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VISUALIZATION OF THE LYMPHATICS OF THE HEART AND THE MEDIASTINAL DRAINAGE PATHWAYS IN THE LIVING CYNOMOLGOUS (MACACA MULATTA) MONKEY

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ABSTRACT

Our interest in the effects of impaired cardiac lymph drainage on coronary atherosclerosis led us to study the cardiac lymphatic anatomy in the monkey, generally considered the ideal experimental animal for examining coronary artery disorders. Short-term and long-term studies to visualize the cardiac lymphatic system and its mediastinal drainage pathways in 14 living monkeys confirmed that the epicardial collecting lymphatic anatomy is comparable to that of man, dog, and pig. These lymphatics, and particular lymphatic drainage to the cardiac lymph node in the right mediastinum, are difficult to visualize, in good part, because lymph uptake of such tracers as India Ink and T1824 blue dye is extremely slow. By modifying our techniques and taking cognizance of the slow lymphatic uptake of the tracers, we have been more successful in visualizing the mediastinal cardiac lymph node. Though our studies confirm that the lymphatic drainage of the monkey heart is similar to that in other mammals, we conclude that the "monkey model" has several drawbacks to study the effects of impaired cardiac lymph flow because of the laborious requirements to visualize successfully the cardiac lymph node. Perhaps the development of new markers would make this lymphatic system more approachable for experimental investigation.

The monkey is generally considered the ideal animal for the experimental study of coronary atherosclerosis. Because we have been interested in the effects of impaired cardiac lymph drainage on coronary artery disorders including atherosclerosis, we initiated a study of the epicardial collecting cardiac lymphatics and the mediastinal cardiac lymphatic drainage pathway in the monkey. In a previous publication (1), we stressed the difficulty of visualizing the cardiac lymphatic drainage system in the living monkey in spite of the fact that histologic studies had revealed the monkey coronary lymphatic and mediastinal drainage system to be similar to that of man and dog. At that time our attempts to visualize the cardiac lymphatic system in the monkey utilized the same techniques that we had previously successfully used in the dog; nonetheless, it was apparent that these techniques were usually inadequate when applied to the cynomolgous (Macaca mulatta) monkey.

In the present paper we summarize our total experience in studying various methods to visualize cardiac lymphatics and their mediastinal drainage system in the living monkey and describe more positive results in depicting the cardiac lymph node by modifying our previous techniques. The aggregate of our experience may help future investigators approach the issue of better visualization of the epicardial lymphatics and draining

TABLE 1
Tracers Used to Visualize Cardiac Lymphatics

• India ink	Higgins Ink Co., Newark, NJ 07103
• Pelikan India Ink	Formerly available from Günther Wagner, Germany, but no longer available. Differs from India ink in the carbon particles being of small standard size.
• CH40	Carbon 40 (50 mgm/ml) containing very small carbon particles (21 nm) (Mitsubishi Chemicals, Tokyo, Japan) combined with 20 mgm/ml of polyvinylpyrrolidone (PVP, K-30 Nakarai Chemicals, Kyoto, Japan).
• T1824 blue dye (Evans)	Available as powder from Sigma-Aldrich Co. Corp., P.O. Box 14508, St. Louis, MO 63178 Reconstituted by adding 22.6 mg to 5.0 ml water.
• Micropulverized barium sulfate	Barium sulfate powder (made into a suspension by adding water) made by Damancy Chemicals, Ltd., England for Nicholas Laboratories, Ltd., Slough, SL 7 4AU, England. Distributed in U.S. by Picker Corp., Cleveland, OH 44143.
• 4% gelatin solution	Prepared by adding 2.0 grams of gelatin powder to 50 ml tap water in small beaker. Stir and allow to stand overnight. On following day, heat gently in a water bath for about 20 minutes until solution is clear.

cardiac lymph node in the monkey with greater insight into technical limitations and accordingly a better chance of success.

