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Pathophysiology of Malignancy: I. Tissue Oxygen Tension of Benign and Malignant Tumors of the Skin.* (22571)

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Many facets of tumor metabolism have been investigated, leading to the conclusion that demonstrable alterations of aerobic and anaerobic respiration exist in malignant cells (1,2). Yet little attention has been paid to the physiologic aspects of oxygen transport and supply in tumor tissue. Thus it seemed appropriate to investigate the parameters of tumor tissue oxygen tension, and study the mechanisms of its control.

In the past, tissue oxygen tension has been mainly estimated from the oxygen content of arterial and venous blood. This method, limited to organs whose rate of capillary circulation can be measured accurately, leads only to gross estimates of tissue oxygen tension changes, and is generally unsuitable for most malignant tumors because of the difficulty of obtaining representative samples of venous blood. Before the introduction of the platinum oxygen cathode, the nearest approach to a satisfactory method for measuring oxygen tension in intact tissues was the one in which gas bubbles, introduced into a tissue, were allowed to come to equilibrium in respect to oxygen, and the gas then withdrawn and analyzed(3). This method is prone to large errors due to compression of surrounding vessels, it cannot measure rapid changes of pO_2 nor is it applicable to estimation of oxygen tension of small volumes of tissue.

The platinum oxygen cathode, described by Davies and Brink(4), has been used extensively for rapid estimation of oxygen tension in intact skin(5), heart(6) and muscle(7). A description of the basic instrumentation and the physiologic variables applicable to its use in skin has been given by Montgomery and Horvitz(5).

Method. Cutaneous oxygen tension was measured *in vivo* by means of the open type oxygen cathode(5). The diameter of the exposed platinum surface was approximately 200 μ. In order to minimize variations in blood flow, all measurements were performed in a warm room (temperature 28° to 30°C) and comparative pO_2 determinations of abnormal and adjacent normal skin were performed simultaneously. The electrodes used were calibrated against each other in saline (equilibrated against air) to be certain that the platinum surfaces exposed were comparable. Three or more consecutive values (obtained by re-insertion of the electrodes into adjacent tumor and normal skin sites respectively) were averaged and compared to each

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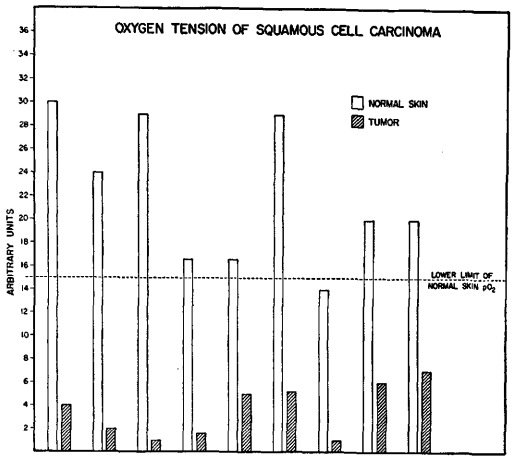

FIG. 1.

other. Biopsies were taken from the areas examined to rule out the possibility of local necrosis at the electrode insertion sites. Each of a pair of electrodes was used alternately in normal and in abnormal skin, so as to further rule out any fixed inherent error.

Apparatus. Platinum microelectrodes were made according to the technic described by Montgomery and Horvitz(5), but using 32 gauge, 90% platinum-10% iridium wire. A silver-silver chloride indifferent electrode was used to complete the circuit. All readings were taken with an applied DC voltage of —0.6 volt, and the current obtained from the electrode system amplified by a specially designed push-pull DC amplifier and fed into a Speedomax x_1-x_2 recorder. Skin temperature was measured simultaneously in some experiments by means of a thermistor needle (inserted into the experimental site) driving the x_2 pen of the recorder. All results were expressed in terms of arbitrary units, each unit corresponding to a current of 3×10^{-11} ampere.

Technic. Patients were placed on a bed in a constant temperature room kept between 28° and 30°C. Electrodes were inserted into the lesions to be studied and into the normal appearing, immediately adjacent skin. The circuit was completed by a finger dipped into a beaker of saline containing the silver-silver chloride anode. Three pO_2 readings were taken at each electrode insertion after equilibrium had been established (denoted by stabilization of electrode current). The electrodes were then re-inserted and the procedure repeated.

