Changes in Oxygen Tension in Yoshida Ascites Hepatoma During Growth.* (32222)

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A fundamental question concerning the metabolism of ascites tumors is whether aerobic or anaerobic conditions prevail in the environment where the ascites cells are living and multiplying, namely, the ascitic plasma. In fact, the belief that the ascites cells are living in the host under conditions near to anaerobiosis is mainly based on indirect data(1,2). In a study on the effect of oxygen on the sensitivity of normal and tumor tissues to alkylating agents, Back and Ambrus(3) measured polarographically the relative changes in oxygen tension (pO_2) in the peritoneal cavity of mice with ascites sarcoma 180 breathing 100% oxygen at various pressures. However, these authors do not report the basal values of tumor pO_2 and do not indicate the age and/or the size of the tumor.

The present work deals with the polarographic measurement of pO_2 in Yoshida ascites hepatoma, and has been undertaken to obtain direct information on the availability of oxygen to the ascites tumors during growth. It was deemed important to use for this investigation a tumor in which glucose availability *in vivo*(4,5) and growth characteristics (5,6) have been already studied.

Materials and methods. The Yoshida ascites hepatoma (AH 130), adapted to Wistar rats, was propagated by injecting intraperitoneally male animals, weighing between 200 and 230 g, with approximately 20×10^6 cells(5,6). Slight anesthesia was produced with 3 to 4 mg of nembutal/100 g body weight intramuscularly and maintained, when necessary, by further injections of 2 mg of the same drug. In a few cases, urethane (150 mg/100 g body weight, given subcutaneously—Ref. 7) or ethyl ether was used instead of nembutal. During the experiment the anesthetized animal was kept in a perspex box which made it possible to change the respiratory atmosphere. To minimize changes of body temperature, the environmental temperature was maintained at 28-30°C. Body temperature, checked by means of a mercury thermometer in the rectum, remained relatively constant during the experiment (maximum changes observed: 37° to 35°C). Generally, the intraperitoneal pO_2 was measured with the oxygen cathode deeply inserted in the lowest part of the abdomen, so that measurements of the rectal temperature provided an estimation of the temperature near the electrode. However, the results were not noticeably affected by placing the electrode in different positions. At the end of the experiment the volume of ascitic fluid and the total cell number were determined. May Grünwald-Giemsa stained smears were also examined.

The pO_2 was measured using either an 'open type' cathode(8-10) and the amplifier previously described(10), or a Clark-type microelectrode (Beckman, 315780) mounted on the Beckman Physiological Gas Analyzer (mod. 160) connected to a recorder. The former apparatus allowed polarographic measurements of pO_2 in the peritoneal cavity even in the absence of ascitic fluid while the latter was better suited to a continuous measurement of pO_2 in the ascitic fluid. The 'open type' cathode was a 300 μ thick Pt-wire, insulated with Araldite and with the end ground flat(9,10) while the anode was a 1 mm thick Ag-wire plated with AgCl, which was inserted subcutaneously. Both the open type and the Clark electrodes were calibrated at 37°C in a constant temperature vessel(10) containing an isotonic buffered saline or ascitic plasma previously equilibrated with gas mixtures containing increasing amounts of oxygen in nitrogen and, in the case of ascitic plasma, 5% CO_2. To calculate pO_2 in the vessel, a correction was applied for vapor pressure of water at 37°C. In a previous work(10) it had been shown that, for

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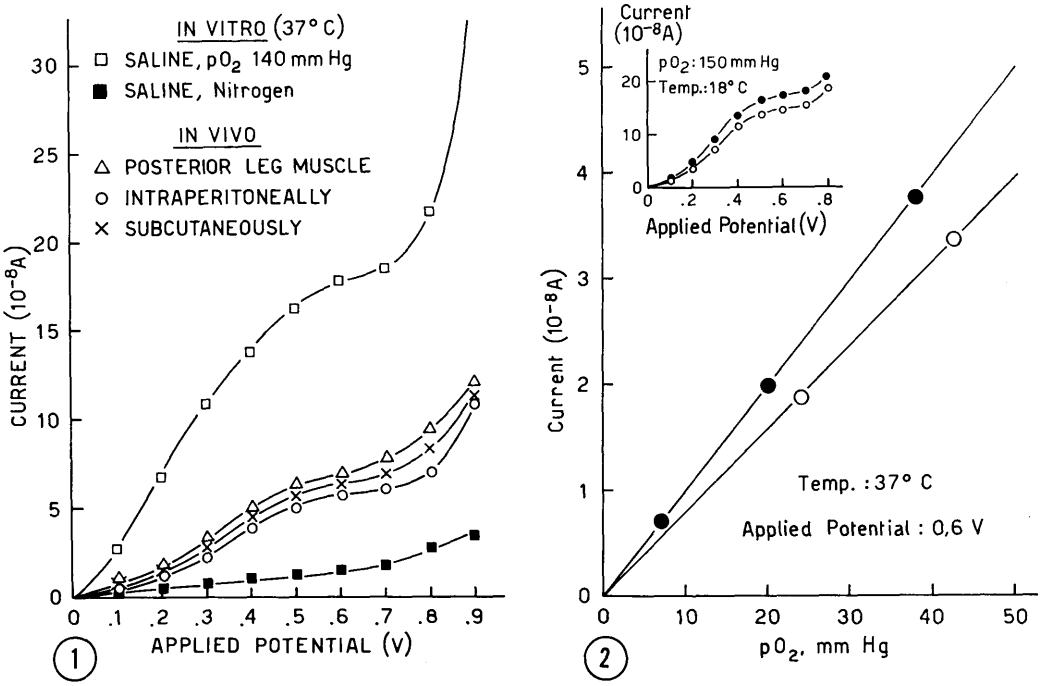

