Experimental Factors Influencing Hepatic Metastases. XII. Effect of Increased Arterial Blood Flow.* (27969)

SIL H. LEE, BERNARD FISHER AND EDWIN R. FISHER
Departments of Surgery, Laboratory of Surgical Research, and Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

In evaluating a variety of factors which might influence the "take" and growth of experimentally induced hepatic metastases, information was obtained concerning the effect of alteration of hepatic blood flow and oxygen supply by vena cava constriction, hepatic artery ligation and portacaval shunt. It was reported(1) that an enhanced incidence and growth of tumors took place in livers that were deprived of arterial blood or were congested as a result of portacaval occlusion, whereas portacaval shunt, though decreasing hepatic blood flow, produced no such effect. While these results suggested that a decreased oxygen supply to the liver promoted development of metastases, it was concluded that the demonstrable liver damage subsequent to alteration of hepatic blood and oxygen supply was probably the responsible factor rather than the decreased oxygen supply *per se* to that organ and/or the tumor. Since publication of that report a technic for increasing arterial blood flow through the rat liver has been developed in this laboratory. This afforded us the opportunity to evaluate the effect of *increasing* the supply of oxygen to the liver (and tumor) upon development of metastases and, consequently, the thesis of Willis(2) that the susceptibility of the liver to metastasis, when compared to other organs, might be due to its poor oxygenation.

Materials and methods. Sprague-Dawley† male rats weighing 330-360 g were housed in individual cages and permitted water and a standard laboratory chow diet *ad libitum.*

Animals whose livers were to be arterialized (Fig. 1) were subjected to a side-to-side portacaval anastomosis employing No. 7-0 continuous silk sutures in a fashion similar to that employed in the human. Ligation of the vena cava between the shunt and the liver resulted in production of a reverse Eck fistula. One week later animals were reoperated upon, tumor cells were injected into the liver *via* the portal vein and increased hepatic arterial flow was accomplished by creation of an arterio-venous fistula between the aorta and vena cava distal to the renal arteries. The latter was accomplished as previously described in detail(3) by producing a 1 to 2 mm longitudinal incision in the anterior wall of the vena cava so that the wall between it and the aorta was identified. Using a small scissors, a 1.5 mm elliptical opening was created between aorta and vena cava. The opening in the anterior wall of the cava was then closed. Following removal of the occlusive clamp the arterial blood could be seen flowing through the fistula.

Control animals were subjected to a sham operation at the time the reverse Eck fistula was produced in the experimental group as well as to a sham arterio-venous fistula following injection of tumor. All operative procedures were performed under light ether anesthesia.

The tumor utilized was the Walker-256 carcinoma. Preparation of tumor cell suspensions and the technic of intraportal injection were identical with those used previously(4). Ten thousand tumor cells contained in ½ ml of a saline solution were injected intraportally *via* a large mesenteric vein. Experiments were devised so that both control and experimental animals were injected with the same tumor cell suspension.

Serum glutamic pyruvic (SGP-T) and oxalacetic (SGO-T) transaminases were measured colorimetrically by the Reitman and Frankel(5) modification of the method described by Wroblewski and Cabaud(6), and values are expressed as sigma-Frankel units.

At sacrifice, 14 days after tumor cell injec-

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† Holtzman Farms, Madison, Wisc.

TABLE I. Effect of Liver Arterialization Upon Hepatic Metastases and Serum Transaminase.

	No. Rats	Metastases No. +	No. —	% +	Grade of tumor growth +1	+2	+3	Serum transaminase* SGO	SGP
Arterialized	40	35	5	88	21	13	1	226 ± 55	61 ± 91
Sham control	43	12	31	28	10	2	0	95 ± 20	13 ± 4

* Sigma-Frankel units.

tion, the patency of all anastomoses was confirmed to assure that livers had been arterialized. That such livers were the recipients of an increased arterial blood flow was evident from gross examination. Livers appeared larger and pinker than controls and possessed the rounded edge and general appearance which was previously observed in dogs subjected to this type of procedure(7). Livers were examined for gross evidence of tumor and metastases were arbitrarily graded as either +1, +2 or +3, depending upon their number and size.

Results. At sacrifice, 14 days after tumor cell injection, hepatic metastases were observed in 35 (88%) of 40 animals with arterialized livers (Table I). Only 12 (28%) of 43 sham operated controls demonstrated tumor. Moreover, 14 (40%) of the tumors were graded +2 or +3 in the former group, whereas only 2 (17%) of the tumor-bearing sham control animals had metastases of such magnitude.