MATERIAL AND METHODS

A total of 14 monkeys, all Macaca mulatta, have been studied since 1987. These monkeys had all been scheduled for euthanasia after completion of non-invasive studies in other university departments. They were housed in the Center for Experimental Animal Resources of the Northwestern University Medical School and were cared for under strict guidelines as defined in the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." The study is reported in two phases: In Phase I were acute experiments and in Phase II were chronic experiments.

Phase I (Acute Experiments)

In seven monkeys, Ketamine and Xylazine were used for preanesthetic sedation, and then Brevital (methohexital sodium) was used to induce general anesthesia. Thereafter, the monkeys underwent endotracheal intubation and anesthesia was maintained with 1.5% halothane delivered by standard equipment. The heart was exposed by a sternal-splitting incision and a pericardial sling was made. After completion of the experimental study, the scheduled euthanasia was accomplished with intravenous pentobarbital and the heart was removed for further examination. Various lymphatic tracers were used in these experiments *(Table 1)*. The longest period of time we waited after the injection of a tracer into the left ventricular apical subepicardium was two hours. Both

TABLE 2
Various Techniques and Tracers Used to Visualize Cardiac Lymphatics in the Cynomolgous Monkey (Macaca mulatta)

- Micropulverized barium sulfate suspension in water injected subepicardially into the left ventricular apex with delayed restudy.
- Micropulverized barium sulfate suspension in water instilled into the pericardial sac with delayed restudy.
- India ink diluted 1:1 with saline injected subepicardially into the left ventricular apex with delayed restudy.
- India ink diluted 1:1 with 4% gelatin solution injected subepicardially into the left ventricular apex with delayed restudy.
- India ink diluted 1:1 with saline injected subepicardially into the left ventricular apex in an acute experiment.
- India ink diluted 1:1 with 4% gelatin solution, Pelikan India ink and/or CH40 carbon suspension injected subepicardially into the left ventricular apex in an acute experiment.
- T1824 blue dye (Evans) injected into the left ventricular apex in an acute experiment.
- Suspension of micropulverized barium sulfate in water injected subepicardially into the left ventricular apex in an acute experiment.

sides of the mediastinum cephalad to the heart were carefully dissected to expose the cardiac lymph node, other possible draining nodes, and connecting lymphatic vessels. Sketches and photographs were made of the visualized lymphatics. The methodology used in this phase of our investigations was similar to that previously used successfully in the dog (2,3).

Phase II (Chronic Experiments)

In these seven monkeys, anesthesia was induced and maintained as described in the acute (Phase I) studies. The initial operation was done under strict aseptic conditions and when completed the pericardium and the chest were closed in the standard way. In four monkeys, India ink diluted 1:1 with saline or 4% gelatin was injected subepicardially into the apex of the left ventricle at the time of the initial operation. Three days after the first surgical procedure the monkeys were reanesthetized and studied to visualize the lymphatic drainage system of the heart by inspection and careful dissection of the right and left mediastinum cephalad to the heart. Sketches and photographs were made of the lymphatic anatomy. Thereafter euthanasia was accomplished and the heart was removed for examination. In three other monkeys, we attempted to visualize the cardiac lymphatic system by instilling a suspension of micropulverized barium sulfate in water into the pericardial sac and/or injecting it into the subepicardial left ventricular myocardium. These three monkeys were restudied several months after the initial surgical procedure. At the time of restudy of each monkey in Phase II, additional tracer injections were made into the left ventricular myocardium to visualize as much as possible of the cardiac lymphatic

