ACTA RADIOLOGICA

PUBLISHED BY THE SOCIETIES OF MEDICAL RADIOLOGY IN DENMARK, FINLAND, NORWAY AND SWEDEN

Vol. 16 Fasc. 1 | THERAPY PHYSICS BIOLOGY | 1977 February

HYPERTHERMIC TUMOUR-CELL DEVITALIZATION IN VIVO

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A number of different observations during recent years give rise to the expectation that heat will turn out to be a useful agent in the therapy of malignant tumours. Clinical and experimental observations suggest that heat either alone or in combination with irradiation or cytostatics may have a curative effect, probably by activation of different mechanisms in the tumour (CAVALIERE et coll. 1967, DIETZEL 1975, OVERGAARD & OVERGAARD 1972 a). This report deals with the uncomplicated heat activity only.

In addition to clinical observations of the disappearance of tumours after intercurrent or induced high-febrile conditions, well-controlled tumours localized on the extremities have been cured by extracorporeal hyperthermic circulation of the region in question.

At the present time the criteria to be fulfilled by the extrinsic heating conditions and the mechanism of the intrinsic heat action are under discussion. Based on existent material an evaluation of these problems is presented.

Heating conditions of tumour

In the existing clinical material it seems impossible to deduce a statement of optimum heating conditions for a curative effect of tumours: High clinical temperatures (about 41°C) for some days in most of the spontaneously cured cases (BUSCH 1866, BRUNS 1887, BOLOGNINO 1908, ROHDENBURG 1918, EVERSON & COLE

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1956), and protracted fever (39 to 40°C) in the toxin-treated tumours in the Coley material (COLEY 1893, NAUTS et coll. 1953, 1959) contrast to regional heating to 42 to 44°C for 2 to 6 hours in recent clinical material (CAVALIERE et coll. 1967) with some complications in normal tissue. Direct heating of superficial tumours at 45 to 46°C for some hours (GOETZE 1932, WESTERMARK 1898) may be considerably reduced when it reaches the focal area of the tumour. Possibly, whole-body heating to about 41.5 to 41.8°C for some hours as used by PETTIGREW et coll. (1974 a, b, HENDERSON & PETTIGREW 1971) may come near to the optimum values.

Some guidance may possibly be sought in animal experiments. A large number of transplanted tumours disappeared after exposure in vivo to local or whole-body hyperthermia; great technical difficulties exist as regards stable and homogeneous heating of tumours and reliable measurements of the temperature (OVERGAARD & OVERGAARD 1972 a, DIETZEL 1975).

In most cases, the tumour temperature is only approximately estimated, and even in continuously controlled series (DICKSON & MUCKLE 1972) instability of the tumour temperature may make the evaluation of the heat dose insufficient or uncertain.

Moreover, most of the tumour systems used were not isologous; possibly an activation of the immune system may have influenced the heat sensitivity of the tumours.

Some general information of the influence of heating temperature and heating time on the tumours was obtained in some previous and recent animal experiments (OVERGAARD & OVERGAARD 1972 a, K. OVERGAARD 1976). Isologous mouse tumours were locally heated in vivo by short-wave diathermy. The intratumoral temperature was continuously checked and automatically stabilized during the defined heating period.

By heating the tumours to the temperature range of 41 to 42.5°C equal reactions were obtained by adjustments of the exposure time in a regular way within 60 to 480 min (Fig. 1.).

In successful cases, the tumours shrank and disappeared within a couple of weeks. A possibly failing effect was ascribed to deficiencies in the heating conditions (OVERGAARD & OVERGAARD 1972 a, K. OVERGAARD 1976).

The results suggest that a curative effect may be obtained at temperature levels presumably tolerable in the treatment of human beings. However, the relative advantages of local or whole-body heating are uncertain, and the technical problems relating to suitable and adequate heating have not been solved.

Internal mechanism of heat action

Evidently, the living tumour in itself may include all conditions—apart from hyperthermia—which are necessary to complete self-destruction, but the nature of these factors and their mechanism of action have not been reliably established.

