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Review Article

The relevance of tumour pH to the treatment of malignant disease

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Introduction

Interest in tumour pH stems from the early part of this century, when the pioneering work of Otto Warburg first suggested that aerobic glycolysis was a characteristic property of malignant cells [134]. Warburg hypothesized that the respiration of tumour cells was "damaged" such that they preferentially metabolized via anaerobic pathways, producing large quantities of lactic acid. This in its turn would render malignant tissue more acidic, which led to the early attempts to measure tissue pH in various extracts and homogenates. These primitive determinations are discussed by Voegtlin et al. [125].

Over the years, and using more refined apparatus, it has now been established beyond doubt that at least some tumours have a more acid interstitial pH than normal tissues. This would appear to be primarily due to the poorly organized vasculature of tumours, which gives rise to sluggish flow and generally poor tissue oxygenation [102,120]. This situation necessitates anaerobic glycolysis producing lactic acid which is inefficiently removed. Correlation between lactic acid content and interstitial pH has been demonstrated in tumours [2,91].

Much work has been performed on the effect of low pH upon cells in tissue culture and a myriad of effects has been found. Low environmental pH has been shown to inhibit cell proliferation, survival and activity [7,26,112]. Both DNA synthesis and glycolysis are inhibited [18,23,96], although the former may be a result of the latter. Effects have also been demonstrated on the distribution of cells in the cell cycle, a shift being seen to G1 [71].

In experimental animal system pH has been implicated as the primary factor in loss of transplantability of tumour cells following incubation with glucose [97]. Low pH has even been implicated in the induction of metastases [115].

Some of these effects may be occurring as a result of changes in the intracellular pH, although the relationship between pHi and pHe is complex and not easily predictable [2,55]. Effects on the cell membrane might, however, also be responsible. Low pH has been shown, under certain conditions, to cause a stiffening of the erythrocyte membrane [99], an effect which may contribute to tumour hypoxia, as stiffening of the red blood cell has been shown to reduce oxygen transport to the tissues [140].

Although some interest in systemic pH and its effects upon tumour growth remains, with some rel-

atively recent work suggesting that metabolic acidosis inhibits tumour growth in experimental tumours [4,49] the emphasis has presently shifted to interest in local tumour pH. Some of the above mentioned pH-induced cellular effects may modify or enhance the tumour reaction to some therapeutic modalities. It is the purpose of this paper to review the literature in this field, especially with a view to the relevance of these findings to the clinical situation.

What is the pH of normal and malignant tissues?

The pH of tissues can be measured by a variety of techniques, which have recently been reviewed by Dickson and Calderwood [24]. The most popular technique is the use of pH electrodes which range from micro electrodes with tip diameters in the order of 1 μm to the much larger "mini" electrodes with tip diameters of up to 5 mm. What is actually measured by such electrodes is generally accepted to be largely dependent upon the pH of the interstitial fluid of the tissue, with an unknown component from damaged cells and blood released from ruptured capillaries. A different method of pH measurement was developed by Gullino et al. [47], who incorporated micropore chambers into the tissues of rats in order to collect the interstitial fluid, the pH of which was determined in vitro. This technique eliminates the tissue injury effects but results in an integrated measurement over a long period of time, and from a relatively large tumour volume. A recent development is the use of ^{31}P-NMR spectroscopy to determine the "apparent pH" of, e.g., tumour tissue [78]. This method has the important advantage that it is non-invasive and therefore does not affect the cellular environment.

Normal tissue pH

A summary of normal tissue pH determinations is presented in Table I. The range of values for subcutis (7.00–8.03) and muscle (7.10–8.06) fall into approximately the same range, although the mean subcutis value for man (7.52) is 0.1–0.2 pH units higher than the mean muscle pH values in rats (7.43) and dogs (7.32). This may be at least partly due to a temperature effect [50]. Experimental animals, however, have skin covered with fur, which would tend to insulate against large temperature gradients. In addition to this, experimental animals are usually under general anaesthesia. This tends to cause metabolic acidosis, especially over long periods of time [16]. The determinations in humans were performed either without anaesthetic or with local anaesthesia only.

The electrode tip size used to obtain the data in Table I varied from 40 μm to very large – but there is no apparent correlation between this and the results obtained.

Tumour pH

The pH of malignant tissue is a subject of great interest to the oncologist and a large number of determinations have been performed, mainly in rodents, in a large variety of tumour sorts. These are listed in Table II. Not surprisingly the range is very wide, in rodents 5.80–7.52, which closely approximates the range measured in human tumours, 5.85–7.68. The measurements of Meyer et al. [72] are not regarded as being relevant as they were determined in excised tissues.

The range of values found is determined by a combination of the following factors:

(i) inter tumour variation – tumour size, ulceration, degree of necrosis, growth rate;
(ii) intra tumour variation – heterogeneity of tissue, proximity to blood vessels;
(iii) possible variations between tumour sorts (pathology).

Inter tumour variation

As a tumour increases in size its vasculature becomes increasingly disorganized and less efficient [102]. This tends to render the tumour more acid. Indeed a relationship between tumour size and pH has been demonstrated experimentally. Jähde et al. [59] found a mean pH of 7.0 in mouse TV1A tu-

TABLE I

Normal tissue pH.

Investigators		Approx. electrode size	Species	Tissue[a]	pH[d] (mean)	*n*	Range
Ashby	(1966)	5 mm	Human	s/m	7.43	6	7.30–7.54
v. d. Berg et al.	(1982)	2 mm	Human	s	7.63	26	7.36–8.03
Harrison and Walker	(1979)	40 μm	Human	s	7.54	40	0.09 SD
Naeslund and Swenson	(1953)	3 mm	Human	s	7.65	5	7.30–8.00
Stamm et al.	(1976)	2–3 mm	Human	s	7.42	10	0.05 SD
Vidyasagar et al.	(1979)	2 mm	Human	s	7.33	11	0.033 SD
Couch et al.	(1971)	large	Human	m	7.38	8	7.30–7.48
O'Donnell et al.	(1975)	?	Human	m	7.37	7	7.32–7.47
Bhat et al.	(1980)	2 mm	Dog	m	7.32	4	0.02 SD
Filler et al.	(1971)	7 mm	Dog	m	7.28	21	0.12 SD
Jennische et al.	(1978)	2 mm	Dog	m	7.37	16	0.04 SD
Lemieux et al.	(1969)	large	Dog	m	7.38	6	7.31–7.45
Steinhagen et al.	(1976)	100–300 μm	Dog	m	7.27	19	0.1743 SD
Wolpert et al.	(1970)	?	Dog	m	(7.15)	10	7.1–7.2
Gullino et al.	(1965)	–	Rat	s	7.37	?	0.007 SD
Tagashira et al.	(1953)	"micro"	Rat	s	(7.1)	?	7.0–7.2
v. d. Berg et al.	(1982)	2 mm	Rat	m	7.59	13	7.20–8.06
Dickson and Calderwood	(1983)	1 mm	Rat	m	7.21	20	0.15 SD
Eden et al.	(1955)	1 mm	Rat	m	7.40	29	0.12 SD
Gebert and Friedman	(1973)	100–300 μm	Rat	m	(7.33)	10	7.25–7.40
Voegtlin et al.	(1934)	1 mm	Rat	m	7.55	33	7.50–7.60
Dickson and Calderwood	(1983)	1 mm	Rat	l	7.32	38	0.11 SD
Eden et al.	(1955)	1 mm	Rat	l	7.40	12	–
Kahler and Robertson	(1943)	1 mm	Rat	l[b]	7.39	12	7.18–7.51
Kahler and Robertson	(1943)	1 mm	Rat	l[c]	7.30	9	–
Tagashira	(1953)	"micro"	Rat	l	(7.0)	?	6.95–7.05
Tagashira	(1953)	"micro"	Mouse	s	(7.1)	?	7.0–7.2
Song et al.[e]	(1982)	50–80 μm	Mouse	m	7.45	?	–
Dunn et al.	(1978)	2 mm	Rabbit	s	7.37	6	0.045 SD
Kost	(1978)	?	Rabbit	m	7.39	20	0.034 SD

[a] s, subcutis; m, muscle or muscle surface; l, liver.
[b] Liver of healthy rats.
[c] Liver of tumour-bearing rats.
[d] Numbers in parentheses are the midrange where the mean pH was not given and could not be calculated from the data reported.
[e] Data presented at the 2nd annual meeting of the North American Hyperthermia Group, Utah, April 1982.

mours of 1–2.5 g and 6.9 in tumours of 4–6 g. Busse et al. [17] working with DS sarcoma in rats found that, as tumours increased in size from 2.1 to 10.5 g, the pH fell from 6.7 to 6.5. However, when tumours were very necrotic the pH increased with increasing size – rising from 7.2 to 7.4 as tumours increased from 1.7 to 25.7 g. Vaupel et al. [120] have also found alkaline values in very necrotic tumours, and more acid values (0.6–0.8 pH units lower) in large ulcerating tumours. In contrast Gullino et al. [47] found no differences between small (3 g) and large (10–20 g) tumours, but "very large" (30–40 g) tumours had a higher interstitial pCO_2 and thus were presumably more acid. These investigators also found that faster growing tumours were more acid. Using ^{31}P-NMR spectroscopy Evanochko et

TABLE II

Tumour pH.

Investigators	Approx. electrode size	Species	Tumour	pH[b] (mean)	*n*	Range
Ashby						
(1966)	5 mm	Human	Malignant melanoma (mostly)	6.81	9	6.63–7.00
v. d. Berg et al.						
(1982)	2 mm	Human	Mammary carcinoma	7.29	22	6.83–7.64
Meyer et al.						
(1948)	?	Human	Various surgical specimens	–	98	5.44–7.96
Naeslund and Swenson						
(1953)	3 mm	Human	Gynaecological tumours	6.94	5	6.40–7.20
Pampus						
(1963)	?	Human	Glioblastoma	6.88	22	6.45–7.35
(1963)		Human	Astrocytoma	6.93	13	5.85–7.10
(1963)		Human	Other brain tumours	6.78	8	6.15–7.25
Wike-Hooley et al.						
(unpubl. data)	2 mm	Human	Mammary carcinoma[a]	7.22	43	6.48–7.58
(unpubl. data)		Human	Other tumours	7.17	32	5.85–7.68
v. Ardenne and Reitnauer						
(1979a)	1–10 μm	Rat	Carcinosarcoma	7.15	20	6.80–7.52
v. d. Berg et al.						
(1982)	2 mm	Rat	Rhabdomyosarcoma	7.15	24	6.91–7.45
(1982)		Rat	Other tumours	7.07	6	7.01–7.16
Dickson and Calderwood						
(1983)	1 mm	Rat	Yoshida sarcoma	7.19	20	0.13 SD
(1983)		Rat	MC7 sarcoma	7.17	22	0.08 SD
(1983)		Rat	D23 carcinoma	7.13	10	0.04 SD
(1983)		Rabbit	VX2 carcinoma	6.99	6	0.15 SD
Eden et al.						
(1955)	1 mm	Rat	Novikoff hepatoma	6.96	39	0.17 SD
(1955)		Rat	3741 sarcoma	6.95	28	0.25 SD
(1955)		Rat	3741 sarcoma	7.13	6	0.16 SD
(1955)		Rat	3741 sarcoma	7.09	6	0.07 SD
(1955)		Rat	Lymphosarcoma	7.00	97	0.19 SD
(1955)		Rat	E2730 sarcoma	7.04	17	0.11 SD
(1955)		Rat	Carcinoma 2226	7.00	32	0.11 SD
(1955)		Rat	Sarcoma 1643	7.01	55	0.16 SD
(1955)		Rat	Fibrosarcoma ACMCA2	6.83	27	0.24 SD
(1955)		Rat	Hepatoma 3924A	7.06	43	0.22 SD
Gullino et al.						
(1965)	–	Rat	Walker carcinoma 2561	7.00	17	0.141 SD
(1965)		Rat	Fibrosarcoma 4956	7.09	7	0.123 SD
(1965)		Rat	Hepatoma 5123	7.19	8	0.078 SD
(1965)		Rat	Hepatoma 3683	7.01	7	0.14 SD
(1965)		Rat	Novikoff hepatoma	6.95	7	0.17 SD
Jähde et al.						
(1982)	10 μm	Rat	TV1A 1–2.5 g	7.0	500	6.8–7.1
(1982)		Rat	4–6 g	6.9	1000	6.7–7.1

TABLE II *(continued)*

Tumour pH.

