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Tumour pH in Human Mammary Carcinoma

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Abstract—*Human tumour pH was investigated using the new Philips C902S tissue pH electrode. In 22 mammary carcinomas a pH of 7.29 ± 0.050 (S.E.M.) was observed, whereas in the human subcutis this value was 7.63 ± 0.034. Tumour pH in some experimental rat tumours was slightly lower than in humans, 7.15 ± 0.029 in rhabdomyosarcoma BA1112 and 7.07 ± 0.024 in a small group of other miscellaneous rat tumours. Rat skeletal muscle was found to have a pH of 7.59 ± 0.070. It is concluded that a small but highly significant* ($P < 0.0001$) *pH difference exists between human mammary carcinoma and human subcutis. This difference is smaller than expected on the basis of animal studies.*

INTRODUCTION

FOR MORE than half a century the interstitial pH of tumours has been thought to be lower than that of normal tissues. This has been demonstrated in several animal tumours [1–7], and some scattered data indicate that this might also apply to human tumours [8–11].

Our interest in the determination of tumour pH stems from the wish to obtain more information about human tumours which are subjected to hyperthermic treatment. Hyperthermia has been shown to be cytotoxic to tumour cells at low environmental pH [e.g. 12–15], and an extensive discussion of this topic has been presented recently by Dickson and Calderwood [6]. On the other hand, low pH seems to offer protection against the cytotoxic actions of both irradiation [16–18] and the chemotherapeutic agent adriamycin [19]. Thus, if tumour pH affects current therapeutic modalities, it would be extremely valuable to assess this parameter in patients, and its determination would form a useful addition to the current diagnostic procedures. Moreover, further evidence of a decreased pH in human tumours may encourage the search for other treatment modalities, such as pH- selective drug therapy [20].

Data on tumour pH in humans are scanty, presumably because the existing assay methods are not very practicable. Recently, however, a new micro-electrode (Philips C902S) has become available which is relatively easy to handle. In this paper the performance of this electrode is evaluated and the assumption that tumour tissue has a lower pH than normal tissue is further investigated. Since human mammary carcinoma (in particular recurrences) is usually readily accessible to a needle-shaped pH probe, this tumour type was selected for this study. Human subcutis was chosen to represent normal tissue. An additional series of measurements was performed on rat tumours and skeletal muscle.

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MATERIALS AND METHODS

pH-Electrode equipment and calibration

In this study the Philips C902S electrode (Philips Nederland B. V., Eindhoven) was used. This electrode is a combined glass/reference electrode with a liquid junction, based on the original design of Stamm *et al.* [21]. The liquid junction is formed by enveloping the glass electrode shaft in a plastic sleeve leaving a capillary layer of reference solution. The sleeve can be removed in order to regularly refresh the reference solution. The maximum diameter of the probe is 2.2 mm. The conical pH-sensitive tip is 1.5 mm long and has a maximum diameter of 1 mm (see Fig. 1).

The pH was measured with a Knick pH-meter (model 645; input impedance $2 \times 10^{12}\Omega$) in conjunction with a chart recorder. The relatively rugged electrode is suitable for clinical investigations, but it is not a simple routine instrument, due to the considerable care

required for calibration. With regular use in tissues the sensitivity declines and response time may increase from 30 sec to as much as 20 min (to within 0.5 mV or 0.01 pH of the final value).

The sensitivity of new electrodes was usually about −57 mV/pH at 20°C. Drift, as measured in buffers, was less than 0.02 pH/hr and the reproducibility was within ±0.01 pH, which conforms to the specifications. The lifetime of the electrodes (when hydrated) varied considerably and ranged from a few weeks to one year. Expressed as operation time in tissues, the lifetime varied from 10 to 120 hr. During this period the sensitivity gradually decreased to a value around −50 mV/pH at 20°C, and then started to decline rapidly while producing progressively unstable recordings. At that point the electrodes were no longer considered reliable. The lifetime of the electrodes could sometimes be extended by reactivation by the manufacturer.