In recent years, two different suggestions on the nature of these relations have been

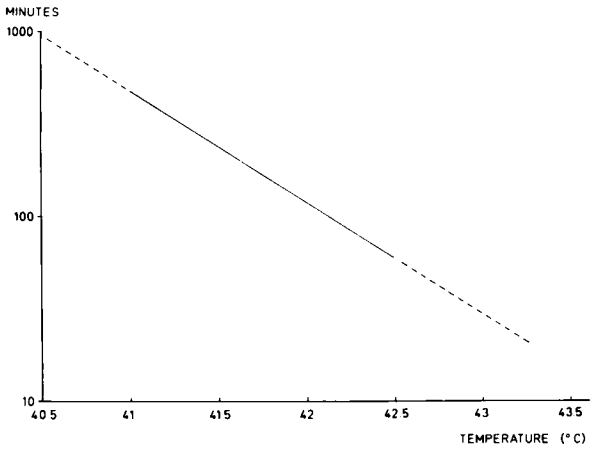


Fig. 1. Semilogarithmic graph indicating combinations of local temperatures and application time (heat dose) resulting in ~20% cures of solid mouse mammary carcinoma (data obtained from Overgaard & Overgaard 1972 a, 1974).

advanced, (1) one stressing lysosomal processes in the cytoplasm as being essential, (2) the other emphasizing irregularities in the synthetic processes of RNA and DNA. A balance between the two concepts may be looked for.

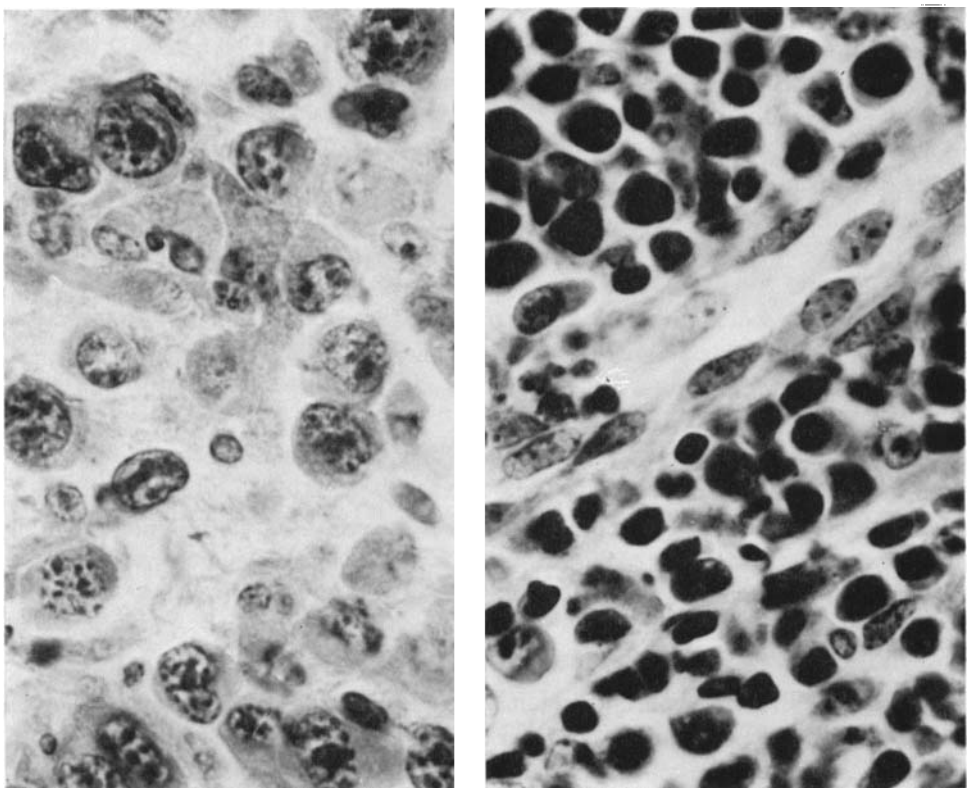
The destructive concept

The first (destructive) concept is mainly based on the morphologic appearance of animal tumours exposed in vivo to local hyperthermia, but some morphologic confirmation in human clinical cases exists (CAVALIERE et coll. 1967, OVERGAARD 1956, PETTIGREW et coll. 1974 b), and it has on certain points been confirmed by biochemical and histochemical observations of heat-treated animal tumours (OVERGAARD & HEYDEN 1974, OVERGAARD & OVERGAARD 1972 a).

Light microscopy may rapidly reveal morphologic alterations (OVERGAARD & OVERGAARD 1972 a): The tumour cells become isolated with distinct cell borders. The cytoplasm decreases and the stainability intensifies. Nuclear chromatin condenses in gradually larger clumps, and the total nuclear structure diminishes. The alterations proceed gradually for some hours. The speed varies both in individual cells and in different tumours, but in successful cases all the tumour cells are destroyed within 24 hours (Fig. 2).

New growth of such cells is never observed. All mitoses are destroyed, and fresh mitoses do not develop. Some hyperaemia in the tumour may be present but not constantly.

