[CANCER RESEARCH 45, 2478–2480, June 1985]

# Vascular Resistance Characteristics of 7,12-Dimethylbenz(*a*)anthracene-induced Rat Mammary Tumors and Normal Tissues as Studied *in Vitro*[1]

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## ABSTRACT

Vascular perfusion characteristics have been studied in dimethylbenz(a)anthracene-induced rat mammary neoplasia and compared with those of skin, skeletal muscle, salivary gland, kidney, spleen, uterus, and brain by means of an artificial perfusion technique. Perfusion of tissues and organs was measured by the microsphere tracer technique. This procedure makes possible a detailed hemodynamic analysis of several tissues under controlled conditions, in this study maximum vascular relaxation, without confounding endogenous vasoregulation.

The maximal perfusion capacity, *i.e.*, during smooth muscle relaxation, of tumors and various tissues was related to perfusion pressure at three levels by means of three differently labeled microspheres. Tumors, especially large ones, have a low maximum perfusion capacity, *i.e.*, high vascular resistance, compared to most other tissues. For the tumors, a relatively high perfusion pressure is required to open up the otherwise collapsed vascular network.

## INTRODUCTION

Contradictory results on total blood flow and vascular reactivity of tumor tissue as comapred to various normal tissues can be ascribed partly to the use of different tumor models and methods of analysis (*cf.* Ref. 1). Most studies are, however, performed *in vivo*, either during anesthesia or under conscious conditions. The central and peripheral hemodynamics thus vary greatly (14), complicating the interpretation of data obtained. The vascular tone in various organs and tissues also varies greatly within the same animal at the time of experimentation, and no base-line vascular tone to which the tumor vasculature can be related can be achieved.

An *in vitro* perfusion technique was therefore developed as a further elaboration of the procedure described by Folkow *et al.* (2). In light of recently published data on blood flow in 7,12-dimethylbenz(*a*)anthracene-induced mammary tumor of the rat (5) and findings of an increased interstitial fluid pressure within these tumors (11), it was considered of interest to elucidate the maximal perfusion capacities in these tumors in relation to various perfusion pressures and to compare these results with findings in various normal tissues.

## MATERIALS AND METHODS

**Tumor Model.** Female Sprague-Dawley rats (Anticimex, Stockholm, Sweden), 50 to 55 days old, were fed by gavage with 7,12-dimethylbenz(*a*)anthracene, 16 mg dissolved in 1 ml of olive oil (4, 13). Multiple mammary tumors became overt from the sixth week after induction, and experiments were performed 4 weeks later.

[1] This investigation was supported by grants from the Swedish Cancer Society (1193-B83-05X) and the Swedish Medical Research Council (B84-12X-06820-01A).

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Received 10/3/84; revised 2/25/85; accepted 3/5/85.

**Perfusion Technique.** The rats were anesthetized with Nembutal (50 mg/kg body weight i.p.). The abdomen was opened in the midline by cauterization, the stomach and intestines were removed, and the vessels and cut ends of viscera were carefully ligated. The thorax was cut open by cauterization, and the aortic root was rapidly connected to a pump system. The right cardiac ventricle was cut open, a cannula was inserted for free outflow, and the perfusion was started. The caudal and one femoral artery were cannulated for measurement of pressure and reference flow, respectively. The femoral vein was cannulated for measurement of venous pressure. Pressures were continuously recorded on a Grass polygraph. The oxygenated perfusate, kept at 38°C, consisted of 6% dextran (Macrodex; AB Pharmacia, Uppsala, Sweden; mean $M_r$ 70,000) and 100 ml of horse serum (normal serum; SBL, Stockholm, Sweden) in 1,000 ml of a salt solution [$Na^+$, 143 mM; $K^+$, 4.3 mM; $Ca^{2+}$, 2.5 mM; $Mg^{2+}$ 0.83 mM; $Cl^-$, 141 mM; $HCO_3^-$; 13.3 mM; $H_2PO_4^-$, 0.46 mM; and glucose, 5.6 mM (9)]. The perfusion system is illustrated in Chart 1. The pump used was a peristaltic constant-flow type Ismatec MP 4. To monitor possible fluid retention, the animal was placed on a balance throughout the experiment. The temperature of the preparation was kept constant at 37°C by a heating lamp mastered from a rectal thermistor. Papaverine was used to obtain maximal smooth muscle relaxation. When no further relaxation could be obtained by repeated papaverine injection, the flow was set to various levels, and arterial and venous pressures were measured. In this way, a flow-pressure relationship was obtained for the whole preparation.

