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# Experimental Study on Lymphatic Vascular Changes in the Development of Cancer

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**Summary**

Sudan Black B and Sudan Blue can partly enter the lymphatics of the small intestine from the lumen with long chain fatty acids. By use of a fat emulsion saturated with them small intestinal and mesenteric lymphatics were clearly delineated. Subserosal and mesenteric lymphatics of the small intestine appeared as blue lines. With this method we studied anatomical changes of the lymphatic vessels during the development of cancer.

As experimental tumor VX2 carcinoma was used. Lymphatic vessels were not be found in cancerous regions by this lymphangiographic procedure, even in the early stages of cancer. Lymphatics passing through the tumorous tissue were completely obstructed and accompanied with peripheral dilatation.

Compared to the morphological changes of the blood vessels, which were studied microangiographically, the lymphatic vessels were more easily affected by the malignant growth.

## *Introduction*

The development and growth of malignant tumors are associated with anatomical changes of the blood vessels, such as increase or decrease in number, and various abnormal patterns as dilatation, stretching and tortuosity. However, our knowledge of the morphological changes of the lymphatic vessels in the development of cancer has lagged far behind that of the blood vessels. To study that problem, an elucidation of the lymphatic vessels, wherein the tumor is developing, is required. Moreover, lymphatic vessels should be elucidated physiologically along with morphological study of lymphatic vessels during the growth of neoplasms. In this study, we have found that small intestinal and mesenteric lymphatics can be clearly delineated by use of a fat emulsion saturated with Sudan Black B and Sudan Blue. With this method we studied anatomical changes of the lymphatic vessels during the development of cancer.

## *Materials and Methods*

Experimental animals and tumors: laboratory rabbits were selected as experimental animals, because satisfactory transplantable tumors are available. The tumor used was the VX2 carcinoma, derived from the Shope virus papilloma (1). This tumor is readily maintained in domestic rabbits. Cancer-cell suspensions were prepared by mincing the solid tumor, developed in the gluteal musculature, with scissors and then pressing it through a finely meshed tea strainer. The cells were diluted with Hank's solution. This resultant supernatant, used for injections, contained predominantly single cells and included $10^6$ viable cells per milliliter.

Rabbits were anesthetized with Nembutal and laparotomy was performed through a midline incision. A tumor suspension (0.1 ml) was injected into the submucosal layer of the small intestine and its mesentery with a 0.25 ml syringe and 30-gauge needle. From 7 days to 21 days after injection small intestinal lymphangiography was carried out.

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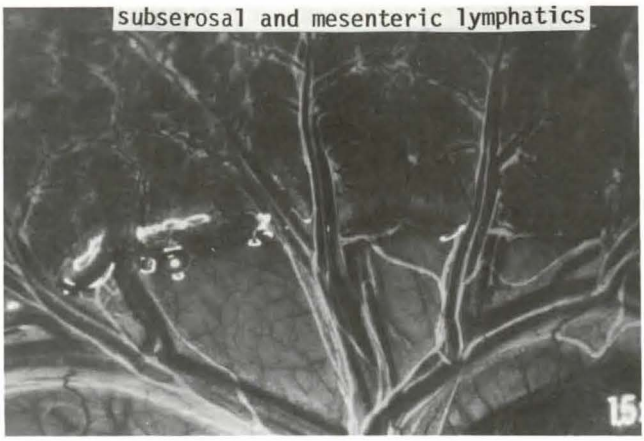



**Fig. 1** Subserosal and mesenteric lymphatics, delineated as white lines in this figure. x 1.5

Lymphangiographic technique: an oil-in-water emulsion was made up of 25% olive oil with saturated Sudan Black B and Sudan Blue, 73% phosphate buffer solution at pH 6.5, and 2% Tween 80. This O/W type emulsion was prepared by the ultrasonification technique. Prior to use of the emulsion, bile juice taken from another healthy rabbit was added at a rate of 1.0 ml/kg of body weight. At the time of intraduodenal infusion of the emulsion, animals were laparotomized through a small midline incision while conscious, because any anesthetic drug might suppress the absorptive activity of the small intestine (2). The volume of the infused emulsion was 10 ml/kg body weight. At 2 hours after infusion, re-operation was performed through a large midline incision under Nembutal anesthesia. Subserosal and mesenteric lymphatics of the small intestine appeared as blue lines, and were photographed with a Medical Nikkor® at 1.5 magnification.

