The Technic for Isolation-Perfusion of the Rat Hind Limb

FRANK W. TURNER, D. MICHAEL TOD, GERALD J. FRANCIS, BRIAN J. GREENHILL, AND CECIL M. COUVES

(Department of Surgery, and Surgical-Medical Research Institute, University of Alberta, Edmonton, Alberta, Canada)

SUMMARY

The technic for isolation-perfusion of the rat hind limb is described. By the standardized technic, limb viability was well maintained, and leakage of anticancer agents into the systemic circulation was negligible. The gross and microscopic response of the Walker 256 carcinosarcoma to perfusion with triethylenethiophosphoramide is described. The preparation permits study of the many variables related to perfusion. The technic is adaptable to other small animals.

In 1957 Creech and his associates introduced the technic of isolation and perfusion of regionally confined neoplasms in an attempt to administer high concentrations of anticancer agents to a tumor-bearing area while protecting susceptible normal tissues (1). This technic is now well established as an adjunct to surgery but is attended with a varying tumor response in humans (2, 4, 9, 11).

Most experimental studies relating to isolation and perfusion have been performed on dogs (5, 8, 10). The dog is satisfactory for studying the mechanics of perfusion and for assessing expected normal tissue tolerance to high dosages of anticancer agents under the conditions of perfusion. Isolation and perfusion of regionally confined tumors in dogs are not convenient because of the lack of naturally occurring or transplantable dog tumors. For this reason we became interested in developing a technic for isolation-perfusion of the rat hind limb bearing the Walker 256 carcinosarcoma; the technic for the rat will be described.

MATERIALS AND METHODS

Rats.—Male Sprague-Dawley rats weighing from 250 to 300 gm. were used. The Walker 256 carcinosarcoma was transplanted into the calf musculature of the right hind limb. Perfusion was possible up to the 10th day after inoculation of the tumor; thereafter the size of the tumor made cannulation difficult.

*This work was supported by Grant No. 248 from the National Cancer Institute of Canada.

Preparation of rats.—Rats were anesthetized with intraperitoneal pentobarbital sodium, 55 mg/

Received for publication July 18, 1961.
The tumor-bearing limb was prepared by being shaved and painted with 2½ per cent tincture of iodine. A long groin incision was made, and the femoral vessels were exposed; the femoral artery was ligated proximally and cannulated in a distal direction, a light 4-0 silk ligature being used to hold the cannula in the artery. The rat was then heparinized through the arterial cannula with 1 mg. heparin, and the cannula was filled with a dilute heparin solution (10 mg. heparin in 10 cc. sterile isotonic saline) and clamped. The femoral vein was then cannulated in a similar manner. A tourniquet, consisting of an ordinary rubber band, was applied around the limb passing beneath the femoral vessels and through separate stab wounds at either end of the main incision. It was anchored to the inguinal ligament with a single 000 silk suture.

Procedure.—The combined blood reservoir, oxygenator, and debubbler was filled with fresh heparinized blood obtained by aspiration of 10–12 cc. of blood from the aorta of a donor rat into a syringe containing 10 mg. heparin. The pump was started to fill the arterial line; the cannulated rat was then connected to the perfusion circuit, the tourniquet tightened by applying small forceps, and perfusion commenced.

In the standard perfusion a flow rate of 0.5 cc/min was maintained, and perfusion was continued for 15 minutes. The temperature in the arterial cannula was maintained at 37° C. The chemotherapeutic agent was introduced at a prepump level to obviate streaming and was administered in equally divided doses at 0, 5, and 10 minutes. At the conclusion of perfusion the pump was stopped, the tourniquet released, and the cannulae removed; the femoral vessels were ligated, and the wound was closed in layers with interrupted 000 silk sutures. Disposable parts (plastic and rubber) were replaced after each perfusion; other parts were cleaned thoroughly with water, aqueous zephiran, and sterile isotonic saline between each perfusion.