**Regional Blood Flow Determination.** At 3 different flow-pressure levels, regional blood flow was determined by injection of polystyrene spheres (3M Co., St. Paul, MN), with diameters of 15 ± 3 $\mu$m (SD), into the tubing connected to the aortic root. The spheres were labeled with $^{141}Ce$, $^{85}Sr$, and $^{51}Cr$, respectively, and were given approximately 150,000 at a time. During and immediately after microsphere injection (90 s), a reference perfusate sample was drawn from the femoral artery at a rate of 0.3 ml/min.

After the perfusion experiment, the tumors and parts of the quadriceps muscle, the paw, spleen, brain, salivary gland, uterus, and kidneys were dissected out, weighed, and placed in vials for activity measurement in a Packard Auto-Gamma spectrometer Model 5019. From the activity in the reference samples, perfusate flow in the tissue samples could be calculated (6).

Care was taken to perform the experiments in a standardized way and as rapidly as possible, since prolongation leads to edema and increased venous pressure. Experiments exhibiting such artifacts were rejected.

**Statistics.** Analyses were performed according to Student's *t* test using a pairing design. Significant differences from the tumor pressure-flow characteristics are indicated by * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) in Chart 1.

## RESULTS

Six successful perfusion experiments were performed, but a larger number of experiments were rejected due to technical failures resulting in edema, increased venous pressure, or uneven distribution of microspheres between the 2 kidneys. In the 6 animals, 24 tumors were analyzed, with a mean weight of 2.9 g. The perfusate flow to the animals was set to produce an initial perfusion pressure (arterial pressure minus venous pressure) of 13.4 ± 0.5 mm Hg, an intermediate pressure of 24.9 ± 0.7 mm Hg, and a final pressure of 34.2 ± 1.6 mm Hg. Absolute perfusate flow data were obtained for each of the above-mentioned tissues in each animal for each of the 3 pressure levels. Pressure-flow data are summarized in Chart 2, from which it will be seen that the flow capacity of tumors is low compared to most other tissues.

Organ vascular resistance is calculated by dividing the perfusion pressure by the perfusate flow, and this measure is considered to represent the functional state of the vascular beds in organs and tissues more adequately. It will be seen from Chart 3 that the vascular resistance is high in tumors and that the resistance seems to decrease more rapidly with increased perfusion pressure in tumors than in most other organs. Another way to express this concept is to present the relative increase in assumed cross-sectional area of the vascular beds, this area being inverse to the square root of the vascular resistance according to Poiseuille's law. These data, related to distending pressure (the mean of the arterial and venous pressure), are presented in Chart 4, from which it will be seen that the cross-sectional area of the tumor vascular bed increases more rapidly than in other tissues.

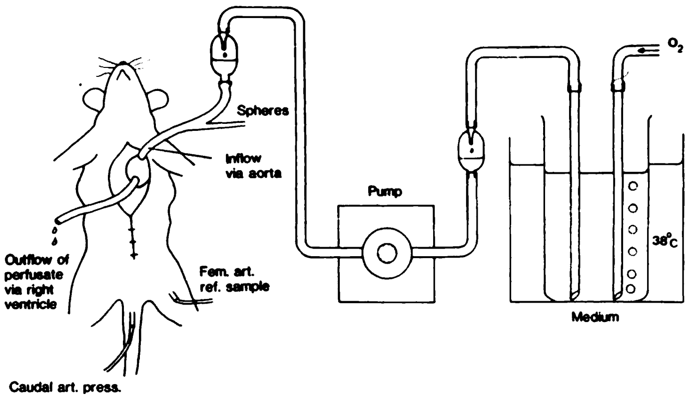


Chart 1. The perfusion set-up. *Fem.*, femoral; *art.*, artery; *ref.*, reference; *press.*, pressure.

