FEATURES AND LIMITATIONS OF THE IN VIVO EVALUATION OF TUMOUR RESPONSE BY OPTICAL MEANS

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Summary.—When a tumour is growing in a thin sheet-like fashion, many useful parameters can be directly determined by optical means. In a “sandwich” system a tumour grows in a thin, separated layer of subcutis on the back of a rodent. The tumour itself is enclosed between two transparent surfaces, one being a glass cover slip. Living tumour cells appear to be relatively transparent; therefore, the blood vessels in the tumour are the most outstanding structures. The assay methods that can be applied for determining tumour response can be divided into three groups: (1) observation and recording of the nature of the tumour vasculature, etc.; (2) dynamic investigations on blood flow; and (3) the use of optical indicators for a relative index of tissue oxygenation state. Especially with regard to the latter methods, recent developments are promising. However, as determinations with these systems do not reveal the clonogenic viability of tumour cells as such, the use of such “observation” systems should be of necessity restricted to answering questions about tumour response that cannot be evaluated by other means.

Many factors are involved in the ways tumour cells can be inactivated by treatment and thus the ultimate response to treatment will depend on the interaction of several variables. For radiation therapy, the most important one seems to be the proportion of hypoxic cells in tumours. The existence of hypoxic cells was predicted by Thomlinson & Gray in 1955 on the basis of histological findings in combination with mathematical models of oxygen diffusion. During the last two decades, hypoxic cells have almost invariably been found by means of bioassays in experimental tumours. The proportion of hypoxic cells in untreated experimental tumours is typically between 10% and 20% of the clonogenic cell mass. In view of its importance for the radio-sensitivity of tumours, information on the mechanisms giving rise to tumour-cell hypoxia should be available. As tumour-cell hypoxia is obviously a phenomenon that is physiological, it should be studied by physiological means. This is especially important, as methods to perform bioassays on human tumours for the determination of hypoxic cells will be difficult to establish.

To obtain information on factors that are of influence in the distribution of oxygen in tumours, several systems have been developed on the basis of Algire’s (1943) observation chamber. Merwin et al. (1950), Yamaura et al. (1971), Yamaura & Matsuzawa (1979), Reinhold (1971) and Reinhold et al. (1979) have adapted this method for use in radiobiological investigations. They have improved the observation chamber technique in such a way that, after inoculation, the tumour is allowed to grow only in a sheet-like fashion. This is generally done by having the tumour grow in an isolated sheet of subcutis, which is enclosed between two narrowly spaced transparent surfaces (Fig. 1). Similar systems for the hamster cheek pouch were designed by Warren et al. (1978). It should be emphasized that, to obtain reliable information from such observation chambers, it is absolutely necessary to have the tumour growing in a thin sheet. The reason is that, for a thickness of 100–200 μm, the tumour tissue is
sufficiently transparent for detailed observation and optical measurements; if the tumour exceeds this thickness, the picture becomes blurred. This not only severely restricts any measurements but it also becomes impossible to judge where supplying vessels come from. The idea behind the "sandwich" type of observation chambers is to perform determinations in what is essentially an equatorial cross section through a tumour. However, one has always to keep in mind that even the most sophisticated "sandwich" preparation still has a thickness of between 50 and 200 μm. This is 4 to 12 cell layers thick.

Viable tumour tissue in these preparations appears surprisingly transparent and clear (Algire, 1943; Kligerman & Henel, 1961). Blood vessels are the most outstanding structures and necrotic areas can be easily distinguished as "cloudy" regions. This is probably due to a change in the refractive index when a tissue area becomes necrotic. It is therefore a simple matter to obtain photomicrographs in which the tumour size, its vascular pattern and, when present, the necrotic areas can be recorded and followed for a number of days (Yamaura & Matsuzawa, 1979) with quantitative analysis.