Investigators	Approx. electrode size	Species	Tumour	pH[b] (mean)	*n*	Range
Kahler and Robertson						
(1943)	1 mm	Rat	Hepatoma 31	6.99	10	6.81–7.10
(1943)		Rat	Primary hepatoma	7.02	6	6.72–7.22
(1943)		Mouse	Hepatoma	6.74	5	6.54–6.93
Müller-Klieser et al.						
(1981)	1–5 μm	Rat	DS carcinosarcoma	6.59	480	6.0–7.3
Tagashira et al.						
(1954)	"micro"	Rat	Fibrosarcoma	(6.88)	?	6.75–7.00
(1954)		Rat	Reticulosarcoma	(6.63)	?	6.55–6.70
(1954)		Rat	Yoshida sarcoma	(6.80)	?	6.65–6.95
(1953)		Rat	OAT hepatoma	6.95	?	–
(1954)		Rat	Ascites hepatoma	6.70	?	–
(1954)		Mouse	Adenocarcinoma	6.25	?	–
(1954)		Mouse	Ehrlich ascites carcinoma	(6.60)	?	6.55–6.65
Voegtlin et al.						
(1935)	1 mm	Rat	Jensen sarcoma	7.11	9	6.92–7.30
(1935)		Rat	Flexner Jobling sarcoma	7.13	5	7.01–7.22
(1935)		Mouse	Mammary carcinoma	6.86	2	6.82–6.90
Evanochko et al.						
(1983)	–	Mouse	16/C mammary adenocarcinoma 1.1–2 g	(7.1)	4	6.8–7.4
Song et al.[c]						
(1982)	50–80 μm	Mouse	SCK mammary carcinoma	6.95	900	6.6–7.3
Vaupel et al.						
(1981)	1 μm	Mouse	CH3 mammary adenocarcinoma	6.73	1453	5.8–7.2

[a] This is a separate group of patients from those reported by v. d. Berg et al.

[b] Numbers in parentheses are the mid-range where the mean pH was not given and could not be calculated from the data reported.

[c] Data presented at the 2nd Annual Meeting of the North American Hyperthermia Group, Utah, April 1982.

al. [29] observed a pH drop from 7.4 to 6.8, in a subcutaneously implanted mouse mammary adenocarcinoma, as the size increased from 2 to 5 g.

Intra tumour variation

Many workers have demonstrated the heterogeneity of single tumours [59,76,120]. One of the main causes, especially when very small electrodes are used, is probably the proximity of the electrode tip to blood vessels. Von Ardenne and Von Ardenne [129] calculated a gradient from the capillary wall into the tumour of 7.2–6.9 at 60 μm distance. Greater distance from the blood supply logically results in lower pH due to restricted oxygen availability, indeed low oxygen tensions in tumours have been measured by Vaupel et al. [120]. Intra-tumour variations of 0.2–0.5 pH have been found in rodent tumours [59,76,120], with the higher pH values being found in the tumour periphery. Measurements within human tumours (Wike-Hooley et al., unpublished data) reveal variations of 0.05–0.36 pH units within single tumours.

One might expect the inter-tumour variation to be greater than the intra-tumour variation, and this is supported by the work of Eden et al. [27]. These workers report an inter-tumour variance in rat lymphosarcomas of 0.0225 compared to an intra-tu-

mour variance of 0.0111. No other investigators have reported similar comparisons, but from a group of 23 paired measurements in various human tumours (Wike-Hooley et al., unpublished data) an inter-tumour variance of 0.039 can be calculated, compared to an intra-tumour variance of 0.016 (one-way analysis of variance, random effects model). Values for human tumour variance based on single point determinations can also be calculated from the data reported by other authors; 0.108 in gynaecological tumours [77], 0.103 in brain tumours [86], 0.009 in malignant melanoma [5], 0.235 and 0.233 in mammary carcinoma [119, Wike-Hooley et al., this paper], and 0.373 in various other tumours [Wike-Hooley et al., this paper].

Tumour pathology

An evaluation of tumour pathology-related pH differences is difficult to make. Particular tumour sorts measured by different workers have in some cases yielded different values (see Table II). The pH values found may be affected by many factors, such as the type of electrode used, the tumour histology, tumour size, and the type of anaesthesia, to give a few examples. Comparisons between tumour sorts measured by any one author are less difficult to interpret. Unfortunately, few workers have performed determinations in assorted tumour types, and when they have done so, the numbers of each tumour sort are often either very small or not given. Some differences can however be found. A students *t* test by the authors on the data of Gullino et al. [47] reveals a significant difference ($2P = 0.0029$) between Novikoff hepatoma and hepatoma 5123, and between Walker carcinoma 256 and hepatoma 5123 ($2P = 0.0014$). The authors also found a significant difference ($2P = 0.0248$) between the Novikoff hepatoma and hepatoma 3924 A as determined by Eden et al. [27]. The differences between the means of these different tumours is however small (maximum difference, pH 0.25) and it is unlikely that this difference could be clinically relevant.

Comparison between human tumours is equally difficult. Pampus [86] measured the pH in several different brain tumours and testing of his data reveals a significant difference ($2P = 0.0045$, student *t* test) between glioblastoma (6.905) and meningeoma (6.40), but only three meningeomas were measured (with a Mann-Whitney U test also significant). There were no differences between the other tumour types. Ninety-seven measurements by Wike-Hooley et al. (unpublished data) and Van den Berg et al. [119] in various human tumours reveal no significant differences between the groups. The mean tumour pH found by Pampus [86], Naeslund and Swenson [77] and Ashby [5] is much lower than found by Wike-Hooley et al. (unpublished data) and Van den Berg et al. [119]. Naeslund and Swenson [77], however, measured the pH following glucose infusion, which lowers the tumour pH. Ashby [5] used an extremely large electrode (5 mm), which must have caused considerable tissue damage and therefore very long equilibration times. Examination of the three traces that he presents shows that in two of them the pH is still rising at the point at which he administered glucose to reduce the tumour pH. The true pH of these tumours may thus have been higher. The low pH values found by Pampus [86] were determined in patients under general anaesthesia, in most cases with artificial respiration, which may have influenced the result. Regulation of the acid-base status of a patient under narcosis is, to a large extent, in the hands of the anaesthetist. Normal tissue pH is known to be a sensitive indicator of the metabolic state and may register changes long before changes in blood pH are seen [20,30,137]. However, to the best of the authors' knowledge, the effect of anaesthesia upon human tumour pH has never been investigated. From these data it is therefore not possible to demonstrate that tumour pathology has a significant effect upon the pH variation between tumours, with the possible exception of brain tumours.

The comparatively high interstitial pH found in some tumours may be the result of nutritional deprivation inhibiting lactate production due to lack of substrate [46], or to the presence in some tumours of an adequate vascularization. It is however evident that some human tumours, though not all, have a relatively low interstitial pH. The possible implications of this fact with respect to cancer therapy are discussed in the following sections.

The influence of pH on the radiation response of mammalian cells

Since the early work of Trowell [114] several reports have appeared on the influence of extracellular pH on the radiation response of mammalian cells. Trowell studied rat lymph nodes cultured in vitro. The other reports all concern cells cultured in vitro. All of the studies on the influence of pH on the radiation response of cells show a slightly decreased radiation sensitivity towards lower extracellular pH [34,52,54,56,94,95]. Both Haveman [52] and Röttinger et al. [94] report data showing that, at low extracellular pH (6.5–6.75 vs 7.3–7.5), the radiation survival curve of cells exhibits a relatively broad shoulder. Freeman et al., [33] report a slight increase in survival of CHO (Chinese Hamster Ovary) cells after irradiation during exposure to low pH (6.7 vs 7.5), but their data do not permit the conclusion that this enhanced survival is the result of a broader shoulder in the radiation survival curve at low pH.

Freeman and Sierra [35] have obtained additional data on CHO cells. These and other data from the literature are summarized in Fig. 1, which shows the α and β values derived from the actual data fitted with the equation $S/S_0 = \exp(-\alpha D - \beta D^2)$ where S/S_0 is the relative survival and D the radiation dose. The parameter α determines the initial slope of the radiation survival curve, and the fraction α/β determines the shoulder width, α being an important parameter when the survival after low dose rate irradiation or multifraction irradiation is considered. The data of both Haveman and Röttinger show that the value of α is significantly influenced by the extracellular pH both during and after the radiation treatment. In contrast to this, Freeman and Sierra [35] report that α is hardly influenced by the environmental pH. The α/β ratios shown in Fig. 1 speak for themselves: when the value of α/β is small the shoulder of the radiation survival curve is large and vice versa. Large changes in the value of α/β under the influence of pH are reported by Haveman and Röttinger et al. and not by Freeman et al.

The relatively broad shoulder in survival curves

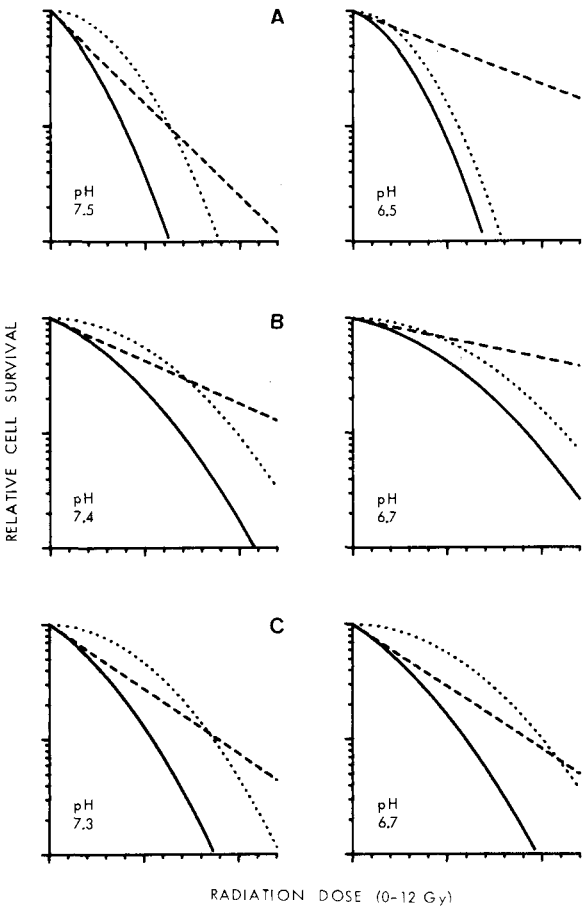

Fig. 1. The effect of reducing environmental pH upon the radiation sensitivity of three different cell lines in vitro. The curves have been drawn on the basis of the α and β parameters, the ratios of which are given below. (A) M8013 cells, α/β ratios of 1.99 and 6.36 at pH 6.5 and 7.5, resp. [52]. (B) Human glioma cells, α/β ratios of 0.89 and 17.3 at pH 6.7 and 7.4, resp. [94]. (C) CHO cells, α/β ratios of 10.87 and 8.38 at pH 6.7 and 7.3, resp. [35]. Dashed line, linear component (α); dotted line, quadratic component (β); solid line, linear-quadratic survival curve.

at low pH, reported by Haveman [52] and Röttinger et al. [94], implies that the influence of pH is more important when cells are treated with fractionated irradiation or low dose rate irradiation. It is clear from the data discussed so far that the changes in radiation sensitivity in response to changes in environmental pH may vary greatly between different cell lines. Holahan et al. [56] report that when CHO cells are kept in "acid" conditions (pH 6.7 instead of 7.4, under normal conditions) only immediately

after X-irradiation, their survival is enhanced compared to cells kept in acid conditions before or during irradiation. They conclude that enhanced survival under acidic conditions after irradiation may be primarily due to either a decrease in fixation of damage in the cells or to an increase in recovery of damage.