FIG. 1. Current-voltage curves obtained with an 'open type' cathode immersed in oxygenated saline or inserted into normal anesthetized animals. All the measurements were made with the same electrode.

FIG. 2. Calibration curve (large graph) and current-voltage curve (inner figure) of an 'open type' cathode in saline (●) and in ascitic plasma (○). In the calibration at 0.6 V the instrument was adjusted to zero with the electrodes immersed in oxygen-free saline or ascitic plasma.

a given pO_2, the increase of the oxygen current produced by raising the temperature from 2°C to 41.5°C is linear and is approximately 2%/1°C if calculated on the basis of the lower temperature tested (2°C). This value is in close agreement with that reported by others(9,11). For our purposes it is important to emphasize that the oxygen current is considerably higher at 37° than at 2°; thus at 37°C a difference of ± 1°C causes approximately a 1% difference in oxygen current. Therefore, the oxygen current measured *in vivo* has not been corrected for the changes in body temperature as the changes observed were not great enough to affect the interpretation of the results.

Results. Fig. 1 shows typical polarograms obtained either *in vitro* (oxygenated saline) or in normal anesthetized rats using the 'open type' cathode inserted intramuscularly, subcutaneously or intraperitoneally. As the electrodes exhibited a 'dark' current (N_2-current) measurable *in vitro* (Fig. 1, lowest curve), the values obtained both *in vitro* and *in vivo* should be corrected for this dark current to estimate the current due to oxygen reduction. It should be said, however, that the values of the dark current can be decreased considerably by applying the polarization potential for several hours before use, as suggested by Rennie *et al*(12). This was done before calibration at the constant potential of 0.6 V.

Fig. 2 shows that the polarographic current was always lower in ascitic plasma than in saline. As the current measured in saline was not apparently affected by previous working of the electrode in ascitic plasma, the lower values found in ascitic plasma appear to be due to the high protein content of this fluid and not to electrode 'poisoning.' Dead ascites cells (killed with either ethanol-acetic acid(13) or formalin) added *in vitro* up to a concentration of 15-20% did not appreciably affect the current. This finding is consistent with results obtained by

Cater *et al*(9) using blood corpuscles. On the whole, the results legitimated the use of ascitic plasma for calibration.

Experiments were then carried out with anesthetized rats bearing Yoshida ascites hepatoma. Using the 'open' electrode and a polarizing potential of 0.6 V, an attempt was made to measure pO_2 intraperitoneally in animals bearing tumors of large size (about 50 ml, 7 days or more after transplantation). The current measured was always too low to be attributed with certainty to oxygen reduction, even when the animals were breathing pure oxygen instead of air. In some cases, however, by substituting nitrogen for air in the respiratory atmosphere a small decrease of the current could be appreciated, suggesting that, at least in these cases, some oxygen was present in the tumor when the animals were breathing air. Measurements have also been made in animals that, having been freed by a syringe of the bulk of ascites, contained only a small portion (a few ml) of the original large tumor. Under these conditions the tumor pO_2 remained low if the animals were breathing air, whilst it raised markedly when the rats were brought to breathe oxygen.

