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SUMMARY

LYMPHOLOGY IN THE FUTURE

Scientific advancements in Lymphology will depend on new approaches to adress specific issues of this area of Biomedicine and also in the application to this old discipline of new methodologies coming from basic research in cellular and molecular biology.

Contemporary concepts in cancer pathology, namely those regarding metastatic phenomena, continue to indicate a pivotal role for the interaction between the lymphatic and blood circulation in determining the evolution and prognosis of cancer. It is also clear today that correct interpretation of lymphodynamic processes requires further histochemical and biochemical data on the permeability of the lymphatic endothelium and on regulatory actions of putative interstitial factors, since it became obvious that lymphatics physiology can not be explained just by the mechanics of pressure interactions. Thus, studies on lymphatic microcirculation will most probably be of great help to understand the pathophysiology of several organs in relation in pathogenic aggressions wherever anatomical and physiological specifies of the lymphatic system are of importance in the modulation of local defense mechanisms.

A promising area of research is certainly that adressing the consequences on lymphedema that result from the activation of macrophages, lymphocytes and fibroblasts. This is because there is solid evidence that, upon activation, these cells secrete molecules, such as enzymes, that may modify the contents and arrangement of the extracellular matrix and of connective tissue fibers. Cellular activation of elements of the lymphatic system have also been implicated in modifying cancer metastization, clearance mechanisms and immune reactions against pathogenic agents.

In my opinion, Lymphology will see in the near future very significant advances that will come primarily from the cooperation of researchers using multidisciplinary approaches to tackle the problems that remain unsolved today.

R. Nuno Grande

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Study of capillary filtration and lymphatic resorption by double labelling albumin and red blood cells in cyclic edema and diabetes.

Etude de la filtration capillaire et de la résorption lymphatique dans l'œdème cyclique et le diabète, par un double marquage de l'albumine et des globules rouges.

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ABSTRACT

Lymphatic resorption was investigated by capillary permeability test, using labelled Albumin and labelled red cells, and FAST FOURIER TRANSFORM analysis of lymphatic fluctuations in 40 patients : 10 healthy subjects, 20 Idiopathic Cyclic Edema patients and 10 Non Insulin Dependant Diabetics patients.

All tests on labelled erytrocytes showed a normal retention rate and normal lymphatic fluctuations mean. In the healthy subjects, there is no albumin retention and lymphatic fluctuations mean is also normal.

In the Idiopathic Cyclic Edema patients under treatment, retention rate is always normal. But lymphatic fluctuations mean is normal when labelled red blood cells were used, and abnormal when labelled Albumin was used.

In Non Insulin Dependant Diabetics patients, the tests on labelled erytrocytes showed a normal retention rate and normal lymphatic fluctuations mean. The tests on labelled Albumin showed a retention rate often normal but lymphatic fluctuations mean was always abnormal.

In Idiopathic Cyclic Edema syndrome, 2 linked disorders, the excess capillary filtration of proteins and the defect in lymphatic resorption of this excess by lymphatic pomps give rise to edema. In Non Insulin Dependant Diabetes, the defect of lymphatic resorption could exist before a capillary permeability trouble.

Key words : Cyclic edema - Diabetes - Landis's test Scintigraphy.

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RÉSUMÉ

Nous avons étudié la résorption lymphatique à l'aide du test de perméabilité capillaire utilisant l'Albumine marquée et les hématies marquées, et les fluctuations lymphatiques à l'aide de la Transformée de FOURIER Rapide, chez 40 sujets : 10 sujets normaux, 20 patients souffrant d'Œdème Cyclique Idiopathique (OCI) et 10 patients Diabétiques non Insulino-Dépendant (DNID).

Tous les tests faits avec des hématies marquées montrent une rétention normale et des fluctuations lymphatiques normales. Chez les sujets normaux, les tests faits à l'Albumine marquée montrent que les 2 paramètres sont normaux.

Chez les patients traités souffrant d'Œdème Cyclique Idiopathique, la rétention est toujours normale. Les oscillations sont normales avec les hématies * et anormales avec l'Albumine *. Chez les diabétiques non Insulino-Dépendant, les tests utilisant les hématies * montrent que les deux paramètres sont normaux. Les tests utilisant l'Albumine * montrent une rétention le plus souvent normale mais des fluctuations lymphatiques moyennes toujours anormales.

