

GROWTH AND METASTASIS OF TUMOR IN ORGAN CULTURE

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Deeper study, trying to get a more general view rapidly to more important

A system that would promote the in vitro growth of malignant tumors in their normal solid configuration would be an invaluable aid in the study of factors influencing metastases, cancer chemotherapy and relationships between tumor and host tissue. Such a system would also open studies of functioning tumors and their relationship with circulating stimulatory substances. A system for the perfusion of isolated, small organs was developed in this laboratory as a by-product of an investigation originally designed to test the efficacy of hemoglobin-plasma solutions as a blood substitute for prolonged extracorporeal perfusion. Crystalline hemoglobin in plasma or serum was perfused through isolated organs, (thyroid, kidney, intestine) for several days in a small apparatus using a 50 cc disc oxygenator, and a roller pump designed for flows of 10-50 cc per minute. The present study was begun when it was noticed that a fragment of mouse melanoma grew ~~swiftly~~ ^{rapidly} when it was implanted into a canine thyroid gland being perfused in this apparatus.

During the past year, twelve canine thyroid lobes have been perfused for periods up to one week. Tumor was implanted in eleven of these and growth, invasion, and metastasis of the tumor was observed. Numerous other observations were made which have not been obtained with ordinary tissue culture techniques.

METHODS

Apparatus

The entire perfusion apparatus was built into an analytical balance case, except for the pump and constant temperature bath (Figures 1 and 2).

FIGURE CAPTIONS

Figure 1. Diagram of Perfusion Apparatus. 1) Organ chamber, 2) Relay which allows new hemoglobin-serum solution to enter oxygenator. 3) Relay which drains system at time it is being replenished with new solution. 4) Oxygenator with discs. 5) Oxygenator motor. 6) Rotary tubing pump. 7) Filters. 8) Water jacket. 9) Reservoir used to spray perfusate over surface tumors so that the number of cells shed suddenly into the circulation could then be counted to give a quick measure of cohesiveness. This technique is not yet perfected and therefore was not included in the present report. 10) Stopcock for collecting samples for circulating cell counts. 11) Stock solution of hemoglobin-serum. Cooling coils not shown here. 12) Collection bottle for discarded perfusate. 13) Stopcock for injecting drugs into system. 14) Cotton filter for oxygen inlet. 15) Thermistor. 16) Water circulating motor. 17) Constant Temperature Bath. 18) Adjusting Screw for lowering venous end of organ chamber.

Figure 2. Perfusion Apparatus Pump and water bath are not shown. Flask with cotton, in right upper ~~hand~~ corner is used to direct warm air against organ chamber before each photograph.

Figure 3. 2 Melanoma Implants. Thyroid gland 3 hours after beginning of perfusion, containing 2 new implants about 1 mm each. These have been implanted about 1/3 the way into the depth of the gland, and this photograph demonstrates the excellent visualization produced by transillumination only. The glass at the bottom of the photograph is the floor of the organ chamber, made from a 1000 ml beaker. The superior thyroid artery is on the right.

Figure 4. Circulating Mast Cell Typical appearance of cells being counted in hemocytometer under ordinary light microscopy on the third days of perfusion. The granules are stained with Trypan Blue which was added only a few minutes before this photograph. The mast cell is about 2X the diameter of the erythrocyte above it, and it compares well to the appearance of those mast cells prepared directly from the original tumor.

Figure 5. Mast Cell Implants After 3 1/2 days of perfusion the subcapsular implant on the right (arrow) has rounded out and has reached about 5x its original size. To the left of it (arrow) A deep implant which was originally not ~~visible~~ visible has begun to grow out of the incision and around the suture which entraps it. A large parathyroid adenoma is also on the right.

Figure 6. Melanoma Implant This is a single deep implant of melanoma which by the third day of perfusion has doubled its size. This view illustrates how excellent visualization of a deep tumor may be obtained by transillumination, ~~whereas such a deep tumor could hardly be seen by direct light.~~ whereas such a deep tumor could hardly be seen by direct light. (Compare with Figure 5). Carotid and superior thyroid arteries are on the right.

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