# Studies in Cancer

## X. Oxidative Capacity of Tumors*

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In previous papers (2, 10) from this laboratory have been reported studies of a series of human and animal neoplasms, concerning biochemical activity in relation to their pathological status. These chemical studies included the cytochrome-oxidase system and succinoxidase activities in tumor slices. Observations were made with the following tumors: sarcoma 180, Yale tumor No. 1, rhabdomyosarcoma, lymphosarcoma $C_3$HED, *p*-dimethylaminoazobenzene hepatoma, and a series of human neoplasms.

The $Q_{O_2}$, that is, the oxygen uptake, of all these tumors was of the same order of magnitude as that of the homologous normal tissues. Their behavior towards paraphenylenediamine and succinate, however, was very different. Whereas the oxygen uptake of the normal tissue slices studied increased definitely, *i. e.*, up to +250 per cent in the presence of the substrates mentioned above, the tumor slices failed to respond or responded very little. This low responsiveness seems to be a characteristic feature of neoplasia. Obviously, the phenomenon may be caused by (a) deficiencies in enzyme systems, or (b) by differences in the permeability to the given substrates (paraphenylenediamine or succinate), or (c) by the loss of a coenzyme factor. To elucidate this point, the behavior of tumor homogenates was compared with the homogenates of homologous tissues. The tissues studied were *p*-dimethylaminoazobenzene hepatoma, rhabdomyosarcoma, lymphosarcoma, Yale tumor No. 1, $V_2$ carcinoma, normal rat liver, and normal rat and mouse leg muscle. Data concerning the behavior of $V_2$ carcinoma slices towards succinate and paraphenylenediamine are also recorded in this paper.

### EXPERIMENTAL METHOD

The biochemical procedure followed in the present investigation was the measure of the oxidative capacity of tissue homogenates in the presence of paraphenylenediamine and succinate. The rate of oxygen uptake was measured at 37° C. The single vessel manometric method of Warburg (16) was used. The test systems used in the experiments to be described were homogenates of tumor or homologous tissue. The homogenates were prepared by grinding the tissues in ice-cold water in a homogenizer. The homogenized suspension was brought to pH 7.3 with phosphate buffer; the final concentration was 0.06*M*. The amount of tissue in the solution was generally 10 per cent for the tumors, 5 to 10 per cent in the case of muscle, and 2.5 to 5 per cent in the case of normal liver. When these concentrations were doubled, the rate of oxygen absorption in the first 30 minutes also was doubled. This fact indicates that the tissue suspension was not too dilute and that the concentration of the substrate was at its optimum.

The final concentration of succinate was 0.018*M*, and that of paraphenylenediamine was 0.009*M*, in the case of hepatoma and of the normal liver homogenates. In the case of the homogenates from other tumors and from muscle, experiments were performed both at the above concentrations and at twice these concentrations. In this latter case there was no increase in the rate of oxygen uptake; indeed, a rather small inhibitory effect was observed.

The materials used were the following: hepatoma produced in Wistar strain rats by means of *p*-dimethylaminoazobenzene; Yale tumor No. 1; rhabdomyosarcoma produced by methylcholanthrene; and lymphosarcoma $C_3$HED. The mouse tumors were propagated in pure strain mice, and $V_2$ carcinoma in brown domestic rabbits. As control tissues, normal rat liver and normal rat and mouse leg muscle were used. In sampling the tumor care was taken to remove all grossly necrotic tissue present.

#### Hepatoma Induced by *p*-Dimethylaminoazobenzene

Rats of the Wistar strain were fed exclusively the diet described by Kensler, Sugiura, and Rhoads (8). This diet contains, per kilogram, 20 cc. of olive oil with 3 per cent of *p*-dimethylaminoazobenzene, mixed with finely ground brown rice. A supplement of fresh carrots was supplied each day.

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It was shown in a previous paper (10) that during the first 50 days the succinoxidase and the cytochrome *c* activities of the livers of rats maintained on this diet remained high and may have even increased somewhat. About the 70th day, however, a rather rapid decline in the activity of each physiological unit set in, and eventually reached the low level characteristic of neoplastic tissues. The first definite tumor appeared on the 163rd day. At that time the succinoxidase and cytochrome system activities had reached the base line. In the present work the evolution of the malignant process was followed by determinations of the succinoxidase and cytochrome *c* system activities of liver slices. The findings were similar to those previously reported (10).