Only the malignant cells are affected, while non-malignant stromal cells, vascular cells and surrounding structures remain uninjured (OVERGAARD & OVERGAARD 1972 a, 1976). The reaction is conditioned by the application of a fully curative dose to the individual tumour cell. On the exposure of a tumour to a somewhat lower heat dose, many tumour cells may in a day or two change (some of them possibly



a b

Fig. 2 a Untreated mouse mammary carcinoma. Large irregular hyperchromatic nuclei dominate. Hematoxylin and Eosin, × 1 200. b) Same tumour 20 hours after heat treatment (42.5°C/60 min). Tumour cells with intense shrinkage of cytoplasm and small dark pyknotic nuclei. Normal morphology of the fibroblasts. Hematoxylin and Eosin, × 1 200.

reversible), but some of the cells may remain viable, and within a few days a multicentric regrowth of the tumour occurs.

Electron microscopy (J. OVERGAARD 1976 a, OVERGAARD & OVERGAARD 1976) has confirmed the cellular isolation and the progressive shrinkage of the cytoplasm and nuclei during the first few hours after the application of a curative heat dose.

In the cytoplasm, a gradually increasing number of larger lysosomes was the most prominent initial feature. Vacuoles and lipid droplets also occurred in this period. The mitochondria changed, first with a dense, shrunken matrix and dilated intracristal spaces; later they were more destroyed with ruptured membranes (Fig. 3).

The cytoplasmic changes continued, and 6 to 12 hours after the treatment an even more extensively destroyed cytoplasm with large lysosomes and autophagic vacuoles dominated together with lipid droplets of myelin figures. At that time, the plasma