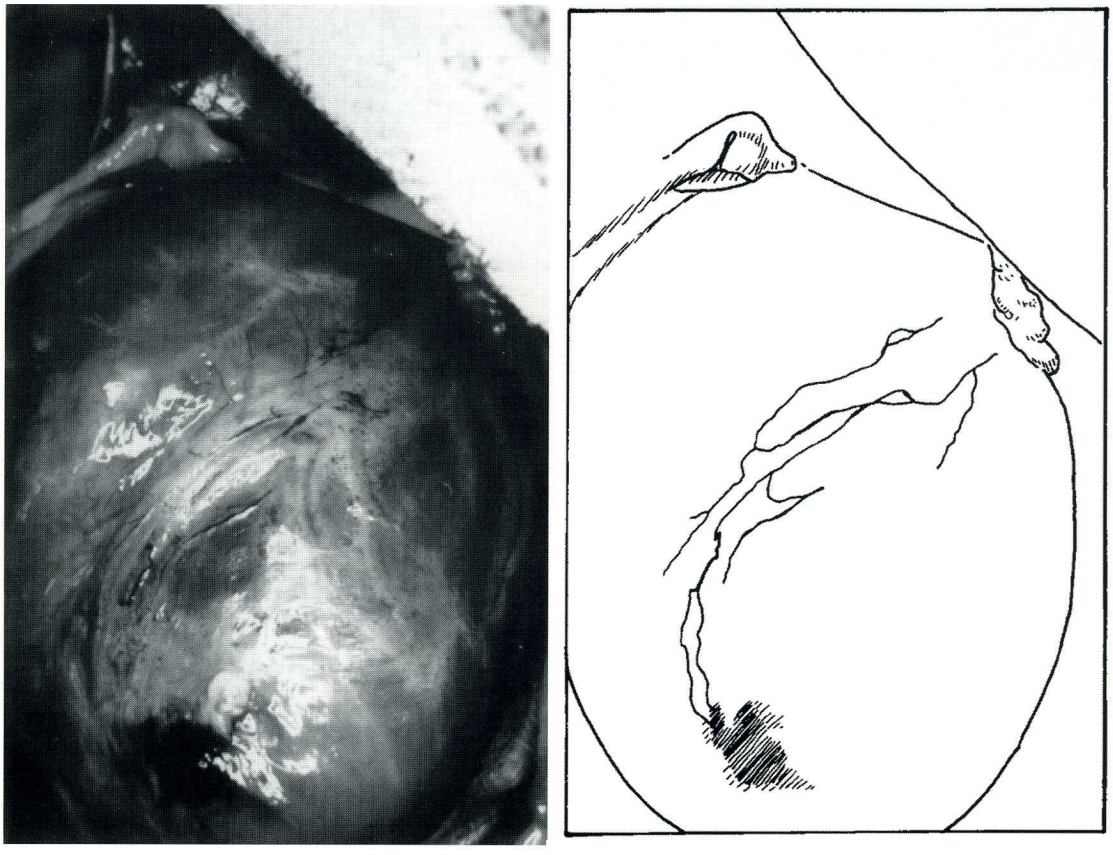

Fig. 1. The ascending interventricular lymphatics are seen as ascending black streaks passing towards the left atrial appendage after the injection of India ink into the apex of the left ventricle. The sketch at the right clarifies the lymphatic patterns, some of which are difficult to visualize in a black and white photograph.

drainage system, including lymphatic drainage into the mediastinum cephalad to the heart.

Table 2 summarizes the various techniques and lymphatic markers used. These techniques and markers were previously found effective in the dog model and have been more recently successfully used in the Yucatan minipig.

RESULTS

Phase I

In each of seven monkeys studied acutely, the ascending interventricular principal lymphatic was consistently visualized *(Fig. 1)*. In two instances, two major collecting lymphatics ascended near the left atrial appendage.

