

Title	CANCER SPREAD VIA EXTRAVASCULAR FLUID PATH
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Citation	日本外科宝函 (1960), 29(6): 1456-1473
Issue Date	1960-11-01
URL	<a href="http://hdl.handle.net/2433/207185">http://hdl.handle.net/2433/207185</a>
Right	
Type	Departmental Bulletin Paper
Textversion	publisher

# CANCER SPREAD VIA EXTRAVASCULAR FLUID PATH

by

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Received for publication Aug. 29. 1960.

Since a local tissue invasion is a prerequisite to the formation of lymphogenic metastasis, knowledge of the pre-lymphovascular fluid path is essential for a satisfactory explanation of the spread of cancer.

This approach was initially represented by Kiharas' report.

It is the purpose of the present report to review our concepts concerning lymphogenic metastasis in the light of knowledge gained from our experiments during the past four years.

## NEWER CONCEPTS ON THE MECHANISM OF INTRAPERITONEAL ABSORPTION

Since v. RECKLINGHAUSEN (1863) discovered that intraperitoneally injected particles were rapidly absorbed through the diaphragm of rabbits, numerous studies have been made on this problem.

Despite these efforts a problem still remains unsolved: Is there any structure existent between the diaphragmatic peritoneal endothelium and the diaphragmatic lymphocapillary may be found.

However, KIHARA and his colleagues have systematically investigated this problem and discovered the fact that the particles absorbed through diaphragmatic peritoneum travel through the sievelike constitution, and enter the diaphragmatic lymph capillary. This sieve-like constitution, that is formed by both collagen and reticular fibers in the subendothelial connective tissues of the diaphragm, gives a spotlike distribution over the diaphragm. This was termed by KIHARA as MACULA cribriformis. Thereafter, this structure was discovered on parietal pleura, pericardium, mediastinal pleura, and omentum, in which were found absorbed particles into lymphatics. In other words, macula cribriformis constitutes the prelymphovascular fluid path. This pre-lymphovascular fluid path was brought to light mor-

phologically. But no functional evidence seems to have been established.

The present study was undertaken in order to study the patho-physiology of intraperitoneal absorption.

## I. HISTOLOGICAL OBSERVATIONS OF THE PERITONEUM PARTICIPATING IN ABSORPTION

The first series of experiments was designed to investigate whether macula cribriformis was detectable with accepted methods in any parts of the peritoneum except for the diaphragm and omentum. By killing rabbits and examining their peritoneal diaphragm, mesenterium, omentum, parietal peritoneum, liver, and lymphnodes in the abdominal cavity at various intervals after intraperitoneal injection with india ink. Itani obtained the following results: 1) The injected particles are removed exclusively by diaphragmatic lymphatics through conspicuously distributed macula cribriformis, 2) On the omentum, some particles are absorbed into venules and lymphatics, being chiefly distributed in the milky spots. 3) A small amount of particle are absorbed from the narrowing ring of coecum, showing dotted or complete circles covering the bowel. They seemed to be a phylogenetic remain of absorption structure, which is active on the serous surface of vertebrates as well as of mammalia. 4) Absorption of particles takes place, in a small amount in the retroperitoneum overlying the fat tissues near the kidney. At the same time, these absorbed particles are found in the retroperitoneal lymphnodes, Lgll. renales and Lgll. iliacae. 5) In the mesenterium, it is believed that a few particles, forming greyish specks near the attachment of the intestine, enter the blood stream through the venule wall. 6) A special structure for absorption i. e. macula cribriformis is not detected anywhere on the mesenterium, or on the parietal peritoneum except on the diaphragm. Nor is it detected on the serosa of digestive organs.

The above mentioned study convinced the present authors that the particulate matter injected into abdominal cavity was absorbed exclusively from the peritoneal diaphragm into lymphatics. Although, slight absorption through the omentum was shown. Moreover, it is presented that absorption through the rest of the parietal peritoneum and mesenteric folds dose not appear to be of much significance in quantity.

## II. CHANGES IN LYMPHATIC ABSORPTION FOLLOWED BY DESTRUCTION OF A MAIN PATHWAY.

The second series of experiments was designed to ascertain whether some changes of the pathway participating in the intraabdominal absorption occurred after the destruction of the main pathway in a diaphragmatic aspect. The following 2 series of experiments were performed by Itani (Table 1): Groupe B. The diaphragmatic surface of the peritoneum was devastated with silver nitrate (Table 2). Group C. The omentum was removed and diaphragmatic surface of the peritoneum was devastated with silver nitrate (Table 3). Each group of animals was injected with india ink intraperitoneally after various postoperative survivals and

Table 1 Group A : Control animals

Rabbit No.	Intervals from Injection to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L.	Pp.L.
32	1/4 hr.	-	+	-	-	-	-	-	-	-
31	1/2 hr.	+	-	-	-	-	-	-	-	-
84	1/2 hr.	+	-	-	-	-	-	-	-	-
83	1 hr.	+	+	-	-	-	-	-	-	-
30	1 hr.	+	-	-	+	-	-	-	-	-
49	1 hr.	+	+	+	+	-	-	-	-	-
85	2 1/2 hr.	+	+	+	+	-	-	-	-	-
34	3 hr.	+	+	+	-	-	-	+	-	-
35	4 hr.	+	+	-	+	-	-	-	-	-
33	24 hr.	+	+	+	+	-	+	-	-	-
36	48 hr.	+	+	+	+	+	+	+	-	-

Notes : C. L. ; Lgll. cardiacae. P. L. ; Lgll. art. pancreatico-lienalis.  
 L. L. ; Lgll. art. hepaticae. M. L. ; Lgll. mesentericae craniales.  
 R. L. ; Lgll. renales. I. L. ; Lgll. iliacaе. A. L. ; Lgll. axillares.  
 Pp. L. ; Lgll. poplitae.