Dans l'Œdème Cyclique Idiopathique, deux anomalies liées, l'excès de filtration capillaire des protéines et le défaut de résorption lymphatique de cet excès par la pompe lymphatique crée un œdème. Chez les Diabétiques non Insulino-Dépendant, le défaut de résorption lymphatique pourrait exister avant l'apparition d'un trouble de la perméabilité capillaire.

LYMPHATIC FLUCTUATIONS

TECHNETIUM 99m-LABELLED RED CELLS TEST

	HEALTHY SUBJECTS		IDIOPATHIC CYCLIC EDEMA PATIENTS	
TEST No	1	2	1	2
L.F.	0.45%±0.25	0.46%±0.24	0.43%±0.22	0.34%±0.19

IODINE 131-LABELLED ALBUMIN TEST

	HEALTHY SUBJECTS		IDIOPATHIC CYCLIC EDEMA PATIENTS	
TEST No	1	2	1	2
L.F.	0.42%±0.27	0.40%±0.23	1.82%±0.61	1.9%±0.63

TABLE 1

LYMPHATIC FLUCTUATIONS

IDIOPATHIC CYCLIC EDEMA PATIENTS UNDER TREATMENT

TEST	99mTc-RED CELLS	99mTc-ALBUMIN
L.F.	0.37%±0.31	2.21%±0.78

NON INSULIN DEPENDANT DIABETICS PATIENTS

TEST	99mTc-RED CELLS	111In-ALBUMIN
L.F.	0.42%±0.40	1.71%±0.78

TABLE 2

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Fistule chylo-duodénale opérée (Résultat après 28 ans).

Marceau SERVELLE
Paris.

RÉSUMÉ

Les malformations des chylifères produites par l'hypoplasie de la citerne de Pecquet entraînent différentes complications : chylopéritoine, chylothorax, chylurie, reflux du chyle dans les lymphatiques lombaires et jambiers et enfin des ruptures des lymphatiques centraux des villosités intestinales d'où une perte de chyle dans la lumière intestinale avec abaissement des protides sanguins. Dans presque tous les cas, la rupture de ces lymphatiques de la muqueuse intestinale siège au-dessous de l'angle duodéno-jujénal. Nous rapportons le seul cas de rupture dans le duodénum de ces chylifères. La maladie débute à 13 ans par une hypoprotidémie marquée. A 18 ans, le tubage gastro-duodénal retire du chyle du duodénum. Mais la lymphographie pédieuse suivie pendant 24 heures montre l'absence de citerne de Pecquet, de gros lymphatiques lombaires et surtout de gros lymphatiques s'injectent devant la 10ème côte droite qui s'évacuent dans le duodénum. A la thoracolaparotomie droite, nous sectionnons entre des ligatures de gros chylifères pré- et rétropancréatiques. Les protides reviennent à la normale, mais 6 semaines plus tard apparaît un chylothorax avec hypoprotéinémie. Par thoracotomie nous suturons un lymphatique diaphragmatique rompu dans la plèvre. Les protides reviennent à la normale. Vingt-huit ans plus tard, le résultat clinique et biologique est excellent.