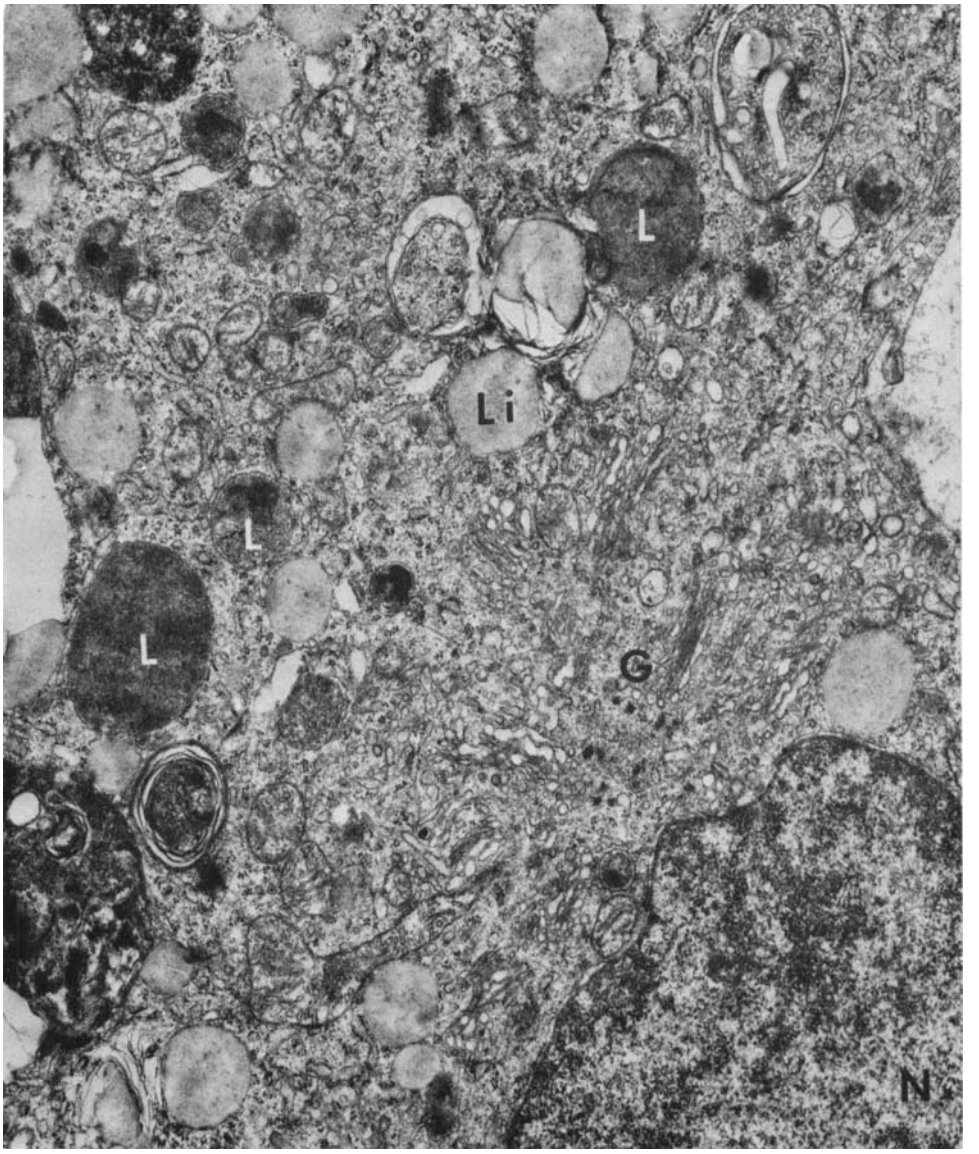


Fig. 3. Electron microscopy. Part of a tumour cell a few hours after treatment with 42.5°C/60 min. Characteristically initial changes in the cytoplasm with a hypertrophic Golgi area (G) with many cisterns and small coated vesicles (primary lysosomes). The surrounding cytoplasm is dominated by small and larger secondary lysosomes (L) and lipid droplets (Li). The nucleus (N) is without major morphologic abnormalities. × 25 000.

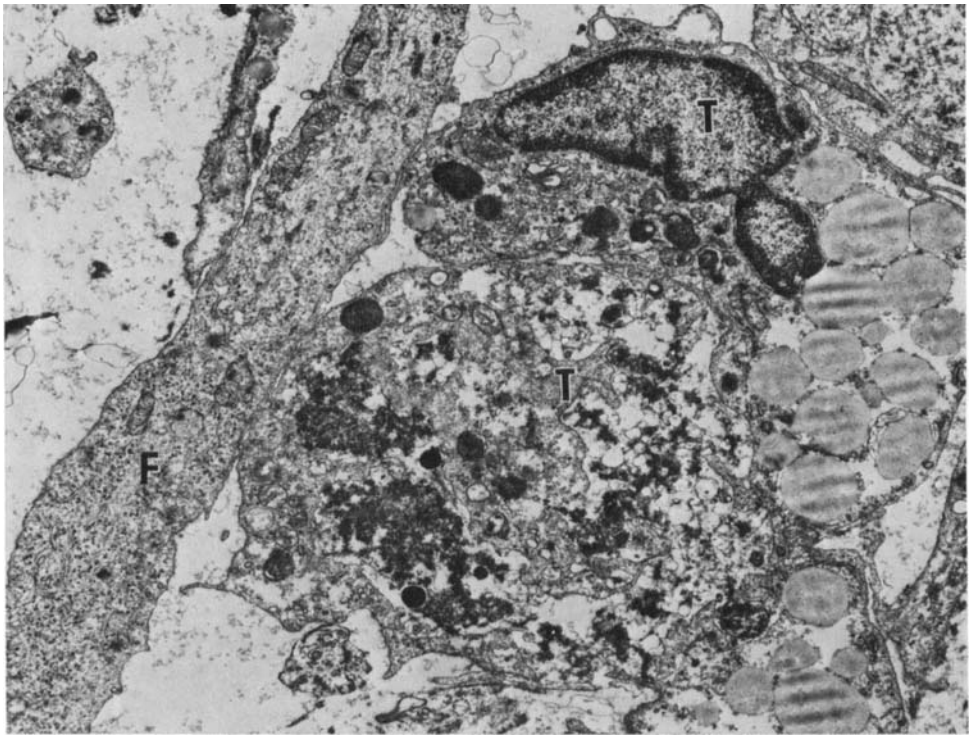


Fig. 4. Electron microscopy. Two peripheral tumour cells (T) with intense cytoplasmic destruction 24 hours after treatment with 42.5°C/60 min. The adjacent fibroblast (F) is without sign of morphologic injury. × 12 000.

membrane may be ruptured in several places. Within 24 hours the vital cytoplasmic organelles were destroyed, and the tumour cells were dying.

Except for the shrinkage, the initial changes in the nuclei following hyperthermic treatment were only sparse and dominated by a more condensed heterochromatin. This feature became later more distinct, and at that time the nucleolar structures were destroyed with a characteristic disappearance of the granular component but, with the fibrillary component intact. In addition it was observed that cytoplasmic polyribosomes shifted to a more monosome dominated configuration. Such alterations are well known and will be discussed in detail later on. Here the course of the alterations is possibly somewhat restricted by the co-existence of the destructive lysosomal process in the cytoplasm.

The intensive destructive alterations are selectively confined to the tumour cells. Stromal and vascular cells sustain only slight and transient injury (Fig. 4).

The morphologic alterations are rapidly accompanied by a rise in the content of some lysosomal enzymes (Catepsin C and acid phosphatases) in the tumour tissue,

gradually decreasing after the end of tumour destruction (OVERGAARD & OVERGAARD 1972 b).

Decrease and abolition of the respiration in the living tumour without alteration of the anaerobic glycolysis were demonstrated by DICKSON & MUCKLE (1972).