Table 2 Group B : Devastation of Diaphragm by Silver Nitrate

Rabbit No.	Intervals from Operation to Injection	Intervals from Injection to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L.	Pp.L.
76	3 1/2 hr.	1 hr.	+	-	-	+	-	-	+	-	-
43	3 ds.	1/2 hr.	-	-	+	+	-	-	-	-	-
59	3 ds.	4 hr.	-	-	-	+	-	-	-	-	-
40	5 ds.	1 hr.	+	-	+	+	-	+	+	-	-
44	5 ds.	4 1/2 hr.	-	-	+	+	-	+	-	-	-
57	7 ds.	1/2 hr.	+	+	+	+	+	+	-	-	-
74	14 ds.	1 hr.	-	-	+	-	-	-	+	-	-
77	23 ds.	1 hr.	+	+	+	+	-	+	+	-	-
90	24 ds.	1 hr.	-	-	+	-	-	-	+	-	-
62	31 ds.	3 hr.	+	+	+	+	+	+	+	-	-

then bled to death at 1/2 to 4 hours. Histological studies on intraabdominal lymphnodes, parietal peritoneum and mesenterium were performed.

A microscopic study also reveals marked differences between normal and treated animals subjected to intraabdominal absorption as follows : 1. The lymphnodes in the epigastric region, Lgll. cardiacae, Lgll. art. pancreatico-lienalis and Lgll. hepaticae, were containing carbon particles from the early stage in both normal and treated animals. However, it seems to be of a great significance that the carbon particles were detected most markedly and most rapidly in the medullary sinuses of Lgll. mesentericae craniales of the treated group (B and C.). A comparison of Group B and Group C with coloured superior mesenteric nodes shows a less extent of carbon particles in the Group B. These differences can not be attributable to

**Table 3** Group C : Devastation of Diaphragm and extirpation of Omentum.

Rabbit No.	Intervals from Operation to Injection	Intervals from Injection to Death	Liver	C. L.	P. L.	L. L.	M. L.	R. L.	I. L.	A. L.	Pp.L.
78	2½ hr.	1 hr.	†	—	—	—	—	—	—	—	—
79	1 d.	1 hr.	+	—	—	—	—	†	—	—	—
45	4 ds.	1 hr.	—	†	†	+	+	—	—	—	—
46	4 ds.	3 hr.	—	+	+	+	+	—	—	—	—
50	7 ds.	1 hr.	+	+	—	—	+	—	+	—	—
47	7 ds.	3 hr.	+	—	+	+	—	—	†	—	—
73	12 ds.	1 hr.	—	+	+	+	†	+	—	—	—
65	26 ds.	1 hr.	—	—	†	—	†	‡	+	—	—
54	29 ds.	1 hr.	—	†	†	†	—	‡	+	—	—
66	30 ds.	3 hr.	—	‡	†	—	†	—	—	—	—
60	57 ds.	1 hr.	—	†	‡	†	+	‡	‡	—	—

the effect of the extirpated omentum. It is explained by the fact that since only small amounts of deposits of carbon particles in the liver were detectable in both B and C Groups, the amounts of absorption through the omentum is out of the question. Moreover, in the retroperitoneal lymphnodes such as Lgll. renales and Lgll. iliacae of the treated one, more rapidly and markedly the dye was found than the control. Briefly speaking, the more perfectly blockage of a diaphragmatic aspect continues, the more evidently colouring of nodes and lymphatics is evidenced. We are of the opinion that these features are analogous to those of Lgll. mesentericae craniales.

In both Group B and Group C, absorption was observed, though in a small degree through the peritoneum, attachment of the intestine, over fat tissues near the kidney, and the narrowing ring of the coecum. Such absorption is more evident and rapid than in control animals.

From the foregoing data he emphasizes a compensative function resulting from a diaphragmatic closure in which Lgll. mesentericae craniales are found containing free carbon particles in their medullary sinuses rapidly and evidently.

### III. SOME DETECTABLE CHANGES OF THE PRE-LYMPH-VASCULAR PATH UNDER VARIOUS TYPES OF PERITONITIS.

Many authors including Opie, Menkin and Bangham, have advocated that an inflammatory reaction in the peritoneal cavity induced by some irritants tends to be localized in situ. Under such conditions, however, no evident histology of the pre-lymphovascular pathway such as macula cribriformis of the diaphragm was obtained. The present study was undertaken to ascertain whether or not macula cribriformis of the diaphragm, acting as a main intraabdominal absorption route, may suffer some morphological changes under such peritonitis. The following two series of experiments with albino rabbits were performed by Itani. The animals

Table 4 Group D : Sterile Peritonitis induced by Turpentine oil.

Rabbit No.	Intervals from Operation to Injection	Intervals from Injection to Death	Liver	C.L.	P.L.	L.L.	M.L.	R.L.	I.L.	A.L.	Pp.L.
67	1 hr.	1 hr.	##	+	+	-	-	+	-	-	-
63	4 hr.	1 hr.	-	-	-	-	-	-	-	-	-
81	10 hr.	1 hr.	-	-	-	-	+	-	-	-	-
56	24 hr.	1 hr.	-	-	-	-	-	-	-	-	-
59	24 hr.	3 hr.	+	+	-	+	+	+	-	-	-
55	28 hr.	1 hr.	-	-	-	-	-	-	-	-	-
68	4 ds.	1½ hr.	-	+	+	-	+	-	+	-	-
70	7 ds.	1 hr.	-	-	-	+	-	-	-	-	-
80	14 ds.	1 hr.	+	-	+	-	+	+	+	-	-

Table 5 Group E : Extirpation of Omentum.