Results. 1. *Oxygen tension of normal skin.* In over 600 individual measurements of the oxygen tension of the normal skin of more than 200 patients, it was found that, provided good blood flow was present, electrode currents exceeded 15 units (4.5×10^{-10} amp.). While there existed marked individual and

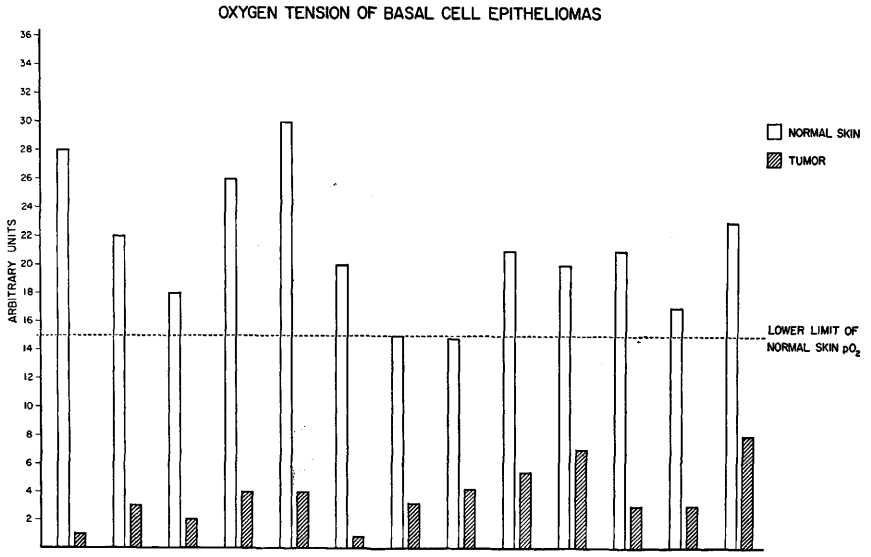

FIG. 2.

daily variations, sometimes approaching 50%, consecutive pO_2 measurements with matched electrodes in any one skin area under closely similar conditions of temperature and blood flow varied no more than 20%.

2. *Oxygen tension of malignant cutaneous tumors.* Eight different varieties of malignant tumors were examined: 9 squamous cell carcinomas, 13 basal cell epitheliomas, 6 metastatic cutaneous melanoma lesions, 2 carcinomas of the breast involving the skin, 2 lesions of dermatofibrosarcoma and 1 tumor lesion each of mycosis fungoides and reticulum cell sarcoma. All bled easily on insertion of the electrodes. In all cases, biopsies obtained from the site of electrode insertion proved the absence of necrosis and the identity of the tumor. In almost all of these malignant lesions, the oxygen tension within the tumor mass was found to be 25% or less of that of the simultaneously examined normal adjacent skin (Fig. 1-3). In several cases, it was possible to outline the extent of tumor infiltration quite accurately, using the low pO_2 as indicator of the extent of involvement.

3. *Oxygen tension of cutaneous lesions characterized by dense cellular infiltrate or acanthosis.* Five dermal cellular nevi on 4 patients, 4 xanthoma tuberosum nodules on 3 patients, 3 sarcoid granulomata on one patient, 2 patches of thickened psoriasis, 3 lichen planus papules on one patient and 2 verruca vulgaris on 2 patients were examined. With the exception of 2 of the nevi, 1 xanthoma tumor and the 2 verrucae, the remaining lesions showed a pO_2 of less than 25% of that of the adjacent normal skin (Fig. 4, 5). All lesions except the nevi and warts bled easily on electrode insertion. In all lesions, dense cellular infiltrate consisting

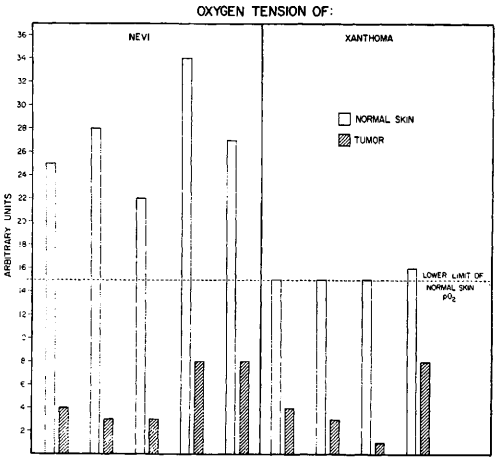

FIG. 4.

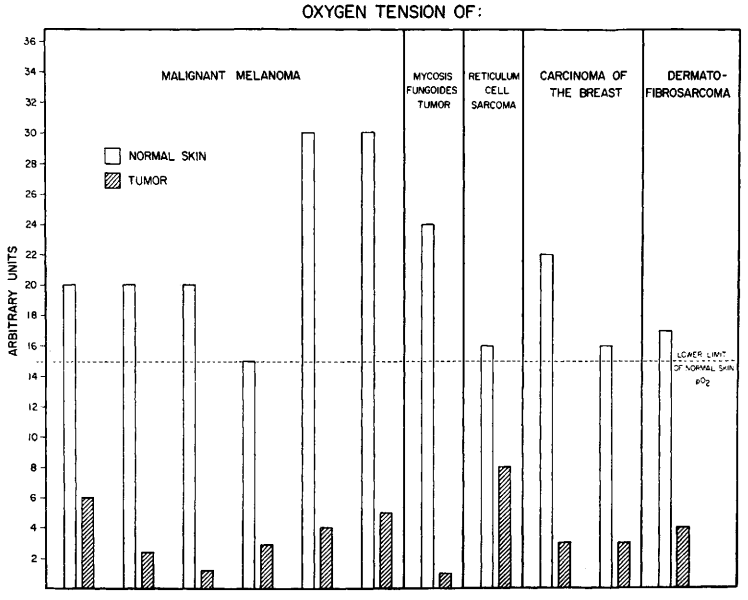

FIG. 3.

