Vascular Distribution of Experimental Hepatomas

by

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CONTENTS

I. INTRODUCTION

Emphasis should be placed on the problem of the blood supply to the hepatomas which originate and grow in characteristic organ receiving dual distribution of the hepatic artery and portal vein from the aspect of their treatment and for understanding of pathophysiology of the hepatomas. Although there have been many publications on this point, the prevailing opinions insist that hepatomas are nourished exclusively by arterial blood, similarly to the tumors originating from other organs. However, experimental methods in these literatures are not always suited to the studies on vascular distribution in vivo. For instance, by resin cast method, vascular distribution could not be clearly visualized in the area of the border of hepatic tissue against tumor, and in the method of dye infusion, the infusion was performed unphysiologically, merely from arterial or venous system and interrelation between infusion pressure and histological findings was not adequately interpreted.

In the present experiment using various strains of experimental hepatomas, vascular distribution of hepatomas were studied with particular attention to keep the condition of experiment as near the physiological one as possible, since there is subtle interference between the hepatic artery and portal vein in vivo.

II. MATERIALS AND METHODS

1. Materials

a). Intrahepatic Inoculation of Transplantable Tumor

For experimental tumors, Walker carcinosarcoma 256, maintained in the Takeda Institute, of following 109th generation weekly by transplanting subcutaneously in the back of rats of Saitama strain. Ten days after the transplantation, subcutaneously growing tu-

mor was extirpated aseptically, which was then ground into pieces and made into cell suspension in saline by the use of glass homogenizer, after removing central necrotic area and fibrous component around. Cell population was adjusted to be 500×10^4 in 0.1cc of the suspension. Fifty rats weighing approximately 120 g were used. With anesthesia of intraperitoneal injection of Nembutal of 3 mg, the abdomen was opened and 0.2 cc of the tumor cell suspension was injected from a branch of the superior mesentric vein. Forty-one rats were subjected to the experiment, after the intrahepatic growth of the tumor had been ascertained by exploration 15 days after transplantation via the portal vein (Fig. 1).

b). DAB-Hepatoma

Sixty rats of Saitama strain weighing approximately 120 g were fed for 100 days with water and diet[8)24)27)] consisted of 1 kg of rice and 20 cc of 3 per cent 3′-methyl-4-dimethylaminoazobenzene olive oil, 29 of which died within 90 days of the feeding. In survival cases, the abdomen was opened around 130 days later, 22 of which, being ascertained the development of tumor in the liver, were subjected to the experiment (Fig. 2).

c). Materials for Vascular Infusion

1. Coloured Gelatin Solution

Gelatin solution was prepared being coloured with *carmine* for the arterial infusion and with *Prussian blue* for the portal infusion. In both occasions, 5 g of the dye was put into 50 cc of distilled water and made into colloidal solution being vigorously stirred in Waring's blender for 5 minutes, which was then mixed with 100 cc of distilled water of 100°C containing 8 g of gelatin. The coloured gelatin solution was preserved at the temperature of 37° C with small amount of crystalline thymol as a preservative. The sedimantation of these gelatin solutions were discarded a few days later. Prior to use the solution was heated to 70°C and used for the infusion at the temperature of 35 to 40°C. If the dye solution in water be used for the infusion, the infused dye is comletely bleached with alcohol used for the dehydration of sections in the process of making paraffin sections, whereas the dye is bleached so slightly by alcohol as to enable the observation, if the dye solution is gelled with gelatin.

ii. Dye Solutions for Vital Staining

As the above mentioned gelatin solution does not reach the small vessels adequately owing to its high viscosity, 1 per cent trypan blue saline solution was used in some rats for the observation of the small vessels.

2. Methods

For the infusion of the dye solution into the hepatic vessels, the apparatus equipped with manometers as shown in Fig. 3 was used. The abdomen was opened in rats having hepatic tumor under anesthesia with ether, and a catheter was inserted into the portal vein. As the direct insertion of catheter into the hepatic artery was technically difficult, it was inserted into the aorta at the level of the beginning of the celiac artery. In order to observe arterial and portal pressures in a condition as near the physiological one as possible, these pressures were determined prior to the following procedures. Then the aorta was ligated cephalad and caudad to the celiac artery, and the inferior vena was

ligated and cut in the thoracic cavity so that the outflow from the liver might not enter the systemic circulation again. The outflow from the liver was introduced extracorporeally through a catheter inserted into the cut end of the inferior vena cava. After washing out the blood within the liver by infusion of saline from the artery and portal vein simultaneously with the similar pressure as observed in the above mentioned procedure, the dye solutions were infused from the artery and the portal vein. At the infusion of the liver with coloured gelatin solution, the entire liver was usually tinctured with the infusion dye within 5 to 10 minutes. The observed arterial pressure ranged from 50 to 80 mmHg, and portal pressure from 90 to 150 mmH_2O. In cases in which the infusion of gelatin solution with these pressures was difficult, the infusion was carried out with higher pressures of the same proportion.

In some rats, trypan blue solution was continuously infused into the portal vein for an hour with simultaneous infusion of saline from the artery in the similar manner. On the contrary, trypan blue solution was infused into the hepatic artery with simultaneous infusion of saline from the portal vein. In this occasion, the infusion was carried out with the same pressures as observed preliminarily, owing to the low viscosity of the solution. For the comparative study of the dye infusion under the physiological pressures with

Tab. 1 Pressures of infusion of red gelatin solution from the hepatic artery and blue one from the portal vein in the liver having DAB hepatoma.

Rat No.	Day after comencement of DAB administration	Hepatic arterial pressure (mmHg)	Portal pressure (mmH_2O)	Infusion pressure for dye solution		Outcome of infusion
				In hepatic artery (mmHg)	In portal vein (mmH_2O)	
2	103	65	135	130	270	good
4	122	55	110	110	220	good
9	100	60	110	120	220	good
12	149	70	140	70	140	good
14	125	70	145	70	145	bad
15	125	65	110	130	220	good
20	128	70	130	140	260	good
21	131	70	125	140	250	good
26	135	50	130	100	260	good
27	136	65	110	130	220	good
31	103	65	125	65	125	bad
33	128	70	130	70	130	bad
35	128	65	120	130	240	good
36	131	60	125	120	250	good
38	136	70	140	140	280	good
42	122	60	100	120	200	good
46	135	70	120	140	240	good
47	136	70	140	70	140	good
50	129	65	125	65	125	bad
54	118	60	120	60	120	good
58	98	65	120	65	120	good
59	149	65	110	65	110	good

that under abnormal pressures, trypan blue solution was infused into the hepatic artery, the portal vein being ligated and interrupted. Moreover, in the aim of clarifying the outflow path from the tumors, dye solution was infused invertedly into the hepatic vein with a pressure of about 150 mmH_2O.

After finishing the infusion, all the vessels from and to the liver were ligated and the entire liver was taken out. The liver with the infusion of gelatin solution was placed in a cool temperature of 4°C for 1 to 2 hours until the infused material was completely gelatinized, and was fixed in 20 per cent formalin solution, and then embedded in paraffin and cut in microscopic sections for double staining of hematoxylin and eosin.

The liver with the infusion of trypan blue solution was fixed in the following solution of ODA and OOTA[16)22)23)] for 30 hours and embedded in paraffin. The microscopic sections were stained with 1 per cent *safranin*.

Solution for the Fixation	cobalt chloride	1.0g
	pycric acid	0.1g
	neutral formalin	5.0cc
	trichlor acetic acid	2.0g
	distilled water	10.0cc

III. RESULTS

1. DAB-Hepatoma

Infusion of coloured gelatin solution was carried out in 22 rats fed with the diet containing 3'-methl-DAB and development of tumor was ascertained. Arterial and portal pressures were as shown in Tab. 1. In 9 cases the infusion was performed with physiological pressures, whereas it was impossible in 13 cases, hence the infusion being performed with higher pressures as twice as much. Excluding 4 cases of unmonotonous appearance of the dye in the microscopic sections, appearance of dyes in the foci of hepatoma was studied in 18 cases.