These observations on Chinese hamster ovary cells have led to further study by Freeman and Sierra [35]. The latter authors confirm that incubation of cells under acidic conditions during and after irradiation leads to a reduced degree of fixation of potentially lethal lesions. An important observation by the same authors is moreover that acid conditions modify survival of exponentially growing cells only, and not of unfed plateau phase cells. Mendonca and Alpen [71] tried to correlate the radioresistance observed at low pH with pH-induced cell cycle effects, and their data seem to indicate some correlation. Further studies, however, are required.

All of the above mentioned studies concern cells under well oxygenated conditions only, and this may mean that the results cannot be used to predict the influence of pH on the radiation sensitivity of cells in vivo. A low pH is often observed in solid tumours concomitant with poor nutritional conditions and relative hypoxia, all the result of poor vascularization. Hypoxia in particular may lead to altered responses at low pH. The ^{31}P-NMR study of Gillies et al. [43] clearly showed that well oxygenated, respiring Ehrlich ascites cells were able to maintain a pH gradient across the plasma membrane, particularly at extracellular pH values below 7.2. These data and those of Gonzalez-Mendez et al. [45] confirm earlier results by Spencer and Lehninger [103]. The pH gradient was observed to collapse immediately upon onset of hypoxia. It may be that cells in tumours where low extracellular pH values have been observed are not able to maintain an internal pH of around pH 7.3, due to hypoxia and possibly also a result of a lack of cellular energy. Indeed, the recent in vivo ^{31}P-NMR study of Evanochko et al. [29] showed that levels of high-energy phosphate in experimental tumours are low, together with a decreased apparent pH and an increased level of inorganic phosphate. Haveman [52] studied the influence of the drug CCP (Carbonyl-cyanide-3-chlorophenylhydrazone) during irradiation of cells at low pH. This drug has proton-conducting properties, and pH gradients across the cellular plasma membrane collapse in the presence of this drug. CCP is, moreover, an uncoupler of oxidative phosphorylation, and the presence of this drug in the cellular medium leads to deprivation of available cellular energy. The presence of CCP at low pH might, to some extent, mimic the situation of cells at low pH under hypoxic conditions: in both cases pools of cellular energy will be depleted and intracellular and extracellular pH will equilibrate. The results of Haveman [52] show that irradiation of cells at low pH, and in the presence of CCP, leads to an increase in radiosensitivity. Based on these results we may speculate that hypoxic cells will be sensitized to radiotherapy by a low environmental pH. Hypoxia will of course, on the other hand, lead to radioresistance which will mask the influence of pH.

Radiation combined with hyperthermia

The radiation response of cells may be enhanced by hyperthermia [8,135]. In vitro experiments with Hela cells [69] have shown that thermal enhancement of the cell killing effect of 7 MeV electrons is only slightly influenced by changing the pH of the cellular medium from 7.4 to 6.7. Changing the pH of the cellular medium has, however, a large influence on the effectiveness of heat treatment of cells. Heat damage responsible for enhancement of radiation effects is thus distinguishable from damage which leads to cell killing after heat treatment alone. Another in vitro study, using Madcap 37 cells [33], showed that modification of the effect of X-irradiation by heat was increased in cells in an acidic environment (pH 6.5 or 6.7 vs 7.5). Moreover, when the heat treatment preceded irradiation the duration of the sensitization appeared to be prolonged at low pH. Studying murine mammary carcinoma (M8013) cells, Haveman [54] also showed that thermal radiosensitization was influenced by pH. The influence of pH in combined heat-irradiation treatment could be explained by neither the

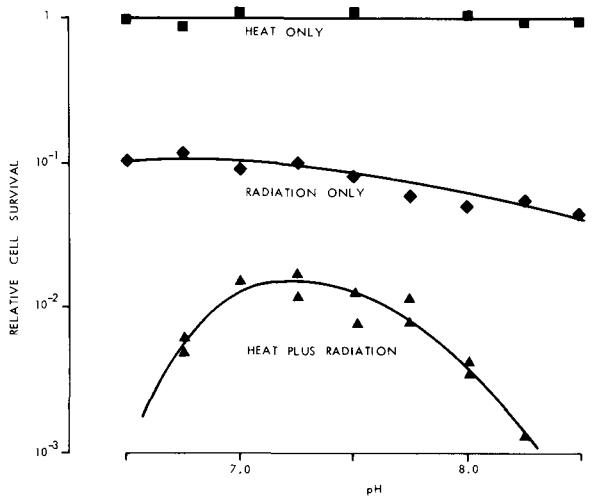

Fig. 2. Influence of environmental pH upon the effectiveness of irradiation and heat treatment of murine M8013 cells. The pH of the cellular medium was maintained by "zwitterion" buffers in Hank's salts solution (HSS). The pH was adjusted prior to sterilization of the medium. Data redrawn from Haveman [54]. ■, effect of 30 min at 42°C in buffered HSS; ◆, effect of 30 min at 37°C and 5.8 Gy in buffered HSS (the medium was changed for normal culture medium immediately after irradiation); ▲, effect of 30 min at 42°C and 5.8 Gy (given half-way during heating) in buffered HSS.

observed influence of pH on the effects of radiation alone, nor of heat alone. Thermal enhancement was relatively strong below pH 7.0 and above 7.75 (see Fig. 2). In common with studies on the influence of pH on the effectiveness of radiation alone, the studies on thermal radiosensitization were also conducted on well oxygenated cells in vitro only. The results may thus be of limited value in predicting the response of cells in a tumour at low pH.

Influence of pH on the cytotoxicity of hypoxic cell radiosensitizers

The radiation sensitizer, misonidazole, and also other, related, electron-affinic compounds have been shown to display a differential cytotoxicity towards hypoxic cells relative to oxygenated cells [108,109]. The hypoxic cell toxicity is related to the electron affinity, the more electron-affinic drug being more cytotoxic [1]. A distinction should, of course, be made between the cytotoxic and radiosensitizing properties of these drugs. The toxicity of electron-affinic compounds can be enhanced by both hyperthermia and low pH. This has been described by Rajaratnam et al. [89]. These workers studied the effects of low pH and temperature upon metronidazole, misonidazole and nitrofurantoin. Lowering the extracellular pH led to enhanced hypoxic cell toxicity with all three compounds. Moreover, at low pH, drug cytotoxicity was enhanced by temperature in all instances. The concentration-time dependence of hypoxic cytotoxicity implies that the critical reactions involved have appreciable activation energies [89]. This is in sharp contrast to the extremely rapid free radical reactions involved in radiosensitization.

The influence of pH observed on the cytotoxicity of radiation sensitizers may have clinical implications. Radiation sensitizers are now being tested in clinical trials with regard to their possible beneficial effect in the radiotherapeutic treatment of tumours with large hypoxic cell fractions.

The importance of pH to hyperthermia therapy

Interest in the influence of tissue pH upon the response of tumour tissue to hyperthermia stems from the origination of two different concepts. Initially, Von Ardenne [126,130] proposed that hyperthermia should be accompanied by acidification of the tumour tissue, achieved by the high-dose intravenous infusion of glucose. Later, once it had become evident that hyperthermia could be employed as a treatment modality, the question arose as to whether the oxygen effect, well known in radiotherapy, was also of importance to hyperthermia-induced cell killing. The latter idea led to several in vitro investigations, such as those by Gerweck, indicating that hypoxia per se was relatively unimportant [37,39] but that the accompanying tissue acidity sensitized the (tumour) cells to hyperthermia [38,82]. As measured by clonogenic assay techniques, there is presently no doubt that a low pH sensitizes cells to hyperthermic treatment, although the mechanisms involved in pH dependent

sensitization are not clear. Possible targets may be the plasma membrane, intracellular proteins, the energy-dependent processes that maintain the intracellular pH, and the lysosomes. The data supporting these various possibilities have been reviewed in a recent book by Hahn [48]. Von Ardenne and Overgaard both proposed that lysosomal damage was instrumental in the hyperthermic insult to cells [83,126]. Subsequently, Overgaard and Poulsen [85] demonstrated that, after treatment at pH 6.4 and 42.5°C for prolonged periods of time (4 to 12 h), proteolysis was increased compared to that at pH 7.2 and 37°C. Similar observations were made by Keech and Wills [63], who found some increase in lysosomal activity at 42.0°C and pH 6.6, compared to values at 37.0°C and pH 7.2. The effect was much stronger at lower pH values, but this is probably not relevant for "in vivo" situations. On the other hand, Haveman [53] demonstrated that the acridine-orange staining of lysosomes, which may be regarded as being related to intra-lysosomal pH, was destroyed by hyperthermia. He concluded that, although a lysosome effect is an important and early event in cellular injury, it is not directly caused by hyperthermia, but activated by other hyperthermia-induced cellular damage.

Intracellular pH reduction

Some of the aforementioned hypotheses on the heat-induced destruction of tumour cells were based on the concept that the intracellular pH decreases as a result of treatment, leading to cell death. Well oxygenated cells have, however, a remarkable ability to maintain their internal pH when the external pH is modified within the physiological range, as has been demonstrated in a number of recent studies using the ^{31}P-NMR technique [43,45]. On the other hand, Hofer and Mivechi [55] demonstrated that once the intracellular pH was decreased the cell viability, estimated from a ^{125}I-URD incorporation assay, decreased strongly after hyperthermic treatment. A possible explanation for interaction between pH and heat has been given by Haveman [51] on the basis of experiments with a proton-conducting drug at low pH. This drug strongly enhanced the effect of hyperthermia at low pH. These experiments clearly indicate that when the intracellular pH is lowered the thermal sensitivity of cells is enhanced.

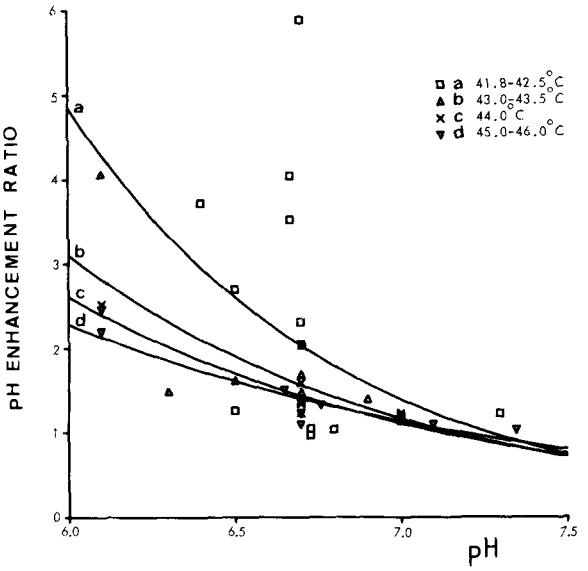

Fig. 3. The enhancement of hyperthermic cell kill by low pH is shown as a function of pH over four temperature ranges. The enhancement ratios plotted are either those given by the authors [41,42,116], or are calculated from the ratio of the D37 values at normal and low pH [21,31,33,37,38,51,68,69,73,79,84].

Enhancement of hyperthermic effect by low pH

With respect to hyperthermic cell survival studies, the effect of pH is usually expressed as a "pH enhancement ratio". This can be defined as the factor by which a heat treatment can be reduced to achieve an iso-effect at two different medium pH values. This factor is frequently expressed as the ratio of the slopes of the "final D_0" values, but some authors present ratios derived from a single iso-effect level, e.g., the 1% level. The pH enhancement factor found by Freeman et al. [31] for CHO cells was found to be dependent upon the pH of the medium. As the pH of the medium was decreased from 7.4 to 6.6, it gradually increased to a factor of 1.5. The exposure time may also be of importance, as Freeman et al. [33] showed that the pH-increased cytotoxicity was most pronounced with exposures ex-

ceeding 100 min, at moderate temperatures. Similar findings were obtained by Gerweck and Richards [41] who investigated the "pH enhancement ratio", expressed as the ratio between the D_0 at pH 7.4 and the D_0 at pH 6.7. They observed a difference between two cell lines, i.e. CHO cells and glioblastoma cells, the latter being more resistant. From their investigations it also became apparent that the "pH effect" increased with *decreasing* temperature. In other words, the enhancing effect of low pH seems to be more pronounced at moderate treatment temperatures. This trend is shown as a function of environmental pH in Fig. 3. The aforementioned observations can be summarized as a sensitizing effect of low environmental pH that becomes important at values below pH 7, and that increases as the pH is lowered.