At the 180th day, when definite tumors had developed, the animals were sacrificed and the livers removed rapidly. The liver homogenate was prepared as described above. The final concentration of paraphenylenediamine was 0.009*M*, that of succinate was 0.018*M*. Control experiments were run with homogenized normal rat liver.

TABLE I: RESPONSE BY TISSUE HOMOGENATE TO SUCCINATE AND PARAPHENYLENEDIAMINE. CU. MM. OXYGEN UPTAKE PER MGM. DRY WEIGHT PER HOUR

| No addition | Succinate 0.018*M* | Δ | p-$C_6H_4(NH_2)_2$ 0.009*M* | Δ |
|---|---|---|---|---|
| | | *p*-DIMETHYLAMINOAZOBENZENE HEPATOMA | | |
| 1.8 | 2.6 | 0.8 | 1.9 | 0.1 |
| 1.8 | 5.2 | 3.4 | 1.4 | —0.4 |
| 5.4 | 6.9 | 1.5 | 5.4 | 0.0 |
| 1.4 * | 17.2 | 15.8 | 10.3 | 8.9 |
| | | NORMAL RAT LIVER | | |
| 1.5 | 30.8 | 29.3 | 17.3 | 15.8 |
| 1.0 | 24.6 | 23.6 | 11.6 | 10.6 |
| 2.4 | 24.0 | 21.6 | 12.0 | 9.6 |
| 3.6 | 30.1 | 26.5 | 14.8 | 11.2 |

* Cirrhosis of the liver.

The results are summarized in Table I. They show that homogenates of hepatoma respond very little to the substrates added whereas homogenates of liver present a very definite response. The results in column Δ express the increase in cu. mm. oxygen uptake for 1 mgm. dry tissue in 1 hour. They express merely the activity of the enzymic systems acting upon the added substrates under the given circumstances. In the case marked by an asterisk, in which a high response was noted, the histological diagnosis was cirrhosis of the liver, not cancer. This particular rat was resistant to the carcinogenic agent; the irritation led only to cirrhosis.

## TRANSPLANTED MOUSE TUMORS

Observations were made with the following transplantable mouse tumors: Yale tumor No. 1, rhabdomyosarcoma produced by methylcholanthrene, and lymphosarcoma $C_3$HED. Yale tumor No. 1 was borne by Bar Harbor strain A, and by Strong strain A mice. The lymphosarcoma and the rhabdomyosarcoma were borne by Bar Harbor C3H mice.

The results with rhabdomyosarcoma and lymphosarcoma were contrasted with data from normal leg muscle of the same murine host and with that of the rat. The final concentrations of paraphenylenediamine were 0.009*M* and 0.018*M*, respectively. Those of succinate were 0.018*M* and 0.045*M*. The data (Table II) show that the tumor homogenates exhibited very little response to either substrate. Normal muscle homogenates, under the same conditions, showed a decided response.

## $V_2$ CARCINOMA

This carcinoma, derived from virus-induced papillomas in domestic rabbits, has been described in detail by Rous and Beard (11) and by Rous, Beard, and Kidd (12). In brief, it appeared in the midst of papillomatous growths and is representative of only one type, *i. e.*, the squamous cell carcinoma. The author is much indebted to Doctor Rous for the original tumor.

In Table III are summarized the data concerning the oxidative capacity of $V_2$ carcinoma slices in mammalian Ringer solution containing 0.015*M* phosphate buffer, at pH 7.3 in the presence of paraphenylenediamine and succinate. The concentration of succinate was 0.018*M*; that of paraphenylenediamine was 0.009*M*. As indicated in the table, the response of $V_2$ carcinoma slices to succinate was only +31.5 per cent and to paraphenylenediamine about −8.9 per cent. This low responsiveness, therefore, harmonizes with the findings for other neoplastic lesions (2, 10). The table demonstrates, likewise, that the homogenized tumor shows a low oxidative capacity to both paraphenylenediamine and succinate.