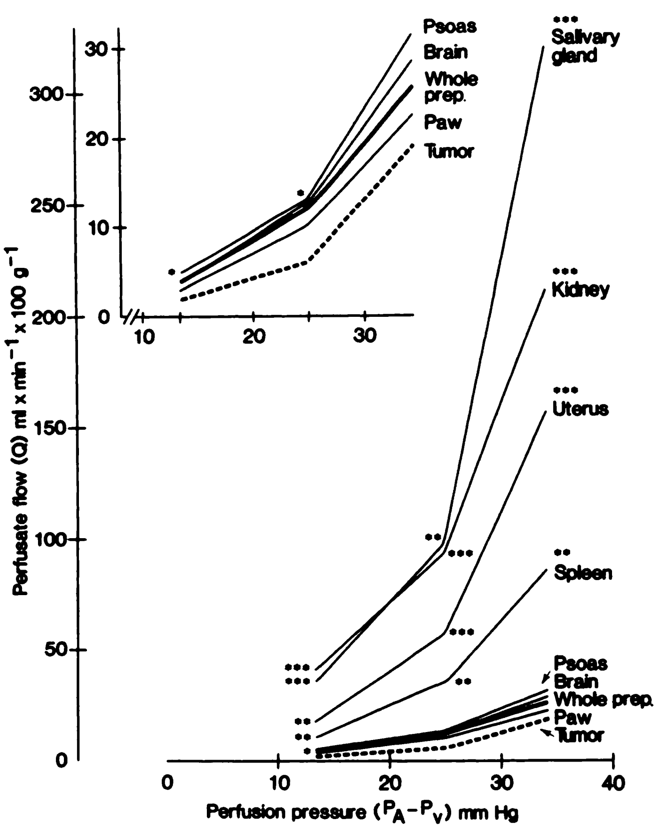


Chart 2. Relationship between perfusion pressure and perfusate flow in various tissues. The *ordinate* is expanded in the *inset* to visualize the low-flow tissues. *, differences significantly different from the tumor graph. For details, see "Materials and Methods." *prep.*, preparation.

## DISCUSSION

This study was undertaken to elucidate 2 aspects of tumor vascular functional morphology and physiology not possible to study in the living animals, *i.e.*, the maximal perfusion capacity during standardized maximal vasodilation of all vascular beds representing the available capillary cross-sectional area and the characteristics of the pressure-flow curves at low perfusion pressures.

*In vivo*, the various regional vascular beds are under undefined and different vasoconstrictor tone, even under so-called resting conditions in the conscious or anesthetized animal. This makes reliable estimation of the blood flow capacity in various tissues under identical vascular constriction, and thus the relative available cross-sectional area of the vascular beds in various tissues, impossible.

An *in vitro* perfusion model was therefore considered useful for the present purposes. A similar perfusion system was used to characterize the vascular bed of rat hind quarters (2). However, only bulk flow as derived from the pump settings was

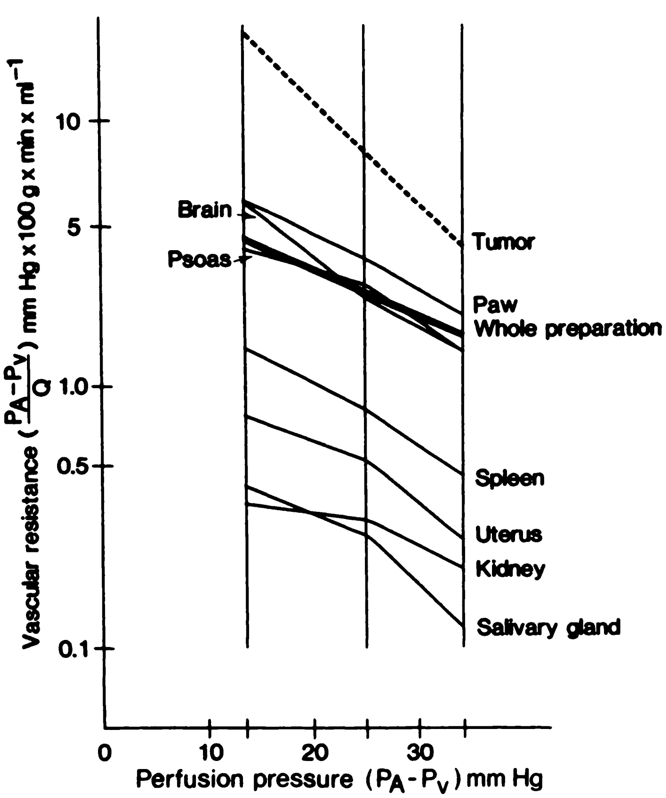


Chart 3. Vascular resistance on a log scale related to perfusion pressure. Significant differences are identical to those in Chart 1.

