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Blood Supply to Hepatic V2 Carcinoma Implants as Measured by Radioactive Microspheres.‡ (29876)

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If intravascular introduction of chemotherapeutic or radioactive materials is to be effective in tumor therapy, the source of blood nourishing metastatic tumors in the liver must be known. Utilizing angiographic techniques in living patients, Bierman(1) has shown that malignant metastatic hepatic deposits derive their blood supply from the hepatic artery. Gans(2) has reached the same conclusion from injection corrosion studies of human livers with metastatic tumors obtained at autopsy. Since V2 carcinoma is used as an experimental model to evaluate effect on tumor growth of agents introduced *via* the vascular route, it is important that the source of blood vessels to this tumor be known.

Materials and methods. Mature virgin female New Zealand rabbits were utilized. A suspension of V2 carcinoma cells obtained fresh from donor rabbits was prepared by mincing in a tissue grinder, adding Tis-U-Sol* at 5°C and diluting to a concentration of 500,000 cells per millilitre. The tumor strain was maintained by injection of tumor cells into the thighs of new donor rabbits each week. Within 2 hours of the time of tumor harvest from the donor, laparotomy under pentobarbital† anesthesia, (25 mg/kg) was carried out in each animal to be studied and one ml of tumor suspension was injected into the appendiceal vein which was then ligated. Fourteen to 19 days later laparotomy was again performed and the livers of rabbits inspected. Those with gross hepatic implants of V2 carcinoma were utilized for microsphere injection and those without obvious tumors were discarded. Forty-two rabbits with obvious metastatic tumors comprised the present study.

Ceramic or plastic microspheres 15 ($\pm$ 3) μ in diameter containing Scandium-46 in tracer doses, 2 microcuries/mg of spheres, were suspended in a 15% solution of low molecular weight dextran giving 2 mg spheres per millilitre dextran. The specific gravity of plastic spheres was 1.5 and that of ceramic spheres 3.0. Occasional clumps of spheres in the dextran were dispersed by exposure to ultrasonic vibration. The mixture was also shaken and inverted immediately prior to injection of a standard 1.5 ml volume.

Hepatic artery injection of microspheres was accomplished using a 25 gauge needle at the end of a polyethylene tube (P.E. 50), and portal vein injection was accomplished by insertion of a 22 gauge needle into a mesenteric vein. Plastic disposable syringes were utilized for all injections.

Three groups of animals were studied. All animals of a group received the initial injection of tumor cells on the same day and from the same donor, and all rabbits of a

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* Tis-U-Sol, Baxter Laboratories, Inc., Morton Grove, Ill.

† Halatal-Jensen-Salsbery Co., Minneapolis, Minn.

TABLE I. Group I. Infusion of Sc^{46} Plastic Microspheres in Rabbits with V2 Carcinoma Hepatic Implants.

Mesenteric vein				Hepatic artery			
Rabbit No.	Mean c/g/m tumor	Mean c/g/m liver	T/L ratio	Rabbit No.	Mean c/g/m tumor	Mean c/g/m liver	T/L ratio
1	616	1,270	.48	12	1,560	434	3.6
2	161	691	.23	13	2,770	379	7.3
3	219	1,146	.19	14	6,524	637	10.2
4	210	1,103	.19	15	2,139	927	2.3
5	437	2,818	.15	16	3,282	604	5.4
6	470	2,524	.19	17	1,390	516	2.7
7	418	2,551	.16	18	1,884	1,114	1.7
8	545	4,075	.13	19	3,070	2,655	1.2
9	559	2,455	.23	20	3,064	2,423	1.3
10	4,677	10,349	.45	21	42,927	8,704	4.9
11	300	1,971	.15	22	25,926	4,421	8.9
Avg	783	2,814	.23	Avg	8,594	2,074	4.2

group also received microspheres on the same day. Animals in each group were sorted into hepatic artery or mesenteric vein studies by random selection. Group I consisted of 22 rabbits. Eleven animals received hepatic artery infusion and 11 of the rabbits received mesenteric vein infusion of Scandium-46 tagged plastic spheres. Group II contained 10 animals which received hepatic artery and 4 which received mesenteric vein infusion of Sc-46 ceramic microspheres. Group III contained 5 animals which received hepatic artery and 3 which received mesenteric vein infusion of Sc-46 plastic spheres. Group III was studied for hepatic distribution of spheres in tumor and normal liver as in Groups I and II but in addition, spill-over into lung and kidney was also determined.