Rabbit No.	Intervals from Operation to Injection	Intervals from Injection to Death	Liver	C.L.	P.L.	L.L.	M.L.	R.L.	I.L.	A.L.	Pp.L.
75	3 hr.	1 hr.	-	-	-	-	-	+	-	-	-
72	1 d.	1 hr.	-	-	-	-	-	-	-	-	-
37	3 ds.	1 hr.	+	-	-	+	+	+	-	-	-
42	3 ds.	5 hr.	+	+	+	-	-	+	-	-	-
41	5 ds.	½ hr.	-	+	+	+	+	+	+	-	-
38	5 ds.	3 hr.	+	+	-	+	-	-	+	-	-
71	7 ds.	1 hr.	+	-	-	+	-	-	-	-	-
53	26 ds.	1 hr.	+	+	+	-	-	+	+	-	-

belonging to Group D (DT) were injected intraperitoneally with turpentine oil (Table 4). In Group E, extirpation of the omentum was performed under ether anaesthesia (Table 5). At various intervals following such procedure, india ink was injected intraperitoneally. Then they were sacrificed 1/2 to 5 hours later. While making a morphological study on the stretched diaphragm, omentum, and other parts of peritoneum, he compared the amounts of carbon particles through the diaphragm with those in retrosternal lymphnodes and liver. He obtained the following results: In Group D showed acute inflammatory changes, such as marked ascites, and abnormally dull colored peritoneum were found. Approximately, 1 or 2 hours after intraperitoneal injection with turpentine oil. DT rabbits disclosed a normal-shaped macula cribriformis which was covered partly with a fibrous mesh. The retrosternal lymphnodes, liver, spleen were accompanied with conspicuous carbon deposits as control animals. The particles which had been injected 5 hours after injection of an irritant, were detained in these organs. This detaining was markedly demonstrated in cases which had turpentine peritonitis for two weeks. Nevertheless, the ten hours or more elapsed cases after injection of an irritant, could reveal

marked changes on the diaphragm i. e. so-called concentric luminal narrowing resulting from the thickened and tortuous collagen fibers with the replacement of fine reticulum fibrils to thick fibers.

#### IV. AN EXTRAVASCULAR LEAKAGE PHENOMENON OF RETROSTERNAL LYMPHATICS.

A recent comprehensive anatomical report on intraabdominal lymph drainage is presented by KIHARA and his co-workers. Carbon particles absorbed into the lymphocapillary of the diaphragm through macula cribriformis from the peritoneal cavity are removed mainly via retrosternal lymphatics or the thoracic duct. However, they discovered an extravascular leakage phenomenon of retrosternal lymphatic vessels that was attributable to a special function of these lymphatics to excrete foreign bodies outwards. And they concluded that the constant existence of the reticulum fibers at the leakage site was definitely located.

A confirmation of this physiological phenomenon is established in the study of a series dealing with pericostal tuberculosis by AOYAGI and his collaborator YAMAMORO (1957). They emphasize that constant occurrence of various sized and extents of the tuberculous lesion is observed in the adipose tissues around the internal mammary lymphatics. This occurrence seems attributable to leakage phenomenon of internal mammary lymphatics.

Physiologically, the present result was in conformity with that of the above mentioned interpretation.

#### AN EXPERIMENTAL STUDY ON LYMPHATIC METASTASIS

The appearance of spontaneous metastasis is quite different from a metastatic growths produced by an intravascular injection method in which tumor cells are injected into lymphatics using excessive pressure. For the purpose of obtaining the easily demonstrable site for the spontaneous metastasis, the authors performed the foregoing studies. In consequence of this, since various aspects of the lymphatic drainage system from the abdominal cavities become evident, the authors select the lymphatic path from the peritoneal diaphragm to the upper retrosternal lymphnodes as a favourable site for studying a lymphatic spread of cancer.

It was obvious, also, that metastasis of tumor when tumor cells were injected intraperitoneally, occurred chiefly in sternal lymphnodes. However, whether or not in anywhere along these paths intervening between diaphragmatic endothelium and their regional superior sternal lymphnodes further tumor metastasis may occur, is the subject of the present study.

#### AN EXPERIMENTAL STUDY ON THE SPREAD OF CANCER IN THE LYMPHATIC SYSTEM

The tumors used were the transplantable YOSHIDA ascites hepatoma in rats, and Ehrlich ascites tumor in mice. For each experiment 0.1-1.0 cc of tumor cell

suspension was used. Counts of tumor cells ranged from 190,000 to 250,000/cmm. By killing the animals and examining histologically the diaphragm, surrounding fatty tissues encircle internal mammary lymphatics, sternal lymphnodes, and the thoracic duct at various intervals from one to ten days after intraperitoneal inoculation, we have obtained the following results: 1) The site of arrest of tumor cell emboli in diaphragm.

The first problem is to ascertain whether or not tumor cells, inoculated in the abdominal cavity, may pass immediately through macula cribriformis of the diaphragm. One to ten days after injection, the diaphragmatic peritoneum was peeled out from the underlying submesothelial connective tissues as thinly possible; stretched preparations were prepared for silver impregnation stain or May-Giemsa stain.

Early changes of these diaphragm revealed the most prominent features: Each sieve of the fenestrated macula cribriformis were densely packed with embolic tumor cells (Fig 5 and 6). Some of them disclosed an evident mitotic division. These embolic deposits gradually developed being accompanied by proliferative reticulin fibrils and confluent with each other. In these cases, on any spot of the diaphragm there are evidently observed remnants of small embolic deposits of tumor cells in the preexistent macula cribriformis. When these foci became older, they tended to be encapsulated by the proliferated reticulum and collagen fibers. A strong conviction for the morphogenesis of these lesions was obtained from serial sections of the diaphragm.