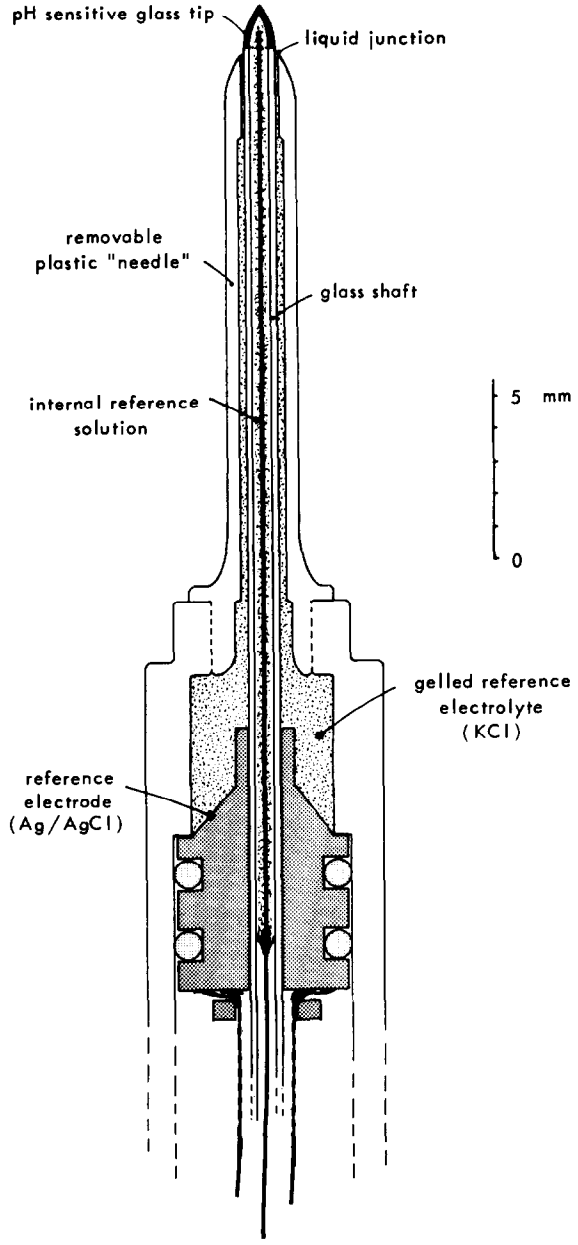

Fig. 1. C902S tissue pH electrode. The glass electrode is housed in a removable plastic sleeve, thus forming a thin layer of reference solution around the glass shaft which enables electrical contact between tissue and reference electrode.

The electrodes were routinely calibrated at room temperature in sterile NBS buffers (pH 6.841 and 7.385; Dr. W. Ingold, A. G.). Room temperature was measured in order to establish the actual pH values of the calibration buffers. It was not considered necessary to perform the calibration at tissue temperature, i.e. 37°C. Although the electrode sensitivity varies with temperature, mV vs pH lines at different temperatures have a common intersection point at approx. pH 7. This implies that close to this pH corrections would be minimal and it can be calculated that, in the physiological range, they would not exceed 0.02 units. Experiments with standard buffers at various temperatures have confirmed this.

The performance of the electrode in a physiological fluid was tested using a blood gas analyser (AVL, Gas Check 938) as a reference instrument and horse serum as the medium. Serum was slightly acidified and maintained at 37°C in a waterbath. As expected, the serum pH drifted to more alkaline values during the experiments. At various intervals samples were taken and placed in test tubes, after which a pH electrode was inserted. As soon as a stable reading was obtained a capillary sample was taken from the test tube and introduced into the blood gas analyser. In a total of 24 paired determinations in the pH range 7.3–7.6 no statistically significant difference was observed between the values obtained with the two instruments (0.002 ± 0.004 units). During this experiment the instrumental drift did not exceed that observed in standard buffers.

Tissue pH measurements

Prior to a tissue pH measurement the electrode was filled with fresh reference solution (gelled electrolyte, as supplied by the manufacturer) and kept in Cidex (Johnson & Johnson, Benelux B. V.) for at least 10 hr to attain sterility (in rat experiments the latter step was omitted). It was then calibrated until a stable reading was obtained. The electrode was carefully inserted into the tissue after a very small incision in the skin had been made. Incision was not necessary when exposed tumours were investigated. The electrode potential was monitored on a chart recorder until the readings

stabilized (20–60 min). After the measurement the electrode was rinsed and again calibrated.

Pre- and post-measurement calibrations often yielded different values. This drift occurred in a positive as well as in a negative direction and rarely exceeded 5 mV (~ 0.09 pH), although occasionally drifts up to 10 mV have been observed. It was assumed that this drift was linear with time, and therefore tissue pH values were routinely estimated on the basis of a linear interpolation with respect to time between the two calibrations. Since most of the pH values presented in this study were obtained from mV-readings taken close to the final calibration, the deviations from results obtained using only the final calibration were small. Furthermore, since positive and negative deviations were about equally distributed, the means appeared to be affected by less than 0.01 pH unit.