Thermotolerance

The development of "thermotolerance", or rather the observation that cells and tissues can become more resistant to heat after a priming treatment or during longer exposure to relatively low hyperthermic temperatures, is presently a subject that is at issue for many investigators. If one assumes that such an induced thermal resistance, as seen in vitro, also develops in human tumours, the question arises as to whether the rate of development is lower in tumours with a low interstitial pH. As a result of their experiments with L1A2 cells Nielsen and Overgaard [79] concluded that the development of thermal resistance, expressed as the Thermal Tolerance Ratio (TTR) – calculated from the ratio of the D_0 values – decreased from a value of 4.1 to 2.4 at pH 6.5, with a smaller decrease at pH 6.8. Similar observations were made by Goldin and Leeper [44] for CHO cells. Gerweck et al. [40,42] showed that the pH effect upon thermotolerance (pH 6.7 vs. 7.4) was greatest at moderate temperatures. Moreover, this effect was more pronounced with fractionated hyperthermia treatment than with a single heat dose. With split-dose treatments at 42°C the pH enhancement ratio derived was a factor of 6. The conclusion, therefore, seems justified that a low interstitial pH in tumours will not only enhance hyperthermic cytotoxicity to tumour cells, especially at moderate temperatures, but will, in addition, help to inhibit the development of thermal tolerance, provided that the interval between heat treatments is sufficiently long for thermotolerance to decay between treatments [40]. These data indicate that (fractionated) hyperthermic treatment may be of additional benefit in eliminating those tumour cells in areas with a low environmental pH.

The effect of pH upon chemotherapy

The question as to whether tumour pH plays a role in determining the effectiveness of chemotherapeutic agents is particularly interesting in view of the notorious unpredictability of individual tumour response to therapy. As most drugs act intracellularly the concentration attained within the cell may limit the effectiveness of some drugs. The pH of the milieu is one of the factors that determines the intracellular drug levels that can be achieved. Transport of antineoplastic agents into the cell is achieved by one of two mechanisms; by active transport via specific carriers, or by passive diffusion through the cell membrane [74]. Both processes may be influenced by changes in the membrane. Hydrogen ion concentration can affect membrane fluidity by altering the transition temperature of polar lipid components of the membrane. This may affect active drug uptake as a result of changes in the kinetics of many transport processes and membrane bound enzymes. Passive diffusion of drugs may be affected by altered membrane permeability. These matters are discussed in detail in a recent book by Hahn [48]. In addition to this, the distribution of a passively diffusing drug on either side of a membrane may be affected by the pH gradient across the membrane [74]. Some drugs dissociate to an extent that varies with the hydrogen ion concentration [93]. The pH at which 50% dissociation occurs is known as the p*K*a of the compound. It has been demonstrated that drugs in the non-ionized form diffuse more easily through the cell membrane [15]. Theoretically, therefore, the concentration that can be obtained in a cell depends upon the pH in the cell.

Ross [92] calculated that for a pH difference of one unit, eight times the concentration of a drug with a p*K*a of 8 can be achieved in malignant tissues as opposed to normal tissues. Further reduction of the tumour pH would enhance this effect.

The discovery that glucose administration can lower tumour pH was the basis of much of the earlier work on selective chemotherapy [19,92, 106,107]. These investigators found that various agents were more effective when used in combination with glucose, although the tumour pH was not measured in these experiments. Care should be taken in interpreting these findings, however. Although glucose administration lowers the extracellular pH, the intracellular pH may be unaffected or may even rise [23,57,88,98]. In addition, glucose itself may affect drug action. As early as 1960 it was demonstrated, by Woods and his colleagues, that 5FU was not metabolized when glucose was not present in the cellular medium [138]. The glucose-mediated potentiation of this drug that they observed in vivo may thus have been due to the presence of glucose rather than to a reduction in tumour pH. A discussion on the effects of glucose upon 5FU uptake and action is presented by Hult and Larson [57]. A pH effect cannot, however, be ruled out, indeed an optimum of pH 6.8 has been found for the action of 5FU upon cultured human mesothelioma cells [80]. Other drugs that have been shown to be more effective at low pH are *N*-oxide mustard [126] and Thio-tepa [28], both upon Ehrlich mouse ascites cells. Enhanced uptake of chlorambucil in transformed hamster cells at low pH has also been demonstrated [74].

A different approach to pH-selective therapy is the use of agents which become active at low pH, as a result of either selective activation or pH-dependent release. In 1969 Stevens and Mosteller [107] reported glucose enhancement of the antitumour action of tetraazatricyclododecane, a compound with a pH-dependent decomposition rate. It hydrolyzes to form formaldehyde and ethylene diamine, a process that occurs more rapidly at low pH. A second possibility was suggested by Bicker [13], who proposed the administration of inactive glucuronides of active drugs. He showed that such compounds could be activated by glucose-mediated enhancement of tumour glucuronidase activity. Using this technique he demonstrated tumour selective uptake of 8-hydroxy quinoline (a disinfectant) and 2-napthol. The effect was attributed to glucose-induced tumour pH reduction activating the lysosomal enzyme, β-glucuronidase. This technique has been used successfully in the treatment of tumour-bearing mice [6]. Although the glucuronide of 5FU was innocuous to normal mice, it effectively retarded tumour growth. This effect was enhanced by prior intraperitoneal injection of glucose. More recently, Yatvin et al. [139] proposed the use of pH-sensitive liposomes to deliver cytostatic drugs selectively to malignant tissues. Liposomes were so constructed that, in regions of low pH, the pH-sensitive lipid incorporated into the vesicle wall underwent a structural change, thus releasing the encapsulated drug.

Low tumour pH may form an incentive to avoid the use of certain drugs. As mentioned earlier, the degree of dissociation of alkylating agents depends upon the pH and p*K*a of the drug. Generally speaking, the lower the pH, the greater the proportion of drug that will be present in the undissociated form. Some drugs, such as basic aliphatic nitrogen mustards, are less reactive in the undissociated form [93], but unacceptable toxicity to normal tissues prevents the use of higher drug dosages. In this case Ross suggested the possibility of pH-dependent protection of normal tissues using readily alkylated sulphydryl compounds (thiols). According to this theory these would be present mainly in the active, ionized form in normal tissues, and mainly in the undissociated form in regions at lower pH. Alkylating agents would thus be selectively deactivated in normal tissues. To the authors' knowledge no further work has been published in this field with respect to chemotherapy. Both Adriamycin and Bleomycin have also been shown to be less effective at lower pH. Born and Eicholtz-Wirth [14] have demonstrated that low environmental pH protects cells against Adriamycin, an effect that is more pronounced in well oxygenated than in chronically hypoxic cells. Although they measured decreased uptake of Adriamycin in both hypoxic cells and those

at lower pH, the difference was insufficient to explain the difference in cytotoxicity. This is perhaps not surprising in view of recent work showing that Adriamycin can be actively cytotoxic without entering the cell, and can thus exert its effect solely by interaction with the cell surface [113]. A similar protective effect at low pH has been observed against both Adriamycin and Bleomycin by Röttinger (unpublished data) in cultured human glial cells. He also found the action of *cis*-Platinum to be pH independent. Evidence for a pH effect upon the action of cyclophosphamide is inconclusive. Early work indicated that pH had a marked influence upon the reaction speed of cyclophosphamide, the alkylating derivatives being formed more slowly under acid conditions [90]. A recent investigation [117] found no enhancement of the cytotoxic effect of cyclophosphamide in vivo following glucose administration, but when it was combined with hyperthermia a substantial enhancement was seen following glucose administration. Only mild normal tissue damage was observed. The authors attribute the improved effect largely to glucose-induced tumour pH reduction, although no pH determinations were performed.

In addition to the direct pH effects on drug uptake and action that have already been discussed, certain pH-related biological effects may also modify drug action. As mentioned earlier, cell growth is influenced by environmental pH. Sensitivity to cytotoxic agents has been shown to be affected by the growth rate of the target cells, slowly growing cells being more resistant [100,118]. As a result of changes in growth rate at lower pH, a large percentage of cells are found in the G1 phase of the cell cycle [71]. The relevance of cell cycle distribution to the effectiveness of antineoplastic agents is well known [10,70].

It is thus evident that tumour pH may have a role in determining response to chemotherapeutic treatment. However, the relevance of these findings to multi-drug combination therapy still remains to be elucidated.

Can the pH of tumours be modified?

The effect of the administration of a variety of agents has been investigated in attempts to modify tumour pH. These can basically be divided into four categories:

(i) acidic compounds;
(ii) sugars;
(iii) other metabolically active compounds;
(iv) various treatment modalities.

Acidic compounds

A limited number of acidic compounds have been administered systemically to experimental animals in attempts to induce either metabolic or selective tumour acidification. The earliest work in which such compounds were administered with concomitant measurement of tumour pH changes was that of Gullino et al. [47]. Using micropore chambers embedded in the tissues of both normal and tumour bearing rats, these workers were able to collect fluid samples from both tumours and normal subcutaneous tissues. They established that selective tumour acidification was possible by either of two techniques. Increasing the carbon dioxide content of inhaled air led to a decrease in tumour interstitial fluid of 0.33 pH units. The corresponding decrease in normal tissue fluid was 0.2 units. A more selective decrease was obtained by administering sodium bicarbonate in the drinking water. Tumour interstitial fluid fell by 0.27 pH units, whilst the fall in normal tissue fluid was minimal. Force feeding with ammonium chloride also caused a pH reduction, but this was of the same order of magnitude in both normal and malignant tissues. The administration of ammonium chloride, particularly when combined with a low calcium diet, has been shown to inhibit the growth of tumours in mice [4]. However, no tissue pH determinations were performed in this study, and considerable reductions in animal body weight were also noted. Similar experiments have

also shown tumour growth inhibition without significant body weight loss following systemic acidification with hydrochloric acid [49]. Once again, no pH determinations were performed and it cannot therefore be determined whether selective tumour pH reduction occurred in these investigations.

Recently, Jähde and Rajewsky [58] attempted to reproduce the effect of sodium bicarbonate reported by Gullino and co-workers. Following administration in the drinking water at the same dosage as given by Gullino's group, they observed no reduction in tumour pH. Their determinations were however performed after only 3 days of treatment, whereas those of Gullino et al. were 10 days after start of treatment, although the latter group did state that changes generally occurred on the third day of treatment.

The above mentioned studies have all been concerned with changes in the extracellular pH. In vitro work indicates, however, that changes in the extracellular environment brought about by acidic compounds, in this case carbon dioxide or lactic acid, can modify the intracellular pH [2]. The relationship between the intra- and extracellular pH for various concentrations of CO_2 and lactate was described by multiple linear regression equations, although the authors conclude that these findings may not be applicable to the in vivo situation.

Sugars

It has been demonstrated by a great number of workers that the administration of glucose can lower the interstitial pH of tumours [5,23,27,61,62, 91,110,125–128,131–133]. A reduction was seen in all but one case, an ascitic hepatoma, as determined by Tagashira et al. [110]. Various doses of glucose (0.05 to approx. 40 g/kg) and administration routes (i.p., i.v. or s.c. injection) have been used. The pH reduction observed varied from 0.02 pH units with very low-dose glucose [62] to 0.8 pH units with high doses [58]. The minimum tumour pH values found following glucose injection vary, falling in the range of 5.50–6.46 for rats, 6.15–6.22 for mice, and 6.29–6.55 for humans.