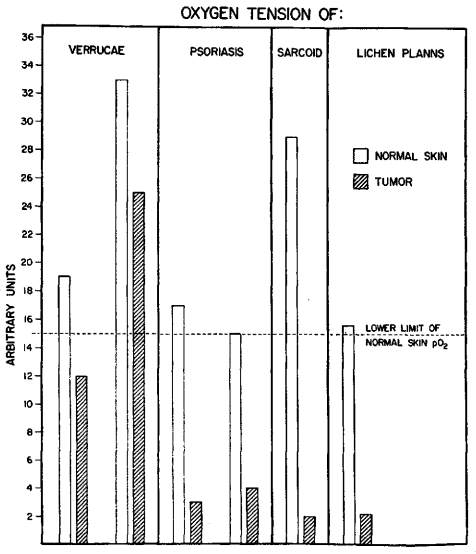

FIG. 5.

of either nevus cells, foam cells or inflammatory cells was noted on biopsy. The psoriatic patches and verrucae showed acanthosis as well as marked round cell infiltration.

4. *Oxygen tension of cutaneous lesions characterized by relatively minor cellular infiltrate and little or no acanthosis.* Six seborrheic keratoses on 4 patients, 8 arsenical keratoses on 2 patients, 1 stasis ulcer, 3 x-ray scars on 3 patients, 1 area of localized pretibial post-hyperthyroid myxedema of the skin and 4 areas of one case of premycosis fungoides were examined. With the exception of 2 arsenical keratoses (where biopsy showed dyskeratosis and early metaplasia) and the x-ray scars (which appeared histologically markedly avascular), the differences between the pO_2 of normal and abnormal skin were not significant (Fig. 6). Only the stasis ulcer bled easily on insertion of the electrodes. In all lesions, the degree of cellular infiltration was minor.

Discussion. Of the various factors controlling skin pO_2, several probably play only minor roles in tumor tissue oxygen tension. Little is known about oxygen diffusion coefficients and solubility in tissue, but it is very unlikely that any differences in these could be of a magnitude great enough to account for the observations reported. Oxygen use by the electrodes decreases as the measured pO_2 falls, and thus would affect readings in the opposite direction from that observed.

The two major determinants of tissue pO_2 are oxygen consumption and oxygen supply. The oxygen consumption of skin tumors has, (with exception of one observation(8)), been reported as increased over that of normal

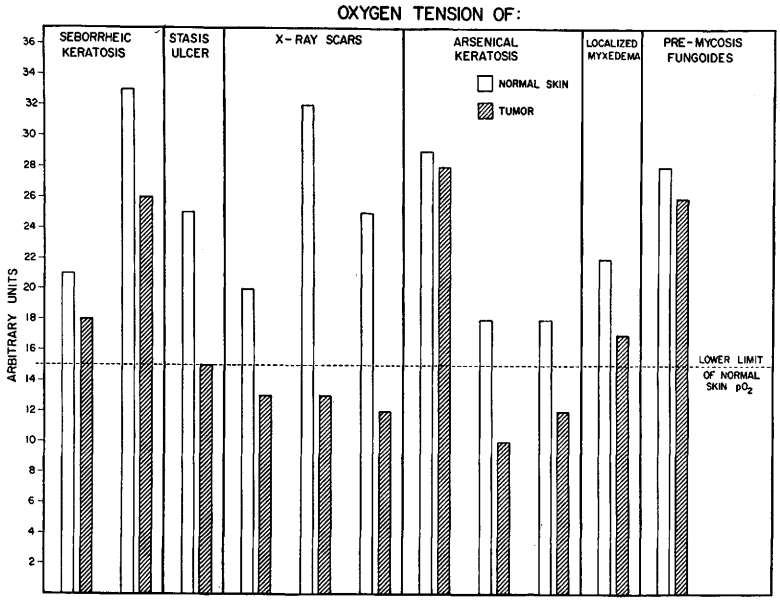

FIG. 6.

cutaneous tissue(9,10). The very low oxygen demand of normal skin(8,11) (QO_2 1.0-2.5) is thought to be due to the relatively small amount of respiring cell mass present, since more than two-thirds of the skin consists of metabolically inactive collagen and elastic fibres(11). Extensive replacement of such inert material by respiring cells (whether of malignant or of benign origin) could increase the oxygen consumption per unit volume of tissue and result in a drop in oxygen tension, provided blood flow remains the same.