In three of these monkeys, the tracers injected into the left ventricular myocardium were micropulvermediastinal lymphatic drainage or the cardiac lymph node seen. In four monkeys, the markers were India ink (Higgins) diluted 1:1 with saline solution and T1824 bue dye (Evans). In one older monkey injected with India ink, the ascending interventricular principal coronary lymphatic was strikingly sclerotic and grossly beaded in appearance. In another monkey, the lymphatic drainage was traced cephalad behind

TABLE 3
Summary of Experience to Visualize Cardiac Lymphatics in the Monkey

Studies	Tracers	Time after injection	Results (visualized)	
Acute	Micropulverized barium sulfate; T1824 blue dye; Higgins India ink; Pelikan India ink; CH40; Czech India ink	up to 2 hours	ascending principal lymphatic mediastinal lymphatics cardiac lymph node	7* 1 2
Chronic	Micropulverized barium sulfate; Higgins India ink; Pelikan India ink	usually 3 days; in monkeys after barium sulfate injection up to 3 months, or longer	ascending principal lymphatic mediastinal lymphatics cardiac lymph node	6 1 6

*Numerals in this column represent number of monkeys

the aorta to a small lymphatic that entered a black cardiac lymph node in the right mediastinum between the innominate artery and the superior vena cava. In two of the "acute" monkeys we injected Pelikan India ink and carbon CH 40 (4) in addition to India ink, but these tracers proved no more effective in depicting the mediastinal lymphatics.

Phase II

In four monkeys, 3 days after the first procedure we injected a tracer before restudy. In each, India ink was injected into the left ventricular apex, either diluted 1:1 with 0.9% saline or with 4% gelatin solution at the time of the initial operation. In three of the four monkeys, we visualized one or more principal coronary lymphatics ascending in the interventricular groove. In one monkey, we traced the ascending lymphatic to beneath the left atrial appendange. In all four monkeys, a black cardiac lymph node was identified in the right mediastinum between the innominate artery and the superior vena cava.

Three monkeys were operated upon and kept for several months before the second procedure and euthanasia. One monkey, four months after the initial procedure, had a 2.0 ml of a suspension of micropulverized barium sulfate instilled into the pericardial sac. At the time of restudy T1824 blue dye was injected into the left ventricular apex. In this monkey, the blue dye promptly visualized the ascending interventricular principal lymphatic, which had a beaded appearance; in addition, the cardiac lymph node became blue. Another monkey was operated upon seven months after the initial operation. At the initial procedure, a suspension of micropulverized barium sulfate was injected subepicardially into the apical area of the left ventricle. At the time of re-operation, there was an adhesive pericarditis and the perietal pericardium was adherent to the heart. The cardiac lymph node was colored white from the barium sulfate. One additional monkey was restudied six months after the initial procedure, which consisted of instilling a