Clinical studies

Tumour pH was investigated in mammary carcinoma of patients referred to the Rotterdam Radio-therapeutic Institute for treatment. Measurements were made only after obtaining the patient's consent. Patients were selected on the basis of tumour accessability. Tumours recurring in heavily irradiated areas were excluded due to the vulnerability of the overlying skin. In some patients subcutis pH was also determined. Additional subcutis pH data were collected with the help of healthy volunteers. Although it would be desirable to perform paired measurements in tumour and normal breast tissues, the normal breast tissue structure renders this impossible. Tumours originate from glandular tissue which is embedded in large amounts of fatty and connective tissue, and it is not possible to ascertain in which tissue the electrode is positioned. Since the subcutis is often used in the literature as a control, we have chosen this as an example of normal tissue. Pairing mammary tumour and subcutis measurements may be unnecessary, as it is doubtful that the minimal systemic pH variations have any significant effect upon either subcutis or tumour pH values. With this in mind and in light of practical problems (often only 1 electrode was available and consecutive measurement would have been unacceptable to patients), paired measurements were not performed in most patients.

Animal studies

Adult male WAG/Rij rats carrying either rhabdomyosarcoma BA1112 or, in a few cases, another experimental tumour were obtained from the Radiobiological Institute TNO, Rijswijk. During pH measurements the animals were kept under light nembutal anaesthesia. Skeletal muscle was taken as a reference tissue in a separate series of animals.

RESULTS

The results of tissue pH measurements in human mammary carcinoma and subcutis are presented in Fig. 2. Human tumour pH was determined in 22 mammary tumours, mainly local recurrences. The mean of all values was 7.29 ± 0.050 (S.E.M.). In the subcutis a value of 7.63 ± 0.034 was found as the result of 26 measurements. The difference between tumour pH and subcutis pH was highly significant ($P < 0.0001$) using Student's t-test.

In addition, measurements were performed in several rat tumours and skeletal muscle. The major part of the rat tumour data was obtained with the rhabdomyosarcoma BA1112 [22]. The mean pH ± S.E.M. of 24 measurements was 7.15 ± 0.029. Six measurements in miscellaneous other tumours yielded a value of 7.07 ± 0.024. These tumours included osteosarcoma, squamous cell carcinoma, mammary carcinoma, anaplastic carcinoma and malignant Schwanoma. Muscle pH was determined in 13 rats. This resulted in a value of 7.59 ± 0.070, which is significantly different from the tumour pH ($P < 0.0001$).

DISCUSSION

The present results confirm the existence of a pH difference between tumour and normal tissue in rats and reveal a similar difference

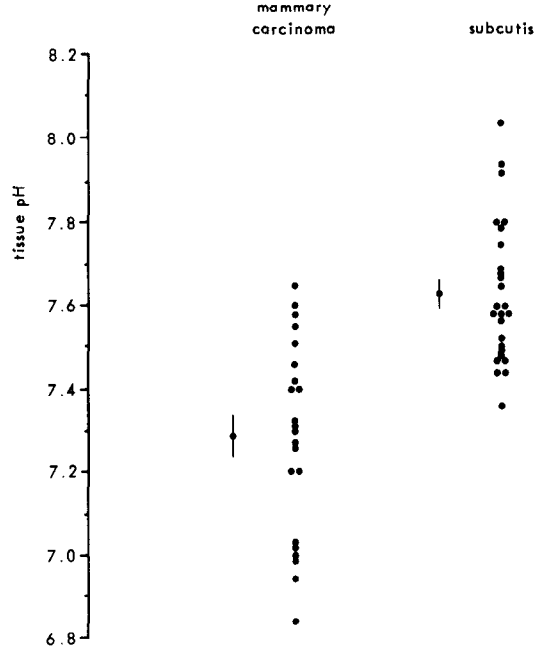

Fig. 2. Tissue pH values observed in human mammary carcinoma and in human subcutis, including the means ± S.E.M.

between human mammary carcinoma and human subcutis.

Low pH values in experimental rat and mouse tumours have been observed by several authors. Values of 7.11 ± 0.034* (Jensen sarcoma, $n = 9$) and 7.13 ± 0.034 (Flexner–Jobling carcinoma, $n = 5$) can be inferred from the date of Voegtlin *et al.* [1]. Kahler and Robertson [2] presented values of 7.00 (6.72–7.22) and 6.7 (6.54–6.93) for rat and mice hepatomas respectively, whereas Tagashira *et al.* [3] reported even lower values ranging from 6.25 to 7.15 in various tumours. Eden *et al.* [4] presented a value of 6.99 as the mean pH of 338 measurements in 156 tumours (range, 6.2–7.5). Gullino *et al.* [5] drained the interstitial fluid of several different rat tumours and observed mean pH values ranging from 6.95 to 7.19. Recently, Dickson and Calderwood [6] found a pH of 7.19 ± 0.034 ($n = 15$) in Yoshida sarcoma, whereas Vaupel *et al.* [7] have observed lower values (6.4–7.1) in C3H mouse mammary adenocarcinoma, with extremely low values of 5.8–6.3 in a large ulcerated tumour. Thus the values of 7.15 ± 0.029 and 7.07 ± 0.024 obtained in this study using rat rhabdomyosarcoma and a few other tumours appear to be close to the upper limit of the values presented in the literature.