Further experiments were carried out with tumors of various sizes using the Clark microelectrode. Typical results are presented in Fig. 3. It is apparent that in anesthetized rats bearing small tumors the intraperitoneal pO_2 was well appreciable, although slightly lower than that found in rats without tumor; breathing oxygen instead of air brought to a marked rise of pO_2, while breathing nitrogen caused a drop in pO_2 to values below the starting level (Fig. 3 A). As shown by Fig. 3 B, pO_2 was much lower in tumors of medium size (5 days after transplantation) than in small tumors; but also in tumors of medium size the pO_2 could be greatly increased by breathing oxygen. The results concerning tumors of large size (7 days or more after transplantation) confirmed those obtained with the 'open type' electrode (see above). Fig. 3 C shows that pO_2 was extremely low in tumors of large size and could not be raised by oxygen inhalation for several minutes. As in the case of smaller tumors, the pO_2 of the ascitic fluid remaining

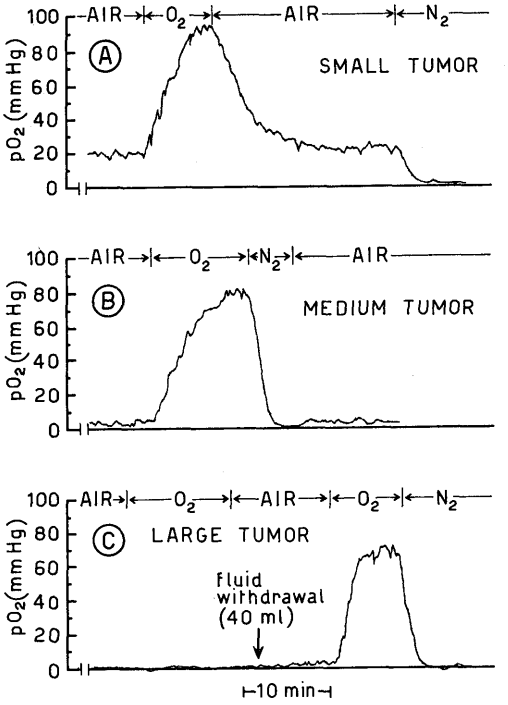

FIG. 3. Effects of the composition of the respiratory atmosphere on PO_2 of tumors of different size. The amplifier (giving directly readings of PO_2 in mm Hg) was calibrated using ascitic plasma equilibrated with gas mixtures of known composition (see Methods). Intraperitoneal PO_2 of normal rats, as measured by the open type electrode (*cf.* Fig. 1) ranged between 30 and 40 mm Hg. A, tumor of 3 days, containing 350×10^6 cells in a volume of about 2 ml. B, tumor of 5 days, containing 800×10^6 cells in a volume of about 6 ml. C, tumor of 8 days, containing $3{,}000 \times 10^9$ cells in a volume of about 50 ml. At the time indicated by the arrow, 40 ml of ascitic fluid were withdrawn with a syringe.

after withdrawal of the bulk of the tumor could be raised and lowered by changing the respiratory atmosphere.

Discussion. The results presented show that during the growth of Yoshida ascites hepatoma the intraperitoneal pO_2 decreases progressively and that, when the tumor has reached a large size, its cells are living under oxygen shortage. Although the lowering of intraperitoneal pO_2 occurs rather early in the life of the ascites tumor, it is apparent that oxygen supply may remain adequate for a longer time, in spite of the increased cell number, as a low pO_2 facilitates oxygen diffusion from blood capillaries of the serosa into the ascitic fluid. The finding that in

tumors of medium size the low pO_2 rises in prompt response to oxygen inhalation is in fact consistent with the view that tumors of this size are not subjected to a substantial oxygen shortage. On the contrary, the lack of response of pO_2 of large tumors to oxygen inhalation clearly demonstrates that in the late stages of tumor life the oxygen supply is far below the amount required to saturate the oxidative capacity of tumor cells. Probably a number of factors cooperate in producing this lack of response of tumor pO_2 to oxygen inhalation. Obviously, a first factor is the increase of cell number, which implies a greater requirement of oxygen. A second factor is the large volume of the ascitic fluid, which dilutes oxygen and may also interfere with its supply by mechanically hindering blood circulation. Finally, some metabolic controls operating in tumor cells may also be of importance in this connection. In particular, if the mutual control between glycolysis and respiration observed in Yoshida ascites hepatoma *in vitro*(14,15) also operates *in vivo*, the respiration of these cells *in vivo* will be inhibited by glycolysis (Crabtree effect) as long as an excess of glucose is available. This is indeed the case of ascites tumors of relatively small size(5). However, in later stages of tumor life, when glucose supply becomes inadequate(4,5), there will be a release of the Crabtree effect and, consequently, a greater disproportion between the amount of oxygen required and that actually available.

Summary. Using polarographic techniques, pO_2 was measured *in vivo* in the peritoneal cavity of adult rats before and after transplantation of Yoshida ascites hepatoma. It was found that the pO_2 in ascites hepatoma undergoes a progressive lowering during the growth of the tumor. While relatively small tumors are not subjected to a substantial oxygen shortage, oxygen supply becomes progressively more inadequate in the late stages of tumor life.

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