When cells of Ehrlich ascites tumor were used, the result was the same as that obtained with YOSHIDA ascites hepatoma (Fig. 8).

In the experiment just described, it seems conceivable that part of the absorbed tumor cells may stopped at the first filter station, i. e. macula cribriformis, which works as an effective barrier to a further spread of cancer.

2) Metastases resulting from cancer cells leaking through retrosternal lymphatics.

The second problem is to ascertain whether or not tumor cells may leak immediately through the internal mammary lymphatics to the surrounding adipose tissue. For this purpose, the anterior portion of the chest wall in the just described experiments which at necropsy appeared to be normal was removed as part of a block resection including the internal mammary chain, intercostal muscles, ribs, costal cartilages, and pleura, but the resection stopped at the sternal margin and was studied by serial section.

Microscopic sections, stained with hematoxylin eosin, revealed in most of the cases, an early tumor growth invariably anywhere in and around the fatty tissues around retrosternal lymphatics. Gradually, these become more remarkable, and occasionally many cancer cells permeated the interstitial tissue of the intercostal muscles and transversus thoracic muscles (Fig. 7). Since we often disclosed vigorous tumor cells in mitosis within these fatty tissues, we pointed out that these results indicated a successful metastasis of the absorbed tumor cells. In early stages, cancer cells involve the perivascular area of the retrosternal lymphatics. However,



they tend to increase in number. It was conceivable, also, that the involvement may result in a leakage through the wall of retrosternal lymphatics, disclosing physiologically their special function i. e. leakage phenomenon, since these lymphatics in the early cases occasionally contained tumor cells showing no direct invasion. Sections with silver stain revealed that cancer cells have a tendency to leak at the internal mammary lymphatics, in which reticulum fibers are arranged.

### A HISTOLOGICAL STUDY ON HUMAN AUTOPSY MATERIALS

A histological study was made on 5 fatal cases of tumor including intraabdominal tumors. Their entire anterior portion of the chest wall including internal mammary lymphatics, the thoracic duct, parasternal nodes, and the diaphragm were removed and dissected. Multiple blocks representing cross sections were prepared for microscopic study. Those preparations which were peeled at the plan of submesothelial connective layer and stained with May Giemsa method, Bielschowsky-Maresch silver impregnation method and haematoxylin eosin showed clearly the pattern between cancer cells and macula cribriformis. The most prominent feature of human autopsy materials was that macula cribriformis was packed with cancer cells. This fact was ascertained also with sections cut in a plane perpendicularly to the surface of the diaphragmatic peritoneum (Fig. 9).

The cancer cells in the anterior portion of the chest wall of human autopsy materials occupy the same sites and show the same relationship to internal mammary lymphatics as observed in animals (Fig. 10). The larger parts of leaked cancer cells into adipose tissues around internal mammary lymphatics were not affected by the destructive and degenerative changes in cytoplasm as well as in nuclei.

We emphasized that the incidence of such involvement in sub-diaphragmatic tumors is not unusual and that the impression of rarity persists because of neglect of study of the diaphragm and internal mammary lymphatics.

### A DEFENSIVE RESPONSE OF MACULA CRIBRIFORMIS TO A CANCER INVASION.

Macula cribriformis of the diaphragm of the mice 24 hours after intraperitoneal injection with Ehrlich ascites tumor cells is nearly always found to inhibit slightly a further passage of tumor cells through them by a luminal narrowing followed

**Table 6** Morphological Changes of Macula Cribriformis induced by Intraperitoneal Injection with Ehrlich Ascites Tumor Cells.

After Injection	Collagen Fibers		Reticular Fibers			Concentric Luminal Narrowing	Coiled Cancer Cells	
	Swelling	Tortuous	Swelling	Tortuous	Proliferation		Number	Degeneration
The First Day	÷	+	÷	+	-	÷	Slightly	-
The Third Day	+	++	÷	++	-	+	Slightly increased	-
The Fifth Day	> ++	++	++	++	-	++	Many	+
The Seven Day								

by a wavy-swelling of collagen and reticulum fibers.

The adjacent fat tissues along the course of retrosternal lymphatics or retrosternal lymph nodes were the site of the metastasis. However, these leaked cancer cells spread more vigorously toward the interstitial connective tissue 5 days after inoculation. These results were summarized in Table 6. From the above mentioned data, the impression was that there was a slight interference with a further spread of cancer through macula cribriformis under a normal condition.

### THE RESPONSE OF HYALURONIDASE ON INTRAABDOMINAL ABSORPTION.

Some workers have postulated a possibility that cancers liberate hyaluronidase and that the pathway for invading cancer cells is opened in consequence of hydrolyzation of hyaluronic acid in connective tissues. However, some workers denied this possibility. Quite apart from the problem whether cancers contain hyaluronidase, or not, in the hope that chemical components in macula cribriformis may be detectable the authors tried to ascertain the effects of hyaluronidase upon the passage of carbon particles through macula cribriformis.

Thirty minutes prior to intraabdominal injection of india ink, rabbits intraperitoneal injected with various units of hyaluronidase. Rabbits were bled to death one hour after intraperitoneal injection with india ink. A histological comparison of the diaphragm and the anterior portion of the chest wall in each group was performed in reference to the absorption quantity of india ink.