Depuis 1962, nous avons étudié 310 malformations des lymphatiques de l'intestin grêle : 130 de ces patients ont été opérés. Le diagnostic est facile grâce à notre test d'hyperlipidémie provoquée (1). La lymphographie pédieuse et celle des lymphatiques de l'intestin grêle (5) réalisée au cours de laparotomies après repas gras mettent en évidence une **hypoplasie de la citerne de Pecquet**, véritable frein à l'évacuation du chyle : il entraîne une dilatation des chylifères et une perte du jeu valvulaire avec reflux du chyle dans les lymphatiques du mésentère et dans ceux des parois de l'intestin grêle. Ces lymphatiques mésentériques dilatés peuvent se rompre dans la cavité péritonéale produisant un **chylopéritoine**. Les lymphatiques centraux des villosités intestinales également dilatés et remplis de chyle peuvent se rompre dans la lumière intestinale d'où une **perte importante de chyle**. Cette rupture des chylifères dans l'intestin fait communiquer la lumière intestinale très septique avec l'intérieur des chylifères, d'où l'apparition de **lymphangites mésentériques**. Parfois, l'hypoplasie de la citerne de Pecquet entraîne une dilatation des 2 troncs lymphatiques lombaires qui perdent leur jeu valvulaire : le chyle reflue à leur intérieur d'abord jusqu'au pli de l'aine, puis dans le membre inférieur : ce n'est pas le cas chez la malade que nous présentons. Enfin, les chylifères ne pouvant s'évacuer par la citerne de Pecquet, des voies de suppléance se développent (lymphatiques pancréatiques, hépatiques, sous-péritonéaux) : elles drainent le chyle vers le muscle diaphragme dont les lymphatiques se dilatent et renferment du chyle : leur rupture dans la cavité pleurale produit un **chylothorax** (chez 50 % de nos opérés de chylothorax, nous avons suturé cette rupture d'un lymphatique sur le diaphragme par où s'écoulait du chyle). Enfin, ces lymphatiques du diaphragme s'évacuent vers la région cervicale par les lymphatiques mammaires internes puis par les lymphatiques médiastinaux inférieurs : par ces derniers, le chyle se rend aux ganglions de la bifurcation trachéale puis aux lymphatiques péritrachéaux. Mais les ganglions de la bifurcation trachéale reçoivent normalement les lymphatiques des 2 poumons. Du fait de la dérivation du chyle, ces lymphatiques pulmonaires se dilatent, perdent leur jeu valvulaire et **le chyle peut refluer dans les lymphatiques des poumons** : les lymphatiques sous-pleuraux peuvent se rompre dans la cavité pleurale d'où l'apparition d'un **chylothorax**.

L'observation que nous rapportons est unique : **les lymphatiques de l'intestin grêle dilatés et remplis de chyle se rompent dans le duodénum** ; dans tous les autres cas, cette rupture se fait plus bas dans la lumière de l'intestin grêle. Voici résumée cette observation.

Mots clés : Protein loosing entheropathy - chylous reflux - duodenum

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OBSERVATION

Mademoiselle Ramb..., 18 ans, nous est adressée le 14 octobre 1964 pour fistules chylo-biliaires. L'observation de cette malade a été rapportée en 1960 par CATTAN, professeur de pédiatrie, à la Société Médicale des Hôpitaux de Paris sous le titre " **Entéropathie avec pertes de protéines et stéatorrhée** ".

Par contre, chez la malade dont nous rapportons l'observation, dès la fermeture de la fistule chylo-duodénale, les protides sont devenus normaux jusqu'à l'apparition du chylothorax (6 semaines). En effet, la fermeture de la fistule chylo-duodénale, en supprimant l'importante fuite chyleuse dans le duodénum, a surchargé les autres voies lymphatiques de suppléance périhépatiques, péripancréatiques et souspéritonéales qui drainent le chyle vers les lymphatiques du diaphragme ; ces dernières se dilatent davantage ; l'un de ces gros lymphatiques diaphragmatiques plein de chyle s'est rompu dans la cavité pleurale. Les ponctions pleurales s'avèrant insuffisantes, nous avons par thoracotomie après repas gras suturé ce lymphatique rompu sur le diaphragme.

Dans tous nos chylothorax opérés, nous n'avons pas vu de récidive du même côté. Un chylothorax avait été opéré au Canada : au retour de la malade en France est apparu un chylothorax du côté opposé et nous l'avons opéré. L'absence de récidives du chylothorax du côté opéré est peut-être dû à la formation d'adhérences pleurales post-opératoires. Cependant, nous avons dû réintervenir pour des chylothorax opérés précédemment car la rupture lymphatique n'avait pas été découverte et donc pas suturée. Plus fréquentes sont des ruptures répétées de chylifères dans la cavité péritonéale : nous avons dû réintervenir 3 fois pour suturer une autre rupture d'un chylifère dans le péritoine.

De toutes les ruptures dans la lumière intestinale des lymphatiques centraux des villosités intestinales, l'observation que nous rapportons est la seule où la fuite se produit au niveau de la muqueuse duodénale. Dans tous les autres cas, la rupture dans l'intestin grèle est située au-dessous de l'angle duodéno-jéjunal. Lorsque la rupture se produit sur la moitié inférieure de l'intestin grèle, on voit apparaître assez souvent des lymphangites mésentériques : une de nos malades en a présenté 52 en 15 ans.