The complex of experimental manifestations may be considered as a rapid and intense activation of acid hydrolases electively in the adequately heat-exposed tumour cells; in fact, it constitutes an intravital duplicate of the normal cadaverous autolysis. Within few hours it leads to total destruction of all vital activity in these cells.

This killing of the tumour cells is the essential element in the process. It is of a fairly uniform character in all tumours examined.

The subsequent elimination of the dead tumour cells and the restoration of the tissue structure are only of secondary importance. This process proceeds in quite the normal way; phagocytosis and enzymatic lysis compete in the elimination, while granulation tissue forms a fibrotic scar, which is normally the final result. Variations in the lytic capacity in different tumours may possibly influence the course.

The repressive concept

A large number of reports on tumour tissue or cells exposed to hyperthermia in vitro have been published.

Investigations are concentrated on the cellular problems sui generis and normal ecological restrictions are non-existent. In this way, direct therapeutic points of view may be reduced and, possibly, replaced by the analysis of cellular behaviour under clinically unrealistic conditions.

Previously the relatively low heat tolerance of tumour cells was demonstrated, and attempts to delineate the quantitative destructive conditions were made.

As the experimental conditions and the parameters used in the assessment varied, it is only natural that the quantitative results as to the size of active heat doses varied considerably.

However, it is remarkable that, in all series dealing with the conditions in a wider range of temperature, (1) a practically regular relation between temperature and time exists, roughly identical with the conditions in in vivo investigations, and (2) that the achievement of severe or lethal cellular injury in in vitro conditions requires higher heat doses than those necessary in in vivo treatment (Fig. 5).

In most reports, only the quantitative heating conditions for tumour destruction are presented (MENDEL 1928, JARES & WARREN 1939).

In some cases normal structures revealed a higher heat tolerance than malignant structures (BENDER & SCHRAMM 1966, CHEN & HEIDELBERGER 1969, GIOVANELLA et coll. 1973., KIM et coll. 1974, KASE & HAHN 1975).

Some experiments demonstrated biochemical or morphologic alterations in the tumour cells referable to heat exposure: decrease or abolition of the cellular respiration in tumour cells without any influence on the anaerobic glycolysis is a constant and characteristic phenomenon in malignant cells whereas normal cells are not

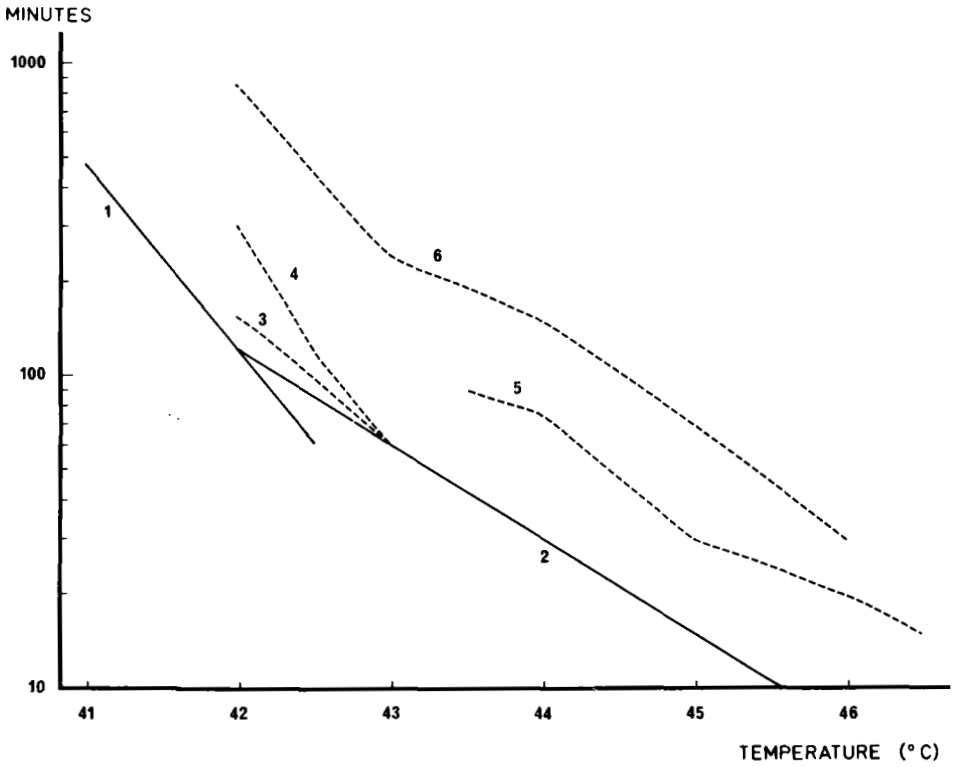


Fig. 5. Semilogarithmic graph. Relation between temperature and time necessary to obtain maximum injury to tumours heated in vivo (——) or tumour cells exposed in vitro (– – –). 1: Overgaard & Overgaard (1972 a, 1974). 2: Crile (1963). Tumour temperature estimated but not controlled. 3: Giovanella et coll. (1970). 4: Palzer & Heidelberger (1973 a). 5: Westra & Dewey (1971). 6: Selawry et coll. (1957). (Experimental conditions and assessing parameters are not identical.)