The results of the recent study by Vaupel *et al.* [7], mentioned above, were obtained using glass micro-electrodes with a very small (1 μm) tip diameter, and demonstrated that experimental tumours may exhibit a remarkably heterogeneous pH-distribution. The variance of single determinations, therefore, represents the sum of inter-tumour and intra-tumour variances, aside from the variance due to instrumental errors. It may be assumed that the use of larger electrodes has an integrating effect on local pH variations, indicating that in many studies, including ours, the spread of single determinations might reflect mainly the variation between tumours. This is supported by the results of Eden *et al.* [4], who found the inter-tumour variance to be twice the intra-tumour variance. Sometimes pH values measured with relatively large electrodes are presented as reflecting interstitial pH. It should be emphasized that this is not necessarily so, although it is conceivable that the measured pH approaches interstitial pH after a sufficiently long equilibration period.

*Literature values in this section represent means ± S.E.M. unless indicated otherwise. These were not always stated as such by the authors concerned, but could sometimes be calculated on the basis of scattered data.

Reports on pH of human tumours are scarce. Naeslund and Swenson [9] monitored pH in gynaecological tumours during glucose infusion, and a value of 6.94 ± 0.147 can be estimated from 5 measurements taken at the start of infusion. Pampus [10] measured pH in miscellaneous brain tumours in patients under anaesthesia and reported a mean value of 6.84 (5.85–7.35). A pH of 6.81 ± 0.038 can be derived from the data of Ashby [11], comprising 9 measurements mainly in melanomas. Very low values (5.44–6.75) were reported by Meyer *et al.* [8], but these were obtained from surgical specimens 5–90 min after their removal from the body. It is not likely that such data reflect physiological conditions. The present results reveal a pH of 7.29 ± 0.050 in human mammary carcinoma. Although this value is lower than the observed subcutis pH as well as human blood pH (normal range 7.35–7.45), it appears to be higher than literature values for either human or experimental tumours. Whether this is a characteristic of human mammary carcinoma remains to be established. Preliminary studies in our department, however, indicate that the tissue pH of several other human tumour types may not be very much different from that observed with mammary carcinoma (unpublished data).

The interstitial pH of rat muscle has been reported to be within the range of 7.2–7.4 [4, 6, 23, 24,], although an earlier study by Voegtlin *et al.* [25] revealed a value of 7.55 ± 0.008 ($n = 36$). Our observations (7.59 ± 0.070) are in agreement with the latter value. In humans, values of 7.65 ± 0.124 ($n = 5$) [9] and approx. 7.5 (in a group of patients under anaesthesia) [26] have been observed in the subcutis, whereas a value of 7.54 ± 0.014 ($n = 40$) has been reported for dermis [27] and 7.4–8.2 for vital granulation tissue in severe burns [28]. The value of 7.63 ± 0.034 obtained in this study apparently fits in this range.

It is somewhat surprising that subcutis pH is higher than the generally accepted range of normal human blood pH (7.35–7.45). This may be partly due to the fact that the temperature of the subcutis is lower than that of blood, since the pH of buffer systems is temperature-dependent to varying degrees according to the buffer system. Harrison and Walker [27] have determined a temperature coefficient of -0.023 pH units/°C for dermis. This would imply that if the dermis temperature were 37°C instead of approx. 33°C, its pH would be 0.09 units lower. Although the value of the coefficient may not be correct (it was determined by relating dermis pH at a depth of

2–3 mm to surface temperature, and only between 27 and 17°C), it indicates that the observed discrepancy between subcutis and blood pH may be at least partly attributed to a temperature difference. Temperature measurements with a thermocouple needle which were performed in our department in connection with hyperthermia treatments showed the normal subcutis temperature to be 32–35°C. Tumour temperatures did not substantially deviate from 37°C.

A possible error in the estimation of tissue pH may arise from coagulation of protein at the interface between reference electrode and test object, which may induce changes in junction potential. Although Salling and Siggaard-Andersen [29] have shown this effect to be negligible in plasma, Hutten and Vaupel [30] demonstrated erroneous pH-estimations when using a glass electrode with a platinum diaphragm junction in protein-containing solutions. In the present study it was concluded that pH values of horse serum determined with the glass electrode were fully compatible with those obtained using a blood gas analyser (see Materials and Methods). Thus it is not likely that the discrepancy between subcutis pH and blood pH can be attributed to interference of proteins with the performance of the electrode.

In conclusion, it is evident that a small but statistically highly significant pH difference exists between human mammary carcinoma and subcutis. This difference, however, is smaller than that observed between rat tumours and rat muscle in this study as well as in various other studies. This indicates that the therapeutic exploitation of such a pH difference, based solely on data from animal studies, may not necessarily be successful in humans.

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