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Perivascular fiber system around lymphatic capillaries.

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ABSTRACT

Lymphatic capillaries are surrounded by a network of filaments and collagen and elastic fibers. Litterature data ascribe to the fiber perivascular apparatus a key role for distending endothelial wall and for opening intercellular junctions in order to permit the passage of fluids and molecules as a consequence of tissue pressure increase. Some hypothesis have been stated about the way these filaments and fibers work.

The fibrillar perivascular system in the myocardium, the skin and the dental pulp, organs with great structural differences have been investigated. The fibrous network turned out to have peculiar morphological characteristics in each organ since its thickness and composition in fiber type differ according to the morphological properties of the tissue. These findings suggest that a single pattern of fibrillar perivascular apparatus is not sufficient to explain the mechanisms of action of filaments and fibers in distending lymphatic endothelial walls.

INTRODUCTION

Lymph capillaries have been generally described as extending in tissue regions where interstitium is rather abundant and the connective fibers form a network around lymphatic vascular walls. This fibrillar network is formed by the so-called " *anchoring filaments* ", inserted in the subtle and discontinuous basal lamina and also by collagen fibrils and elastic fibers. An important role has been ascribed to anchoring filaments and collagen and elastic fibers for the endothelial wall distension and for the opening of the intercellular junctions in order to permit the passage of fluid and molecules and the formation of the lymph (26, 14, 16, 5, 6, 7, 24, 27, 28, 2, 9, 12). The fibrillar component would be submitted to a tension because of the interstitial pression increase ; it causes the stretching of the filaments anchored to the endothelial wall and the following distension of it.

Morphological and chemical characteristics of anchoring filaments have been investigated in order to clarify their function. High magnification observations reveal a structure with a clear central core and a more dense peripheral outline ; furthermore, they present cross striations (16). Histochemical investigations evidentiated that anchoring filaments are rich in disulfide groups (3), glycoconjugated sulphate groups and carbohydrates containing -glycol groups (13). Because of these morphological and histochemical characteristics, the anchoring filaments resemble the microfilaments of the elastic fibers (15, 16, 3) and the oxitalan fibers (4, 12, 13).

Litterature data have supplied quite similar patterns in order to explain the " *pulling function* " that anchoring filaments and collagen and elastic fibers exercise on the lymphatic vascular walls (6, 7, 9, 12). These patterns obtained from observations in some organs have been consequently applied to all the organs. In our investigations on the lymphatic capillary network in organs with very different structural and functional components, we observed differences in the distribution and the composition of the fibrillar apparatus surrounding the lymphatic capillaries (17, 18, 19, 20, 21, 22, 25). Therefore, we believe useful to discuss wether one unitary pattern is available to explain the organization and the functional modalities of the fibrillar apparatus around the lymphatic capillaries.

Key words : Lymphatic capillaries, heart, derma, dental pulp, perivascular fibers.

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MATERIAL AND METHODS

Lymphatic capillaries were investigated in human skin, myocardium and dental pulp in normal conditions. Cardiac samples were obtained from 4 normal hearts that were not used for transplantation because of receiver problems (20). Skin samples were taken from 6 lower leg biopsies of normal volunteers (25). Dental pulps were obtained from 20 non carious teeth extracted

In addition, it must be underlined that, in the endothelial wall of the myocardial and pulpal lymphatic capillaries, the micropinocytotic vesicles are very abundant. These, according to the vesicular theory (10, 1), are supposed to be the main responsible structures to the passage of fluids and macromolecules through the endothelial wall during the lymph formation. We believe that both the micropinocytotic vesicles and the intercellular junctions have an important role in the lymph formation. Either the former or the latter prevail in different organs and in different functional conditions. The opening of the intercellular junctions is the main mechanism for the passage of substances through the endothelial wall in those organs which require an intense lymphatic drainage or with tissue pressure increase. In these conditions, the stretching function of the fibrillar apparatus on the endothelial wall would result fundamental.