Ten microscopic sections were taken from the various parts of hepatoma in a single animal, and observation was carried out on histological character of the tumors. The tumors were classified into 3 types from the histological view.

i. Hepatoma Type

Principal structure resembles hepatic cells being sharply bordered, and tumor cells with irregular and huge nuclei are arranged in cords, being surrounded by thin reticular or collagenic fibers and facing to the sinusoidal channel of blood stream. The sinusoidal channels were sometimes wide and sometimes narrow (Fig. 4).

ii. Cholangioma Type

Cylindric or cubic tumor cells showed glandular struture having endothelium cells as the basement, and containing a large amount of connective tissue (Fig. 5).

iii. Mixed Type

This showed intermediate histological character of hepatoma and cholangioma or mixture of cholangioma and hepatoma (Fig. 6, 7).

Among 18 rats studied, most of them had mixed type tumor, in 9 cholangioma type was predominant, in 4 hepatoma type was predominant and in 5 the both was equally observed, as shown in Tab. 2.

Tab. 2 Appearance of dyes in infusion of red gelatin solution from the hepatic artery and blue one from the portal vein in the liver having DAB hepatoma.

Rat No.	Histological appearance of various type		Appearance of dyes in tumor tussiue		
	Hepatoma	Cholangioma	B. *(%)	R. ** (%)	B.+R.***(%)
26		+	76	15	9
38	+	+	83	10	7
15	−	+	52	41	7
20	+	+	57	36	7
4	+	++	12	81	7
12	+	++	13	79	8
21	+	++	9	83	8
35	+	++	10	83	7
47	+	++	14	79	7
9	+	++	11	79	10
27	+	+	25	65	10
42	+	+	28	63	9
54	+	+	23	69	8
58	+	+	22	67	11
59	+	+	27	64	9
2	++	+++	4	94	2
36	++	+++	0	100	0
46	+++	+++	2	98	0
Average			26	67	7

* : Vessels filled with blue gelatin solution.
** : Vessels filled with red gelatin solution.
*** : Vessels filled with both solutions.

Ten sections of hepatoma were taken from each of 18 rats regardless of the histological types of tumor. Furthermore, in 10 microscopic fields arbitrarily from each section, 1800 microscopic fields in all, kind of dyes contained in the vessels was studied by microscopic power of 150 time enlargement. Sixty- seven per cent of the vessels was filled with carmine infused into the artery, 26 per cent of the vessels was filled with Prussian blue infused into the portal vein and 7 per cent of the vessels was filled with both dyes. During this study, it was clarified that the pattern of dye filling in the vessels differed depending on the histological character of tumors, as follows.

In tumor tissue which shows histological picture of typical cholangioma, dye infused into the artery only could be observed in the vessels of stromal connective tissue and dye infused into the portal vein could not be observed at all (Fig. 8).

In tumor tissue of hepatoma type, large amount of dye infused into the portal vein could be observed in the sinusoidal path way of blood, at the same time containing small amount of dye infused into the artery. Coexistence of both dyes infused into the artery and portal vein could be recognized even in narrow part of the sinusoids (Fig. 9).

Peculiarities of above mentioned dye spread were similar in tumor tissue of mixed type which was most frequently observed in rats examined here. In the area with predominance in character of cholangioma, most vessels contained merely the dye infused

into the artery, whereas in the area with histological picture of hepatoma the dye infused into the portal vein could be more or less observed (Fig. 10).

When a small number of the vessels containing the dye infused into the portal vein could be observed even in tumor tissue seemingly assumed to be cholangioma type, detaild observation of the adjoining tumor cells revealed mass of tumor cells principally resembled liver cells, obviously different from tumor cells in other parts. Thus, entrance of the dye infused into the portal vein in the tissue of hepatoma was invariably observed.

2. Intrahepatic Inoculation of Transplantable Tumor

Tumor cell suspension of Walker carcinosarcoma 256 was transplanted into the liver via the portal vein in rats. In 41 rats, intrahepatic tumor growth could be ascertained by exploratory laparotomy performed 15 days after the transplantation, in 20 cases of which coloured gelatin solution was used for the infusion and in the remaining 21 cases trypan blue solution was used.

Histological picture of Walker carcinosarcoma 256 transplanted into the liver via the portal vein was consisted of a number of tumor cell mass of various sizes. In large mass of tumor cell, sometimes central necrosis or scarring was observed and marginal area of the mass immediately faced to the surrounding liver tissue, without having capsule, layer of connective tissue or infiltration of round cells around. In some area, infiltration of tumor cells into the mass of parenchymal cells of surrounding liver tissue (Fig. 12, 13, 14).

a). Infusion of Coloured Gelatin Solution

Tab. 3 Pressures of infusion of red gelatin solution from the hepatic artery and blue one from the portal vein in the liver having tranplanted hepatic tumor.

Rat No.	Hepatic artertal pressure (mmHg)	Portal pressure (mmH_2O)	Infusion pressure for dye solution		Outcome of Infusion
			In hepatic artery (mmHg)	In portal vein (mmH_2O)	
61	70	150	140	300	good
62	80	140	160	280	good
63	60	120	60	120	bad
64	65	120	130	240	good
65	65	125	65	125	good
66	70	140	140	280	good
67	70	120	70	120	bad
68	75	125	150	250	good
69	50	90	50	90	bad
70	75	135	75	135	good
71	65	100	130	200	good
72	70	120	70	120	bad
73	65	130	130	260	good
74	75	130	150	260	good
75	65	120	130	240	good
76	60	120	120	240	good
77	65	130	65	130	bad
78	80	145	160	290	good
79	70	140	70	140	good
80	60	120	60	120	bad

Arterial and portal pressures in 20 rats are represented in Tab. 3. In 9 cases, carmine gelation solution and Prussian blue gelatin solution were infused into the artery and portal vein respectively under the same pressures as previously measured. In 11 cases, the infusion was carried out under a higher pressures twice as much. Six cases out of these 20 were excluded from the experiment since the filling of colored gelatin solution was not monotonous all over. Sections were taken from various part of the liver of the remaining 14 cases, and 10 tumor masses from an individual rat were chosen including masses of various sizes. Distribution of dyes was examined in these materials.

Distribution of dyes in the intrahepatic transplanted tumor mass is tabulated in Tab. 4. To summarize these findings, most of the large tumor masses developed in the liver parenchyma had central necrosis, and in this necrotic foci neither the dye infused into the artery nor that infused into the portal vein could be found. Excluding such area, the dye infused into the artery could be observed widely in a shape of dots or bands in tumor tissue all over. Appearance of the dye infused into the artery was observed to be dense in the central area of the tumor tissue and it tended to become more sparse in the pe-

Tab. 4 Appearance of dyes in the tissue in infusion of red gelatin solution from the hepatic artery and blue one from the portal vein in the liver having transplanted hepatic tumor.