disturbed in that way (WESTERMARK 1927, MENDEL 1928, DICKENS et coll. 1936, CAVALIERE et coll. 1967, MONDOVI et coll. 1969 b, MUCKLE & DICKSON 1971, MUCKLE et coll. 1971, MUCKLE 1973). Some difference in lysosomal heat resistance in tumour and normal cells has been suggested (TURANO et coll. 1970).

Gradually, more detailed problems as to the mode of heat action on tumours have turned up (SELAWRY et coll. 1957), and recent investigations have shown that, in most cases, hyperthermia given under in vitro conditions may induce injury to nuclear acid synthesis (DEWEY et coll. 1976, MONDOVI et coll. 1969 a).

Incorporation of labelled uridine and thymidine indicates that the RNA synthesis is the first and main target in the process (DICKSON & SHAH 1972, PALZER & HEIDELBERGER 1973 b, WAROCQUIER & SCHERRER 1969).

This observation may be morphologically confirmed by light microscopy and especially electron microscopy which reveals nucleolar changes indicating a primary defect in the RNA synthesis (SIMARD & BERNHARD 1967, SIMARD et coll. 1969, HEINE et coll. 1971, J. OVERGAARD 1976 a, b, LOVE et coll. 1970).

In the nucleolus the granulated component disappeared while the fibrillar component was intact.

The reactions are identical in tumour cells and in non-malignant cells, and in both cases most of the alterations are of a reversible character and usually repaired within about one or two days.

In such cells, a number of lysosomal structures are visible, but no irreversible lysosomally induced destructions have been reported (HEINE et coll. 1971).

As preserved viability (cloning ability, isotope uptake, etc.) is generally used as the criterion in recent investigations, the direct interest in cellular decay is low. No general suggestions as to the mechanism of cell killing are given.

The heat sensitivity of proliferating cells may vary according to the different stages in the cell cycle, but generally late S phase and mitotic phase seem to be the most sensitive (WESTRA & DEWEY 1971, PALZER & HEIDELBERGER 1973 b; SISKEN et coll. 1965, DEWEY et coll., 1976, JUUL & KEMP 1933, MARTIN & SCHLOERB 1964, SELAWRY et coll. 1957, SAPOZINK et coll. 1973).

However, recent investigations of non-proliferating density-inhibited tumour cells indicate that these cells may be even more sensitive to heat than cells in active proliferation (HAHN 1974, SCHULMAN & HALL 1974.)

The reversible RNA alterations may scarcely have any direct cytocidal effect, but they may possibly indirectly be lethal through deficiency in vital elements such as chromosomal DNA or other proteins, or heat denaturation of such material (PALZER & HEIDELBERGER 1973 b, DEWEY et coll. 1976), including the mitotic spindle (DEWEY et coll. 1971).

Also the abolition of cellular respiration may possibly be lethal (WESTERMARK 1927, MENDEL 1928, DICKSON & SHAH 1972). This may explain some tumour-specific activity.

As the mentioned variations in kinetic conditions just as in metabolic (HAHN 1974, KASE & HAHN 1975), oxidating (SCHULMAN & HALL 1974, HARISIADIS et coll. 1975, KIM et coll. 1975) and acidic (OVERGAARD & OVERGAARD 1975 a, J. OVERGAARD 1976 b, OVERGAARD & BICHEL 1976) conditions may be present at relatively low temperatures (41–43°C) the possible interference of factors other than heat may be discussed.

Some observations indicate that cell-killing may be a prolonged process (GIOVANELLA et coll. 1973, PALZER & HEIDELBERGER 1973 a), and possibly depends on two or more focal cellular lesions (PALZER & HEIDELBERGER 1973 a, b).

Variations in experimental conditions make comparison between different series uncertain, and as hyperthermic investigations on cultured cells are performed in a wide range of temperatures, possibly the influence of some important factors may be modified at the top and bottom of this range.

It is remarkable that evident cellular decay and severe mitotic irregularities are observed at low temperatures (41°C) (SELAWRY et coll. 1957), and that variations in kinetic, metabolic and environmental conditions may influence the sensitivity in similar moderate heat ranges.