**Table 7** The Response of Hyaluronidase on Intraabdominal Absorption.

		Diaphragm		Leaked Carbon Particles in Adipose Tissue along the Retrosternal Lymphatics
		Pars Tendinosum	Pars Musculorum	
Hyaluronidase	100 Unit	###	###	++
"	200 "	###	###	##
"	1000 "	+	÷	+
Control (Distilled Water)		###	###	++

The results as illustrated in Table 7. were obtained. In spite of the author's anticipation, however, the results suggest that high units of hyaluronidase cause the concentric luminal narrowing resulting from the swelling and torsion of the collagen fibers in macula cribriformis, and consequently intraabdominal absorption was unexpectedly reduced.

### DISTRIBUTION OF THE ABSORBED TUMOR CELLS IN THE INTERNAL MAMMARY LYMPHATICS AND THORACIC DUCT FOLLOWING INTRAPERITONEAL ADMINISTRATION.

To determine if tumour cells passed through the macula cribriformis of the diaphragm, a suspension of Ehrlich ascites tumour cells was injected into the abdominal cavity of rabbits directly upon the inferior surface of the diaphragm. Thirty

minutes later a drop of lymph was obtained from main excreting collecting lymphatic channels (the starting point of the internal mammary lymphatic) on either side adjacent to the sternum by exposing the pleural surface of the diaphragm, and simultaneously from the thoracic duct just above the level of the VIIIth vertebrae.

This lymph was then observed under a phase contrast microscope. Simultaneously, lymph was put into a R. B. C. pipette and diluted to 1:100 concentration with acet-gentianaviolett solution and transferred to a counting chamber. The result was given in table 8. Table 8. strongly suggests a higher absorptive distribution of tumour cells in the internal mammary lymphatics than in the thoracic duct. Furthermore, it becomes evident that the tumour cells in the internal mammary lymphatics indicated no degenerative changes in shape of mitochondria. On the contrary, the tumour cells contained in the thoracic duct indicated various types of cellular degeneration; degenerating cells with vesicular mitochondria or semilunar type of mitochondrial degeneration. From this fact, it is strongly suggested that the internal mammary lymph seemed to be a more suitable living medium for tumour cells than the thoracic lymph which contained various factors related to the digestion. As stated by TAKEDA, the reason to choose the changes of the mitochondrial shape as a susceptible sign of the cellular degeneration is that the changes in the mitochondrial shape are in parallel with the cellular degenerative changes.

Next, the impression was gained that there was some interference with absorption by macula cribriformis. This impression was reinforced by the histological evidence that the areas of macula cribriformis of this rabbits were packed with cancer cells.

**Table 8** Shows the Distribution of Tumour Cells According to Number in Lymph.

	Peritoneal Cavity	Internal Mammary Lymphatics	Duct. Thoracicus
Tumor Cell Number/cmm.	308,000	54,000	10,000
Features of Phase-Contrast Microscope		No degeneration	Degeneration Remarkable

### PREVENTING AGAINST METASTASIS.

A complete prevention against metastasis is the crux of the problem of survival. Before the cause and cure of cancer can be determined, it necessary to use methods available at present to prevent metastasis. The authors had the following experiment to ascertain whether or not an immediate passage of tumor cells through the macula cribriformis and the spread followed by the leaked tumor cells through the retrosternal lymphatics following administration of some anti-cancer agents could be prevented. Mice, weighing approximately 20g. were used. The series of experiments shown in Table 9. were performed. The tumors used were the transplantable Ehrlich ascites tumors in mice. By killing the animals at various

intervals, histological studies were performed.

Table 9 Method of Proceeding.

Group	Nitromin	Anti-Cancer Agent			Colcemid	Ehrlich Ascites Tumor
		Carzino-philin	Sarcomycin	Thio-TEPA		
1	1 mg/kg	100 U/kg	0.02 g/kg	1 mg/kg	0.01mg/kg	
2	2 "	200 "	0.04 "	3 "	0.02 "	
3	3 "	300 "	0.06 "	5 "	0.04 "	
4	Solvent.	Solvent.	Solvent.	Solvent.	Solvent.	
5	2 mg/kg	200 U/kg	0.04 g/kg	1 mg/kg	0.01mg/kg	Tumor cells Inoculated at 24 Hours after Final Injection with Anti-Cancer Agents
6	"	"	"	3 "	0.02 "	48 Hours
7	"	"	"	5 "	0.04 "	72 Hours
Intervals		7 Days				7 Days after inoculation

## RESULTS.

In the first group of experimentation, animals under normal condition are submitted to intraperitoneal injection with anti-cancer agents of various degrees, and the following results were obtained: There were no appreciable differences of diaphragmatic macula cribriformis between control mice and mice treated with the following dose: Sarcomycin 0.02 gr./kg., Nitromin 1mg./kg., Carzinophilin 200 units/kg., Thio-TEPA (Triethylene Thio-phosphoramidate) 1 mg/kg., Colcemid (Colchitin) 1mg/kg. However, Sarcomycin 0.04gr/kg. or more, Carzinophilin 300 units/kg. or more, Nitromin 2mg/kg. or more, Tespamin 5mg/kg. or more. Colcemid 0.01mg/kg. or more, showed a typical response to a further spreads of cancers. The structural elements of the macula cribriformis changed by these drugs more or less consisted of severely altered collagen fibers. The collagen fibers disclosed the fibrinoid swellinglike changes, i. e., very thickend and somewhat tortuous fibrils. The most pronounced feature was the various degrees of the overproliferation of fine reticular fibrils in all series, except for Carzinophilin. These tendencies were most remarkably shown in Colcemid and Sarcomycin and less weak in Nitromin and Thio-TEPA. Carzinophilin had no such response (Table 10 and 11). Such features suggest an extreme inhibition of intraabdominal absorption through the diaphragm. In fact, the inhibition of cancer cell passage through macula cribriformis was essentially due to the concentric luminal narrowing followed by these fibrosis.