Rat No.		Large tumor foci		Small tumor foci	
		Internal area	Marginal area	Internal area	Marginal area
61	H.A.*	##	##	+	+
	P.V.**	−	##	##	##
62	H.A.	##	##	+	+
	P.V	−	##	##	##
66	H.A.	##	##	+	+
	P V	−	##	##	##
78	H.A.	##	##	+	+
	P V.	−	##	##	##
64	H.A.	##	+	+	+
	P.V.	−	##	##	##
68	H.A.	##	+	+	+
	P.V.	−	##	+	##
73	H.A.	##	+	+	+
	P.V.	−	+	+	##
74	H.A	##	+	−	+
	P.V.	−	+	+	##
76	H.A.	##	+	−	+
	P.V.	−	+	+	##
65	H.A.	+	+	−	−
	P.V.	−	+	+	##
70	H.A.	+	+	−	−
	P.V	−	+	+	+
71	H.A.	+	+	−	−
	P.V.	−	+	+	+
76	H.A.	+	+	−	−
	P.V	−	+	+	+
79	H.A	+	+	−	−
	P V	−	+	+	+

* : Hepatic artery filled with red gelatin solution.
** : Portal vein filled with blue gelatin solution.

ripheral area. In the border of the tumor tissue against liver parenchyma, the dye from the portal vein could be found in most sections, and in some tumor foci this dye was observed in relatively internal area of the tumor foci. Particularly in the area where tumor cells infiltratively invaded from the marginal area of the tumor foci into the surrounding liver parenchyma, the dye infused into the portal vein was observed densely, while that infused into the artery was less frequently observed. Tumor foci of moderate size without having central necrosis could be observed with considerable frequency, and the dye infused into the artery could be seen in the central area, which was, however, sparse. Similarly to large tumor foci, appearance of the dye infused into the artery was predominant in the internal area whereas in most tumor foci the dye infused into the portal vein could be observed in the marginal area likewise. In small tumor foci, appearance of the dye infused into the portal vein was more remarkable than that infused into the artery, Prussian blue densely appearing all over the tumor foci and carmine being observed at most sparsely in dots. Some tumor foci of this size were deprived of appearance of this dye (Fig. 11).

b). Infusion of Trypan Blue Saline Solution

Arterial and portal pressures in 14 cases of infusion of trypan blue saline solution are tabulated in Tab. 5 and 6. In 7 cases, trypan blue saline solution was infused for 1 hour into the portal vein with the same pressure as was previously measured, at the same time saline solution being infused into the artery with the same pressure as was previously measured. In other 7 cases, trypan blue solution was contrariwise infused into the artery, at the same time saline solution being infused into the portal vein. Owing to low viscosity of trypan blue saline solution, the infusion could be carried out in all cases with the same pressures as were previously measured in the artery and portal vein, respectively. As in most cases of infusion of trypan blue solution the dye did not satisfactorily remain within the vessels of the liver, appearance of the dye in intrahepatic tumor foci was examined in 3 cases of infusion of trypan blue solution into the portal vein and in 2 cases of infusion of trypan blue solution into the artery. Ten microscopic sections were prepared from each individual liver and studies were carried out in 10 tumor foci of various sizes selected arbitrarily from an individual liver specimen.

Tab. 5 Appearance of dye in infusion of trypan blue solution from the portal vein with simultaneous infusion of 0.9 per cent salt solution from the hepatic artery in the liver having transplanted hepatic tumor.

Rat No.	Hepatic arterial pressure (mmHg)	Portal pressure (mmH_2O)	Infusion pressure for dye solution		Appearance of dye in tissues		
			In hepatic artery (mmHg)	In portal vein (mmH_2O)	Internal area of tumor foci	Marginal area of tumor foci	Liver tissue
81	65	130	65	130	−	−	−
82	75	140	75	140	−	+	+
83	70	135	70	135	−	+	++
84	65	110	65	110	−	−	−
85	60	110	60	110	−	+	++
86	65	130	65	130	−	−	−
87	65	135	65	135	−	−	−

Tab. 6 Appearance of dye in infusion of trypan blue solution from the hepatic artery with simultaneous infusion of 0.9 per cent salt solution from the portal vein in the liver having transplanted hepatic tumor.

Rat No.	Hepatic arterial pressure (mmHg)	Portal pressure (mmH_2O)	Infusion pressure for dye solution		Appearance of dye in tissues		
			In hepatic artery (mmHg)	In portal vein (mmH_2O)	Internal area of tumor foci	Marginal area of tumor foci	Liver tissue
88	65	125	65	125	+	+	−
89	60	120	60	120	−	−	−
90	75	130	75	130	−	−	−
91	70	135	70	135	+	+	+
92	65	120	65	120	+	+	+
93	65	140	65	140	+	−	−
94	70	110	70	110	+	−	−

In cases of infusion of saline into the artery and trypan blue solution into the portal vein, abundant network of capillaries could be observed around large tumor foci, being filled with trypan blue and fine branches of the network entering the tumor tissue here and there. Thus, the dye could be found in the interspaces of the tumor cells (Fig. 15, 16).

The network of the capillaries was not so marked around small tumor foci, but the dye was frequently found in the internal area of the tumor tissue (Fig. 17).

In cases of infusion of saline into the portal vein and trypan blue solution into the artery, the dye remained very little within the vessels of the liver and it was scarcely found within the sinusoids of the liver parenchyma, appearing slightly in the internal area of the tumor foci (Fig. 18).

c). Infusion of Trypan Blue into the Hepatic Artery under Interruption of Portal Flow

In order to explore the change in appearance of the dyes in the hepatic tumor under infusion with pressures of abnormal relationship in the artery and portal vein, trypan blue solution was infused into the artery alone with the interruption of portal flow in 3 rats. Ten microscopic sections were taken from each individual liver specimen and studies were carried out in 10 tumor foci of various sizes selected arbitrarily, finding of which is represented in Tab. 7. Different from the occasion with simultaneous infusion of saline into the portal vein with physiological pressure difference, the dye appeared well all over the tumor tissue, at the same time appearing in the marginal area adjoining the liver

Tab. 7 Appearence of dye in infusion of trypan blue solution from the hepatic artery under interruption of portal flow in the liver having transplanted hepatic tumor.

Rat No.	Hepatic arterial pressure (mmHg)	Infusion pressure for dye solution (mmHg)	Appearance of dye in tissues		
			Internal area of tumor foci	Marginal area of tumor foci	Liver tissue
95	65	65	+	+	+
96	60	60	++	+	+
97	60	60	++	++	+

parenchyma (Fig. 20).

d). Infusion of Trypan Blue into the Hepatic Vein

In the aim of clarifying the out-let path of blood in hepatic tumor, trypan blue solution was infused backwards in 4 rats into the catheter inserted into the cut end of the inferior vena cava for external drainage from the liver. In this occasion, the infusion into the hepatic vein was performed with the similar pressure as portal pressure and the hepatic artery and portal vein were cut and let opened at the liver hilum. After accomplishment of the infusion, 10 microscopic sections were taken from each of individual liver specimen, and the studies were carried out in 10 tumor foci of various sizes selected ad libitum. The results are represented in Tab. 8. In these sections, the dye was observed to appear in the marginal area of the tumor foci (Fig. 19).

Tab. 8 Appearance of dye in infusion of trypan blue solution from the hepatic vein with the cut and opened hepatic artery and portal vein in the liver having transplanted hepatic tumor.