Until recently the pH drop seen following glucose loading was thought to be a result of the stimulation of glycolysis. Gullino et al. [46] showed that glucose levels in tumours were very low, but could be raised by glucose infusion. The increase in available energy substrate was thought to enable cells to metabolize more rapidly, thus producing larger quantities of lactic acid. Indeed, higher levels of lactic acid have been measured in tumours following glucose injection [23,61,125]. The observation that administration of non-metabolizable sugars, such as galactose, did not result in pH changes was further evidence for this hypothesis [125]. However, as long ago as 1951 it had been shown that glucose caused a reduction in tumour blood flow [3]. To test whether this might be a mechanism for pH reduction, Eden et al. [27] injected lymphosarcoma bearing rats with a compound that was thought to selectively reduce tumour blood flow (podophyllotoxin), and found no change in pH values. It was also reported by von Ardenne [130] that glucose caused a reduction in blood flow. He attributed this effect to glucose-induced pH reduction resulting in stiffening of the erythrocyte membrane (as observed by Schmid-Schonbein, [99]), the resulting erythrocyte aggregation leading to blocked capillaries thus reducing blood flow. He thus believed that blood flow changes were secondary to pH changes. Earlier work had demonstrated, however, in rats bearing lymphosarcoma, that injection of the non-metabolized sugar galactose could indeed cause a drop in tumour pH [62], in contrast to the work of Voegtlin's group. The authors supposed that this indicated a differential ability of some tumours to metabolize certain sugars.

Only recently has this question been resolved. In a series of reports beginning in 1979, Dickson and Calderwood have shown that glucose injection reduces the extracellular pH of the Yoshida sarcoma, associated with an accumulation of lactate. This is accompanied by a decrease in both glycolysis and tumour blood flow. However, the pH change occurs more slowly than and *after* the change in blood flow. It is proposed that a change in blood viscosity, as a direct result of glucose, initiates a vicious circle in which progressive congestion of tumour capillaries occurs, resulting in vascular stasis. This leads

to reduced clearance of metabolites and a pH drop that is secondary to the decrease in blood flow. To test this hypothesis further they substituted galactose for glucose, and found vascular stasis without tumour pH reduction. They concluded that blood flow changes and pH changes were independent. Glucose reduced the blood flow, and led to a decrease in glycolysis. As a result of reduced perfusion and inhibited lactate removal, the pH falls.

These conclusions are not at variance with the previously mentioned work by Eden and co-workers and Kahler and Moore [27,62]. The injection of either podophyllotoxin or galactose may have reduced tumour blood flow, but in the presence of very low levels of glucose the metabolic rate may have been too low for this to cause a pH drop in the first case, but sufficient for the reduced blood flow to lead to a build up of metabolic products in the second.

Other metabolically active agents

In a series of reports beginning in 1965, von Ardenne has propounded his conceptual "Krebs Mehrschritt-Therapie" or Cancer Multistep therapy. The basis of this approach is the carefully timed application of a combination of agents. One of the important steps in the proposed schedule is the attainment of "optimized tumour acidification" which in turn causes a decrease in the tumour microcirculation, supposedly enabling hyperthermia to be administered effectively at moderate temperatures. Although von Ardenne achieves tumour acidification using high-dose glucose infusion, he has combined this with a variety of agents in attempts to further lower the tumour pH. Using amygdalin and β-glucosidase he obtained a pH decrease of 0.97 pH units beyond the decrease achieved with glucose alone, the total decrease being of up to 1.6 pH units. The values in healthy tissue remained unchanged [127]. These authors have also reported improved acidification following the administration of certain anti-hypertensive drugs such as sodium nitroprusside or NAD [128,133].

Injection of insulin has been shown to induce pH changes in rat tumours, once more over and above those induced by glucose alone [91]. This is presumably a result of the effect of insulin upon glycolysis.

Finally, intracellular acidification has been achieved in tumour slices by the presence of oxamate in the incubation medium [57]. This compound inhibits the enzyme lactic dehydrogenase, thus causing a build up of acidic glycolytic intermediates.

Treatment modalities

The treatment modality upon which the greatest amount of work has been performed with respect to pH changes in tumours is hyperthermia (see Table III). Several workers have determined tumour pH either during or immediately following hyperthermia treatment. In most cases a fall was seen [12,101,121,122,131,132]. The authors account for these changes by variations in blood flow and tissue oxygenation during treatment, and that such variations occur has also been demonstrated [12,122]. Two groups have, however, been unable to demonstrate pH changes as a result of hyperthermia [23,136]. It has been suggested that this could be a question of temperature [122]. Indeed, the groups reporting pH drops all used treatment temperatures above 42°C. Of the groups that saw no change, Wike-Hooley and co-workers gave a treatment of 2 h at 41.8°C (whole body hyperthermia), and Dickson and Calderwood subjected Yoshida sarcoma of rats to 42°C × 60 min. Interestingly, a different group [122] heated the latter tumour to 44°C for 60 min and observed a modest fall in pH (−0.24 pH units), which lends support to the theory that treatment temperature may play a role. In one case, however, tumour pH reduction has been reported during treatment at temperatures under 40°C [12]. Scrutinization reveals that the tumour temperature in the example given never exceeded 34°C, and this cannot therefore be considered to be an effect induced by hyperthermia. That there should be a temperature "threshold" effect is not surprising in view of the work performed on the effects of hyperthermia upon blood flow and tissue oxygenation. It has been demonstrated that as tum-

TABLE III

Changes in tumour pH induced by hyperthermia.

Tumour	Species	pH determination (HT)	Temp. °C	HT duration (min)	pH decrease (mean)	*n*	Ref.
DS carcinoma	Rat	During	42–43	?	−0.41	12	131
DS carcinoma	Rat	During	42–43	130–195	−0.47	12	132
Yoshida sarcoma	Rat	After	42.0	60	0	5 or 6	23
C3H mammary carcinoma	Mouse	After	43.0	60	−0.55	96/108	12
Walker 256 carcinoma	Rat	During	43.0	60	−0.16[a]	1	101
Walker 256 carcinoma	Rat	During	46.0	60	−0.38[a]	1	101
SCK mammary carcinoma	Mouse	During	43.5	30	−0.38[a]	1	101
Dunn osteosarcoma	Mouse	After	47.0	15	−0.25[a]	1	78
Dunn osteosarcoma	Mouse	After	47.0	30	−0.62[a]	1	78
C3H mammary carcinoma	Mouse	After	43.0	60	−0.54	142/136	121
Yoshida sarcoma	Rat	After	44.0	60	−0.24	440/450	122
16/C mammary carcinoma	Mouse	After	47.0	15	−0.1[a]	1	29
16/C mammary carcinoma	Mouse	After	47.0	30	−0.5[a]	1	29
Various	Man	During	41.8	120	0	11	136

[a] Where values for only one tumour were reported the maximum pH drop is given.

our temperature increases the tissue oxygen tension and blood flow also increase up to temperatures of about 41–42°C [12]. At higher temperatures these parameters are rapidly reduced. A similar effect was observed by Vaupel and co-workers [122]. No change in blood flow was seen most frequently in tumours treated at 40–42°C for short periods, whereas a progressive decrease in flow was frequently seen in tumours heated to 44°C or higher. At intermediate temperature doses a biphasic course was seen. A corresponding change in tissue oxygenation was observed. Examination of the few measurements of tumour pH performed during hyperthermic treatment reveals that heating seems to cause an initial short pH rise, presumably due to increase in blood flow and oxygenation during "warming-up" followed by a continual, gradual decrease in pH once the tissue is at the treatment temperature [101,132]. The exception to this pattern is a Walker tumour heated to 43°C in which a continual pH rise was seen during the first hour of treatment [101]. A second treatment at the same temperature did however result in a pH drop. This may be explained by the fact that Song's group found no change in blood flow in this tumour following 1 h at 43°C. In all other studies of hyperthermia-induced pH changes where pH reduction has been observed, the pH determinations have been performed prior to and immediately following treatment (see Table III), so no conclusions as to the moment at which the change occurs can be drawn.

To the best of the authors' knowledge no reports on radiation therapy-induced pH changes, and only two reports (both from the same group) concerning chemotherapeutically induced changes have appeared in the literature. In the latter case the pH determinations were performed by ^{31}P-NMR spectroscopy prior to and following treatment of tumour bearing mice with BCNU or Adriamycin [29,78]. In both cases a pH rise was observed shortly following drug administration; from 7.2 to 7.3 22 h after administration of BCNU, and from 6.8 to 7.4 27 h after treatment with Adriamycin. In both cases the values were given for only one animal. The changes in pH occurred before any change in tumour volume was seen, although the first mouse gave a complete response and the second a tumour mass reduction of about 70%.

Therapy can thus induce acute changes in tumour pH. Tumour regression with accompanying changes in tissue perfusion and oxygenation can

also, however, result in long-term changes. This has been demonstrated for combined hyperthermia and radiation therapy [136]. This study reported a mean increase of 0.23 pH units in human tumours following a series of combined treatments given over a period of up to 3 weeks. These changes were ascribed to changes in tissue perfusion and oxygenation as have already been reported following radiotherapy alone [87]. The two pronged attack with radiotherapy attacking the well oxygenated, actively metabolizing cells and hyperthermia destroying hypoxic cells should logically lead to tissue normalization with a subsequent rise in interstitial pH. Whether a similar rise in tumour pH occurs following radiotherapy alone is not yet known.

Summary and conclusions

The wide range of tumour pH values that have been determined in human tumours is shown in Fig. 4. It can be seen that tumour pH values may be very low, or may fall in the same range as the values found in normal tissues. This means that pH-mediated modification of therapeutic effectiveness will be patient specific, rather than a general phenomenon.

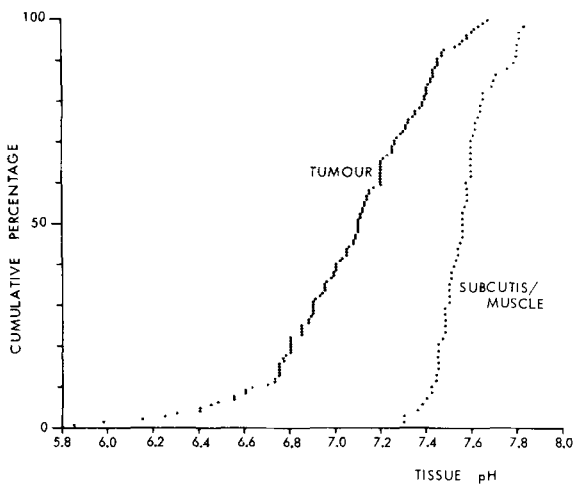

Fig. 4. A cumulative distribution of all the human tumour pH determinations reported to date (see Table I) and the corresponding values in normal subcutaneous or muscle tissue. In cases where two simultaneous determinations were performed the mean value has been plotted.

That the pH of the cellular environment might influence the effectiveness of various therapeutic agents is not a new idea. The data published in this field to date concerning such effects have been discussed extensively and are summarized in Table IV. Here we can see that low pH leads to decreased cell survival following treatment with hyperthermia, radiotherapy combined with hyperthermia, radiosensitizers and various chemotherapeutic agents. Conversely, low pH affords some protection against radiation and some drugs. Most of these data were, of necessity, derived from in vitro studies. In vivo studies are in most cases not feasible due to the difficulty of isolating the effect of one selected factor. Low tumour pH is, in vivo, generally assumed to be closely interlinked with tissue hypoxia and low blood-flow levels, each of which may individually influence the experimental outcome. Moreover, most of the aforementioned in vitro studies were conducted under well-oxygenated conditions. As previously mentioned, euoxic cells can, under certain conditions, maintain a pH gradient over the cell membrane. This collapses with the onset of hypoxia, leading to intracellular acidification. Low oxygen levels have been shown to be characteristic of many tumours. Within these limitations it is thus evident that tumour pH values could have far-reaching consequences for therapy. If the in vitro findings should prove to be relevant to the clinical situation various applications are possible. Pre-selection of patients less likely to respond to certain (toxic) chemotherapeutic agents, or conversely selection of agents that are more likely to be effective in the pH range of the tumour to be treated are two examples. Alternatively, the exploitation of low tumour pH values is a possibility. Agents that form or release toxic derivatives in areas of low pH, e.g., pH-sensitive liposomes, will work selectively in such areas. Tumour selective therapy may also be possible in patients with higher tumour pH values if the tumour pH can be lowered. This has been achieved experimentally by the administration of hyperthermia at temperatures above 42°C, or by the administration of glucose. The latter method may, however, have further consequences, such as reduced tumour blood flow and

TABLE IV

The influence of pH reduction upon the effectiveness of various treatment modalities.