In conclusion, our findings on the cardiac tissue, the skin and the dental pulp have marked the importance of the structure provided by the filament and fiber network delimiting the lymphatic capillary wall. But it has also been demonstrated that thickness and composition in fiber type of the fibrillar perivascular system differ according to some morphological properties of the tissue and of the lymphatic endothelial wall. So we believe that a key role in distending the endothelial wall and in opening intercellular junctions following tissue pressure increase is played by the fiber perivascular network. However in our opinion, a single pattern of filament and fiber organization is not sufficient for all the organs.

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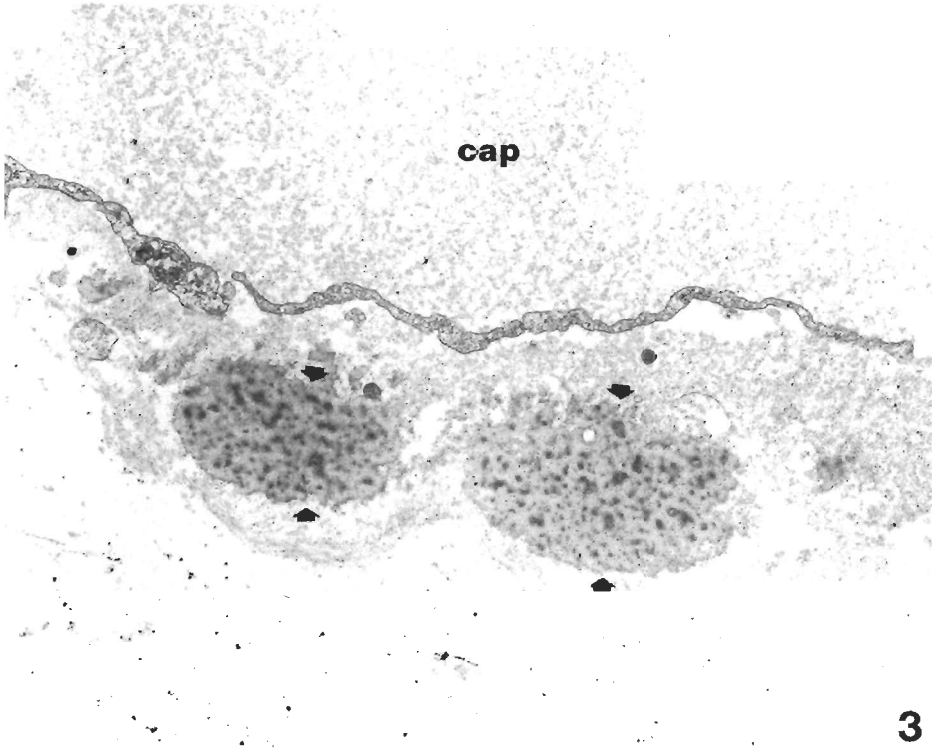