Rat No.	Infusion pressure for dye solution (mmH_2O)	Appearance of dye in tissues		
		Internal area of tumor foci	Marginal area of tumor foci	Liver tissues
98	150	−	+	++
99	150	−	+	+
100	150	−	+	+
101	150	−	+	++

IV. DISCUSSION

Since WRIGHT[29] reported in 1937 that in hepatoma blood is supplied from the hepatic artery and flows out through the portal vein, according to the results of his experiment of infusion of coloured gelatin solution or coloured oil emulsion into the hepatic artery and portal vein, studies on blood supply to hepatic tumors have made a great progress being followed by many reports. MANN and WAKIM[20], in 1953, reported on vascular distribution in hepatoma developed on the basis of liver cirrhosis in hemochromatosis, using hepatic vascular cast method. In 1954, BREEDIS and YOUNG[1] studied blood supply to metastatic hepatoma in men, DAB hepatoma in rats and Vx_2 carcinoma transplanted in the liver of rabbits by the use of infusion of dye solution and vascular cast method. MURTHY[19] studied in 1959 vascular distribution following the process of inducement of DAB hepatoma by the use of infusion of india ink into the portal vein. MIYAKE and OKUHIRA[21] investigated in 1962 vascular distribution using cast preparation technique and infusion of coloured gelatin solution respectively in a single case of hepatoma accompanied by liver cirrhosis, cholangioma, liver metastasis from esophageal carcinoma. These reports invariably asserted that hepatic tumor receives blood supply exclusively from the hepatic artery and opinion that portal blood does not enter the tumor tissue has been prevailing. However, the portal vein conveys blood to the liver 3 to 4 times as much compared with the hepatic artery, as reported by GRINDLAY and HERRICK[9] that portal blood flow was 148 to 505 cc/min., whereas hepatic arterial blood flow 44.6 to 163 cc/min. On the other hand, according to BRADLEY[2], different from systemic venous

blood, portal blood contains as much oxygen as arterial blood does. Thus considering, it is questionable that portal blood, which occupies 60 to 80 per cent of total hepatic blood flow with high oxygen content in addition does not participate at all in nourishing tumors originating and growing in the liver.

Concerning interrelationship between arterial blood flow and portal blood flow in hepatic circulation, HONJO[10)11)32)] insisted that portal blood flow is seriously disturbed with resulting intrahepatic congestion, when the hepatic arterial flow is interrupted in normal liver. GRINDLAY[4)] also reported that following experimental constriction of the portal vein, portal blood flow is decreased with simultaneous increase in hepatic venous flow. From these observations, increase in hepatic arterial flow is readily presumed to develop at disturbance of portal blood flow, and it is assumed that arterial and portal flows are closely related with one another in hepatic circulation[25)28)]. At present, there is no established method to study vascular distribution in hepatic tumors under physiological condition and infusion technique is chiefly applied. However, exquisite attention should be paid to the technique to reproduce with as much fidelity as possible the conditions of circulation in hepatic tumors in organism, when the infusion technique is used for the study of vascular distribution in hepatic tumors.

Reflecting, however, upon method of investigation of researchers hitherto been reported, it seems that, little attention has been paid to the pressure of infusion of dye solutions. For instance, FISHER[5)] studied vascular distribution of hepatic tumor by infusing india ink into the hepatic artery with simultaneous ligation of the portal vein at the liver hilum and contrariwise infusing india ink into the portal vein with the ligation of the hepatic artery, and from the results of his experiment he concluded that hepatic tumor is nourished solely by arterial blood. MURTHY[19)] also made experiments of india ink infusion into the superior mesenteric vein alone, and reported that appearance of dye in hepatic tumor foci could not be observed. In experiment of MANN and WAKIM[20)] using resin cast method, pressure of the infusion was appropriately changed depending upon resistance at infusion. In experiments of BREEDIS and others[1)], india ink was infused into one of the two vessels in living organism and the liver was extirpated before recirculation of the infused india ink. In cases of necropsy in his experiments, infusion was carried out simultaneously into the hepatic artery and portal vein with the physiological pressure of 80 to 150 mmH_2O. In some parts of the present experiment, difference in appearance of dye in hepatic tumor foci due to method of infusion was studied by infusing dye solution solely into the hepatic artery under ligation of the portal vein, and by infusing dye solution into the hepatic artery with simultanenous infusion of 0.9 per cent salt solution under physiological pressure difference. In the former occasion, dye was widely observed in tumor foci, whereas in the latter occasion it was observed to be sparse in marginal area of tumor foci. From these findings also, it is assumed that exquisite attention should be paid to the pressure of infusion in the method of infusion of dye solution.

In the present experiment, portal and arterial pressures were measured prior to various procedures such as thoracotomy or vessel ligation in every experimental animal immediately after laparotomy, and dye solution was infused simultaneously into the both vessels with the similar pressures as measured respectively. In most cases of infusion with col-

oured gelatin solution, pressures for the infusion were required to be somewhat increased in the proportion of physiological difference since the infusion was difficult. At the infusion of trypan blue solution, however, the solution was infused with the similar pressures as measured very accurately in the abdominal aorta and the portal vein. Thus, the present experiment was carried out with attention to keep the conditions as near as physiological one, even though it was not perfect.

In DAB hepatoma, tumors of typical picture of cholangioma type was supplied with arterial blood alone, while in tumors of hepatoma type and those showing more or less histological feature of liver cell carcinoma, participation of the portal vein in vascular distribution could be observed. There is no definite view, at present, concerning cell origin of tumors of hepatoma type and cholangioma type in DAB hepatoma. However, the findings that hepatoma resembling normal liver cells in structure receives distribution of both of the hepatic artery and portal vein and cholangioma with the character of rather carcinoma of the extrahepatic bile duct receives distribution of the hepatic artery alone are accepted to be quite rational from the standpoint of pathogenesis of hepatic tumors. POPPER[6)25)] classified primary hepatoma of men into hepatic carcinoma and carcinoma of intrahepatic bile duct based on the comparative study of histological and clinical findings. In the findings on vascular distribution in DAB hepatoma in the present experiment, it is accepted to be interesting findings that hepatoma and mixed type tumor which show the histological picture resembling normal liver cell cord and interlobular peripheral bile duct reveal outstanding contrast to typical cholangioma which shows the histological picture resembling rather carinoma of the extrahepatic bile duct, in respect to the participation of portal blood.

WRIGHT[29)] carried out simultaneous infusion experiment from the hepatic artery and the portal vein in 15 corpses of liver metastasis originating from carcinoma of the large intestine, stomach, pancreas or breast, and he observed that portal blood did not enter tumor tissue, but with slight pressure on the hepatic vein, portal blood entered the tumor, forming junction with the hepatic artery. Based on these findings, he considered that blood streams tumor tissue from the hepatic artery and flows out via the portal vein in metastatic tumors. In transplanted tumor in the present experiment, however, when trypan blue solution of low viscosity was infused into the portal vein with the similar pressure as measured previously in this vein and 0.9 per cent salt solution was simultaneously infused into the hepatic artery with the similar pressure as measured previously in this artery, trypan blue appeared in the interspace of tumor cells in marginal area of tumor foci without pressure on the hepatic vein. This finding was interpreted to indicate that the portal vein is not always the outlet vessel of blood stream in tumor tissue. As the reason of absence of the portal vein in most hepatic tumor foci of transplanted tumor, BREEDIS[1)] postulated that the portal and hepatic veins are readily invaded and obstructed by tumor cells compared with the hepatic artery, and a small number of intrahepatic portal branches observed in the marginal area are nothing but the vessels of remnant liver parenchyma in the tumor growth.

HIRONO[13)] carried out experiments of ligation of the portal branches entering 70 per cent area of liver parenchyma including hepatic lobe transplanted with tumor, at various

stadiums after implantation of various experimental tumors in the left hepatic lobe in rats. In his experiments, all the animals of control with simple laparotomy died of tumor growth, whereas prolongation of survival time could be observed in animals with portal branch ligation, and an atrophy was observed in the area of the interruption of portal blood flow, at the same time herein existing tumor revealing a tendency of retardation and decrease in growth and metastasis formation. Incidence of the tumor regression was histologically proved to be 13.3 to 26.1 per cent. He also reported that significant difference could not be observed between the animals of ligation of the branches of the hepatic artery entering the liver area including tumor foci and control animals. KRAUS and BELTRAN[17] implanted Walker carcinosarcoma 256 in the right lobe of the liver and ligated the portal branches entering 30 per cent liver area including the lobe of implantation 7 days later. He could observe regression of tumor growth in 31.2 per cent of the animals. As the cause of such inhibition of tumor growth brought about by the interruption of portal blood to the hepatic area of tumor growth, atrophy of the liver parenchyma, of course, as the soil of tumor growth cannot be neglected. On the other hand, however, this is interpreted to suggest participation of portal blood in nourishment of hepatic tumors to some extent, and the portal branches entering the marginal area of transplanted tumor come to possess an important sigificance as those enclosed in tumor tissue. Providing that tumor cells actually participating in growth and metastasis are those of marginal area, significance of portal blood should be emphasized even more[3)4)15)18)30)31)].

