Differential Growth of Metastatic Tumors in Liver and Lung*

Experiments with Rabbit V_2 Carcinoma

B. Lucké, C. Breedis, Z. P. Woo, L. Berwick, and P. Nowell

(*Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.*)

It is a common occurrence that in man metastases in the liver grow rapidly and often become huge, whereas in the lung they rarely attain such massive proportions. Hence, of the two organs most often involved in the spread of cancer—liver and lung—the liver is widely regarded as offering a particularly favorable environment for the growth of metastases. However, the hypothesis that liver supports tumor growth better than does the lung has not been previously tested by controlled experiments. This has been done in the present investigation. Living tumor cells were injected simultaneously into the afferent vessels of liver and lung to ascertain whether the resultant tumors grow equally well in these two organs or whether, on the contrary, there are significant differences between them in their ability to support tumor growth.

For these purposes the V_2 rabbit carcinoma (7) is suitable. The rabbit is a sufficiently large animal for injections into small vessels, such as the hepatic artery, to be possible. Suspensions containing many viable tumor cells can be easily prepared. The tumor grows rapidly in various organs (1). The metastatic tumors in liver and lung are approximately spherical, sharply circumscribed, and of distinctive pale color, and hence can be accurately measured.

For comparison with the results of these experiments on rabbits, measurements will be given of metastatic tumors observed at autopsy in liver and lung of man.

MATERIALS AND METHODS

The V_2 carcinoma was maintained by serial transplantation in the thigh muscles of rabbits. Suspensions of tumor cells were prepared from healthy portions under sterile conditions. Small bits were placed in Tyrode solution and squeezed with a glass rod through a fine-meshed (40 wires per inch) stainless steel sieve. The larger fragments were allowed to settle out. Individual cells and small cell aggregates per cubic millimeter of the suspension were counted in a hemocytometer. The concentration was adjusted by dilution. In Experiments I and II the number of tumor cells was varied, as shown in Table 1. In all other experiments there were from 0.5 to 1.3 million individual cells and from 1,000 to 15,000 cell aggregates/0.5 ml, the amount used for injection. The suspension was kept in a small flask on an ice plate and, to insure uniformity, was gently swirled before it was taken into the syringe for each injection.

Large albino rabbits, weighing from 2.4 to 2.9 kg., were used.

TABLE 1

RELATION BETWEEN NUMBER OF TUMOR CELLS INJECTED AND NUMBER AND SIZE OF RESULTANT TUMORS IN LIVER AND LUNG

Experiment no.	No. tumor cells ($\times 10^3$) and route of injection		Number of tumors		Size Geometric Mean Volume ($mm.^3$)	
	Portal vein	Systemic vein	Liver	Lung	Liver	Lung
I	7,000	7,000	900	1,100	102	1.4
	70	70	94	110	151	1.5
	70	7,000	17	1,000	76	3.2
II	280	2.8	600	127	284	1.4
	2.8	280	24	1,350	28	2.2
	2.8	280	40	1,600	112	2.4

Under ether anesthesia, laparotomy in the midline was performed so as to expose the portal vein or hepatic artery. The former was injected through one of the large mesenteric veins; the hepatic artery was injected directly. All animals received suspensions of tumor cells, freshly prepared for each experiment, first into either portal vein or hepatic artery and, about 1 minute later, into one of the large ear veins, through a 27-gauge needle. Thus, in each animal, tumor cells were deposited at very nearly the same time in both liver and lung. Bleeding, which was sometimes severe after puncture of the hepatic artery and less so after injection of the portal tributary, was controlled by compression with warm saline sponges.

The animals were sacrificed at various intervals from 12 to 35 days after inoculation; the liver and lungs were removed, hardened in formalin, and then cut into serial slices not larger than 2 mm. in thickness. Size and number of tumors were determined by the hand lens or naked eye, with care that the same tumor was not counted or measured more than once. When the number of tumors exceeded 135 (which happened in only three rabbits), the total number was estimated from counts in representative slices. Measurements with a finely

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calibrated scale were made of the two largest diameters of the tumors to an accuracy of 0.5 mm. Since most of the tumors were approximately spherical, their volume was readily computed. After counts and measurements, ten samples of tissue were removed at random from each organ, and sections were prepared for microscopic examination. Because of the presence of coccidioidal granulomas in liver or of pneumonic consolidations in lung, which could not with certainty be differentiated in measurements or counts from tumors, a few rabbits had to be eliminated. This left 25 rabbits for comparison.