**Fig. 2** Mesenteric lymphatics, appeared as black lines in this figure. They had a characteristic beaded appearance. x 1.5

*Results*

The lymphatic vessels usually ran alongside the veins and were more numerous than veins (Fig. 1). Mesenteric lymphatics ran in the mesentery from the intestinal border to the lymph node situated at the root of it. They had a characteristic beaded appearance due to valves (Fig. 2). Just before entry into the lymph node, the mesenteric lymphatic vessels divided into branches (Fig. 3). All of the lymphatics running nearby in the mesentery did not always enter the same lymph node. Some bypassed it and entered another adjacent node. Fig. 4 illustrates the interrelationship between the mesenteric lymphatic vessels and minute VX2 carcinomas transplanted in the mesentery. Although these tumors were very

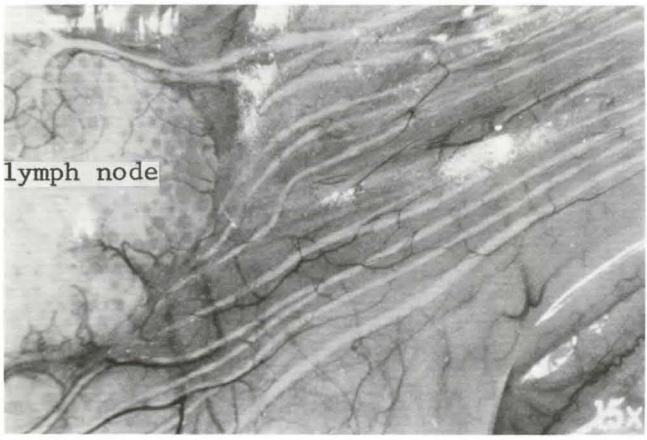



**Fig. 3** Lymphatics of the root of mesenterium. Lymphatics were stained white and blood vessels stained black in this. Just before entry into the lymph node, they divided into branches. x 1.5

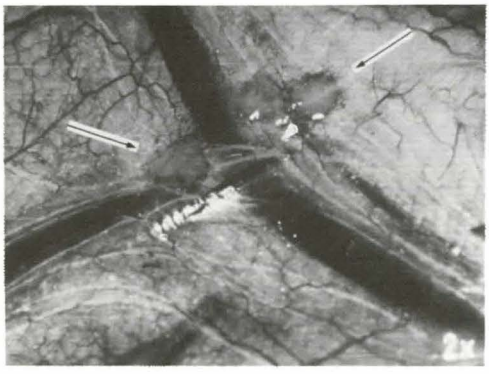


**Fig. 4** Interrelationship between the mesenteric lymphatic vessels and minute VX2 carcinomas, indicated by arrows, in the mesentery. No lymphatic could be found in them. x 1.5

small; under 1.5 mm in diameter in size, blue lines cannot be visualized in them. The tumor had been transplanted into the submucosal layer of the small intestine. When it had grown up to over 1 cm in diameter, and had not invaded the serosal layer, serosal lymphatic streams were preserved with their normal architecture. However, when the tumor infiltrated the serosal layer, subserosal lymphatics passing through the cancerous region were completely obstructed and accompanied with peripheral dilatation. In the tissue surrounding the tumor the blue color gradually increased with the development of cancer, probably because of the intensive lymph formation (3) (Fig. 5). The regional lymph nodes constitute a true filter which mechanically and tem-