Some reports on diversified effects of administration of carcinostatics by way of the vessels for hepatic tumor are instructive, on the other side, in consideration of vascular distribution in hepatic tumors. In clinical case of liver metastasis from carcinoma of the lung, FUKUYAMA[7] administered carcinostatics from the hepatic artery, autopsy finding of 37th day revealed complete scarring in central area of the metastatic tumor foci remaining no tumor cell, while in the marginal area tumor cells could be as yet observed confronting with surrounding normal liver tissue. ITO[14] administered carcinostatics from the hepatic artery in 5 cases of primary and metastatic hepatomas, and made comparative study of histological findings before and after the administration. In a case of liver metastasis from stomach cancer, second look operation carried out 3 weeks later revealed disappearance of tumor cells in the central area of tumor foci, with some tumor cells remaining in the marginal area. He reported that effect of carcinostatics administration from the hepatic artery could not be demonstrated histologically in some of primary hepatomas. The diverse effect of carcinostatics administered from the hepatic artery depending on characters and site of tumor come to be readily comprehended by the aid of the findings of the present experiment that vascular distribution shows diversity in DAB hepatoma so much depending on histological pictures, and particularly in transplanted hepatic tumors portal blood enters the marginal area of tumor foci where tumor tissue is immediately adjoining the surrounding normal liver tissue, suggesting that there exist some hepatic tumors in which participation of portal blood cannot be denied.

V. SUMMARY

Blood supply to DAB hepatoma and transplanted hepatic tumor was studied using

dye solution infusing technique.

Infusion experiment was carried out in 18 rats with DAB hepatoma which appeared after 100 days feeding with diet containing 3′-methyl-4-dimethylaminoazobenzene in 0.06 per cent, and 41 rats with tumor growth in the liver ascertained 15 days after transplantation of Walker carcinosarcoma 256 via the portal vein.

Infusion apparatus equipped with manometers was used, and the infusion of dye solution was simultaneously from the hepatic artery and portal vein carried out either with the same pressures as measured previously in the hepatic artery and portal vein or with the pressures of the similar proportion. Carmine gelatin soluton was infused into the hepatic artery and Priussian blue gelatin solution was infused into the portal vein. In some rats, trypan blue saline solution was infused into the portal vein or hepatic artery. In this occasion, saline solution was simultaneously infused from another vessel with the pressure of physiological difference. The liver infused with dye solutions were extirpated and microscopic sections were taken for the study of appearance of dye solution in tumor foci, and the results obtained are summarized as follows.

1). Diverse appearance of dye in tumor foci of DAB hepatoma was observed depending on histological characters.

a). In the area of typical cholangioma type tumor, merely dye infused into the artery could be observed.

b). In tumor foci of hepatoma type, appearance of dye infused into the portal vein was predominant, slightly mingling with the dye infused into the artery.

c). In tumor foci of mixed type, both dyes infused into the portal vein and hepatic artery were observed, and dye infused into the artery was predominant in area of cholangioma character, while in area of hepatoma character, dye infused into the portal vein could be observed.

2).

a). When colored gelatin solution was infused in transplanted hepatic tumor, in the internal area of large tumor foci excluding that of central necrosis, dye infused into the hepatic artery could be observed, and in marginal area dye infused into the portal vein existed. In small tumor foci, appearance of dye infused into the portal vein was predominant, dye infused into the hepatic artery appearing slightly.

b). When trypan blue solution was infused in cases of transplanted hepatic tumor, abundant net-work of the portal capillaries could be observed around large tumor foci, showing inflow of portal blood in marginal area of tumor foci. In small tumor foci, dye infused into the portal vein entered as far as the internal area.

From above described findings, it is assumed that hepatic tumor does not always receive blood supply solely from the hepatic artery but in some tumor the portal vein well participates in vascular distribution of hepatic tumor, depending on its histological picutres.

Accomplishing the present paper, the author is so deeply indebted to Prof. Dr. Ichio Honjo for his kind advices and supervision, and the author is also grateful to Dr. Kozaka and the members of our clinic for their kind helps.

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Fig. 1 Liver surface of rat at 15 days after transplantation of cell suspension of Walker carcinosarcoma from the portal vein. Transplanted tumor appears greyish white. Rat No. 81

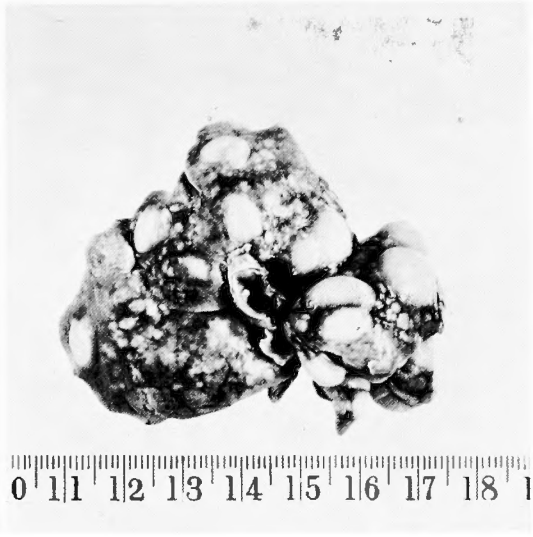

Fig. 2 Macroscopic finding of DAB hepatoma appeared after 100 days' feeding with diet containing 3'-mehtyl-DAB in 0.06 per cent. Rat No. 14

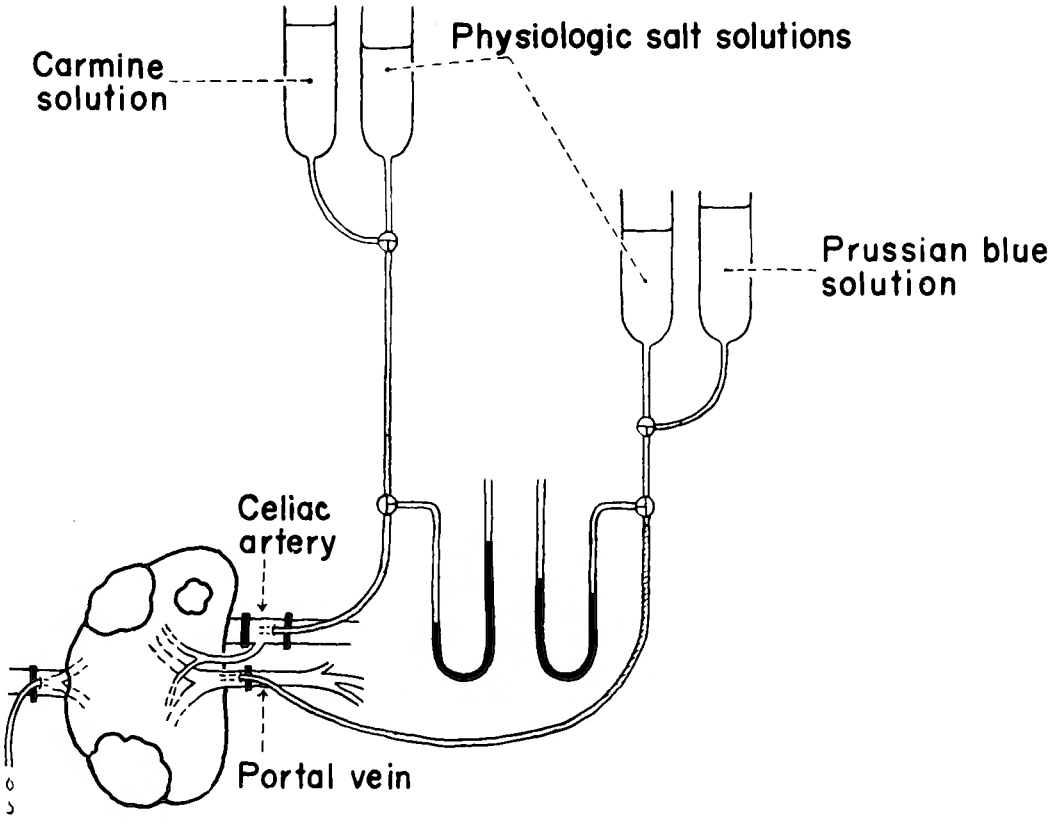

Fig. 3 Apparatus for simultaneous infusion into the portal vein and hepatic artery with various pressures.

