A New Method of Lymph Collection in Animals

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Analysis of the available literature leads one to conclude that the methods used at present to collect lymph from animals, including the most common one, thoracic duct drainage, are fraught with shortcomings. They are laborious and largely ineffective and require general anesthesia, often with preliminary contrasting of the lymph bed or keeping the animals starving, all of which to a certain extent reduces the reliability of the results. Other drawbacks are the complicated surgical technique and the topographoanatomical features of the thoracic duct, its cervical portion in particular [1-3, 7-11].

The present study was undertaken to develop an effective way of collecting lymph in animals superior to existing methods.

Our tasks were as follows: 1) to choose a species of animal for investigation; 2) to select the anesthesia; 3) to gain access to the thoracic duct cisterna; 4) to devise an instrument for lymph collection.

The suggested method for lymph collection was developed using chinchilla rabbits weighing 2-3.5 kg. After the experiments the animals were killed by injection of sodium thiopental aqueous solution into the ear vein. The experiments were carried out in November. Rabbits show certain advantages over other animals: 1) their pain sensitivity threshold is high, this permitting local infiltration anesthesia at all stages of the proposed method of lymph collection; 2) their abdominal cavity is sufficiently large, this facilitating access to the thoracic duct cisterna, the largest portion of the duct [4,9]. The thoracic duct cisterna in rabbits is reached by freeing the left kidney and its vascular appendages (renal vein and artery, ureter, and extraorganic lymph vessels), as well as the regional lymph nodes and medial transposition thereof. The tissues fixing the kidney are stratified in this case along its lateral edge to the origin of the renal artery, this corresponding to the localization of the thoracic duct cisterna [5]. The described approach was registered as Innovation № 716 of April 28, 1987, at the Novosibirsk Medical Institute. For puncture of the thoracic duct cisterna and lymph collection from it, glass micropipettes were used (manufactured according to Russian Patent № 1495076) with a channel diameter of 200-500 μ and the tip pointed at an acute angle to the longitudinal axis. Such glass micropipettes are superior to the currently available micropipettes and metal injection needles: lymph collection can be visually controlled, the puncture of the cisterna wall is atraumatic, no potential difference occurs, followed by redox reactions at the pricking site of the micropipettes (which greatly improves the reliability of the results), and at the same time, the micropipettes are hard enough to puncture lymph and blood vessels (Fig. 1).

The method of intravitam lymph collection in animals is as follows. The rabbit is fixed supine by the fore- and hind limbs. The anterior abdominal wall is routinely treated and anesthetized along the white line with 0.25% procaine solution at the sites of the left kidney projection and of an incision approximately 7 cm long. A layer-by-layer

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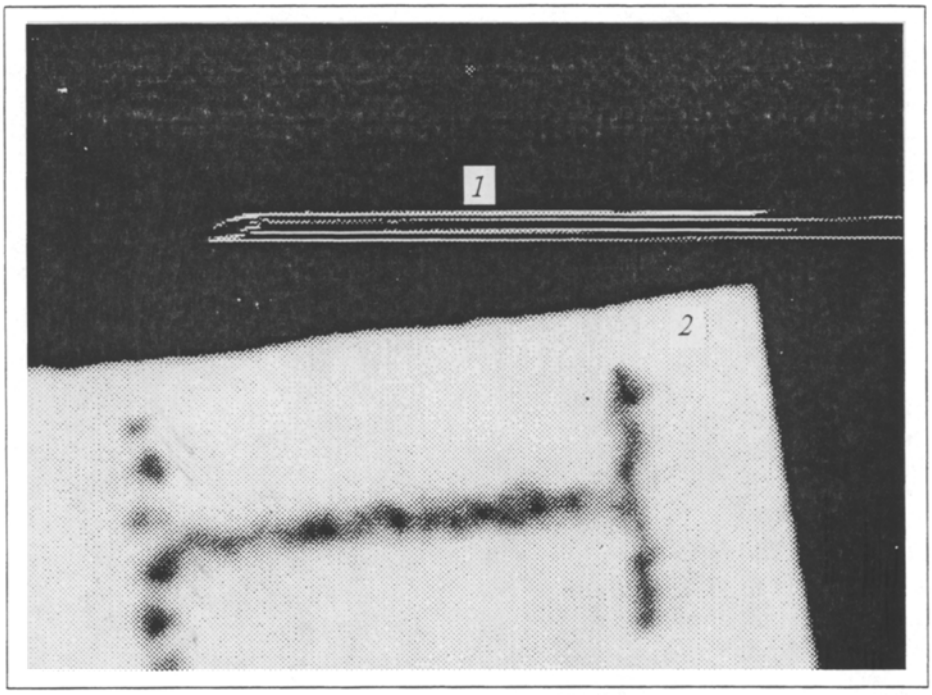