DISCUSSION AND RESULTS

In the course of standardization of the technic and in related investigative work some 800 perfusions were completed. Several factors emerged as being of great importance in preserving a viable limb. Although the use of aseptic technic was mandatory, of equal importance was absolute cleanliness of all equipment, since even the smallest particles of foreign material acted as emboli and resulted in gangrene of the perfused limb; similarly, silicone embolization occurred if excess silicone was left in the debubbler. The venous return was the best indicator of the adequacy of the perfusion and required constant observation. If difficulty was encountered in establishing a good venous return (greater than 0.4 cc/min) the procedure was abandoned, since gangrene almost inevitably resulted. Over-perfusion could be prevented by making connections which were a good fit but not excessively tight; in the event of incipient over-perfusion the pressure build-up caused the connections to "blow."

![Diagrammatic representation of perfusion apparatus.](chart1)

A. Blood reservoir, oxygenator, and debubbler.
B. Sigamotor pump.
C. Heat exchanger.
D. Femoral artery and vein cannulated with thermometer in arterial line. Tourniquet in position deep to femoral vessels.
By this technic studies undertaken to date have largely related to standardization of the method; these have included studies of flow rates, perfusate temperature, limb viability, leakage, tumor response and metastatic spread, animal survival, and the histopathology of perfused Walker 256 tumors. In all these studies the chemotherapeutic agent used was triethylenethiophosphoramide (TSPA) at a dosage of 4 mg/kg body weight.

**Flow rates.**—Flow rates above 1.0 cc/min were unsuccessful because of obvious over-perfusion; flow rates above 3.0 cc/min uniformly resulted in destroyed limbs, and the immediate tumor response was no greater than with a physiologic flow. A flow-rate of 0.5 cc/min is now regarded as optimal.

**Perfusate temperature.**—Perfusions were performed with arterial line temperatures of 20°, 37°, and 42° C. It was found that with the low flow rates used the temperature of the tumor was not significantly altered, even after perfusing for 30 minutes. Limb viability was not affected.

**Limb viability.**—Using the standardized technic described above limb destruction was negligible. In the last 200 consecutive perfusions, three limbs developed minimal gangrene, and in no case did this involve more than the tips of two toes.

**Leakage.**—Studies with radioactive iodinated serum albumin indicated virtually complete isolation of the limb over the 15-minute period of the perfusion.

**Animal survival and tumor response.**—The effect of perfusion of the Walker 256 tumor with TSPA under standard conditions, as measured by survival time of the rat, is shown in Table 1. These figures were obtained from an unselected group of rats. At the 10th day the number of these small cells had greatly increased, and numerous large irregularly shaped cells with bizarre nuclei had appeared which were thought to represent a residual radiomimetic effect.

**Histopathology.**—Microscopic studies were performed to determine the post-perfusion condition of the rat limb. No changes were seen in the leg muscles in specimens examined from 5 days to 2½ months after perfusion.

Changes in the histology of the tumor resulting from perfusion with TSPA were also followed over the 10 days immediately after perfusion. Twenty-four hours after perfusion details of nuclear structure became blurred in all except a few cells, and there was a marked tendency for cells to adopt a fusiform shape; mitotic figures, normally plentiful, became difficult to find. At 48 hours the nuclear changes due to the radiomimetic effects of TSPA were clearly seen, consisting mainly of abnormal residua of mitosis and clumping of chromatins; there was considerable inter- and intracellular edema. By the 6th day it was difficult to identify tumor cells, although a few small cells with dark-staining nuclei occasionally in mitosis were apparent. At the 10th day the number of these small cells had greatly increased, and numerous large irregularly shaped cells with bizarre nuclei had appeared which were thought to represent a residual radiomimetic effect.

**Isolation-perfusion of the rat hind limb would seem to offer many advantages in the investigation of perfusion technics; it is a simple and relatively cheap procedure which permits study of the effect of many variables in terms of the response of a well characterized anaplastic tumor.** It would also seem to be of value in assaying new anticancer agents. It is presently being used in this laboratory for further studies on perfusate temperature, pH, and oxygen tension. The technic is adaptable, and successful perfusions have been performed on the hind limb of hamsters weighing as little as 80 gm.

**ACKNOWLEDGMENT**

The authors wish to thank Mr. Alex Gecey for his invaluable technical assistance.
REFERENCES

The Technic for Isolation-Perfusion of the Rat Hind Limb


Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/22/1_Part_1/49

Sign up to receive free e-mail alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.