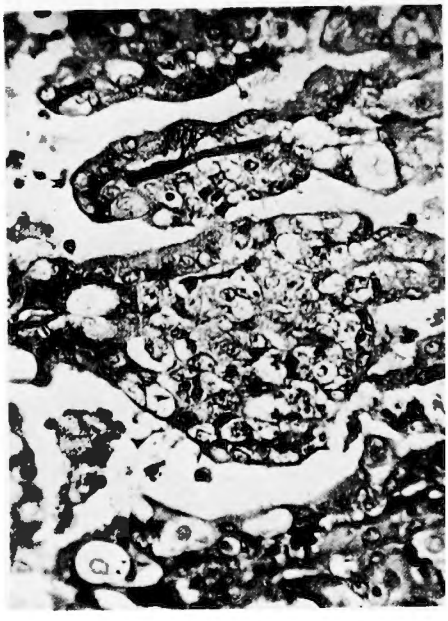

Fig. 4 3′-methyl-DAB hepatoma. Histological picture of hepatoma type.
H. E. ×400 Rat No. 14

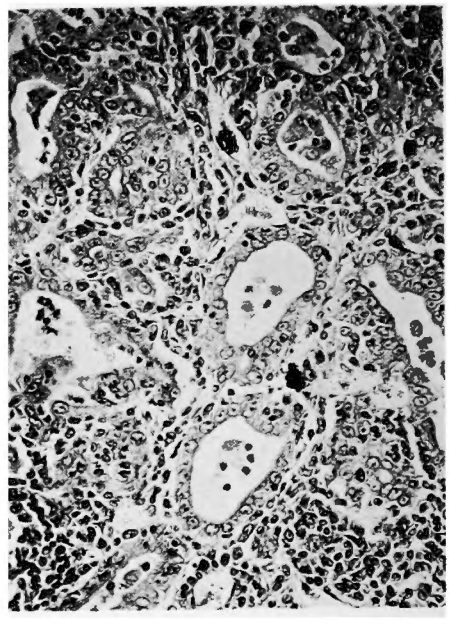

Fig. 5 3′-methyl-DAB hepatoma. Histological picture of cholangioma type.
H. E. ×300 Rat No. 31

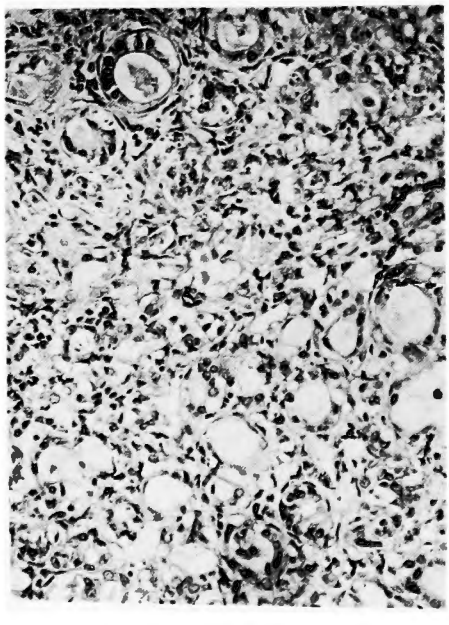

Fig. 6 3′-methyl-DAB hepatoma. Histological picture of mixed type, chiefly consisted of hepatoma type cells at the same time forming glandular lumen. H. E. × 300 Rat No. 50

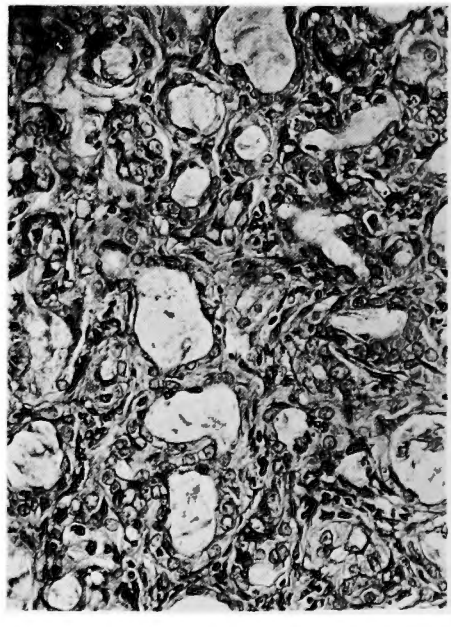

Fig. 7 3′-methyl-DAB hepatoma. Histological picture of mixed type, containing tumor cells of hepatoma type within the structure of cholangiomal type. H. E. ×300 Rat No. 33

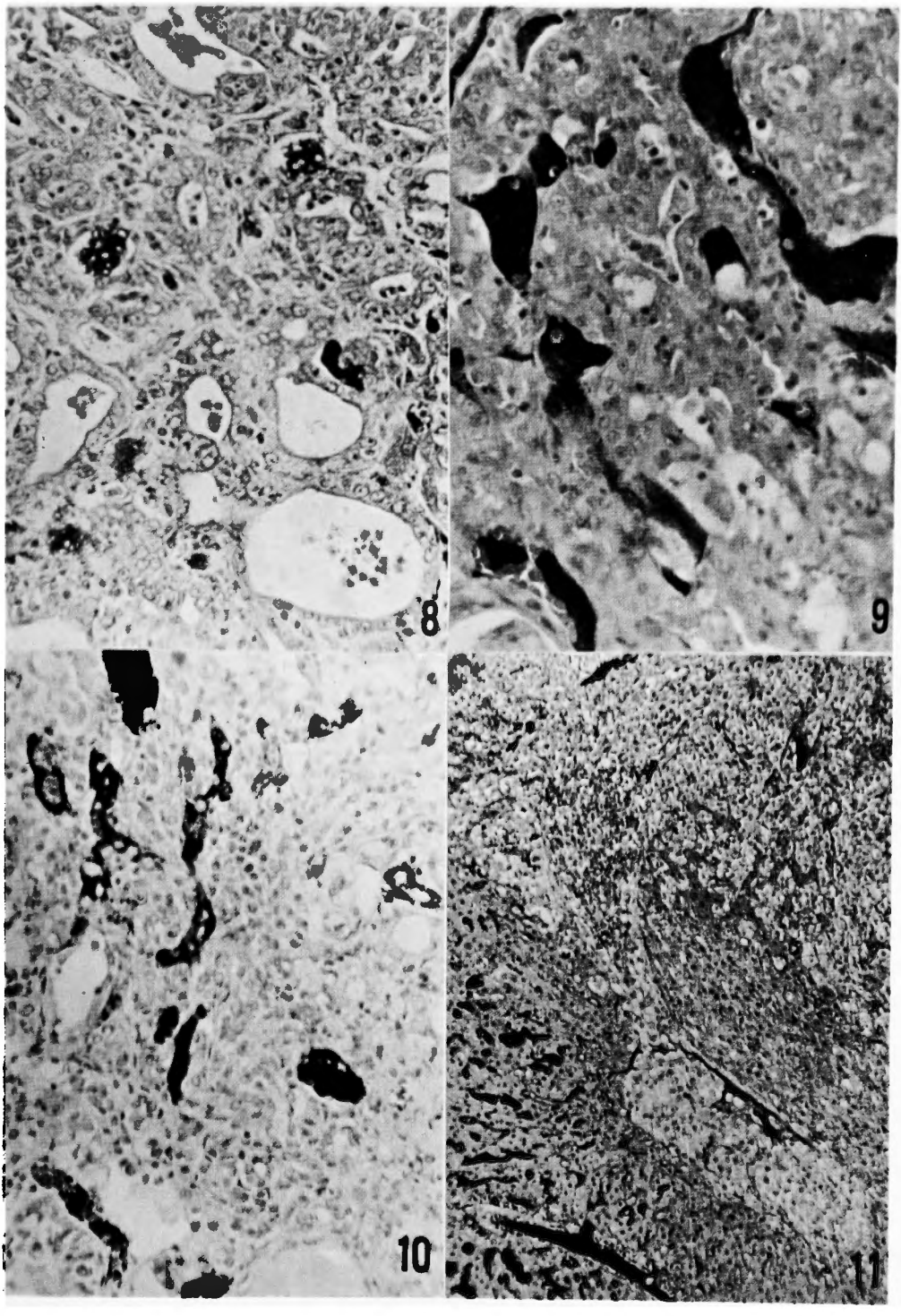
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Fig. 8 3′-methyl-DAB hepatoma. Appearance of dye in the tumor tissue of cholangioma type. Prussian blue was infused into the portal vein and carmine into the hepatic artery, using the apparatus as illustrated in Fig. 3. H. E. ×300 Rat No. 36.