When the intraabdominal pre-administration of anticancer agents is superimposed on intraabdominal inoculation with Ehrlich ascites tumor cells, the lodged cancer cells in macula cribriformis are coiled around by proliferating reticular fibrils and then fallen into degeneration. Moreover, such cases failed to yield leaked metastasis

through the retrosternal lymphatics. The results of X-ray irradiation on rats were in accord with the response of Nitromin.

**Table 10** Morphological Changes of the Diaphragmatic Macula Cribriformis Induced by the Anti-Cancer Agents.

Group	Dosis/kg.	Diaphragm						
		Collagen Fiber			Reticulum Fiber			
		Swelling	Tortuous	Dispersion	Swelling	Tortuous	Dispersion	Increasing
nitromin	1 1 mg.	+	-	-	+	-	-	-
	2 2 mg.	+	+	-	+	+	-	##
	3 3 mg.	+	+	-	+	+	-	##
	4 Solvent.	+	+	-	+	+	-	-
carzinophilin	1 100U.	##	-	+	##	-	-	-
	2 200U.	##	-	##	##	-	##	÷
	3 300U.	##	-	##	##	-	##	÷
	4 Solvent.	##	##	-	##	##	-	-
sarcomycin	1 0.02g.	+	+	-	-	-	-	+
	2 0.04g.	+	+	-	-	-	-	##
	3 0.06g.	+	+	-	-	-	-	##
	4 Solvent.	+	+	-	+	-	-	-

**Table 11** Morphological Changes of Fiber of Macula Cribriformis Induced by the Anti-Cancer Agents

	Increasing	Swelling	Tortuous	Inhibitory Effects to the Passage of India Ink through Macula Cribriformis
Colcemid	##	+	##	##
Sarcomycin	##	+	+	##
Nitromin	+	+	+	##
X-Ray Radiation	+	+	+	##
Thio-TEPA	+	+	±	+
Carzinophilin	±	##	±	+

## DISCUSSION

A recent comprehensive concepts on metastasis of neoplasms is presented by Zeidman and Coman et al. They emphasized that the development of a blood-born metastasis involves three major aspects i. e. (I) invasion, (II) embolism, (III) development of arrested emboli. As they described, it is conceivable that at least a major factor responsible for invasion is decreased adhesiveness, ameboid motility and penetrability of cancer cells against the vascular wall. However, it is the authors' opinion that a successful resolution of fundamental changes that occur in the primary tumor cells and metastatic cells is quite necessary. But, quite apart from this consideration, it is necessary to study the mode of tumor cells which invade the prelymphovascular fluid path or extravascular fluid path. No experimental work on the invasiveness of the cancer cells to the pre-lymphovascular fluid

path does not seem to have been so far reported. The present experimental results indicate that cancer cells in part are caught by prelymphovascular fluid path, where macula cribriformis or reticular fibers were arranged. They occupy an intraluminal position, where they come into close relationship with reticular and collagen fibers. At least part of them had the degenerative changes. It was considered that the factors responsible for retrogression of the embolic cancer cell deposits are attributable to inhibitory agents of the proliferating reticular fibers. This result may suggest the effectiveness of the macula cribriformis as a temporary barrier to a further spread of cancers.

While, the paramount interesting thing was that the cancer cells discharged into lymphatics leak into the surrounding environment at a definite site in the course of lymphatic passage. Sometimes, these leaked cells or embolic deposits cancer cells in the macula cribriformis seem to have remained dormant in the adipose tissue or in the macula without ever producing remarkable symptom. Sometimes after such a resting period the tumor cells may regain their vigor and flare up anew with widespread through out the body. However, leaked cancer cells or deposited cancer cells in the macula cribriformis in its relation to surrounding tissues, may be more complex than hitherto imagined. Still left entirely unexplained, however, is the problem in this field.

The soil hypothesis seems to have been widely accepted. If cited the soil hypothesis, it is conceivable that the adipose tissue encircle internal mammary lymphatics may be the fertile soil suitable for growing the cancer cell. But, still left entirely unresolved.

Since these extravascular leaked implants were observed, an attempt was made to carry out the foregoing experiment to prevent such metastatic mechanism. Any reasonable, practical measure which may reduce the incidence of cancer metastasis is suggested for use, until the cause and cure of cancer can be determined. But, complete inhibitory response the ultimate aim of the authors' attempt, were not obtained from our data. In any way, it is interesting that these anti-cancer agents that are regarded only as a radiomimetica responsible for cancer cell destruction, show considerable effects on the prelymphovascular fluid path or lymphatics.

However, concerning the passage of tumor cells through macula cribriformis, it is considered desirable to make a quantitative measurement of the differences in the discharged agent into lymphatics between animals treated with anti-cancer agents and controls. The above mentioned experiments strongly suggest that these anti-cancer agents may inhibits such form of metastasis to some extent although not completely.

## SUMMARY

A study is made of the development of metastasis regarding the passage of tumor cells through the prelymphovascular fluid path in reference to the phenomenon of their extravascular leakage.

Part of the tumor cells which are intraperitoneally transplanted deposit on the specifically featured sites, i. e., macula cribriformis and the area of lymphatic

leakage. It is considered that such involvement may be found more widely on a more careful observation. We emphasize that the extravascular fluid path may participate in cancer spread.

We are much indebted to Emeritus Prof. Dr. Takusaburo Kihara and Prof. Dr. Fukuzo Oshima, for their kind guidance throughout this experimental study.