Treatment	pH[b] effect	Para-meter	Cell line	Derivation	O_2 status[c]	Serum present	Refs.
Radiotherapy	–	pH e	Lymph nodes	Rat	E	+	114
	–	pH e	M 8013S	Mouse mammary carcinoma	E	–	52, 54
	+	pH i	M 8013S	Mouse mammary carcinoma	EH	+	52
	–[a]	pH e	Glial	Human astrocytoma	E	+	94
	–	pH e	Glial	Human astrocytoma	E	+	95
	0	pH i&e	BP8 ascites	Mouse sarcoma	E	+	75
	0	pH i&e	BP8 ascites	Mouse sarcoma	AH	+	75
	–	pH e	CHO	Mouse sarcoma	E	+	34
	–	pH e	CHO	Mouse sarcoma	E	+	56
Hyperthermia	+	pH e	LIA2 ascites	Mouse lung	E	–	83
	0	pH i	SDB	Rat mammary carcinoma	E	–	22
	+	pH e	CHO	Chinese hamster	E	+	38
	+	pH e	CHO	Chinese hamster	AH	+	39
	+	pH e	CHO	Chinese hamster	CH	+	39
	+	pH e	CHO	Chinese hamster	E	+	40, 42
	+	pH e	Glioblastoma	Human	E	+	41
	+	pH e	PNJ ascites	Mouse mammary carcinoma	E	+	11
	+	pH e	CHO	Chinese hamster	E	+	31
	+	pH e	CHO	Chinese hamster	E	+	32
	+	pH e	CHO	Chinese hamster	E	+	34
	+	pH e	Madcap 37	Mouse mammary carcinoma	E	+	73
	+	pH e	M 8013S	Mouse mammary carcinoma	E	–	51
	+	pH i	M 8013S	Mouse mammary carcinoma	EH	–	51
	0	pH e	CHO HAI	Chinese hamster	E	–	68
	+	pH e	CHO HAI	Chinese hamster	E	+	68
	+(?)	pH e	BP8 ascites	Murine sarcoma	E	–	55
	+	pH i	BP8 ascites	Murine sarcoma	E	–	55
	+	pH e	Fibrosarcoma	Mouse	E	+	116
	+	pH i&e	BP8 ascites	Murine sarcoma	E	+	75
	+	pH i&e	BP8 ascites	Murine sarcoma	AH	+	75
	+	pH e	Glial	Human astrocytoma	E	+	95
	+	pH e	M 8013S	Mouse mammary carcinoma	E	–	54
Radiotherapy and hyperthermia	+	pH e	Hela cells	Human cervix carcinoma	E	+	69
	+	pH e	Madcap 37	Mouse mammary carcinoma	E	+	33
	+	pH e	CHO	Chinese hamster	E	+	34
	+	pH i&e	BP8 ascites	Mouse sarcoma	E	+	75
	+	pH i&e	BP8 ascites	Mouse sarcoma	AH	+	75
	+	pH e	M 8013S	Mouse mammary carcinoma	E	–	54
Chemotherapy							
Aromatic nitrogen mustards							
basic methylam.	+	g.a.[c]	Walker carcinoma	Rat	IV		92
melphalan ester	+	g.a.[c]	Walker carcinoma	Rat	IV	92	

TABLE IV *(continued)*

The influence of pH reduction upon the effectiveness of various treatment modalities.

Treatment	pH[b] effect	Parameter	Cell line	Derivation	O_2 status[c]	Serum present	Refs.
neutral (CB1074)	0	g.a.[c]	Walker carcinoma	Rat	IV		92
zwitterionic, melphalan	0	g.a.[c]	Walker carcinoma	Rat	IV		92
acidic, chlorambucil	–	g.a.[c]	Walker carcinoma	Rat	IV		92
Chlorambucil	+	pH e	GD 248 lymphoid V79	Chinese hamster	E	–	74
5FU	+	g.a.[c]	Flexner-Jobling ca.	Rat	IV		65
5FU	0	pH i	Walker 256 (slices)	Rat	E	–	57
5FU	+	pH e	Mesothelioma	Human	E	+	80
Triethylene melamine	+	g.a.[c]	Walker carcinoma	Rat	IV		19
Aromatic nitrogen mustards (CB 3039)	+	g.a.[c]	Walker carcinoma	Rat	IV		19
Others	0	g.a.[c]	Walker carcinoma	Rat	IV		19
Nor-HN2	0	g.a.[c]	Walker carcinoma	Rat	IV		19
Diepoxides	0	g.a.[c]	Walker carcinoma	Rat	IV		19
Azo-compound	0	g.a.[c]	Walker carcinoma	Rat	IV		19
Cyclophosphamide	0	g.a.[c]	Fibrosarcoma	Mouse	IV		117
Tetraazatricyclododecane	+	g.a.[c]	Ehrlich ascites	Mouse	IV		107
N-oxide mustard, Mitomen	+	pH e	Ehrlich ascites	Mouse	H	?	126
Thio-tepa	+	pH e	Ehrlich ascites	Mouse	E	–	28
Adriamycin	–	pH e	B14FAF28	Chinese hamster	E	+	14
Adriamycin	–	pH e	B14FAF28	Chinese hamster	CH	+	14
Adriamycin	–	pH e	Glial	Human	E	+	[d]
Bleomycin	–	pH e	Glial	Human	E	+	[d]
Cis-Platinum	0	pH e	Glial	Human	E	+	[d]
Radiosensitizers							
Metronidazole	+	pH e	V79-379A	Chinese hamster	H	+	89
Nitrofurantoin	+	pH e	V79-379A	Chinese hamster	H	+	89
Misonidazole	+	pH e	V79-379A	Chinese hamster	H	+	89

[a] Low dose rate.

[b] –, Protection, increased survival at low pH; 0, no effect; +, sensitization, decreased survival at low pH.

[c] Glucose administered i.p. or i.v. to reduce tumour pH, minimum dose 5 g/kg.

[d] Röttinger, unpublished data.

[e] E, euoxic; H, hypoxic; AH, acutely hypoxic; CH, chronically hypoxic; EH, equivalent to hypoxic conditions due to the addition of CCP (carbonylcyanide-3-chloro-phenylhydrazone), an uncoupler of oxidative phosphorylation; IV, in vivo.

hence impaired drug delivery to the tumour.

Any of the above mentioned approaches to therapy requires an idea of the acidity of the tumour that is to be treated. This necessitates individual assessment of tumour pH. At the moment very few pH electrodes suitable for clinical use are commercially available. Although the Philips C902S tissue pH electrode has proven to be sufficiently robust

for clinical use, its comparatively large size (tip diameter 2 mm) renders repeated determinations unfeasible, due to the long stabilization times that are required. However, as it has been shown that therapy can affect tumour pH, repeated determinations may be necessary when different therapies are tried. Non-invasive determination by ^{31}P-NMR spectroscopy would be an ideal solution, if this can be adapted for clinical use. It is to be hoped that a suitable technique will be developed in the near future, so that tumour pH determination can become a simple, routine procedure in the assessment of malignant disease.

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References

1 Adams, G. E., Stratford, I. J., Wallace, R. G., Wardman, P. and Watts, M. E. Toxicity of nitro compounds towards hypoxic mammalian cells in vitro. Dependence on reduction potential. J. Natl. Cancer Inst. 64: 555, 1980.

2 Albers, C., van den Kerkhoff, W., Vaupel, P. and Muller-Klieser, W. Effect of CO2 and lactic acid on intracellular pH of ascites tumor cells. Respir. Physiol. 45: 273–285, 1981.

3 Algire, G. H. and Legallais, F. Y. Vascular reactions of normal and malignant tissues in vivo. IV. The effect of peripheral hypotension on transplanted tumors. J. Natl. Cancer Inst. 12: 399–408, 1952.

4 Anghileri, L. J. Tumor growth inhibition by ammonium chloride-induced acidosis. Int. J. Clin. Pharmacol. 12: 320–326, 1975.

5 Ashby, B. S. pH studies in human malignant tumours. Lancet ii: 312–315, 1966.

6 Baba, T., Kidera, Y., Kimura, N. T., Aoki, K., Kamura, T., Taniguchi, S. and Nishikawa, K. 5 Fluorouracil *O*-β-D-Glucuronide as a newly synthesized chemically modified, non toxic anticancer drug. Gann 69: 283–284, 1978.

7 Bear, M. P. and Schneider, F. H. The effect of medium pH on rate of growth, neurite formation and acetylcholinesterase activity in mouse neuroblastoma cells in culture. J. Cell. Physiol. 91: 63–68, 1977.

8 Belli, J. A. and Bonte, F. J. Influence of temperature on the radiation response of mammalian cells in tissue culture. Radiat. Res. 18: 272–276, 1963.

9 Bhat, R., Braverman, B., Raju, T. N. K., Justice, P. and Vidyasagar, D. Changes in tissue (tpH) and blood lactate during hypoxia and recovery in puppies. Pediat. Res. 14: 591, 1980.

10 Bhuyan, B. K. Cell cycle-related cellular lethality. In: Growth Kinetics and Biochemical Regulation of Normal and Malignant Cells, pp. 373–375. Editors: B. Drewin K.O. and R. M. Humphrey, Williams and Wilkins. Baltimore, MD, 1977.

11 Bichel, P. and Overgaard, J. Hyperthermic effect on exponential and plateau ascites tumor cells in vitro dependent on environmental pH. Radiat. Res. 70: 449–454, 1977.

12 Bicher, H. I., Hetzel, F. W., Sandhu, T. S., Frinak, S., Vaupel, P., O'Hara, M. D. and O'Brian, T. Effects of hyperthermia on normal and tumour microenvironment. Radiology 137: 523–530, 1980.

13 Bicker, U. Application of β-D-glucuronides and glucose together suggests a new direction for cancer chemotherapy. Nature 252: 726–727, 1974.

14 Born, R. and Eicholtz-Wirth, H. Effect of different physiological conditions on the action of Adriamycin on Chinese hamster cells in vitro. Br. J. Cancer 44: 241–246, 1981.

15 Brodie, B. B. and Hogben, C. A. M. Some physico-chemical factors in drug action. J. Pharm. London 9: 345–380, 1957.

16 Buelke-Sam, J., Holson, J. F., Bazare, J. J. and Young, J. F. Comparative stability of physiological parameters during sustained anaesthesia in rats. Lab. Anim. Sci. 28: 157–162, 1978.

17 Busse, J., Muller-Klieser, W. and Vaupel, P. Intratumour pH distribution – a function of tumor growth stage? Pflugers Arch. 389: R55, 1981.

18 Ceccarini, C. Effect of pH on plating efficiency, serum requirement and incorporation of radioactive precursors into human cells. In Vitro 11: 78–86, 1975.

19 Connors, T. A., Mitchley, B. C. V., Rosenoer, V. M. and Ross, W. C. T. The effect of glucose pre-treatment on the carcinostatic and toxic activities of some alkylating agents. Biochem. Pharmacol. 13: 395–400, 1964.

20 Couch, N. P., Dmochowski, J. R., van de Water, J. M., Harken, D. E. and Moore, F. D. Muscle surface pH as an index of peripheral perfusion in man. Ann. Surg. 173: 173–183, 1971.

21 Dewey, W. C. and Freeman, M. L. Rationale for use of hyperthermia in cancer therapy. Ann. N.Y. Acad. Sci. 335: 372–378, 1980.

22 Dickson, J. A. and Oswald, B. E. The sensitivity of a malignant cell line to hyperthermia (42°C) at low intracellular pH. Br. J. Cancer, 34: 262–271, 1976.

23 Dickson, J. A. and Calderwood, S. K. Effects of hyperglycemia and hyperthermia on the pH, glycolysis and respiration of the Yoshida sarcoma in vivo. J. Natl. Canc. Inst. 63: 1371–1381, 1979.

24 Dickson, J. A. and Calderwood, S. K. Thermosensitivity of neoplastic tissue in vivo. In: Hyperthermia in Cancer Therapy, pp. 63–140. Editor: F. K. Storm. G. K. Hall, Boston, MA, 1983.

25 Dunn, L. K., Redstone, D., Roe, H. L., Steer, P. J. and Beard, R. W. The relationship between tissue and arterial pH in hypercarbic rabbits. Arch. Gynecol. 226: 31–36, 1978.