Fig. 9 3′-methyl-DAB hepatoma. Appearance of dye in the tumor tissue of hepatoma type. Colored gelatin solution was similarly infused as in occasion of Fig. 8. H. E. ×300 Rat No. 38.

Fig. 10 3′-methyl-DAB hepatoma. Appearance of dye in the tumor tissue of mixed type. Colored gelatin solution was infused similarly as in occasion of Fig. 8. H. E. ×300 Rat No. 15.

Fig. 11 Appearance of dye in the tissue of transplanted hepatic tumor obtained 15 days after infusion of cell suspension of Walker carcinosarcoma 256 via the portal vein. Colored gela tin solution was similarly infused as in occasion of Fig. 8. Large tumor foci in upper part and small tumor foci in liver tissue in lower half. H. E. ×100 Rat No. 68.

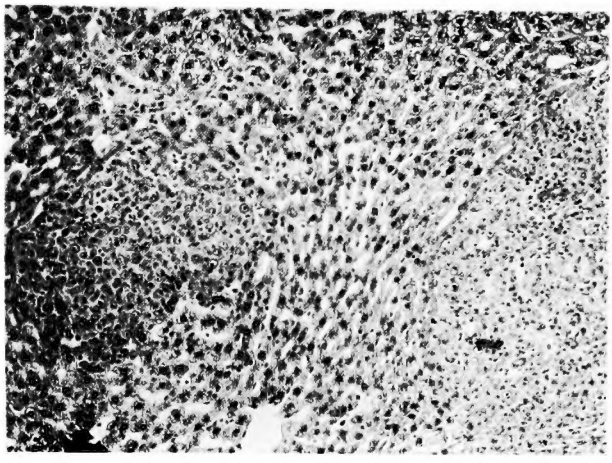


Fig. 12 Histological picture of transplanted hepatic tumor obtained 15 days after infusion of cell suspension of Walker carcinosarcoma 256 via the portal vein. Tumor focus in the right half and small tumor foci in liver tissue in the left half.
H. E. ×100 Rat No. 72

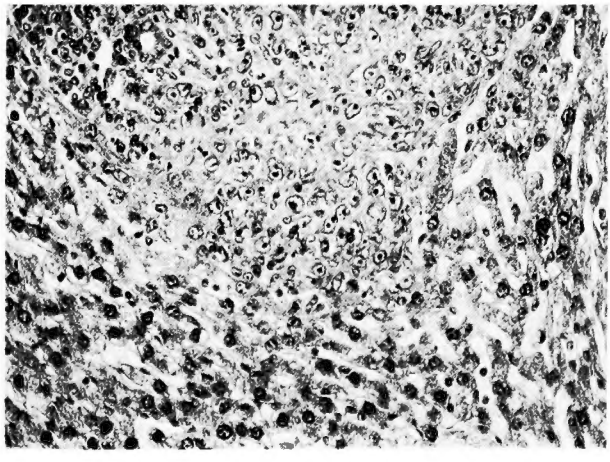


Fig. 13 High power enlargement of Fig. 12. Marginal area of tumor focus in upper half. H. E. ×300 Rat No. 72

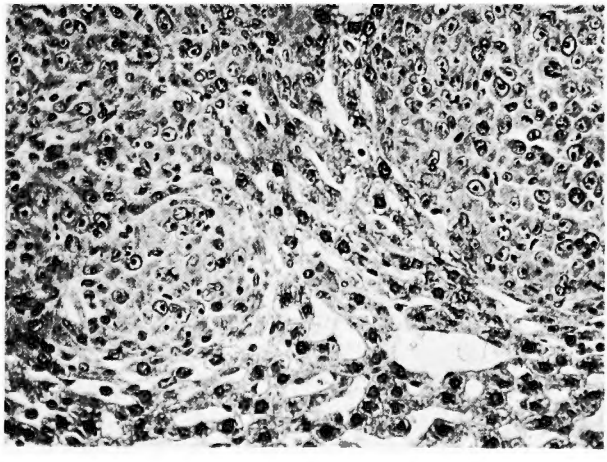


Fig. 14 High power enlargement of Fig. 12. H. E. ×300 Rat No. 72

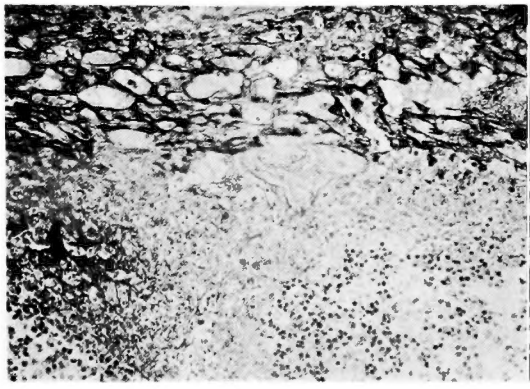


Fig. 15. Transplanted tumor tissue obtained 15 days after infusion of cell suspension of Walker carcinosarcoma via the portal vein. Trypan blue solution was infused from the portal vein with simultaneous infusion of saline from the hepatic artery with pressures as measured respectively. Relatively large tumor focus in lower half.
Safranin ×100 Rat No. 83

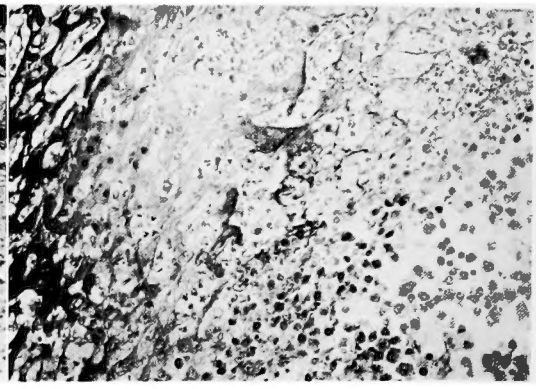


Fig. 16. High power enlargement of marginal area of tumor focus in Fig. 15. Marginal area in the right half. Safranin ×300 Rat No. 83