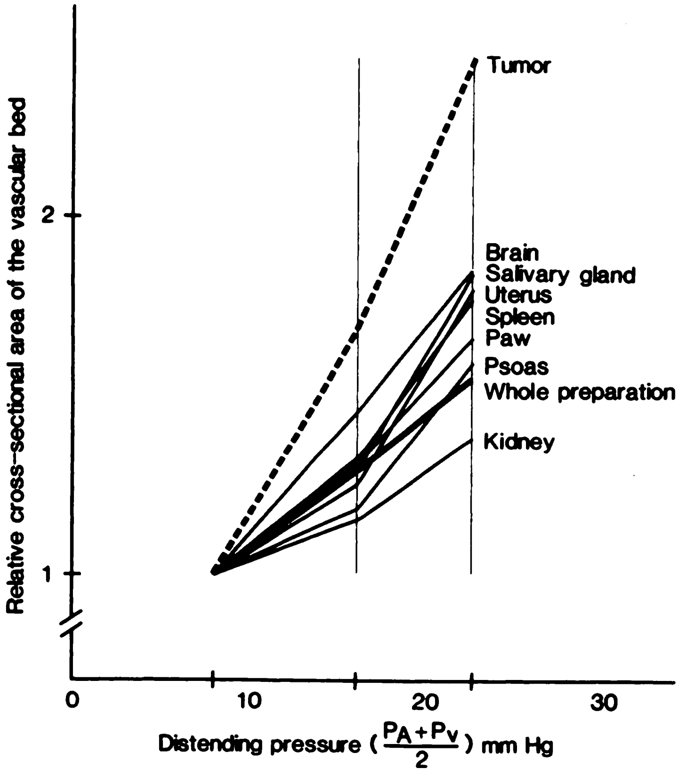


Chart 4. Relationship between cross-sectional vascular area and distending pressure in various tissues. At the lowest perfusion pressure, all areas have been normalized to unity.

recorded, giving information on pressure flow characteristics of the whole preparation but not on regional perfusion. It was therefore decided to combine the perfusion procedure with the labeled microsphere technique generally used in the *in vivo* situation. This combination opens up possibilities of obtaining pressure-flow curves for an unlimited number of tissues but has certain practical limitations. Due to a tendency towards edema formation, perfusion experiments should be completed within a short time; otherwise, the vascular resistance will gradually increase, especially at high perfusion pressures. The venous outlet must not be compromised; otherwise, the venous pressure will increase.

The microsphere tracer technique involves some complications when used in this system. Since the aortic root is cannulated, there is a risk of insufficient mixing of spheres within the perfusate, especially at low flow rates. Extremely low perfusate flow rates therefore cannot be used, and a perfusion pressure between 10 and 15 mm Hg was considered the lower limit. Even mixing of spheres was ensured by rejecting experiments with more than 20% difference in perfusate flow between the 2 kidneys. The number of spheres used in the successive injections should be limited since vascular plugging will otherwise give rise to increased vascular resistance. It seems that the *in vitro* situation is more susceptible to this artifact than is the *in vivo* situation.[3] The number of observations along the pressure-flow curve will therefore be limited in this work to 3 points: a lowest pressure limited to 13.4 mm Hg, for reasons stated above; an intermediate pressure of 24.9 mm Hg; and an upper pressure of 34.2 mm Hg. The latter may seem low, but it should be realized that maximal vasodilation is rarely present under *in vivo* conditions, thus exposing the capillary bed to this pressure in full. The maximal perfusion capacity at any perfusion pressure is low for tumor tissue. *In vivo*, measurement of resting blood flow in this animal model (5) showed comparatively high flow values in tumor tissue compared to various other tissues. This indicates that the tumor vascular bed is probably maximally dilated under resting conditions, in contrast to most other vascular beds. The unrestrained exposure of the tumor capillary bed to a high blood perfusion pressure might result in edema and increased interstitial fluid pressure, which was actually recorded in these tumors (11). Recently published findings of decreasing interstitial fluid pressure within these tumors upon noradrenaline infusion further substantiate this concept (10).

The curves in Chart 4, showing the relative cross-sectional area of the vascular beds in relation to distending pressure show that this area increases more rapidly for a certain increment in distending pressure in tumor tissue than in the other vascular beds studied. This finding can probably be attributed to an extravascular, interstitial tissue pressure counteracting the intravascular distending pressure. Thus, the "critical closing pressure" (7), not studied here due to technical difficulties in measuring perfusate flow at perfusion pressures below 13 mm Hg, is probably higher in tumor tissue than elsewhere. This finding indicates an impact on vascular perfusion of the increased interstitial fluid pressure recently recorded in these tumors (11) as well as in other tumors (3, 8). The hypothesis of a "compartment syndrome" taking part in the development of tumor hyponutrition and necrosis is substantiated.

[3] Unpublished observations.

## ACKNOWLEDGMENTS

We are indebted to Iris Jansson for excellent technical assistance.

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*Cancer Res* 1985;45:2478-2480.