However, some of the questions one would like to answer with systems like the one described are: Why, when and where are the hypoxic cells located in these tumours? How do they respond to therapy? Recent developments in methods for the optical analysis of metabolic processes as well as highly sophisticated methods for quantitative determinations on the microcirculation will no doubt yield a large body of information regarding tumour physiology that can be used to gain insight into the processes in human tumours. In addition to the aforementioned photographic recording of the vascular pattern and necrosis with their quantitative evaluation, the quantitative application of fluorescence microangiography (Reinhold, 1971), the topical determination of the local redox state (Gosalvez et al., 1972; Reinhold et al., 1979) and the topical analysis of erythrocyte velocity and regional tumour blood flow are all possible (Endrich et al., 1979b).
To the exciting future applications belongs the distribution of oxygen assayed by fluorescent probes like pyrene butyric acid. Mitnick & Jobsis (1976) have used this indicator for determination of the oxygen tension in brain. The recently developed optical indicators for tissue pH (Lubbers et al., 1977; Visser et al., 1979) might also be adapted for use in “sandwich” tumour systems. This would be of great importance for investigations on the response of tumours to hyperthermia. In addition, it must be possible to estimate the rate of diffusion of chemotherapeutic agents with fluorescent properties, such as adriamycin, into the tumour tissue. Finally, the determination of the oxygenation state of the blood by absorption spectrometry (Vaupel et al., 1978) seems to be within the range of possibilities.

Some, but not all, of the aforementioned methods have been used for the determination of tumour response to irradiation, none, to the authors’ knowledge, to chemotherapy and only two methods for hyperthermia. Merwin et al. (1950) observed vascular changes in the original Algire-type of observation chamber, while Yamaura & Matsuzawa (1979) more recently pointed to the unexpected occurrence of regrowth foci after irradiation from the periphery of the tumour rather than the (hypoxic) centre. Reinhold (1971) has followed the microcirculation in tumours by means of microangiography and found an improvement in the circulation during fractionated radiation therapy. The oxygenation state of the tissue also responded with an improvement, followed by a dip and a subsequent slight recovery (Fig 2) (Reinhold et al., 1979), after a dose of 20 Gy to “sandwich” tumours in the
rat. This pattern was almost identical to
the one described by Thomlinson (1969)
with a regrowth delay method. A decrease
in blood velocity in a tumour during
hyperthermia was found by Endrich et al.
(1979a). This supported the finding of
Reinhold et al. (1977) that stoppage of
the microcirculation in the central parts of
"sandwich" tumours may take place after
prolonged hyperthermic exposure.

"Sandwich" tumour preparations are
laborious to make and require a high de-
gree of skill for making the preparations
as well as for performing the determina-
tions. Moreover, the preparations are
fragile; this means that the risk of losing
animals, e.g. during anaesthesia, depends
greatly on the experimental procedure.
The majority of the determinations, such
as velocity measurements or NAD(H)
redox determinations, are also time-
consuming. As a result, the number of
animals as well as the number of deter-
ninations that can be performed are
necessarily restricted. On the other hand,
only with these kind of microphysiological
methods can effects such as the occurrence
of fluctuation in blood supply (Intaglio-
letta et al., 1977) or NAD(H) fluores-
cence (Reinhold et al., 1977) be demonstrated.

Obviously, one has to balance the
efforts expended to perform a limited
number of detailed physiological deter-
ninations against the more easily obtain-
able data from bioassays or histology.
While gain in the quality of information
obtained with bioassays may be achieved
by using in vivo/in vitro methods (Barend-
sen, this symposium), gain in the quality
of the optical determinations will have to
come from better application and detect-
of, e.g. fluorescent probes, as well as
from improved data acquisition and
handling. For example, "sandwich"
tumours are presently being analysed by
single-line scanning for oxygenation
(pyrene butyric acid fluorescence quench-
ing) as well as for the NAD(H) hypoxic
response. This means in practice that two
different parameters are analysed at the
same site, the numbers of sites per tumour
ranging from 200 to 400. These numbers
allow the construction of histograms for
individual tumours for hypoxic NAD(H)
response as well as, hopefully, values for
the tissue oxygenation. The application of
the linearity of the latter is presently
being scrutinized (Lubbers & Opitz, 1976).
Generally speaking, there must be possi-
blities to perform many of the afore-
mentioned determinations at the same
time in the same tumour, generating a
two-dimensional image of a great number
of factors concerned with the response of
tumour microphysiology to treatment.

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