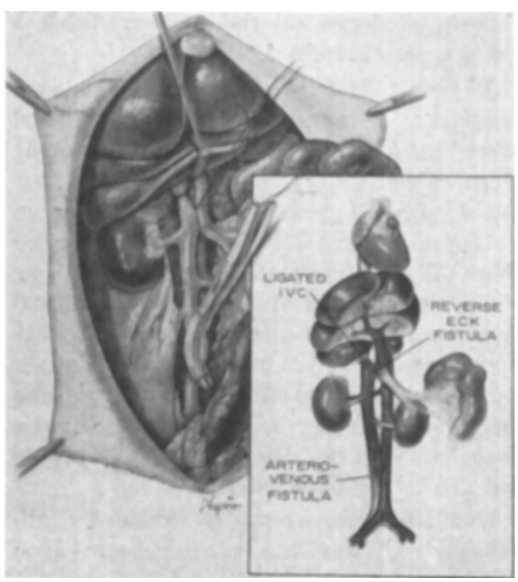

FIG. 1. Arterialization of the liver.

Interference with the normal hepatic blood flow resulted in a significant increase in both SGO-T and SGP-T. At 48 hours after injection of tumor cells and arterialization the SGO-T and the SGP-T were 226 ± 55 and 61 ± 91 units, respectively, whereas these values in unarterialized sham operated controls were 95 ± 20 and 13 ± 4 units, respectively.

Discussion. The increased incidence and growth of hepatic metastases following increased hepatic oxygenation in this study are comparable to that noted with the converse state induced by vena caval constriction and hepatic artery ligation. As with these latter, hepatic arterialization resulted in significant elevation of serum SGOT and SGPT activities which, in the absence of overt alteration in other organs, strongly suggests the presence of hepatic damage. Production of hepatic damage by various modalities has been demonstrated previously to augment hepatic metastases in this experimental model. Recognition of comparable effects on hepatic metastases attendant with increased or decreased hepatic oxygenation makes it unlikely that the results obtained following arterialization are due to an effect on the tumor cells *per se*. These considerations appear to invalidate the popular concept which relates the marked susceptibility of the liver to metastases to its relatively poor oxygenation.

Summary and conclusions. Previous studies demonstrated that a reduction in arterial blood supply to the liver resulted in an augmentation of experimentally induced hepatic metastases. It was concluded that the alteration of the "soil," *i.e.*, liver damage, subsequent to alteration of hepatic blood and oxygen supply was responsible. From the reported experiments it was demonstrated that following an increase in arterial blood supply to the liver *via* arterialization of that organ a

similar increase in "take" and growth of tumor occurs which likewise may be related to hepatic trauma. From these observations it would seem unlikely that the susceptibility of the liver to metastases, when compared with other organs, might be due, as has been proposed, to its relatively poor oxygen supply.

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Effect of a Low Calcium Diet on Bone Crystallinity and Skeletal Uptake of Ca^{45} in Rats.* (27970)

J. MENCZEL,† R. SCHRAER,‡ G. PAKIS, A. S. POSNER AND R. C. LIKINS

National Institute of Dental Research, National Institutes of Health, Public Health Service, U. S. Dept. of HEW, Bethesda, Md.

It has been suggested that primary osteoporosis is due to increased bone resorption which produces a negative calcium balance (1-4). Radiotracer kinetic studies in humans indicate that in this condition the rate of bone formation is not impaired(5-7). In rats made osteoporotic through administration of a calcium deficient diet containing an adequate amount of vitamin D, the skeletal uptake of injected stable strontium was found to be increased and it was concluded that bone formation was abnormally rapid (8).

Several studies have shown that the calcium balance in osteoporosis can be improved by increasing calcium intake(2,9,10). A similar effect was observed following administration of sodium fluoride to osteoporotic patients(11,12). There is also an indication that the prolonged ingestion of 8 ppm fluoride in drinking water may have a beneficial effect in counteracting osteoporotic changes in humans(13). Since it was shown that the crystallinity of bone apatite improves with an increase of fluoride content(14,15,16), it is possible that the kinetics of bone resorption is affected by fluoride administration.

The purpose of the present study was to investigate the effect of a low calcium diet on calcium turnover in relation to bone crystallinity. The effect of fluoride on the bone changes produced by the calcium deficient diet was also examined.

Methods. Male Sprague-Dawley rats were assigned at 35 days of age to 2 groups and offered *ad libitum* a low fluoride (<1 ppm) prepared diet(17,18) and drinking water containing either 0 ppm F (Group I) or 50 ppm F (Group II) for 320 days. All were then given 0.5 ml of a solution containing 20 mc of Ca^{45} and 1.5 μg of Ca by intraperitoneal injection and killed after one hour. At sacrifice approximately 5.0 ml of blood were obtained by cardiac puncture from each rat and the plasma was analyzed for radiocalcium by methods previously described(19). One tibia and one femur were removed.

Each tibia was arbitrarily sectioned to divide the ends from the cylindrical portion of the shaft, the ends were pooled and with the

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† NIH Visiting Scientist from Hebrew Univ. and Hadassah Med. School, Jerusalem, Israel.

‡ Biophysics Laboratory, Dept. of Physics, Pennsylvania State University, University Park, Pa.