26 Eagle, H. The effect of environmental pH on the growth of normal and malignant cells. J. Cell. Physiol. 82: 1–8, 1973.

27 Eden, M., Haines, B. and Kahler, H. The pH of rat tumours measured in vivo. J. Natl. Cancer Inst. 16: 541–556, 1955.

28 Euler, J., Sauerman, G., Priesching, A. and Klöekler, K. Wirkung von Temperatur, pH und Thio-Tepa auf Angehraten und Thymidineinbau van aszitestumorcellen. Wien. Klin. Wochenschr. 86(8): 211–219, 1974.

29 Evanochko, W. T., Ng, T. C., Lilly, M. B., Lawson, A. J., Corbett, T. H., Durant, J. R. and Glickson, J. D. In vivo ^{31}P NMR study of the metabolism of murine mammary 16/C adenocarcinoma and its response to chemotherapy, X-radiation and hyperthermia. Proc. Natl. Acad. Sci. U.S.A. 80: 334–338, 1983.

30 Filler, R. M., Das, J. B., Haase, G. M. and Donahoe, P. K. Muscle surface pH as a monitor of tissue perfusion and acid-base status. J. Pediat. Surg. 6: 535–542, 1971.

31 Freeman, M. L., Dewey, W. C. and Hopwood, L. E. Effect of pH on hyperthermic cell survival. J. Natl. Cancer Inst. 58: 1837–1839, 1977.

32 Freeman, M. L., Raaphorst, G. P., Hopwood, L. E. and Dewey, W. C. The effect of pH on cell lethality induced by hyperthermic treatment. Cancer 45: 2291–2300, 1980.

33 Freeman, M. L., Holahan, E. V., Highfield, D. P., Raaphorst, G. P., Spiro, I. J. and Dewey, W. C. The effect of pH on hyperthermic and X-ray induced cell killing. Int. J. Radiat. Oncol. Biol. Phys. 7: 211–216, 1981.

34 Freeman, M. L., Boone, M. L. M., Ensley, B. A. and Gillette, E. L. The influence of environmental pH on the interaction and repair of heat and radiation damage. Int. J. Radiat. Oncol. Biol. Phys. 7: 761–764, 1981.

35 Freeman, M. L. and Sierra, E. An acidic extracellular environment reduces the fixation of radiation damage. Radiat. Res. 97: 154–161, 1984.

36 Gebert, G. and Friedman, S. M. An implantable glass electrode used for pH measurement in working skeletal muscle. J. Appl. Physiol. 34 (1): 122–124, 1973.

37 Gerweck, L. E., Gillette, E. L. and Dewey, W. C. Killing of Chinese Hamster cells in-vitro by heating under hypoxic or aerobic conditions. Eur. J. Cancer 10: 691–693, 1974.

38 Gerweck, L. E. Modification of cell lethality at elevated temperatures. The pH effect. Radiat. Res. 70: 224–235, 1977.

39 Gerweck, L. E., Nygaard, T. G. and Burlett, M. Response of cells to hyperthermia under acute and chronic hypoxic conditions. Cancer Res. 39: 966–972, 1979.

40 Gerweck, L. E., Jennings, M. and Richards, B. Influence of pH on the response of cells to single and split doses of hyperthermia. Cancer Res. 40: 4019–4024, 1980.

41 Gerweck, L. E. and Richards, B. Influence of pH on the thermal sensitivity of cultured human glioblastoma cells. Cancer Res. 41: 845–849, 1981.

42 Gerweck, L. E., Dahlberg, W. K. and Greco, B. Effects of pH on single or fractionated heat treatments at 42–45°. Cancer Res. 43: 1163–1167, 1983.

43 Gillies, R. J., Ogino, T., Shulman, R. G. and Ward, D. C. 31P Nuclear magnetic resonance evidence for the regulation of intracellular pH by Ehrlich ascites tumour cells. J. Cell. Biol. 95: 24–28, 1982.

44 Goldin, E. M. and Leeper, D. B. The effect of reduced pH on the induction of thermotolerance. Radiology 141: 505–508, 1981.

45 Gonzalez-Mendez, R., Wemmer, D., Hahn, G., Wade-Jardetzki, N. and Jardetski, O. Continuous flow NMR culture system for mammalian cells. Biochim. Biophys. Acta 720: 274–280, 1982.

46 Gullino, P. M., Clark, S. H. and Grantham, F. H. The interstitial fluid of solid tumours. Cancer Res. 24: 780–797, 1964.

47 Gullino, P. M., Grantham, F. H., Smith, S. H. and Haggerty, A. C. Modifications of the acid-base status of the internal milieu of tumours. J. Natl. Cancer Inst. 34: 857–869, 1965.

48 Hahn, G. M. Mechanisms of heat action. In: Hyperthermia and Cancer, pp. 87–130. Plenum Press, New York and London, 1982.

49 Harguindey, S., Henderson, E. S. and Naeher, C. Effects of systemic acidification of mice with sarcoma 180. Cancer Res. 39: 4364–4371, 1979.

50 Harrison, D. K. and Walker, W. F. Micro-electrode measurement of skin pH in humans during ischaemia, hypoxia and local hyperthermia. J. Physiol. 291: 339–350, 1979.

51 Haveman, J. The pH of the cytoplasm as an important factor in the survival of in vitro cultured malignant cells after hyperthermia. Effects of carbonylcyanide 3-chlorophenylhydrazone. Eur. J. Cancer 15: 1281–1288, 1979.

52 Haveman, J. The influence of pH on the survival after X-irradiation of cultured malignant cells. Effects of carbonylcyanide 3-chlorophenylhydrazone. Int. J. Radiat. Biol. 37 (2): 201–205, 1980a.

53 Haveman, J. The capacity of lysosomes of cultured mammalian cells to accumulate acridine orange is destroyed by hyperthermia. Cell Tiss. Res. 213: 343–350, 1980b.

54 Haveman, J. Influence of pH and thermotolerance on the enhancement of X-ray induced inactivation of cultured mammalian cells by hyperthermia. Int. J. Radiat. Biol. 43: 281–289, 1983.

55 Hofer, K. G. and Mivechi, N. F. Tumour cell sensitivity to hyperthermia as a function of extracellular and intracellular pH. J. Natl. Cancer Inst. 65: 621–625, 1980.

56 Holahan, E. V., Stuart, P. K. and Dewey, W. C. Enhancement of survival of CHO cells by acidic pH after X irradiation. Radiat. Res. 89: 433–435, 1982.

57 Hult, R. L. and Larson, R. E. Dissociation of 5-Fluorouracil uptake from intracellular pH in Walker 256 carcinosarcoma. Cancer Treat. Rep. 60: 867–873, 1976.

58 Jähde, E. and Rajewsky, M. F. Tumor-selective modification of cellular microenvironment in vivo: effect of glucose infusion on the pH in normal and malignant rat tissues. Cancer Res. 42: 1505–1512, 1982.

59 Jähde, E., Rajewsky, M. F. and Baumgärtl, H. pH distributions in transplanted neural tumors and normal tissues of BDIX rats as measured with pH micro electrodes. Cancer Res. 42: 1498–1504, 1982.

60 Jennische, E., Enger, E., Medegard, A., Appelgren, L. and Haljamae, H. Correlation between tissue pH, cellular transmembrane potentials and cellular energy metabolism during shock and during ischemia. Circ. Shock 5: 251–260, 1978.

61 Kahler, H. and Robertson, W. B. Hydrogen ion concentration of normal liver and hepatic tumours. J. Natl. Cancer Inst. 3: 495–501, 1943.

62 Kahler, H. and Moore, B. pH of rat tumours and some comparisons with the Lissamine-green circulation test. J. Natl. Cancer Inst. 28: 561–568, 1962.

63 Keech, M. L. and Wills, E. D. The effect of hyperthermia on activation of lysosomal enzymes in Hela cells. Eur. J. Cancer 5: 1025–1031, 1979.

64 Kost, G. J. Medial gastrocnemius and soleus muscle surface pH responses to hemorrhagic shock. Fed. Proc. 37 (3): 774, 1978.

65 Kung, S. S., Goldberg, N. D., Dahl, J. L., Parks, R. E. and Kline, B. E. Potentiation of 5-Fluorouracil inhibition of Flexner-Jobling carcinoma by glucose. Science 141: 627–628, 1963.

66 Lemieux, M. D., Smith, R. N., Couch, N. P. and Macey, A. M. Surface pH and redox potential of skeletal muscle in graded hemorrhage. Surgery 65: 457–461, 1969.

67 Li, G. C. and Hahn, G. M. A proposed operational model of thermotolerance based on effects of nutrients and the initial treatment temperature. Cancer Res. 40: 4501–4508, 1980.

68 Li, G. C., Shiu, E. C. and Hahn, G. M. Recovery of cells from heat-induced potentially lethal damage: effects of pH and nutrient environment. Int. J. Radiat. Oncol. Biol. Phys. 6: 577–582, 1980.

69 Lunec, J. and Parker, R. The influence of pH on the enhancement of radiation damage by hyperthermia. Int. J. Radiat. Biol. 38: 567–574, 1980.

70 Madoc-Jones, H. and Mauro, F. Site of action of cytotoxic agents in the cell cycle. In: Antineoplastic and Immunosuppressive agents I. Handbook of Experimental Pharmacology XXXVII, pp. 205–219. Editors: A. Sartorelli and D. G. Johns. Springer-Verlag New York, NY, 1974.

71 Mendonca, M. and Alpen, E. L. Extracellular pH and the cell-cycle: possible links to radioresistance. In: Proc. 7th Int. Congr. Radiat. Res. Amsterdam, pp. B7–15. Editors: Broerse J. J., Barendsen, H. B., Kal, H. B. and Van der Kogel, A. J. Martinus Nijhoff Publishers, 1983.

72 Meyer, K. A., Kammerling, E. M., Amtman, L., Koller, M. and Hoffman, S. J. pH Studies of malignant tissues in human beings. Cancer Res. 8: 513-518, 1948.

73 Meyer, K. R., Hopwood, L. E. and Gillette, E. L. The thermal response of mouse adenocarcinoma cells at low pH. Eur. J. Cancer 15: 1219–1222, 1979.

74 Mikkelsen, R. B. and Wallach, D. F. H. Transmembrane ion gradients and thermochemotherapy. In: Biomedical Thermology, pp. 103–107. Editors: Gautherie, M. and Albert, E. Alan R. Liss Inc., New York, 1982.

75 Mivechi, N. F., Hofer, K. G. and Hofer, M. G. Influence of hypoxia and acidity on thermal radiosensitization and direct heat-induced death of BP-8 sarcoma cells. Radiology 138: 465–471, 1981.

76 Müller-Klieser, W., Busse, J. and Vaupel, P. Tissue pH-distribution within malignant tumors as measured with antimony microelectrodes. Adv. Physiol. Sci. 25: 253–254, 1981.

77 Naeslund, J. and Swenson, K. Investigations on the pH of malignant tumours in mice and humans after the administration of glucose. Acta Obstet. Gynecol. Scand. 32: 359–367, 1953.

78 Ng, T. C., Evanochko, W. T., Hiramoto, R. N., Ghanta, V. K., Lilly, M. B., Lawson, A. J., Corbett, T. H., Durant, J. R. and Glickson, J. D. 31P NMR Spectroscopy of in vivo tumours. J. Magn. Reson. 49: 271–286, 1982.

79 Nielsen, O. S. and Overgaard, J. Effect of extracellular pH on thermotolerance and recovery of hyperthermic damage in vitro. Cancer Res. 39: 2772–2778, 1979.

80 Nissen, E. and Tanneberger, S. T. Influence of pH and serum on the effectivity of antineoplastic agents in vitro. Arch. Geschwulstforsch. 51: 152–156, 1981.

81 O Donnell, T. F. Measurement of percutaneous muscle surface pH. Lancet ii: 533, 1975.

82 Overgaard, J. Effect of pH on response to hyperthermia. In: Proc. Symp. Cancer Ther. Hyperthermia Radiat., Washington, April 1975, p. 43.

83 Overgaard, J. Influence of extracellular pH on the viability and morphology of tumour cells exposed to hyperthermia. J. Natl. Cancer Inst. 56: 1243–1250, 1976.