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Fig. 1 Normal appearance of the diaphragmatic Macula Cribriformis (Rabbit) ×100

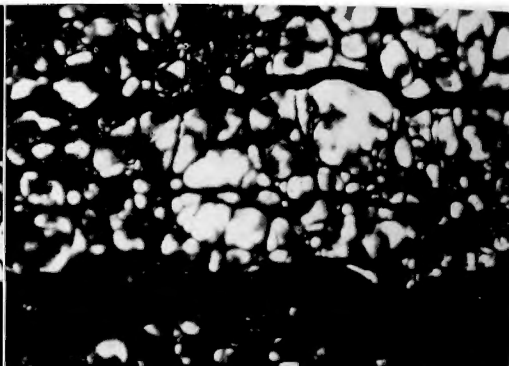


Fig. 2 Normal appearance of the diaphragmatic Macula Cribriformis (Rabbit) ×400



Fig. 3 Normal appearance of the diaphragmatic Macula Cribriformis (Human) ×100

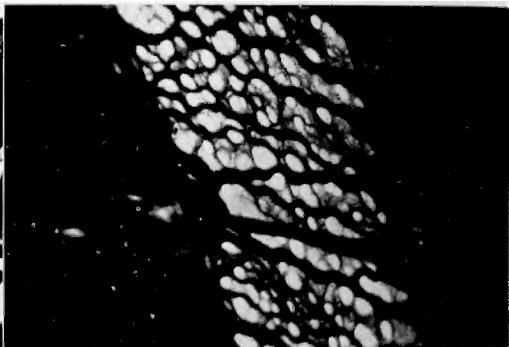


Fig. 4 Normal appearance of the diaphragmatic Macula Cribriformis (Mouse) ×200



Fig. 5 Each sieve of the fenestrated Macula cribriformis were densely packed with embolic Yoshida ascites hepatoma cells (silver impregnation stain) ×100

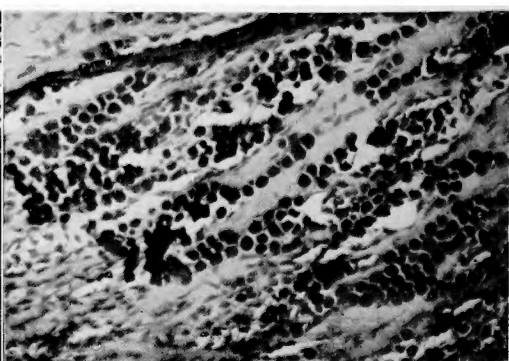
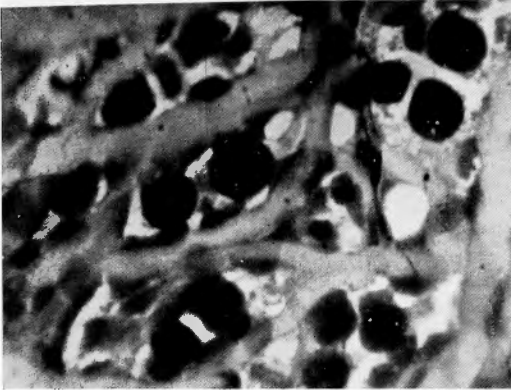
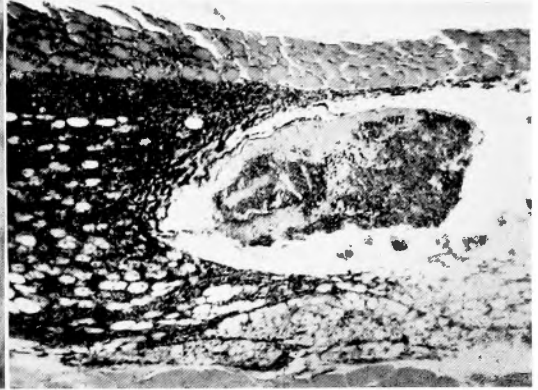


Fig. 6A Each sieve of the fenestrated Macula cribriformis were densely packed with embolic Yoshida ascites hepatoma cells (May-Giemsa Stain) ×200





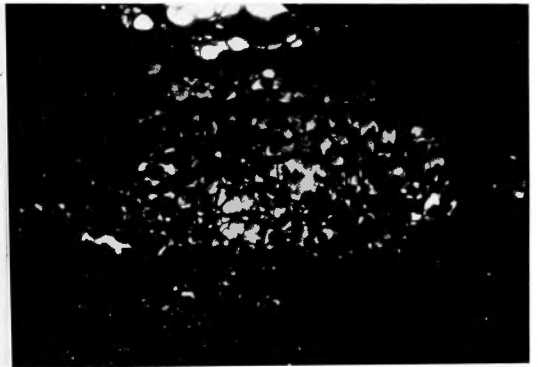
**Fig. 6B** Each sieve of the fenestrated macula cribriformis were deusely packed with embolic Yoshida ascites hepatoma cells (May-Giemsa stain)  $\times 400$



**Fig. 7A** Leaking tumour cells in and around the fatty tissues around the internal mammary lymphatics (Yoshida sarcoma Rat)  $\times 100$



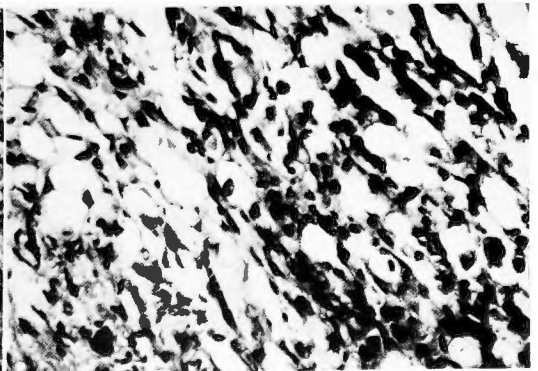
**Fig. 7B** Leaking tumour cells in and around the fatty tissues around the internal mammary lymphatics (Yoshida sarcoma Rat)  $\times 200$