The thin-walled 25 gauge needle was inserted into the hepatic artery and the polyethylene tubing was allowed to fill with blood, eliminating bubbles. A syringe was then connected and microspheres injected manually at a rate slow enough that blood was visible in the hepatic artery distal to the needle. Too great injection pressure stopped or reversed blood flow in the artery. A similar technique was employed for infusing microspheres into the mesenteric vein. Injection was followed by flush of one millitre of normal saline. Fifteen minutes after microsphere injection the rabbits were sacrificed and the livers excised. All tumors were carefully dissected from the liver and cut into one-gram samples. The remaining liver was finely sliced in two directions to avoid missing any tumors and then separated into one-gram samples. Each gram of tumor and of normal liver from each rabbit was then counted in a well-type scintillation counter for one minute. A mean c/g/m was calculated for tumor and for liver. The resulting mean count-per-gram-per minute of tumor was compared with the mean count-per-gram-per minute of liver for each rabbit. In Group III, the lungs and kidneys were excised, divided into one-gram samples and counted in addition to liver and tumor.

Results. Results are expressed as a ratio of mean count-per-gram-per minute for tumor to mean count-per-gram-per minute for liver $\frac{c/g/m(T)}{c/g/m(L)}$ or a tumor/liver ratio (T/L ratio. The results of Group I are illustrated in Table I. The overall average counts in liver tissue were similar whether injection was by hepatic artery or mesenteric vein. However, the overall average counts in tumor produced by hepatic artery injection were much higher than counts in tumor produced by mesenteric vein injection.

Group II is illustrated in Table II showing that ceramic microspheres gave T/L ratios very similar to those seen with plastic microspheres.

Group III animals which had received plastic microspheres were examined for radiation in lungs and kidneys as well as liver. The results are recorded in Table III. Once again the T/L ratios compare very closely

TABLE II. Group II. Infusion of Sc^{46} Ceramic Microspheres in Rabbits with V2 Carcinoma Hepatic Implants.

A. Mesenteric vein infusion				B. Hepatic artery infusion			
Rabbit No.	Mean c/g/m tumor	Mean c/g/m liver	T/L ratio	Rabbit No.	Mean c/g/m tumor	Mean c/g/m liver	T/L ratio
23	134	151	.89	27	1,285	648	2.0
24	566	1,586	.36	28	1,811	1,512	1.2
25	85	668	.13	29	6,008	2,491	2.4
26	399	3,360	.12	30	9,577	5,736	1.7
				31	3,333	421	7.9
Avg	296	1,441	.37	32	8,270	1,695	4.9
				33	6,192	1,222	5.1
				34	5,912	702	8.4
				Avg	5,298	1,803	4.2

TABLE III. Group III. Infusion of Plastic Microspheres in Rabbits with V2 Carcinoma Hepatic Implants.

A. Mesenteric vein						B. Hepatic artery					
Rabbit No.	Mean c/g/m tumor	Mean c/g/m liver	T/L ratio	Mean c/g/m lungs	Mean c/g/m kidneys	Rabbit No.	Mean c/g/m tumor	Mean c/g/m liver	T/L ratio	Mean c/g/m lungs	Mean c/g/m kidneys
35	155	514	.3	33.2	*	38	5,199	1,002	5.2	*	*
36	67	619	.12	*	*	39	6,685	779	8.6	*	*
37	460	831	.55	*	*	40	2,575	603	4.3	*	*
						41	1,750	1,482	1.2	*	*
Avg	227	655	.32			42	1,723	793	2.2	206	*
						Avg	3,586	932	4.3		

* Background count only.

with the other two groups. The amount of radiation reaching the lungs and kidneys was negligible.

