis of the parameters investigated in this study.

*Physiologisches Institut, Mainz University, F.R.G., in collaboration with Dr F. Kallinowski and Prof. P. Vaupel.

Determination of tumour pH on the basis of a single point measurement has, of course, its limitations. The large variation in tumour pH values is due to a summation of two factors — the variation between different tumours and the variation within a single tumour. Tumours are not homogeneous structures and may have both necrotic areas and areas that are well supplied with blood [8]. Local variations in pH are to be expected, and that these do indeed occur has been demonstrated in experimental tumours [9-11]. Theoretically, at least, these local differences could explain the wide variation in pH values found in this study.

However, analysis of 25 paired determinations revealed that the variance between different tumours is larger than that within tumours, by more than a factor of two. This means that despite intratumoural heterogeneities it should be possible to classify a tumour as more, or less, acid on the basis of a limited number of determinations.

A common criticism of pH measurement using an invasive probe is that disruption of the tissue, in particular damage to blood vessels, leads to erroneous determinations.

There can be no doubt that the introduction of a probe into tissue causes some local damage, and this is presumably the cause of the 'acid shift' typically seen in the first few minutes of a determination. This is followed by a 'recovery phase' after which the electrode reaches a stable value. We assume that during this phase the fluid around the electrode tip is equilibrating with the surrounding interstitial fluid. This process requires varying lengths of time, generally a shorter period when measuring in normal tissue. This may be explained by the more efficient blood flow in healthy tissue [8]. As the vasculature of tumours is chaotic and of lower integrity than that of normal tissue, one might expect the disruption in tumours to be greater than in normal tissue. Although this may indeed be the case, the experimental tumour preparations, in which the blood vessels were visualised after introduction of a dummy electrode, show that this disruption is within acceptable limits (Fig. 4).

In conclusion, this study shows that although the average pH of human tumours is lower than that of normal tissue, no assumptions regarding the pH of individual tumours can be made. The acidity of tumours could not be estimated on the basis of their histology, degree of differentiation, size, type of lesion or any other parameter investigated in this study.

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