Furthermore, malignant tumors are known to have a highly abnormal vascular system, characterized by lack of vessel differentiation, increase in volume of the vascular bed, and presence of very wide sinusoidal capillary channels(12-14). It is also probable that a significant amount of tumor blood bypasses the cell mass through arterio-venous anastomoses(13,15). This vascular anomaly probably results in a low blood flow (and reduced oxygen supply), resulting in decreased hemoglobin saturation and a steeper fall in oxygen tension within the capillaries. Hence the mean capillary oxygen tension (and similarly the tissue tension) will be lower. Experiments to be reported later demonstrate that tumor capillary blood in fact has a much lower mean pO_2 than that flowing in the capillaries of normal skin, which is probably near arterial.

As far as the non-malignant skin diseases examined, it has been shown by manometric methods that those lesions associated with inflammatory exudate or acanthosis have an increased aerobic respiration(16,17) while those lacking increase in cellularity have a normal or even reduced respiration(10). It has also been shown histochemically that, at least in psoriasis, the increased oxygen consumption is due to the increase in the number of epidermal cells and to the inflammatory cellular infiltrate(18).

It thus seems proper to conclude that the marked decrease of tissue oxygen tension observed in malignant cutaneous tumors is due to a combination of diminished capillary blood flow and a relative increase in the oxygen consumption of the diseased skin. In benign lesions characterized by acanthosis or cellular infiltration, but without the vascular anomalies of malignant tissue, the relative increase of oxygen consumption appears to be the major factor responsible for lowering tissue pO_2.

The relationship of our observation of a very low tumor tissue pO_2 to Warburg's theory of anoxic causation of carcinogenesis(19) is as yet unclear. If it could be demonstrated that local hypoxia precedes the development of malignant tumors, this hypothesis would be supported. Preliminary studies in our laboratories suggest that vascular changes leading to tissue hypoxia actually may precede chemical tumor induction in mice. However, until such experiments are completed and confirmed, judgment must be withheld.

One immediate application of our observations might be made in the field of radiobiology, since it has been shown that lowering of tissue oxygen tension protects against radiation injury(20). Thus the low tumor pO_2 would afford malignancies some measure of protection against therapeutic irradiation. If our observations can be extended to include noncutaneous malignancies, it appears reasonable that raising the low tissue oxygen tension might make some tumors more sensitive to the effects of radiation. Experiments to evaluate this procedure are now in progress.

Summary. 1. The tissue oxygen tension of a variety of benign and malignant cutaneous tumors was measured *in vivo* by means of a platinum oxygen cathode. 2. Malignant tumors, and benign lesions characterized by marked cellular infiltration or acanthosis, showed a very low oxygen tension as compared to that of the immediately adjacent normal skin. Benign lesions characterized by little or no increase in cellularity showed normal tissue pO_2. 3. The marked decrease of tissue oxygen tension of malignant tumors is probably due to a combination of an abnormality of the vascular system (insufficient oxygen supply) and a relative rise of oxygen consumption (resulting from a marked increase of respiring cell mass as compared to that of the normal skin). In benign cellular lesions, the latter factor is probably of primary importance. 4. The possible relation-

ships of our observations to the hypoxia theory of carcinogenesis and to tumor radiosensitivity are discussed.

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Intestinal Absorption of Unhydrolyzed Tripalmitin.* (22572)

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One of the oldest and most important questions on the mechanism of fat absorption is the degree of absorption, if any, of unhydrolyzed triglycerides(1). Several years ago this laboratory published results of a study of absorption of C^{14} glycerol labeled conjugated trilinolein(2). Only about 60% of the labeled glycerol appeared in the lymph fat. It was concluded, therefore, that at least 40% of ingested triglycerides were completely hydrolyzed. The relative amounts of labeled glycerol in the saturated and unsaturated triglycerides of lymph fat after ingestion of mixtures of labeled fat with unlabeled saturated triglyceride, led to the conclusion that the remaining 60% of the ingested triglycerides were absorbed as monoglycerides.

The design of the above experiment was such that the absorption of relatively small amounts of unhydrolyzed triglycerides could not have been detected. The present study is an effort to determine more accurately if small amounts of unhydrolyzed triglycerides may be absorbed.

Methods. If a mixture of 10% saturated triglyceride and 90% unsaturated triglyceride are completely hydrolyzed and resynthesized by random distribution, the resultant fat will contain 0.1% saturated triglycerides. Any saturated triglycerides in the resultant fat above 0.1% will be equal to the quantity of

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