**Fig. 8** Macula cribriformis of the human autopsy materials were packed with cancer cells. (extension preparat)



**Fig. 9A** Leaking cancer cells through the internal mammary lymphatics (Gastric cancer human materials)  $\times 100$



**Fig. 9B** Leaking cancer cells through the internal mammary lymphatics(Gastric cancer human materials)  $\times 200$

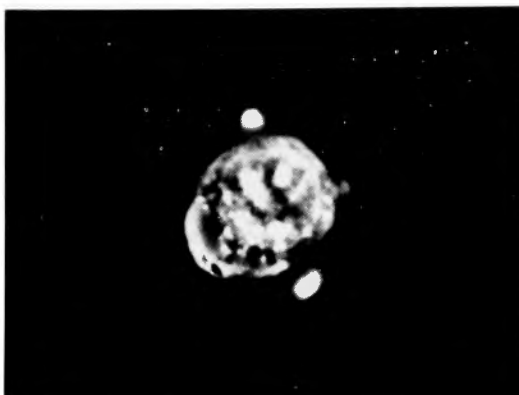


Fig. 10 The phasecontrast microscopic features of the internal mammary lymph containing the tumour cells. No degeneration

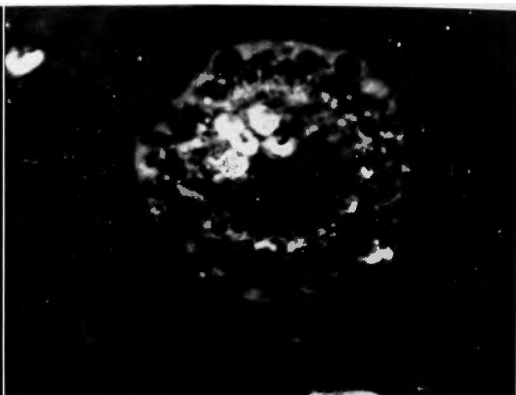


Fig. 11 The phasecontrast microscopic features of the thoracic duct lymph containing the tumour cells. Cellular degeneration remarkable.

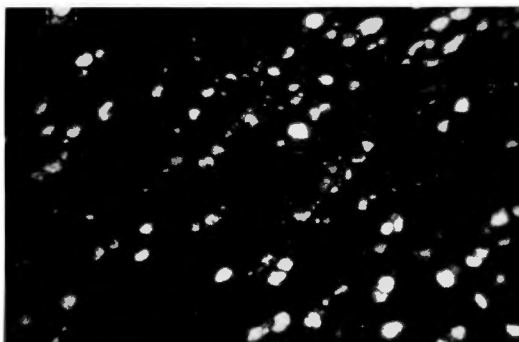


Fig. 12 The luminal narrowing of the macula cribriformis of the mouse treated with Colcemid. These changes were mainly induced by the overproliferation of the reticular fibrils.



Fig. 13 The luminal narrowing of the macula cribriformis of the mouse treated with Colcemid. These changes were mainly induced by the overproliferation of the reticular fibrils. with Sarcomycin.

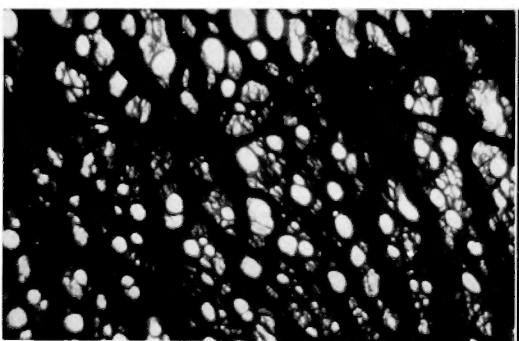


Fig. 14 The luminal narrowing of the macula cribriformis of the mouse treated with Colcemid. These changes were mainly induced by the overproliferation of the reticular fibrils. with Nitromin.

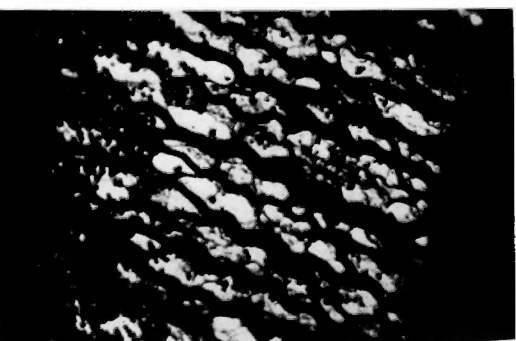


Fig. 15 The luminal narrowing of the macula cribriformis of the mouse treated with Colcemid. These changes were mainly induced by the overproliferation of the reticular fibrils. with Carzinophilin.

## 和文抄録

## 脈管外通液路系による癌の拡がり方

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吾々は、木原名誉教授の発見された脈管外通液路系が癌細胞の移動に際し如何なる態度をとるかを追求した。先づ井谷の腹腔内吸収に関する基礎実験の結果前リンパ管通液路系を形成する横隔膜篩状斑の病態生理面を知り得た。次いで吉田腹水癌、マウスのエールリツヒ腹水癌、等による実験の結果横隔膜篩状斑が抑留増殖の場を形成し得る事実や内胸リンパ管から周囲組織内に癌細胞漏出の見られる事実を確認し得た。同時

に腹腔内癌腫症の剖検例に際し上記同様の個所を研索せる結果上記動物実験と全く同一の結果を得た。同時に篩状斑の抑留機構の解明やその機能に関する実験を行い殊に制癌剤に対する篩状斑の態度を検討し2.3の興味ある新知見を得た。以上により癌細胞は脈管外通液路就中前リンパ管通液路にて抑留増殖の場を形成し得る事、更には傍リンパ管通液路系による癌細胞の拡大進展の起り得る新事実を解明し得た。