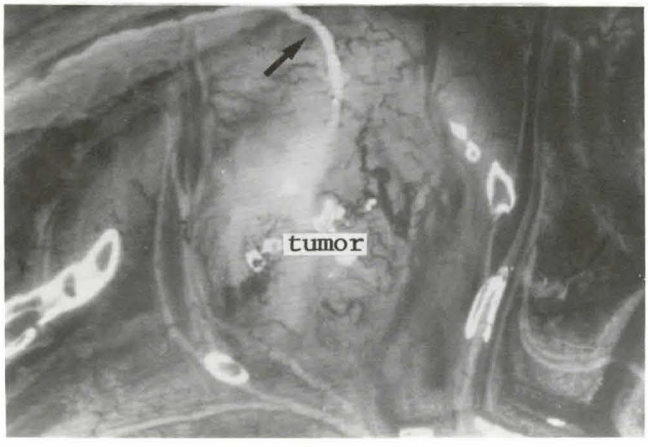



**Fig. 5** Subserosal lymphatics passing through the cancerous region were completely obstructed and accompanied with peripheral dilatation, as indicate by arrows. x 1.5

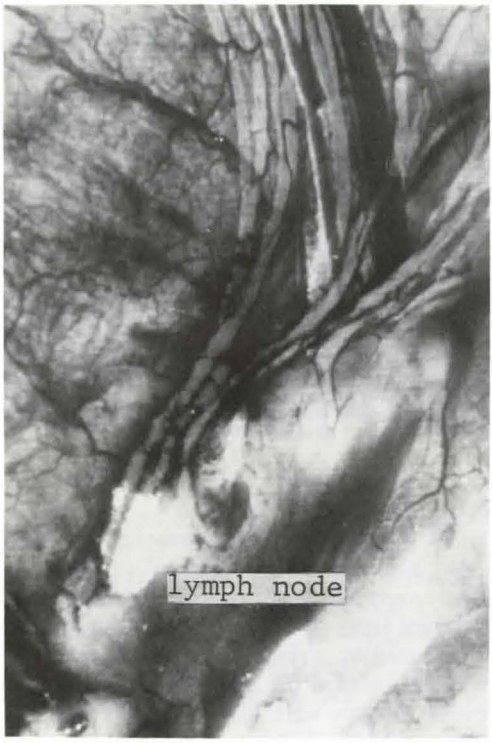



**Fig. 6** At the advancement of lymph node metastases, the afferent lymphatics were strikingly dilatated, because of obstruction of lymph stream

porarily arrest the spread of cancer. In our experiment, the area of lymph node metastases was pale, while adjacent uninvolved areas were stained by the dye. When a lymph node was extensively involved by metastatic disease, the afferent lymphatics to it were strikingly dilated and did not empty into it (Fig. 6).

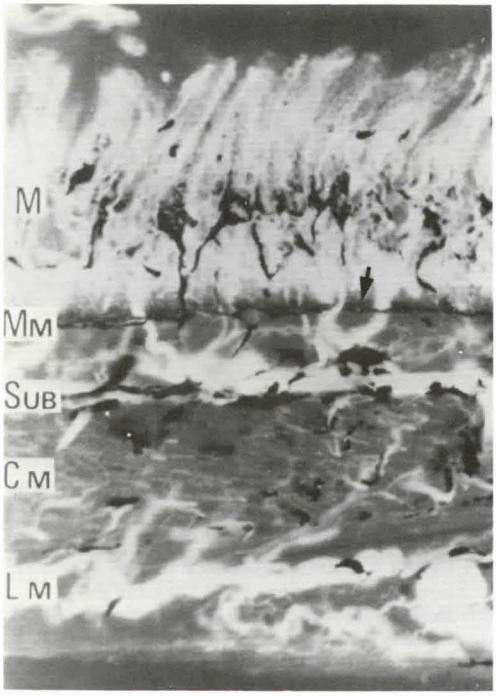
*Discussion*

Certain dyes and other high molecular substances, interstitially injected, can be selectively absorbed by the lymphatics. Many workers have used these agents for the demonstration of the lymphatics (4, 5, 6, 7). Also in our previous paper, India ink was employed as an injection dye for the study of the regeneration of intestinal lymphatics (8). There are some disadvantages, however, to this kind of indirect lymphangiography for physiologic study; for instance, the filling of the lymphatic system is not complete and the caliber of demonstrated lymphatics is sometimes so wide that it seems likely that it is an artifact. Since the historic report of *Aselli* (9), fatty foods, such as butter, cream and milk, have been used for demonstration of the mesenteric lymphatics of the small intestine. However, that demonstration has never been so clear that intestinal lymphangiographic studies have developed in the past.