84 Overgaard, J. and Bichel, P. The influence of hypoxia and acidity on the hyperthermic response of malignant cells in vitro. Radiology 123: 511–514, 1977.

85 Overgaard, J. and Poulsen, H. K. Effect of hyperthermia

and environmental acidity on the proteolytic activity in murine ascites tumour cells. J. Natl. Cancer Inst. 58: 1159–1161, 1977.

86 Pampus, F. Die Wasserstofionenkonzentration des Hirngewebes bei raumfordernden intracraniellen Prozessen. Acta Neurochir. 11: 305–318, 1963.

87 Pappova, N., Siracka, E., Vacek, A. and Durkovsky, J. Oxygen tension and prediction of the radiation response. Polygraphic study in human breast cancer. Neoplasma 29: 669–674, 1982.

88 Poole, D. T. Intracellular pH of the Ehrlich Ascites Tumor cells as it is affected by sugars and sugar derivatives. J. Biol. Chem. 242: 3731–3736, 1967.

89 Rajaratnam, S., Adams, G. E., Stratford, I. J. and Clarke, C. Enhancement of the cytotoxicity of radiosensitizers by modest hyperthermia: the electron affinity relationship. Br. J. Cancer 46: 912–917, 1982.

90 Rauen, H. M. Die Bildungsgeschwindigkeiten von *N*-β-Halogenäthylaziridinen aus Bis (β-halogenäthyl)-aminen unter physiologischen Bedingungen. Arzneim. Forsch. 14: 855–859, 1964.

91 Rauen, H. M., Friedrich, M. and Norpoth, K. Messungen zur Manipulation der Glucose — abhängigen Tumorsäuerung in vivo. Z. Naturforsch. 236: 1461–1475, 1968.

92 Ross, W. J. C. Increased sensitivity of Walker tumours towards aromatic nitrogen mustards carrying basic side chains following glucose pre-treatment. Biochem. Pharmacol. 8: 235–240, 1961.

93 Ross, W. J. C. Biological Alkylating Agents, pp. 29–31, 143–147, 195. Editor: R. W. Raven. Butterworth, London, 1962.

94 Röttinger, E. M., Mendonca, M. and Gerweck, L. E. Modification of pH induced cellular inactivation by irradiation-glial cells. Int. J. Radiat. Oncol. Biol. Phys. 6: 1659–1662, 1980.

95 Röttinger, E. M. and Mendonca, M. Radioresistance secondary to low pH in human glial cells and Chinese hamster ovary cells. Int. J. Radiat. Oncol. Biol. Phys. 8: 1309–1314, 1982.

96 Rubin, H. and Fodge, D. Interrelationships of glycolysis, sugar transport and the initiation of DNA synthesis in chick embryo cells. In: Control of Proliferation in Animal Cells, pp. 801–816. Editors: B. Clarckson and B. Baserga Cold Spring Harbour Conf. on Cell Proliferation, Vol I, Cold Spring Harbour Lab. 1974.

97 Sahler, C. P. Glucose-induced pH changes in Ehrlich ascites tumor cells. Gann 60: 657–660, 1969.

98 Schloerb, P. R., Blackburn, G. L., Grantham, J. J., Mallard, D. S. and Cage, G. K. Intracellular pH and buffering capacity of the Walker-256 carcinoma. Surgery 58: 5–11, 1965.

99 Schmid-Schönbein, H., Volger, E., Weiss, J. and Brandhuber, M. Effect of *O*-(β-hydroxyethyl)-rutosides on the microrheology of human blood under defined flow conditions. Vasa 4: 263–269, 1975.

100 Shackney, S. E., McCormack, G. W. and Cuchural, G. J. Growth rate patterns of solid tumours and their relation to responsiveness to therapy: An analytical review. Ann. Intern. Med. 89: 107–121, 1978.

101 Song, C. W., Kang, M. S., Rhee, J. G. and Levitt, S. H. The effect of hyperthermia on vascular function, pH and cell survival. Radiology 137: 795–803, 1980.

102 Song, C. W. Physiological factors in hyperthermia. Natl. Cancer Inst. Monogr. 61: 169–176, 1982.

103 Spencer, T. L. and Lehninger, A. L. L-lactate transport in Ehrlich ascites-tumour cells. Biochem. J. 154: 405–414, 1976.

104 Stamm, O., Latscha, U., Janecek, P. and Campana, A. Development of a special electrode for continuous subcutaneous pH measurement in the infant scalp. Am. J. Obstet. Gynecol. 124: 193–195, 1976.

105 Steinhagen, C., Hirche, H. J., Nestle, H. W., Bovenkamp, U. and Hosselman, I. The interstitial pH of the working gastrocnemius muscle of the dog. Pflügers Arch. 367: 151–156, 1976.

106 Stevens, C. D., Quinlin, P. M., Meinken, M. A. and Koch A. M. Sulfapyrazine precipitated in cancer tissue upon repeated glucose injections. Science 112: 561, 1950.

107 Stevens, C. D. and Mosteller, R. C. Enhancement by glucose of the inhibition of an Ehrlich ascites tumour by tetraazatricyclododecane. Cancer Res. 29: 1132–1136, 1969.

108 Stratford, I. J. and Adams, G. E. Effect of hyperthermia on differential cytotoxicity of a hypoxic cell radiosensitizer RO-07-0582 on mammalian cells in vitro. Br. J. Cancer 35: 307–313, 1977.

109 Sutherland, R. M. Selective chemotherapy of non-cycling cells in an in vitro tumour model. Cancer Res. 34: 3501–3503, 1974.

110 Tagashira, Y., Yasuhira, K., Matsuo, H. and Amano, S. Continual pH measuring by means of inserted microglass electrode in living normal and tumour tissues (1st report). Gann 44: 63–64, 1953.

111 Tagashira, Y., Takeda, S., Kawano, K. and Amano, S. Continuous pH measuring by means of microglass electrode inserted in living normal and tumour tissues (IInd report), with an additional report on interaction of SH-group of animal protein with carcinogenic agent in the carcinogenetic mechanism. Gann 45: 99–101, 1954.

112 Taylor, A. C. Responses of cells to pH changes in the medium. J. Cell Biol. 15: 201–209, 1962.

113 Tritton, T. R. and Yee, G. The anticancer agent Adriamycin can be actively cytotoxic without entering cells. Science 217: 248–250, 1982.

114 Trowell, O. A. The effect of environmental factors on the radiosensitivity of lymph nodes cultured in vitro. Br. J. Radiol. 306: 302–309, 1953.

115 Turner, G. A. Increased release of tumour cells by collagenase at acid pH: A possible mechanism for metastasis. Experientia 35: 1657–1658, 1979.

116 Urano, M., Gerweck, L. E., Epstein, R., Cunningham, M.

and Suit, H. D. Response of a spontaneous murine tumour to hyperthermia: factors which modify the thermal response in vivo. Radiat. Res. 83: 312–322, 1980.

117 Urano, M. and Kim, M. S. Effect of hyperglycemia on thermochemotherapy of a spontaneous murine fibrosarcoma. Cancer Res. 43: 3041–3044, 1983.

118 Valeriote, F. and Van Putten, L. Proliferation-dependent cytotoxicity of anticancer agents: a review. Cancer Res. 35: 2619–2630, 1975.

119 Van den Berg, A. P., Wike-Hooley, J. L., van den Berg-Blok, A. E., Van der Zee, J. and Reinhold, H. S. Tumour pH in Human Mammary Carcinoma. Eur. J. Cancer Clin. Oncol. 18: 457–462, 1982.

120 Vaupel, P. W., Frinak, S. and Bicher, H. I. Heterogeneous oxygen partial pressure and pH distribution in CH3 mouse mammary adenocarcinoma. Cancer Res. 41: 2008–2013, 1981.

121 Vaupel, P. Einfluss einer lokalisierten Mikrowellen-Hyperthermie auf die pH-Verteilung in bosartigen Tumoren. Strahlentherapie 158: 168–173, 1982.

122 Vaupel, P., Müller-Klieser, W., Otte, J., Manz, R. and Kallinowski, F. Blood flow, tissue oxygenation, and pH distribution in malignant tumours upon localized hyperthermia. Strahlentherapie 159: 73–81, 1983.

123 Vidyasagar, D., Bhat, R., Raju, T. N. K., Asonye, U. and Papazafiratou, C. Continuous tissue pH (tph) monitoring in sick neonates. Pediat. Res. 13 (4): 509, 1979.

124 Voegtlin, C., Fitch, R. H., Kahler, H. and Johnson, J. M. The hydrogen-ion concentration of mammalian voluntary muscle under various conditions. Am. J. Physiol. 107: 539–550, 1934.

125 Voegtlin, C., Fitch, R. H., Kahler, H., Johnson, J. M. and Thomson, J. W. Experimental studies in cancer. I. The influence of the parenteral administration of certain sugars on the pH of malignant tumours. Natl. Inst. Health Bull. 164: 1–14, 1935.

126 Von Ardenne, M. Selective multiphase cancer therapy: conceptual aspects and experimental basis. Adv. Pharmacol. 10: 339–380, 1972.

127 Von Ardenne, M. and Reitnauer, P. G. Verstärkung der mit Glukose infusion erzielbaren Tumorübersaüerung in vivo durch Amygdalin und β-Glukosidase. Arch. Geschwulstforsch. 45 (2): 135–145, 1975.

128 Von Ardenne, M. and Reitnauer, P. G. Verstärkung der mit Glukoseinfusion erzielbaren Tumorübersaüerung in vivo durch NAD. Arch. Geschwulstforsch, 46 (3): 197–203, 1976.

129 Von Ardenne, M. and Von Ardenne, A. Berechnung des pH-Profils im Interkapillarraum der Krebsgewebe für die Falle mit und ohne Langzeit-Glukose-Infusion. Res. Exp. Med. 171: 177–189, 1977.

130 Von Ardenne, M. Prinzipien und Konzept 1977 der Krebs-Mehrschritt-Therapie. Arch. Geschwulstforsch. 48 (6): 504–520, 1978.

131 Von Ardenne, M. and Reitnauer, P. G. Amplification of the selective tumour acidification by local hyperthermia. Naturwissenschaften 65: S. 159, 1978.

132 Von Ardenne, M. and Reitnauer, P. G. Verstärkung der mit Glucoseinfusion erzielbaren Tumorübersäuerung durch lokale Hyperthermie. Res. Exp. Med. (Berlin) 175: 7–18, 1979a.

133 Von Ardenne, M. and Reitnauer, P. G. Verstärkung der mit Glukoseinfusion erzielbaren Tumorübersauerung in vivo durch Natriumnitroprussid. Pharmazie 34: 447, 1979b.

134 Warburg, O., Posener, K. and Negelein, E. Uber den Stoffwechsel der Carcinomzelle. Biochem. Z. 152: 309–344 1924.

135 Westra, A. and Dewey, W. C. Variation in sensitivity to heat shock during the cell cycle of Chinese hamster cells in vitro. Int. J. Radiat. Biol. 19: 467–477, 1971.

136 Wike-Hooley, J. L., van der Zee, J., van Rhoon, G. C., van den Berg, A. P. and Reinhold, H. S. Human tumour pH changes following hyperthermia and radiation therapy. Eur. J. Cancer Clin. Oncol. 20: 619–623, 1984.

137 Wolpert, P. W., Noller, K., Shaughnessy, D., Houchin, D. N., Baccari, M. E. and Miller, F. A. Tissue pH: a new clinical tool. Arch. Surg. 101: 308–313, 1970.

138 Woods M. W., Hunter, J. C. and Burk, D. Glucose enhancement of inhibition by fluorinated and normal pyrimidines of tumour glycolysis and transplantability. Proc. Am. Assoc. Cancer Res. 3: 163, 1960.

139 Yatvin, M. B., Kreutz, W., Horwitz, B. A. and Shinitzky, M. pH sensitive liposomes: possible clinical implications. Science 210: 1253–1254, 1980.

140 Zander, R. and Schmid-Schönbein, H. Influence of intracellular convection on the oxygen release by human erythrocytes. Pflügers Arch. 335: 58–73, 1972.