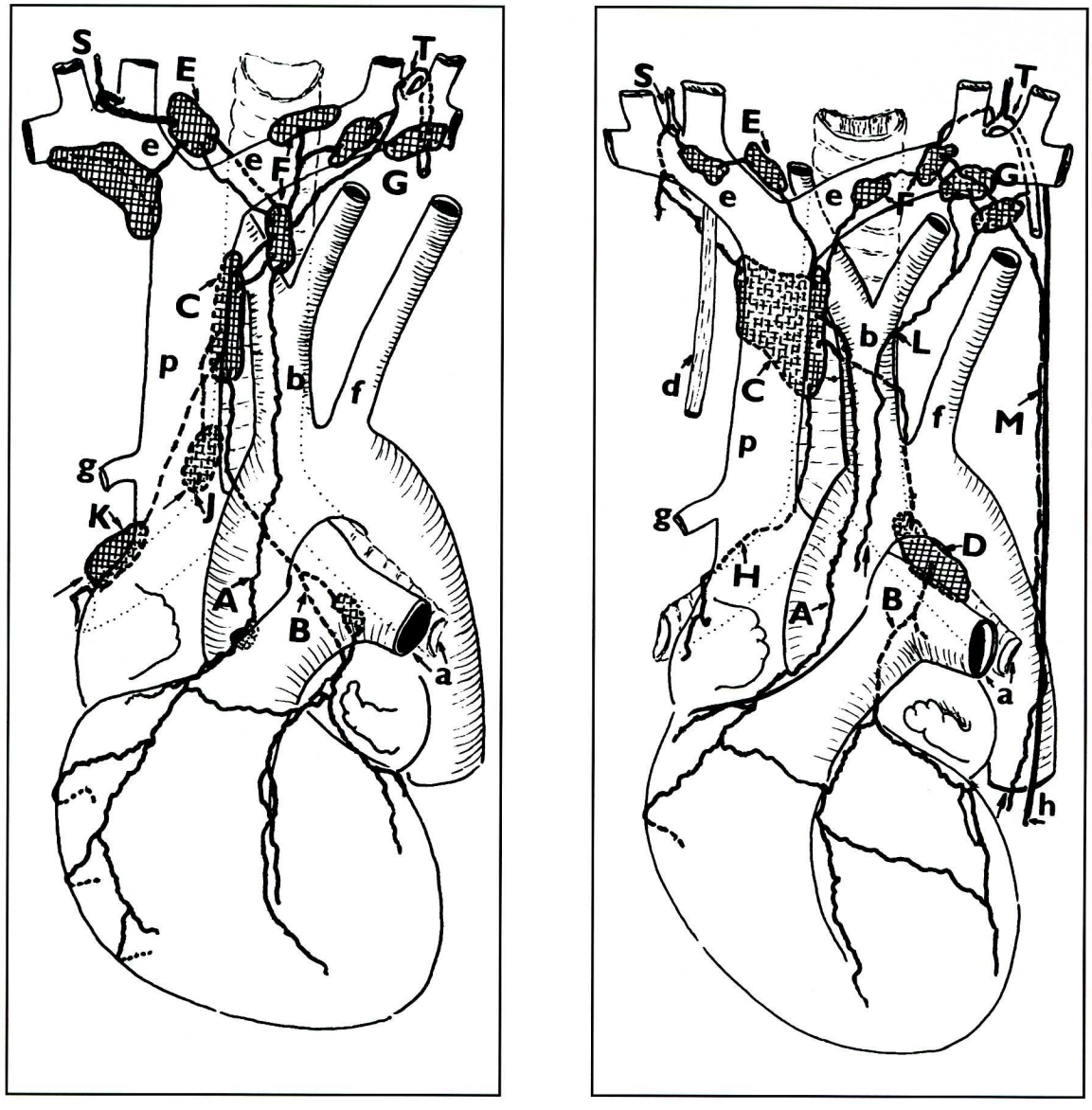

Fig. 2. These diagrams from the paper by Eliskova et al (1) show the details of the cardiac lymphatic drainage system in the monkey as determined by careful histologic study. The system is quite similar to that seen in other mammals that have been studied, including man. [Reprinted from Lymphology 25 (1992), 69-74.]

A=right coronary lymphatic; B=left coronary lymphatic; C=cardiac lymph node; D=left superior tracheobronchial node; E=nodes of anguli innominati-subgroup of right anterior mediastinal nodes; F=anterior transverse mediastinal nodes; G=left anterior mediastinal nodes; H=lymphatic from the area of the crista terminalis; J=paratracheal node receiving lymphatics from the pericardium; K=right superior tracheobronchial node receiving lymphatics from the pericardium; L,M=lymphatics from the pericardium; S=right lymphatic duct; T=thoracic duct; a=left pulmonary artery adjacent to bronchus; b=common arterial trunk (truncus communis); d=vagus nerve; e=right and left brachiocephalic veins; f=left subclavian artery; g=azygos vein; h=phrenic nerve; p=superior vena cava.

suspension of micropulverized barium sulfate into the pericardial sac. At the time of restudy, barium was detected in the cardiac lymph node. India ink injected into the apex of the left ventricle demonstrated an ascending interventricular principal coronary lymphatic.

Table 3 summarizes the procedures used to visualize the lymphatics and the outcomes in Phases I and II.