## COMMENT

Several investigators have reported that a deficiency of cytochrome *c* is characteristic of neoplasia (3, 5, 6, 7, 15). Other investigators have reported that several enzymes are poorly represented in tumor tissue. That certain new growths are low in succinic dehydrogenase was first reported by Elliott and Greig (4), and later by Potter (3, 13). Cytochrome oxidase also was reported to be low in tumors by Schneider and Potter (13) and by Shack (14). However, tumor slices possess the same oxygen uptake rate in Ringer solution as do the homologous tissue slices. If succinate or paraphenylenediamine are added, the rate of oxygen uptake by normal tissues increases up to +250 per cent, while the tumor slices fail to respond or respond very little.

TABLE II: RESPONSE BY TISSUE HOMOGENATE TO SUCCINATE AND PARAPHENYLENEDIAMINE. CU. MM. OXYGEN UPTAKE PER MGM. DRY WEIGHT PER HOUR

| No addition | Succinate 0.018*M* | Δ | Succinate 0.045*M* | Δ | p-$C_6H_4(NH_2)_2$ 0.009*M* | Δ | p-$C_6H_4(NH_2)_2$ 0.018*M* | Δ |
|---|---|---|---|---|---|---|---|---|
| | | | | YALE TUMOR | | | | |
| 2.0 | 3.6 | 1.6 | 3.3 | 1.3 | 3.0 | 1.0 | 2.0 | 0.0 |
| 1.6 | 3.8 | 2.2 | 3.0 | 1.4 | 2.7 | 1.1 | 2.7 | 1.1 |
| 1.6 | 4.3 | 2.7 | 4.3 | 2.7 | 2.7 | 1.1 | 2.8 | 1.2 |
| 2.0 | 3.0 | 1.0 | | | | | | |
| | | | | LYMPHOSARCOMA | | | | |
| 3.2 | 4.8 | 1.6 | 4.3 | 1.1 | 2.4 | —0.8 | 1.7 | —1.5 |
| 0.9 | 3.0 | 2.1 | 3.2 | 2.3 | 2.0 | 1.1 | 2.0 | 1.1 |
| 2.5 | 4.5 | 2.0 | 4.1 | 1.6 | 2.0 | —0.5 | 1.7 | —0.8 |
| 3.7 | 4.6 | 0.9 | 4.4 | 0.7 | 0.2 | —3.5 | 0.9 | —2.8 |
| 3.2 | 4.9 | 1.7 | 4.3 | 1.1 | 2.4 | —0.8 | 1.7 | —1.5 |
| | | | | RHABDOMYOSARCOMA | | | | |
| 3.1 | 5.6 | 2.5 | 5.0 | 1.9 | 4.4 | 1.3 | 5.8 | 2.7 |
| 1.1 | 2.8 | 1.7 | 2.5 | 1.4 | 2.7 | 1.6 | 2.3 | 1.2 |
| 3.6 | 5.0 | 1.4 | 4.3 | 0.7 | 3.6 | 0.0 | 3.6 | 0.0 |
| 1.0 | 3.3 | 2.3 | 3.2 | 2.2 | 3.0 | 2.0 | 3.5 | 2.4 |
| 2.2 | 4.6 | 2.4 | | | 2.2 | 0.0 | | |
| 0.7 | 3.3 | 2.6 | | | 1.0 | 0.3 | | |
| 2.9 | 5.4 | 2.5 | | | 2.9 | 0.0 | | |
| | | | | RAT LEG MUSCLE | | | | |
| 2.1 | 11.3 | 9.2 | 11.8 | 9.7 | 5.3 | 3.2 | 7.5 | 5.4 |
| 1.4 | 10.8 | 9.4 | 9.2 | 7.8 | 5.4 | 4.0 | 8.0 | 6.6 |
| 0.8 | 10.6 | 9.8 | 10.5 | 9.7 | 6.0 | 5.2 | 7.7 | 6.9 |
| 1.3 | 9.7 | 8.4 | 8.6 | 7.3 | 4.8 | 3.5 | 3.6 | 2.3 |
| | | | | MOUSE LEG MUSCLE | | | | |
| 1.2 | 9.7 | 8.5 | 9.7 | 8.5 | 5.5 | 4.3 | 6.6 | 5.4 |
| 0.4 | 8.3 | 7.9 | | | 4.3 | 3.9 | 6.3 | 5.9 |
| 0.9 | 9.3 | 8.4 | 9.7 | 8.8 | 5.2 | 4.3 | 7.2 | 6.3 |