RESULTS

The relative size of tumors in liver and lung.—In the first two experiments the concentration of the tumor cell suspensions was varied, the liver or lung receiving either the same number of tumor cells or one organ receiving 100 times as many cells as the other. As shown in Table 1, the number of resultant tumors was roughly proportional to the concentration of the cell suspension, but in all animals the mean size of the tumors was greater in the liver than in the lung.

TABLE 2

NUMBER AND SIZE OF TUMORS IN LIVER AND IN LUNG RESULTING FROM INTRAVASCULAR INJECTION OF EQUAL AMOUNTS OF TUMOR CELL SUSPENSION*

(*a*) Into portal vein and a systemic vein (rabbits 1–14) or (*b*) Into hepatic artery and a systemic vein (rabbits 15–21)

Vessels into which tumor cells were injected	Rabbit no.	Days after intravascular injection of tumor cells	Number of tumors		Size of tumors	
			Liver	Lung	Liver	Lung
Portal vein and ear vein	1	12	21	36	2.6	0.6
	2	14	121	135	7.1	1.1
	3	14	38	63	3.8	0.9
	4	15	10	65	7.6	0.6
	5	21	19	14	19	0.6
	6	21	16	104	13	0.6
	7	27	24	13	10	0.5
	8	27	33	85	65	1.5
	9	31	18	24	17	0.9
	10	32	900†	1,100†	102	1.4
	11	32	94	110	151	1.5
	12	34	47	550†	538	3.0
	13	35	38	135	440	2.2
	14	35	85	300†	390	3.9
Hepatic artery and ear vein	15	12	7	88	4.0	0.6
	16	21	17	76	8.1	0.7
	17	27	11	60	7.2	1.7
	18	27	9	75	17.0	1.2
	19	29	19	62	25.0	0.9
	20	34	27	92	60.0	2.8
	21	35	12	130	790.0	3.4

* Animals are arranged according to length of survival after injection.
† Estimated from counts of representative slices.

In the other experiments, any given animal received the same concentration of tumor cells in the liver and in the lung. In fourteen rabbits the size of the tumors in the liver after injection by the portal system was compared to the size of tumors in the lung, the animals having been sacrificed at various intervals. In seven rabbits, a similar comparison was made after introducing tumor cells into the liver through the hepatic artery. In both groups, the mean size of the metastases in the liver exceeded the mean size of the metastases in the lungs at every stage of growth. In other words, the liver tumors always exceeded the lung tumors in size, whether the emboli reached the liver through the portal vein or the hepatic artery. These data are summarized in Table 2, where size is expressed as geometric mean volume in mm.[3] Representative photographs of the tumor in six rabbits are given in Figure 1.

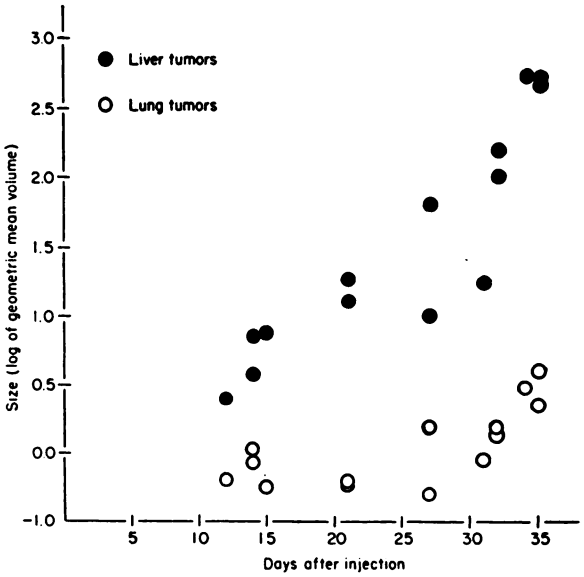

CHART 1.—Relation between the sizes of the tumors in liver and lung after injection of equal numbers of tumor cells into the portal vein and into a systemic vein (data from Table 2, rabbits 1–14).