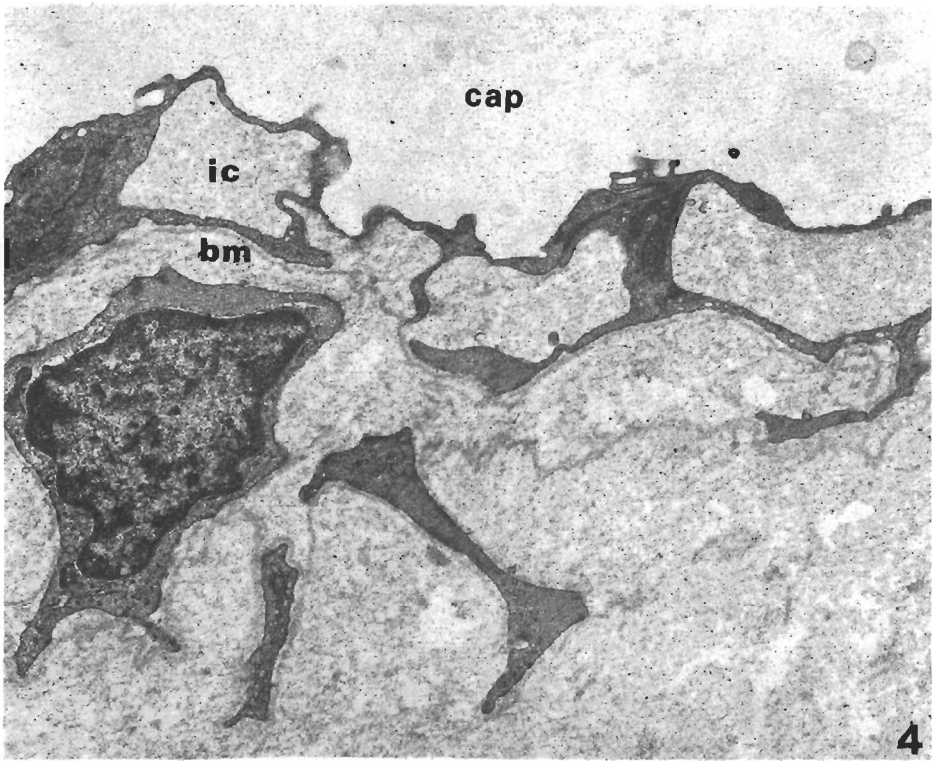

Fig. 3 — LYMPHATIC CAPILLARY (cap) IN THE SKIN.
Perivascular fibrillar system is characterized by numerous large elastic fibers orcein stained (◗).
× 10000

Fig. 4 — LYMPHATIC CAPILLARY (cap) IN THE DENTAL PULP.
Endothelial wall, rich in interendothelial channels (ic) is covered by an evident basal membrane (bm).
× 11000

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Enzyme-histochemical study of lymphatic capillaries in lung parenchyma.

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ABSTRACT

We have visualized the lung lymphatics using the 5'-nucleotidase (5'-Nase) histochemical staining method. The specificity of the 5'-Nase reaction was controlled through the inhibition of non-specific alkaline phosphatase (ALPase) by including L-Tetramisole in the substractum incubating medium. The 5'-Nase method allowed us to identify the lymphatics of the superficial subpleural network as well as the lymphatic vessels associated with the deep lung tissue. Intense reaction products of 5'-Nase activity were deposited in the endothelial cells of the wall of the lymphatic vessels and in the endothelial cells at the valves. The blood vessels of the lung did not show any positive reaction for the 5'-Nase. Thus, 5'-Nase detection is an excellent method to study the microanatomy of lung lymphatics and it has some advantage when compared with other methods proposed, to differentiate lymphatic vessels from blood vessels, like the immunohistochemical detection of the presence of laminin and the toluidine blue staining of the lumen of lymph vessels.

Our data showed that : (1) the alveolar region of the lung is devoid of lymphatic capillaries, (2) the superficial and deep networks of lung lymphatics presented similar histochemical reactivity to the 5'-Nase method.

Key words : Lung, Lymphatics, 5'-Nucleotidase, Histochemistry.

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INTRODUCTION

Lymphatic invasion is an important prognosis factor in malignant disease of the lung system. The distinction between small venules and lymphatic capillaries, and between smaller veins and lymphatic collecting vessels is, however, difficult or impossible to make by light microscopy. Several methods have been recently developed to facilitate the distinction between these two different types of vascular structures by light microscopy, namely histochemichal (1) and immunohistochemical ones (2, 3).

Here, we decided to investigate the topography of lung lymphatics using the 5'-Nase histochemical method as it was described by WERNER, SCHUNKE and TILLMAN in 1987. This is a pertinent endeavour since the fine distribution of lymphatics at the alveolar level is still controversial, namely in pathological conditions such as mitral stenosis and pulmonary hypertension. In these situations, some authors have reported that lymphatic capillaries appear at the alveolar wall (4, 5, 6).

Our data on the arrangement of pleuropulmonary lymphatics as viewed by the 5'-Nase staining documented that the alveolar wall does not contain lymphatic capillaries. These vessels form well-developed plexus around the air conducting channels.

MATERIALS AND METHODS

The lungs of ether-anesthetised Wistar rats of both sexes weighing from 150 to 200 g were fixed by perfusion via the pulmonary artery with cold formaldehyde-$CaCl_2$ fixative (6 % paraformaldehyde, 1 % $CaCl_2$) in 0,1 M cacodylate buffer (7 % sucrose).