Among fat-soluble dyes, Sudan Black B and Sudan Blue can enter the lymphatics of the small intestine from the lumen with long chain fatty acids. The transport of long chain fatty acid occurs through the intestinal lymphatics. Median and short chain fatty acids, in contrast, appear to pass directly into the blood stream via the portal venous system. Triolenic acid is best absorbed in the intestine relative to several types of long chain fatty acids. In this study, therefore, olive oil was selected from a number of oily media, because it includes mostly triolenic acid. The oil-in-water type emulsions were prepared from them.

This method of lymphangiography can be applied not only to the rabbit, but also to other experimental animals, such as the dog, cat and rat. Figures 7a and 7b are lymphangiograms of the canine intestinal wall, the former by the indirect puncture method described in our previous report (8) and the latter by the present experimental method. The lymphatic network above the muscularis mucosae can be also visualized by this experimental method.

Lymphatic vessels were not be found in cancerous regions by this lymphangiographic procedure, even in the early stages of cancer. Fig. 9 shows a small tumor, seen only by histologic examination. Erythrocytes were seen in the cluster of VX2 tumor cells, although the dye outlined but did not penetrate the tumor. The finding of a lack of lymphatic vascular supply in tumors has also been reported by others, who injected dye into the lymphatic vessel or the interstitial space (10, 11). However, there is no other report on this subject using this kind of physiological lymphangiography. Observations made by them tend to support our experimental results.



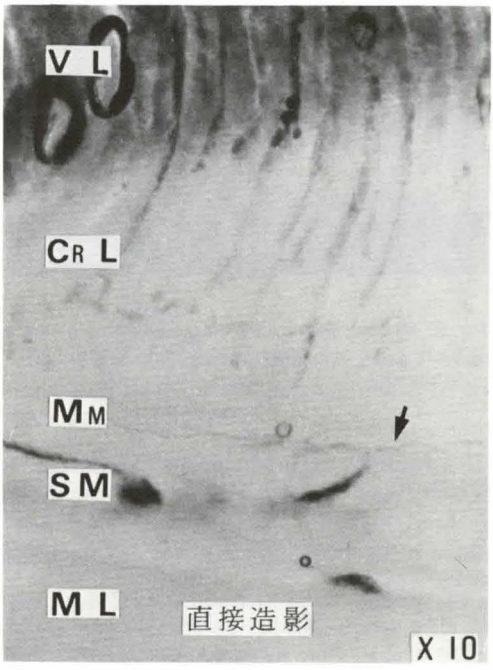




**Fig. 7a** Lymphangiogram of the canine ileal wall by the indirect puncture method. Xylol transluent specimen, India ink, Bac sulfate. x 9

**Fig. 7b** Lymphangiogram of the canine ileal wall by the present experimental method. Glycerin translucent specimen, Sudan Black B and Sudan Blue. x10

A horizontal network above the muscularis mucosae is characteristically found in both figures as indicated by arrows. M: mucosal layer; Mm: muscularis mucosa; Sub: submucosal layer; CM: circular muscle layer; LM: longitudinal muscle layer; ML: muscle layer

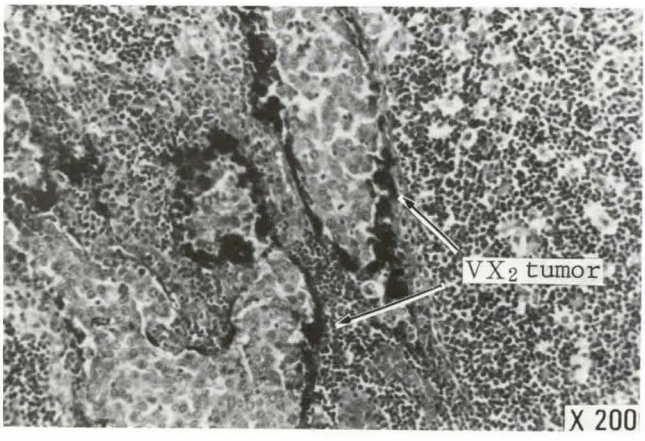



**Fig. 8** Histological specimen of a small VX2 carcinoma in the vermiform appendix. This indicates a lack of lymphatic supply in tumors. Hematoxyline-Eosin stain. x 200