Discussion

Although many details are far from being clear, existing observations suggest that two different heat-provoked processes may delete the tumour cells, one by a relatively rapid lysosomal destruction of the vital cytoplasmic organelles, the other by a possibly RNA-dependent restraint of the synthetic activity of vital elements or in other ways. The determining cellular points of attack are different, but in each of the reactions some elements of the alternative reaction may be identified.

Obviously the heating conditions may determine the reaction type: The 'destructive' form has been observed only on heat exposure in vivo. The 'repressive' type is mainly reported in in vitro experiments in cultured tumour cells. An analysis of the differences in cellular conditions in the two types of treatment may perhaps explain the differences in the reactions.

In cell cultures, the cellular conditions are relatively clear. The composition of the suspending and nutrient medium may be varied arbitrarily to imitate natural conditions, and unlimited possibilities to vary one or more of the milieu-forming conditions exist. But it is not certain that all relevant conditions have been fulfilled. Specifications as to the acidity of the medium are not given in all cases, but as cultivation conditions are usually implied, presumably buffering in the pH range of 7.4 to 7 was used (DICKSON & MUCKLE 1972, MONDOVI et coll. 1969 a). A fully homogeneous and controlled heat application to all cells is possible in the cultures.

In vivo experiments present quite different relations. Technical conditions may counteract a fully homogeneous heat application to all tumour cells (at best only 25 to 30 per cent of the cases are considered successful in the experiments by OVERGAARD & OVERGAARD 1972 a). As regards the cellular milieu the conditions given in the natural tumour structure must be considered but unfortunately the metabolic conditions in tumour tissue and cells are insufficiently known. According to the GULLINO three-compartment concept (GULLINO et coll. 1964), an intimate interaction exists between the biochemical conditions (1) in the interior of the tumour cells, (2) in the intracapillary content and (3) particularly, in the interstitial fluid of the tumour structure. WARBURG et coll. (1924) and BURK et coll. (1967) have stated that malignant cells, in addition to respiration, have a considerable glycolysis, and produce lactic acid. Some of the acid is drained through the circulation, but a clear amount is present in the interstitial fluid, varying somewhat in different tumours (GULLINO et coll. 1964, KAHLER & ROBERTSON 1943), and presumably according to the nutritional state (VOEGTLIN et coll. 1935, NAESLUND & SWENSON 1953, TAGASHIRA et coll. 1953, 1954). All existing information indicates an acid state in the interstitial fluid in tumour structures under 'normal' conditions (MEYER et coll. 1948, NAESLUND & SWENSON 1953, EDEN et coll. 1955).

According to Poole and co-workers (POOLE et coll. 1964, POOLE 1967), presumably a mutual relation exists between the intra- and extracellular pH. In an acid milieu this may give a lower pH inside tumour cells as compared with an alkaline milieu.

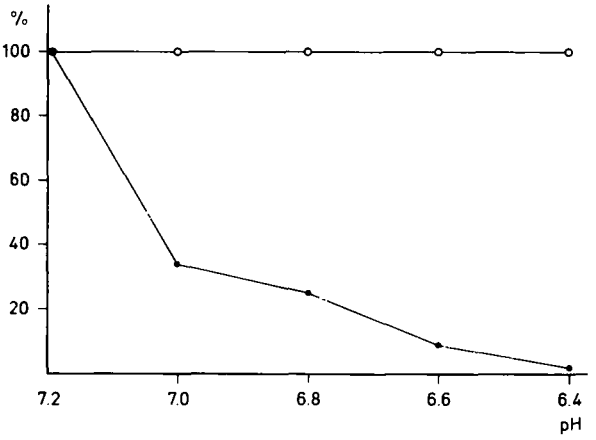


Fig. 6. Ability of ascites tumour cells to produce tumours in mice (viability in per cent) after incubation in vitro under normothermic (37°C ○—○) or hyperthermic (42.5°C/60 min ●—●) conditions at different environmental pH. (From Overgaard & Overgaard 1975 a.)

The activation of lysosomal enzymes may be an intracellular process favoured by a low pH.

This difference in the cellular milieu may be important: Before heating, the state is tolerably stable in the culture. The lactic acid continuously formed leaks out into the medium and is neutralized in the buffer. Also the living tumour may be in a stable state in an acid milieu.

These observations suggest that heat initially may effect some identical alterations in all the tumour cells: The decrease in or abolition of respiration with continued production of lactic acid, the disturbances in nucleolar RNA and some lysosomal formation have been described under both conditions. Possibly, pre-existing differences in cellular conditions may be responsible for the subsequent course.

In the culture (basic milieu), the buffering capacity stabilizes the continued acid production; the high pH prevents any intense lysosomal activity, and cellular injury is essentially confined to the synthetic regression.