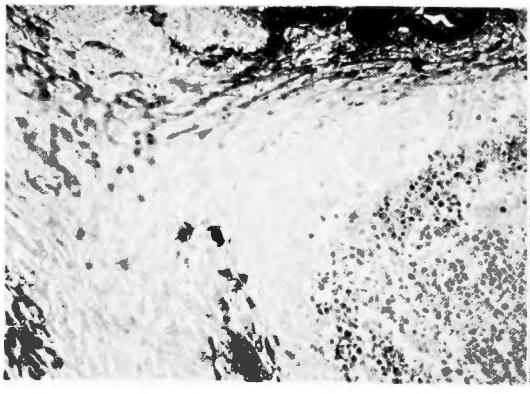


Fig. 17. Relatively small tumor foci obtained by infusion of cell suspension of Walker carcinosarcoma 256 via the portal vein. Tryapn blue solution was infused from the portal vein with simultaneous infusion of saline from the hepatic artery with pressures as respectively measured.
Safranin ×100 Rat No. 83

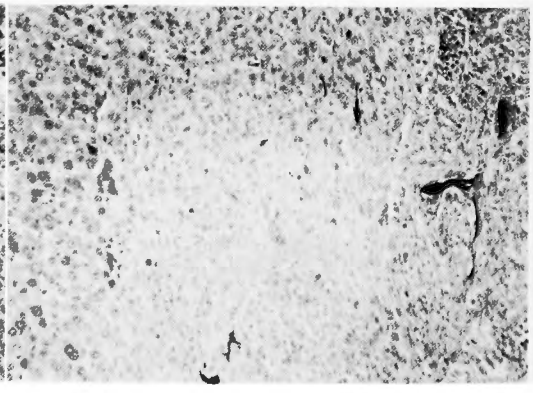
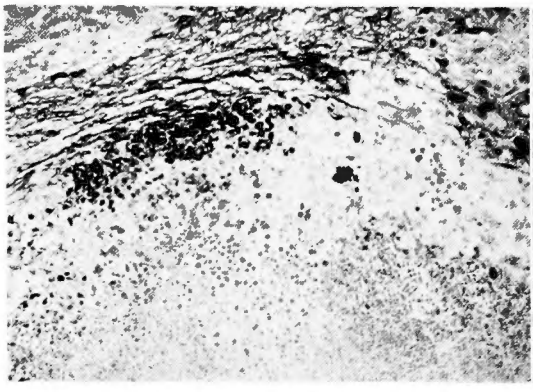


Fig. 18. Transplanted tumor tissue obtained by infusion of cell suspension of Walker carcinosarcoma 256 via the portal vein. Trypan blue solution was infused from the hepatic artery with simultaneous infusion of saline from the portal vein with pressures measured respectively. Tumor tissue in the right 3 quaters.
Safranin ×100 Rat No. 88



Fg. 19 Transplanted tumor tissue obtained by infusion of cell suspension of Walker carcinosarcoma 256 via the portal vein. Trypan blue solution was infused backwards from the portal vein with the pressure of 150 mmH_2O, the hepatic artery and portal vein being cut and severed. Safranin ×100 Rat No. 95

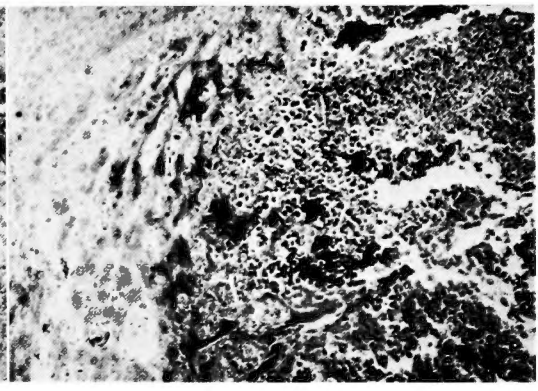


Fig. 20 Transplanted tumor tissue obtained by infusion of cell suspension of Walker carcinosarcoma 256 via the portal vein. Trypan blue solution was infused from the hepatic artery with pressure as measured previously, the portal flow being interrupted.
Safranin ×100 Rat No. 98

Homburger-Fishman's The physiopathogy of cancer. 85, A Hoeber-Harper Book, 1959.
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(* Written in Japanese)

和文抄録

実験的肝腫瘍の血行支配

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松 村 晴 夫

動脈血と門脈血の二重支配をうける特殊な臓器を発生増殖の場とする肝腫瘍が，如何なる血行支配をうけているかということは，肝腫瘍の病態生理を理解する上に，また実地臨床上肝腫瘍の治療の上にも充分考慮されなければならない問題である．従来までの諸報告ではすべての肝癌腫瘍は動脈血のみで栄養されているとする見解が有力である．

著者は生体においては肝動脈血行と，門脈血行とがたがいに微妙な関連をもつているので，なるべく生理的な肝の循環状態という点に留意し，Walker carcinosarcoma 256の rat 肝内移植腫瘍ならびに，3′-methyl-DAB肝癌を対象とした色素注入法により，肝腫瘍の脈管支配の再検討を試みた．

manometerを装備した注入装置を用い，個々のratにつき予め動脈圧と門脈圧を測定し，これらと等しい注入圧あるいは圧の比率で，同時に肝動脈ならびに門脈から色素溶液を注入した．その結果を要約すれば，次の如くである．

1) 実験的肝原発性腫瘍

門脈より Prussian blue gelatin 溶液，肝動脈から Carmine gelatin溶液を注入し，その腫瘍巣の色素流入分布状況を観察するに，その組織学的性状により異なつていることを認めた．

a) 典型的cholangioma type の部分では，動脈性注入色素のみが流入している．

b) hepatoma type の腫瘍巣では門脈性色素の存在が有力であり，動脈性色素も混在している．

c) mixed type では門脈性色素，動脈性色素の両者が存在し，cholangioma の性格が強ければ動脈性注入色素が有力であり，hepatomaの組織像と共に門脈性注入色素の存在が認められる．

2) 実験的肝移植腫瘍

Prussian blue ならびに Carmine にて着色せる gelatin 溶液注入ならびに，Trypan blue 溶液を注入し，腫瘍巣への色素流入状況を観察した．

a) 着色gelatin溶液注入では比較的大きい腫瘍巣内部全般には，肝動脈性色素のみが存在するが，肝組織に接する腫瘍巣辺縁部には門脈性色素の存在を認め，小さい腫瘍巣では全体に優勢に門脈性色素が流入している．

b) Trypan blue 溶液を門脈から，生食水を肝動脈から予め測定せる肝動脈圧と門脈圧に等しく同時に注入した場合，大きい腫瘍巣の周辺に Trypan blue で充された豊富なる毛細血管網の存在を認め，処々から腫瘍巣に向つてその末梢枝が入り込み，腫瘍細胞の間隙に色素が流入している．

c) 肝静脈より Trypan blue 溶液をほぼ門脈圧と等しい圧にて逆行性に注入し，肝動脈，門脈を切断開放すれば，腫瘍巣の辺縁部に色素の流入を認めた．

以上の結果より肝臓腫瘍の血管支配は従来の諸報告の如く常に肝動脈のみによるものでなく，腫瘍の組織性状によつて様相が異なり，肝腫瘍のなかには肝動脈のみならず，門脈血も関与する場合のあることが判明した．従つて肝腫瘍の発生病理の研究あるいは，肝腫瘍に対して制癌剤の局所投与を行なう場合などに上述の知見を充分考慮すべきであると考える．

（尚，本論文の要旨は，第1回日本癌治療学会総会，ならびに第23回日本癌学会総会において発表した．）