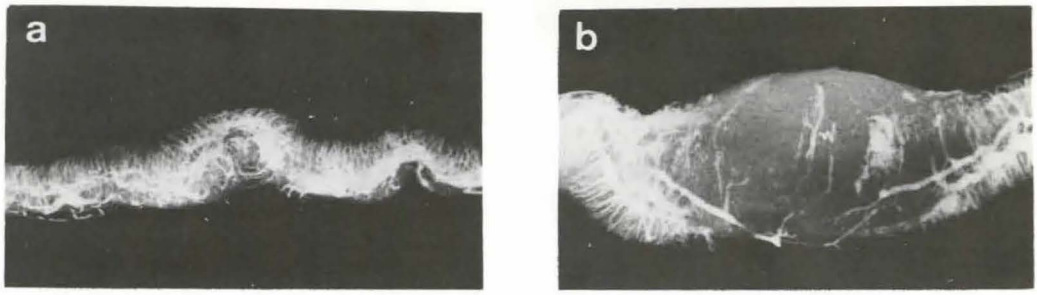



**Fig. 9** Microangiogram of VX2 carcinoma of the small intestine.
a) Early stage of tumor development, under 1 cm in diameter
b) Advanced stage of it

To investigate the anatomical changes of blood vessels in the development of VX2 carcinoma, microangiography was performed. Our technique of microangiography was described previously (8).

In early stages of tumor development, under 1 cm in diameter, there was an increase in the blood vessel number due to neoformation with dilatation of the vascular lumen (Fig. 9a). In advanced cancers the tumorous tissue, pressing the blood vessels and damaging the walls of capillaries, affected the blood supply to the central node of malignant growth, and the central portions of the tumor became hypovascularized (Fig. 9b). Therefore, it is possible to conclude that the lymphatic vessels are more easily damaged by the malignant growth than the blood vessels.

*References*

1 *Kidd, J.G., P. Rous:* A transplantable rabbit carcinoma originating in a virus-induced papilloma and containing the virus in masked or altered form. J. Exper. Med. 71 (1940) 813–838
2 *Marshal, F.M.:* Effects of halothane on gastrointestinal motility. Anesthesiology 22 (1961) 363–370
3 *Idanov, D.A., N.V. Krylova:* Macromicroscopic studies on vascular system of malignant tumors. Arkh. Anat. 43 (1962) 27–50
4 *Gerota, D.:* Zur Technik der Lymphgefäßinjektion. Anat. Anzeiger 12 (1896) 216–228
5 *Hudack, S., P.D. McMaster:* The lymphatic participation in human cutaneous phenomena. J. Exper. Med. 57 (1933) 571–585
6 *Kamei, Y.:* The distribution and relative locations of lymphatic and blood vessels in the mucosa of the rabbit colon. Nagoya Med. J. 15 (1969) 223–238
7 *Rhoads, J.E., Jr.:* Intraoperative demonstrations of the mesenteric lymph channel. Surg. Gynec. Obstet. 140 (1975) 951–957
8 *Satomura, K., N. Tanigawa, S. Magari:* Microlymphangiographic study of lymphatic regeneration following intestinal anastomosis. Surg. Gynec. Obstet. 146 (1978) 415–418
9 *Aselli, G.:* De Lactibus Sive Lacteis Veins, Quarto Vasorum Mesarai corum Beneve nove inveto. Milano J.B. Bidellium Mediolani (1927)
10 *Zeidman, I., B. Copeland, S. Warren:* Experimental studies on the spread of cancer in the lymphatic system. II. Absence of a lymphatic supply in carcinoma. Cancer 8 (1955) 123–127
11 *Gilchrist, R.K.:* Surgical management of advanced cancer of the breast. Arch. Surg. 61 (1950) 913–929

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