For ease of inspection, the data are represented graphically. Charts 1 and 2 show the relation between the size of the tumors in liver and lung after injecting equal numbers of tumor cells into the portal vein or the hepatic artery, respectively, and into a systemic vein. In order to reduce the data to a convenient scale, logarithms of the geometric means have been used (3). Both graphs make it evident that the rate of increase in the size of tumors in the two organs differed. The rate of tumor growth was obviously much faster in the liver than in the lung. If more than one animal was examined on a given day, as, for example, on the fourteenth day (Chart 1) and on the 27th day (Chart 2), the least mean size of the liver tumors always exceeded the greatest mean size of the lung tumors. Since this relation held, whether the portal vein or the hepatic artery was the route by which the tu-

mor cells were introduced into the liver, the results of these experiments have been combined to show more clearly the ratio between the size of liver tumors and of lung tumors. In Chart 3, the logarithms of the ratios, mean liver tumor volume to mean lung tumor volume, are plotted against time. The right ordinate gives the proportionality scale

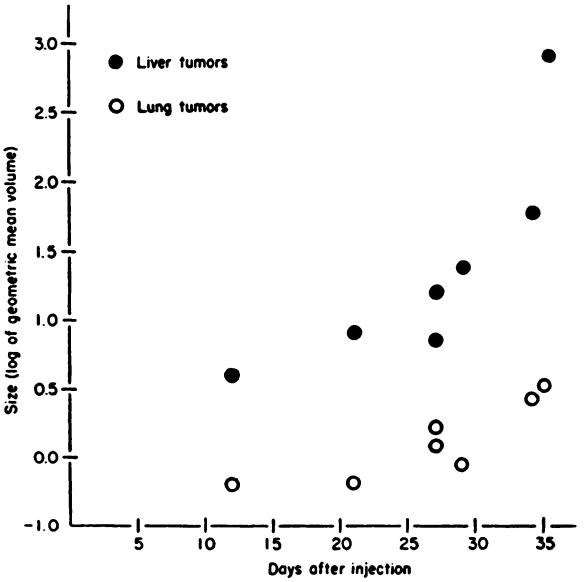

CHART 2.—Relation between the sizes of the tumors in liver and lung after injection of equal numbers of tumor cells into the hepatic artery and into a systemic vein (data from Table 2, rabbits 15–21).

of this ratio. At the beginning of the observations, 12 days after inoculation, the liver tumors were about 5 times greater in actual volume than the lung tumors; the difference in ratio steadily increased so that at the end of the experiments, 35 days after inoculation, the liver tumors were from 100 to 250 times greater in size.

The relative number of tumors in liver and lungs.—As shown in Table 2, there were usually more tumors in the lung than in the liver, especially when the hepatic artery was injected. These differences can perhaps, but not with certainty, be accounted for by the bleeding, particularly when injections were made into the hepatic artery. Because of the loss of blood, it is thought that a variable number of tumor cells failed to reach the liver. It is also possible that other factors come into play, such as differences in passage of cells through liver to lung or through lung to liver. Also, there may be differences between these organs in the percentage of arrested tumor emboli that become established. However, differences in the number of tumor cells reaching liver and lung, as is shown in Table 1, influenced only the number of the resultant tumors, but not their size.

Comparative size of metastatic cancers in liver and lung of man.—It is of interest to compare the results of these experiments with a cancer of the rabbit to measurements made at autopsy of metastatic cancers in the liver and lungs of man. Routine measurements at human autopsies are seldom as precise as those made in experimental animals for a specific purpose; moreover, the actual size of every tumor present is rarely stated; hence, average size cannot be computed. Nevertheless, in many protocols there is a statement as to the shape and size of the *largest* tumor. In our own protocols, we found recorded 154 cases in which the measurements appeared to be sufficiently exact for computation of tumor volume. To find out whether comparison based upon *largest* size, rather than mean size, gives any information of value, we have first computed the ratio of largest liver tumor volume to largest lung tumor volume in the experimental rabbits. As shown in Table 3, the largest liver tumors in the rabbit were on the average 39 times greater than those in the lung. A similar preponderance of tumor size in liver over lung was found in the human material. In 29 cases with involvement of both organs, the ratio of liver tumor

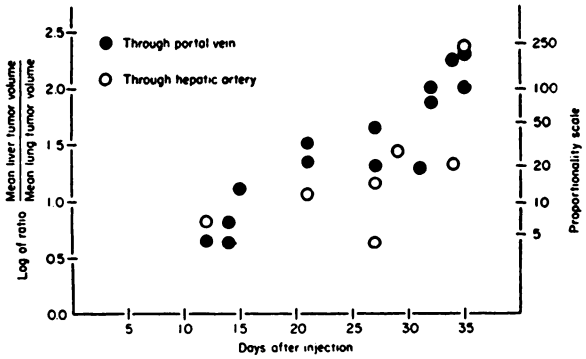

CHART 3.—Ratio of sizes of liver tumors to lung tumors in 21 rabbits injected with equal amounts of tumor cell suspension, either into the portal vein and a systemic vein (solid circles) or into the hepatic artery and a systemic vein (open circles).