Fig. 1. Glass micropipette. *1*) pricking part; *2*) Optima typewriter "unit" type. ×32.

incision is made along this line. The surgical wound is widened with a hook towards the left kidney. The intestinal loops are medially drawn aside and held with a cloth. The left kidney is situated on the posterior abdominal wall with its vascular formations and regional lymph nodes located dorsal to the renal artery. The pararenal tissues are then anesthetized with 0.25% procaine. The pricking is performed cranially and caudally vis-a-vis the renal vein and artery. The left kid-

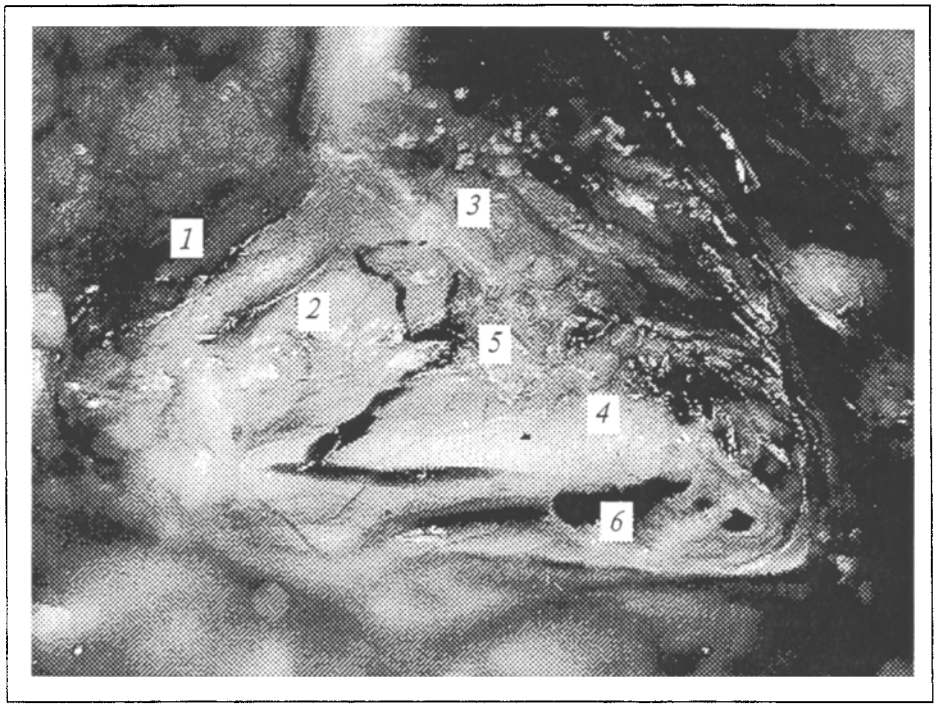

Fig. 2. Topography and anatomy of thoracic duct cisterna and adjacent organs after creation of access to it. *1*) left kidney; *2*) ureter; *3*) left renal artery; *4*) abdominal aorta; *5*) renal regional lymph node; *6*) thoracic duct cisterna. The extraorganic lymph bed of the left kidney was contrasted by injection of a black ink aqueous suspension. The picture was taken with transfer rings.

ney and its vascular formations and regional lymph nodes are then freed by layering the soft tissues along the lateral margin of the kidney as far as the origin of the renal artery and medially drawn aside. The thoracic duct cisterna is situated dorsally somewhat to the left of the abdominal aorta and cranially in relation to the renal artery origin (Fig. 2). The cisterna is punctured with a glass micropipette (or cannulated) and the lymph from the cisterna is drawn into the micropipette channel in the required volume, which depends on the type of further investigation. This method of lymph collection in animals is going to be patented: the application № 4944468/30-14, dated July 14, 1991, has been approved.

The procedure can be carried out by one worker; if local anesthesia is replaced by general anesthesia, the method is applicable o virtually any animal species. Data on the cellular composition of the lymph may be available as soon as 1-1.5 h after starting the operation. This method made it possible to detect for the first time dendritic cells and Mott's cells in rabbit central lymph [6,7].

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