Discussion. These findings demonstrate convincingly that radioactive microspheres lodge in tumors in higher concentrations than surrounding liver when infused by the hepatic artery. Just the opposite is true if the microspheres are infused by the portal system. This adds increasing weight to previous demonstrations by other techniques(1,2) that metastases in liver receive their blood supply from the hepatic artery and not from the portal vein. The use of radioactive microspheres for blood flow studies has been proven a valid technique by several authors in various organs(3,4,5). Lindseth(5) has found excellent correlation with the deuterium oxide technique.

On the basis of these experiments it can be concluded that if cancercidal or radioactive materials are to be studied for effect on V2 carcinoma, the agent should be administered *via* the hepatic artery. It has been shown that effective cancercidal doses of pure Beta irradiation can be delivered to experimental hepatic metastases without destruction of the remaining liver(6).

This experiment was designed to allow statistical analysis of the difference between the mesenteric vein group and the hepatic artery group. In every rabbit with mesenteric vein infusion the tumor had fewer counts than the liver, while in every rabbit with hepatic artery infusion the tumor had more counts than the liver. The difference between the hepatic artery rabbits and the mesenteric vein rabbits is highly significant using the Chi-square test ($P<<0.001$).

In addition, the design was to afford a statistical evaluation within each rabbit of the difference in counts between the one-gram tumor fragments and the one-gram liver fragments using counts as measuration data. Analysis of this parameter was found to be impractical due to uneven distribution of counts producing a markedly skewed curve. Therefore, although it is not true that each

and every cubic centimeter of tumor tissue received more microspheres than each and every cubic centimeter of liver tissue after hepatic artery injection, the overall distribution of microspheres showed a much higher average concentration in tumor tissue than in liver tissue. The converse was true of portal system infusion. Unevenness of microsphere distribution has been observed in other organs and may represent segmental and alternating variations in blood supply with time(5).

Conclusions. 1. Radioactive microspheres reach a concentration in hepatic metastases averaging 4 times the concentration in surrounding liver parenchyma when infused *via* the hepatic artery. 2. When delivered into the portal system radioactive microspheres reach metastases in only an average of one-third their concentration in the surrounding liver. 3. Spill-over of radioactive microspheres 15 μ in diameter from liver to lungs and kidneys is negligible. 4. Arteriovenous shunts in liver and in V2 carcinoma are either few in number or are less than 15 μ in diameter.

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Subcellular Distribution of Serotonin in Rabbit Platelets.* (29877)

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Although serotonin (5-hydroxytryptamine) is known to be present in platelets(1), its subcellular localization has not been clearly established. Hughes and Brodie(2) reported that serotonin was not attached to subcellular particles. On the other hand, Baker *et al*(3) stated that most of the serotonin was concentrated in "cytoplasmic granules." Buckingham and Maynert(4) studied platelet fragments obtained by differential centrifugation after ultrasonic disruption and concluded that serotonin was stored either within "granules" or "some kind of subcellular particle."

The recent development of a method for separating subcellular platelet particles on a continuous sucrose gradient(5) has made it possible to examine the subcellular distribution of this biologic substance in a precise manner. The efficacy of the separation of the platelet granules and membranes by this technique has been confirmed by electron microscopy. Pilot experiments indicated that the procedure, originally designed for human platelets, could be utilized equally well for the study of rabbit platelets.

Methods. 1. Fractionation of rabbit platelets. Fifteen rabbits were bled from the carotid artery through a polyethylene cannula. To achieve rapid cooling, the blood was introduced into a large volume of mineral oil maintained at 0°C. Heparin-saline was provided to give a final concentration of 5 units/ml. The heparinized blood was processed at 5°C throughout. It was initially spun in a refrigerated centrifuge (International PR-2) at 100 $\times$ *g* for one hour. After decanting the supernatant, the sediment was resuspended in heparin-saline, 5 units/ml, and again centrifuged at 100 $\times$ *g* for one

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