to lung tumor was 7:1. In 59 cases in which metastatic cancers occurred in the liver but not in perceptible form in the lung (although secondary growths were often present in other organs), and in 66 cases where the reverse relation obtained, the combined ratios were again about 7:1. In contrast to the single type of cancer used in the experiments, the human material comprised a great variety of cancers. A comparison such as the above is suggestive but not conclusive. It is noteworthy,

however, that, despite the variable nature of this material, human liver tumors were found to be, on the average, much larger than lung tumors.

DISCUSSION

From the results of these experiments we can now attempt to answer the questions proposed in the "Introduction." In all experiments the growth of cancer in the liver exceeded that in the lung by a wide margin. This finding bears out the widely held belief, based upon clinical and autopsy observations, that the liver affords a particularly favorable environment for cancerous metastases. (For discussion and references to the literature, see especially Willis [9, 10], Walther [8], and Katz [5].) The nature of the factors, physical or chemical, that make hepatic tissue a superior environment for growth of cancer are unknown. Because of the liver's great metabolic activity, it seemed possible that the temperature within the liver parenchyma might play a part; but, although in recent thermocouple measurements the temperature within the livers of rabbits was found to be consistently higher than the temperature within the substance of the lung, the average difference was only 0.18° C. (2). Such a small difference in temperature cannot account for the great difference between the liver and lung in their ability to support growth of metastases.

TABLE 3

COMPARISON OF SIZES OF THE *Largest* TUMORS IN LIVER AND LUNG OF RABBITS, AND OF SIZES OF THE *Largest* METASTATIC TUMORS IN LIVER AND LUNG OF MAN

	No. cases	Liver Geometric mean of largest tumors ($mm.^3$)	Lung Geometric mean of largest tumors ($mm.^3$)	Approximate Ratio of liver tumor volume to lung tumor volume
Rabbit tumors in *both* liver and lung	21	537	13.8	39:1
Human tumors in *both* liver and lung	29	5,600	780	7:1
Human tumors in *either* liver or lung	59 in liver 66 in lung	5,400	790	7:1

Of chemical factors peculiar to the liver, the following have been especially considered as favoring growth: rich carbohydrate content and poor oxygenation (9), the exceptionally high concentration of amino acids (11), the presence of growth-promoting substances (4). All these factors may play a part, but definite knowledge is still lacking. Meanwhile, these hypotheses are valuable in suggesting lines for further investigation of an important subject.

SUMMARY AND CONCLUSIONS

The ability of liver and lung to support growth of cancer was compared by injecting simultaneously suspensions of V_2 rabbit carcinoma cells into either the portal vein or the hepatic artery, and into a systemic vein. Thus, in each animal, cancer cells were deposited in both liver and lung. The animals were sacrificed at various intervals of time, from 12 to 35 days after inoculation, and the number and size of the tumors were determined.

In all 25 animals available for comparison it was found that the mean size of the liver tumors exceeded the mean size of the lung tumors, whether the liver was injected through the portal vein or the hepatic artery. At the beginning of the observations, 12 days after inoculation, the liver tumors were about 5 times greater in volume than the lung tumors. At the end of the observations, 35 days after inoculation, the liver tumors were from 100 to 250 times greater in size.

According to the present experiments, tumors, once established, grew faster in the liver than in the lung. Therefore, the liver is the better environment for supporting tumor growth.

Comparing the sizes of metastatic cancers in liver and lungs in a series of 154 human autopsies in which adequate measurements were recorded, the mean size of the largest tumors was 7 times greater in the liver than in the lung.

The experiments support the hypothesis originally based on clinical and autopsy observations that, in the liver and lung at least, the factor of tissue environment is of importance in the problem of metastasis.

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FIG. 1.—Representative photographs showing size of tumors in liver and lung of six rabbits sacrificed 32–35 days after intravascular injection of tumor cells. Each rabbit had received equal quantities of cell suspensions both in a systemic vein and in either portal vein or hepatic artery. The resulting tumors were invariably much larger in the liver than in the lung. (Actual size.)