The lungs were excised and immediately frozen in liquid nitrogen. Sections (15-18 μm thick) were cut in a cryostat and mounted on gelatin coated slides. The sections were then immersed in the same fixative for 10 min. After washing in cacodylate buffer, they were incubated for 30-60 min. in a lead based stan-

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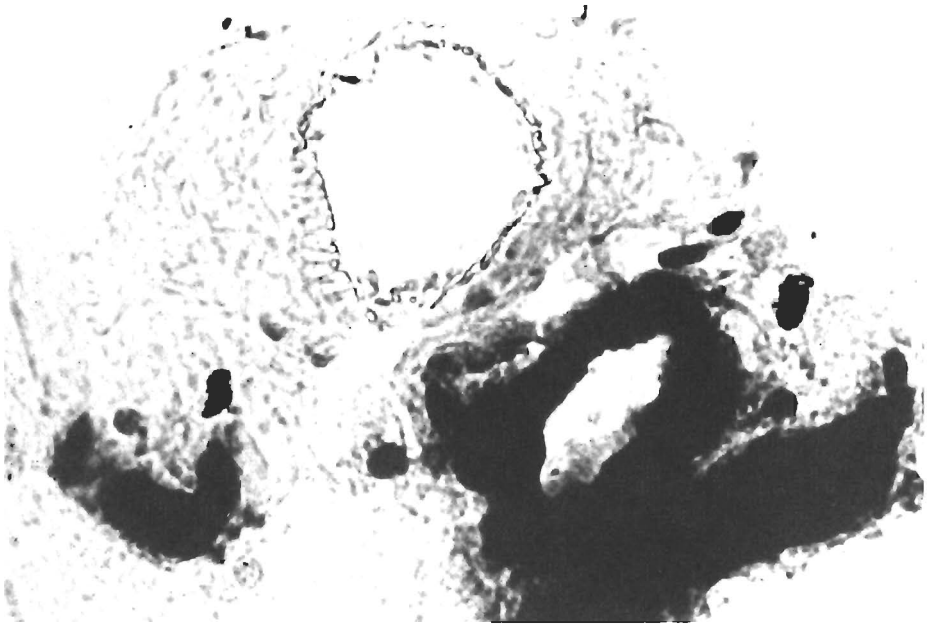

FIGURE 3
Light micrograph illustrating small collecting lymphatic vessels located in the adventicia of a bronchial structure.

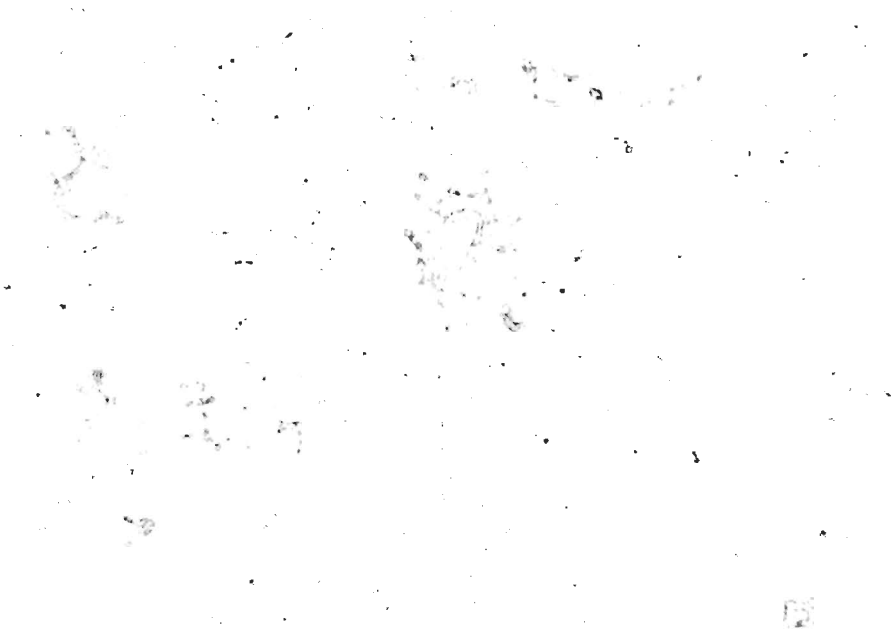

FIGURE 4
Light micrograph showing absence of 5'-Nase reaction product at the alveolli of the lung thus indicating that the deep region of the normal lung parenchyma is deprived of lymphatic vessels.