In the living tumour (acid milieu), not all the lactic acid can disappear, and intracellular acidity possibly increases. The lysosomes are activated, and the destruction of organelles escalates. The RNA alterations are moderate and overtaken by the cytoplasmic destruction, which may be complete even at low temperatures.

If a mutual interaction between intra- and extracellular pH levels has a general validity, it may be possible to imitate the in vivo results in vitro by the use of appropriate pH relations. In fact, a constant heat dose did effect a gradually more intense destruction in tumour cells exposed to heating in media of gradually lower pH levels (Fig. 6) (OVERGAARD & OVERGAARD 1975 a, OVERGAARD & BICHEL 1976). Also some ultramicroscopic alterations of the 'destructive' type were identified in such cells (J. OVERGAARD 1976 b).

At present, no concrete knowledge can explain the gradual difference in heat effect reflected in the falling pH; possibly intermediate reaction grades exist.

Also some reported variations in heat sensitivity between cultured cells under varying medium conditions (possibly affecting differences in buffering (HAHN 1974)) or under varying kinetic or metabolic conditions (HAHN 1974, SCHULMAN & HALL 1974) may possibly be explained in a similar way by local acidifications caused by poor shifts in the medium in dense parts of the culture.

In fact, the present knowledge of the exchange of medium and the intracellular pH conditions in cultures is extremely limited.

Final remarks

Available information indicates that heat exposure within the range of 41 to 41.5°C for some hours probably has a curative effect on malignant tumours in animals and man. The exposure time and conditions of heating may in practical cases depend on the technical conditions as to the homogeneity of heat, which will be considered in greater detail in a subsequent report.

As regards the explanation of the in vivo mechanism of the heat action all observations confirm the morphologic, biochemical and histochemical concept described as 'destructive'.

However, also evident 'repressive' alterations in the RNA activity have been observed. These alterations may under certain conditions be of importance in tumour reactions; but as the action may be clearly overtaken by the rapid cytoplasmic destruction, no importance can be ascribed to these alterations in the reaction.

It must be stressed that a curative effect requires a well-defined minimum heat dose. On exposure to doses considerably below this minimum, many cells are microscopically affected to a varying degree. Only cursory descriptions of such alterations exist (OVERGAARD & OVERGAARD 1972 a). The presence of two conspicuous types of cells are noteworthy: cells with 'great dark nuclei' and cells at arrested metaphase. On heat doses within this low range, corresponding ultramorphologic alterations (dispersion of nucleolar RNA and delayed mitoses) are commonly reported.

As heat doses in the same fractional ranges have shown a clear synergism in combination with subtherapeutic roentgen doses (OVERGAARD & OVERGAARD 1974, 1975 b), the importance of the 'repressive' alterations deserves a thorough analysis. Provided such work is performed with a therapeutic aim, it is of importance that natural tumour conditions are considered.

SUMMARY

A review of the morphologic, biochemical and clinical effects of hyperthermia on malignant cells indicates the presence of two principally different heat-induced alterations. (1) A 'destructive' lysosomal dependent cytoplasmic reaction dominates the tumour-cell devitalization in vivo, probably influenced by the characteristic tumour cell environment. (2) 'Repressive' nuclear abnormalities may be observed, but seem to be secondary in the in vivo reaction. However, under certain conditions (combined treatment modalities) this nuclear effect may be of importance.

ZUSAMMENFASSUNG

Eine Übersicht über die morphologischen, biochemischen und klinischen Effekte von Hyperthermie auf malignen Zellen deutet auf das Vorhandensein von zwei prinzipiell verschiedenen Wärme-verursachten Veränderungen. (1) Eine destruktive, lysosomal-abhängige cytoplasmische Reaktion dominert die Devitalizierung der Tumorzelle in vivo, wahrscheinlich beeinflusst durch den Charakter der Tumorzellumgebung. (2) Repressive nukleare Abnormitäten können beobachtet werden, diese scheinen jedoch sekundär zu den in vivo Reaktionen zu sein. Jedoch kann unter gewissen Bedingungen (kombinierte Behandlungsmodaliteten) der nukleare Effekt von Bedeutung sein.

RÉSUMÉ

La revue des effets morphologiques, biochimiques et cliniques de l'hyperthermie sur les cellules malignes montre qu'il y a deux types principaux d'altérations induites par la chaleur. (1) Une réaction 'destructive' du cytoplasme dépendante des lysosomes domine la dévitalisation de la cellule tumorale in vivo, influencée probablement par l'environnement caractéristique de la cellule tumorale. (2) On peut observer des anomalies nucléaires 'répressives' qui cependant paraissent secondaires dans la réaction in vivo. Cependant dans certaines conditions (modalités de traitements associés) cet effet nucléaire peut être important.

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