TABLE III

RESPONSE BY $V_2$ CARCINOMA SLICES TO SUCCINATE (0.018*M*)

| $Q_{O_2}$ Succ. | $Q_{O_2}$ | Change, % |
|---|---|---|
| 7.05 | 5.65 | +23 |
| 7.50 | 6.40 | +17.2 |
| 4.45 | 2.80 | +58.9 |
| 3.60 | 3.30 | +26.9 |
| 5.65 | 4.53 | +31.5 |

RESPONSE BY $V_2$ CARCINOMA SLICES TO p-$C_6H_4(NH_2)_2$ (0.009*M*)

| $Q_{O_2}$ p-$C_6H_4(NH_2)_2$ | $Q_{O_2}$ | Change, % |
|---|---|---|
| 4.85 | 5.65 | —14.2 |
| 5.30 | 5.30 | —17.2 |
| 3.35 | 3.20 | + 4.7 |
| 4.50 | 4.71 | — 8.90 |

RESPONSE BY $V_2$ CARCINOMA HOMOGENATE TO SUCCINATE AND PARAPHENYLENEDIAMINE. CU. MM. OXYGEN UPTAKE PER MGM. DRY WEIGHT PER HOUR

| No addition | Succinate 0.018*M* | Δ | p-$C_6H_4(NH_2)_2$ | Δ |
|---|---|---|---|---|
| 0.6 | 1.6 | 1.0 | 1.5 | 0.9 |
| 0.7 | 1.4 | 0.7 | 1.5 | 0.8 |

This phenomenon may represent real differences in the enzyme systems, when compared with normal homologous tissue slices. However, when dealing with enzymes contained in tissue slices or intact tissue several facts have to be borne in mind. Failure of response to a substrate may mean lack or deficiency of the enzyme that should act on the given substrate, or impermeability of cell membranes to the given substrate. It may also be interpreted as due to the loss of a coenzyme factor, which could diffuse from the tissue into the solution. The permeability of tumor cells is still discussed. Some authors find the permeability increased (9, 17). In tissue culture, the permeability of normal and sarcomatous fibroblasts for water has been found not to differ (1). These facts might imply that the permeability of tumor cells may be changed for some substances while not for others.

The results presented above indicate that homogenates of tumor tissue exhibit a low responsiveness to succinate and paraphenylenediamine. They behave as do the corresponding tumor slices.

Homologous normal tissue homogenates exhibit the definite response mentioned above to both succinate

and paraphenylenediamine, as shown by the slices of the same tissue. These results suggest, therefore, that the response of slices of tumor or of normal tissue to succinate and paraphenylenediamine reflects the cytochrome oxidase system and succinoxidase activities.

As carried out, the test in these experiments yields an indication of the over-all activity of the entire oxidative systems involved:

cytochrome oxidase → cytochrome *c* → succinic dehydrogenase → succinate

The similar behavior of slices and homogenates shows that, in this particular case, the limiting factor is not the impermeability or the diminished permeability to the substrates mentioned above, or the loss, through diffusion in the surrounding medium, of a coenzyme factor.

## SUMMARY

Comparisons have been made on the oxidative capacity of a series of homogenates from animal neoplasms and the oxidative capacity of homogenates of normal tissues. The chemical studies included cytochrome oxidase system and succinoxidase activities.

The homogenates behave as do the slices, *i. e.*, tumor homogenates show little or no response either to paraphenylenediamine or to succinate, whereas normal tissue homogenates show considerable response to both substrates.

The results indicate that the low response to paraphenylenediamine and succinate is the same in slices as in homogenates; accordingly, it reflects the over-all activity of the respective oxidative systems involved, and is not due to limiting factors such as permeability.

The oxidative capacity of $V_2$ carcinoma slices towards succinate and paraphenylenediamine shows that low responsiveness to these two substrates is a property of this neoplasm.

The oxidative capacity of the homogenate from $V_2$ carcinoma in the presence of the two substrates in question is very low. The homogenate behaves as do the slices.

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