DISCUSSION

There are two studies that report experimental interference with cardiac lymph flow in monkeys. In 1976 McKinney described that Patus monkeys developed ventricular endocardial changes after ablation of the cardiac lymphatics and the feeding of a plantain diet (5). Unfortunately, he provided no information on how cardiac lymph flow was impaired. Lemole described coronary artery changes in Rhesus monkeys after interference with cardiac lymph flow (6). He informed us (personal communication) that he and Dr. Elizabeth V. Lautsch injected a carbon suspension into the epicardial fat at the cardiac apex, and that about 45 minutes later they detected lymphatics entering "nodes between the pulmonary artery and the aorta and on the pericardial reflection behind the pulmonary artery and the left atrium." Unfortunately, Lemole was unable to provide further information concerning the techniques used and the details were never published.

We know from careful histologic studies *(Fig. 2)*, that the monkey has a well developed cardiac lymphatic system comparable to that of other mammals (2). The rate of migration of the India ink tracer injected into the left ventricular apex in the living monkey is slow, probably because of the very small size of the initial lymphatics in cardiac muscle. The cardiac lymph node was visualized three days after India ink was injected into the left ventricular muscle.

When a micropulverized barium sulfate suspension was injected into the ventricular myocardium or instilled into the percardial sac, the cardiac lymph node became white. We do not know how long it took for this coloration to occur, but it likely took at least many hours. It appears that T1824 blue dye (Evans) is more effective in visualizing the cardiac lymphatics after the system has been exposed to micropulverized barium sulfate. Perhaps the barium partially blocks the lymphatics, and such partially obstructed lymphatics are possibly more efficient in taking up the T1824 blue dye.

It should be emphasized that all monkeys used in our studies have previously been used for non-surgical experiments and that almost all of them were chronologically old. Perhaps some of the difficulty in acutely visualizing cardiac and mediastinal lymphatics with India ink or T1824 blue dye relates to the older age of these monkeys.

From these series of experiments, we conclude that (a) the initial lymphatics of the heart in the monkey are very small, (b) visualization of the right-sided mediastinal cardiac lymph node with India ink in the monkey is slow, occurring over many hours or days, (c) experiments designed to obstruct the cardiac lymphatic system in the monkey must be undertaken with due recognition of the difficulty in adequately visualizing the mediastinal drainage system and the cardiac lymph node, (d) though the demonstration of the cardiac lymphatic system in the monkey is slow, the anatomical arrangement is comparable to that found in man, dog, and pig, (e) it remains desirable to develop a more effective and consistent method of visualizing the mediastinal drainage lymphatics of the heart in the cynomolgous (Macaca mulatta) monkey in order to encourage the use of this animal for studying the effects of cardiac lymph drainage. In the meantime, the relative ease of visualizing porcine cardiac lymphatics makes the pig the current species of choice for investigations on the effects of cardiac lymphatic blockade on the coronary arterial system under varying experimental conditions.

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REFERENCES

1. Eliska, O, M Eliskova, AJ Miller, et al: The efferent cardiac lymphatic pathways in the Macaque monkey. Lymphology 25 (1992), 69-74.
2. Miller, AJ: *The Lymphatics of the Heart.* Raven Press, New York City, 1992.
3. Miller, AJ, S Jain, B Levin: Radiographic visualization of heart muscle and pericardial sac in the dog. Chest 59 (1971), 271-275.
4. Hagiwara, A, T Takahashi, K Sawai, et al: Lymph nodal vital staining with newer carbon particle suspensions compared with India ink: Experimental and clinical observations. Lymphology 25 (1992), 84-89.
5. McKinney, B: Endocardial changes produced in Patus monkeys by ablation of cardiac lymphatics and the administration of a plantain diet. Amer. Heart J. 91 (1976), 484-491.
6. Lemole, GM: The role of lymphostasis in atherogenesis. Ann. Thorac. Surg. 31 (1981), 290-293.

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