THE LYMPHATIC PATHWAY FROM THE NOSE AND PHARYNX

The Absorption of Dyes

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Plates 25 to 27

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Schulz, Warren, and Drinker (1) noted that Type III pneumococci, instilled into the nose of rabbits, could often be found within an hour in the cervical lymph. This observation formed the starting point of our present experiments on the cervical lymphatic pathway.

The lymphatic vessels of the head and neck, like those of the limbs, fall into a superficial and a deep group. The collecting trunks of the superficial vessels accompany the external jugular vein. The tributaries of the deep group flow into the cervical lymph duct, which runs down the neck on the lateral side of the common carotid artery and internal jugular vein. The superficial vessels empty into the main cervical duct, though the level at which this takes place varies in different species; in the cat and monkey it occurs low down in the neck, in the dog not far from the angle of the jaw. The cervical lymph duct on the left side joins the terminal portion of the thoracic duct, just before it opens into the junction of the internal jugular and subclavian veins. On the right side the cervical duct either joins the right lymph duct, or may open independently into the junction of the right subclavian and internal jugular veins. In the present series of experiments we have cannulated the cervical lymph duct low down in the neck, immediately before it joins the venous system.

When the cervical duct is cannulated, one of three things may happen. (*a*) There may be a spontaneous flow of lymph. This is usually the case in the monkey, and presumably, therefore, in man.

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(*b*) In the absence of a spontaneous flow, gentle massage of the duct empties its lymph into the cannula, after which the duct fills up again and the procedure may be repeated. (*c*) Occasionally the flow of lymph is negligible. No satisfactory explanation has yet been offered for this occasional "dryness."

If, in an animal whose cervical duct has been cannulated, one instils into the nose a solution of trypan blue or T-1824[1] in physiological saline, the dye quickly passes through the mucous membrane and enters the lymph. It appears in the cannula within 20 to 30 minutes in the monkey, cat, and rabbit, rather longer in the dog, in concentrations which are weak at first but subsequently undergo progressive increase until a fairly constant level is reached.

An experiment of this kind serves a double purpose. It affords a means of investigating the mechanism whereby the living and intact lining of the nose and pharynx permits the rapid passage of these dyes. It also enables one, on subsequent dissection, to obtain a beautifully stained preparation of the entire cervical lymphatic pathway (Figs. 1, 2, and 3). It is a method of delineating a living and functioning system of vessels while actually at work, and is free from the disadvantages of the injection methods usually employed.

Material and Technique

The experiments, summarized in Table I, have been performed on 1 monkey (*Macaca mulatta*), 14 cats, 2 dogs, and 6 rabbits. The dyes employed were trypan blue, in a 2 per cent, 3 per cent, or 5 per cent solution in physiological saline, and T-1824 in 1 per cent or 5 per cent solution. In three experiments Hydrokollag, a fine graphite suspension, was used. The dyes, in the concentrations used, had no irritant action on the living tissues, as tested by dropping in the nose and by subcutaneous injection in the authors.

In most of the experiments a cannula was tied in the trachea and the esophagus ligatured. The trachea was cannulated in order to prevent the dye from entering the lungs and being absorbed into the blood through the pulmonary capillaries,

[1] The dye T-1824, from a lot prepared by Drs. Hartwell and Fieser of Harvard University in 1936, was furnished us through the great kindness of Professor Magnus I. Gregersen. This sample is free from salt and contaminating isomers. Efforts have been made to call the compound "Evans blue," but to avoid confusion we have preferred the original designation which is also the one used by Gregersen and Gibson (2) in their paper on the behavior of certain vital dyes in plasma.

and in order to block submucous lymphatics. It is of interest that in control experiments in which the trachea and esophagus were not interrupted, dye absorption was equally rapid. All animals were completely anesthetized by nembutal.

The Anatomical Pathway

The superficial lymphatics of the head drain the skin, salivary glands, and the mucous membrane around the buccal margin and nostrils. The deep lymphatics drain the mucous membrane of the mouth, nose, accessory air sinuses, and pharynx. In the cat and dog the deep lymphatics empty into the large superior deep cervical node, close to the bifurcation of the common carotid artery. From the posterior border of this node the cervical duct takes origin, descending to the outer side of the carotid sheath, usually close to it but occasionally situated more laterally. The cervical duct is single as a rule, but it may be double, and occasionally may even be replaced by a plexus of vessels. The duct usually empties on the left side into the thoracic duct, on the right side into the right lymph duct, although many variations are possible.

The cervical lymph duct stands out in our dye experiments as a blue cord, beaded on account of the distension of the segments between the regularly placed valves. It runs down from the posterior border of the superior deep cervical node. Before dye can enter the cervical duct it must first pass through the node, which it therefore colors, usually more deeply at the upper pole than the lower. In the dog one sometimes sees the coloration limited to the posterior portion of the node, with a fairly sharp line of demarcation between this and the unstained anterior portion. In a well marked case this functional segmentation is very striking. In the dog the origin of the cervical duct from the posterior border of the large superior deep cervical node is very evident.

Above the upper pole of the superior deep cervical node, and converging to it, are a number of lymphatic vessels. The largest, two or three in the cat, four or five in the dog, can be traced upwards as far as the pterygoid hamulus, and then between this and the eustachian tube. On removal of the hamulus they can be dissected further as they run forward on the floor and side wall of the nose.

The tonsil was never colored in our experiments, even where the surrounding mucous membrane was obviously stained. One or two small lymph vessels were sometimes seen dorsal to the tonsil or caudal to its lower pole.

All the lymph vessels from the nasopharynx entered the superior deep cervical node. No vessels were seen running down directly into the cervical duct without first of all passing through the node. This accords with the conclusion of Drinker, Field, and Ward (3) on the filtering capacity of lymph nodes, which held that lymph does not return to the circulation without passing through at least one node.

In the cat the cervical duct occasionally passes through an additional node low down in the neck. This occurs even more frequently in the rabbit. It is in the monkey, however, that the interposition of extra nodes along the course of the cervical duct reaches its most advanced degree of development. (Figs. 1,

TABLE I

Summarized Data of Experiments

Experiment No.	Animal	Dye used	Concentration	Amount instilled in nostrils		Time dye placed in nostrils	Time first noted in lymph	Remarks
				Right	Left			
			per cent	*cc.*	*cc.*			
1	Cat	Trypan blue	Unknown	1.0	1.0	10:00	10:20	
2	"	" "	2	0.5	0.5	3:15	3:40	
3	"	" "	5	0.5	0.5	3:10	4:20	Duct not cannulated; found only after being colored by dye
4	"	" "	3	None	1.0	2:45	3:00	
5	"	" "	3	1.0	1.0	10:30		Cervical ducts not cannulated. Killed at 2:05 for examination of interior of cranium. No blue found
6	Dog	" "	3	2.0	2.0	2:04	2:55	
7	Cat	T-1824	5	1.5	1.5	2:40	3:00	
8	"	"	5	0.5	1.0	12:15	12:37	
9	"	"	5	1.0	1.0	11:30	11:55	
10	"	"	5	2.0	—	10:05	10:10	Dye observed in afferent vessels to superior deep cervical node
11	Dog	"	5	1.0	1.0	11:57	12:50	
12	Rabbit	"	5	1.0	—	1:45	1:59	
13	"	"	5	1.5	1.5	Left in nose 2 hrs. and 35 min. Cranium then opened and interior of skull examined, particularly region above cribriform plate. No dye found. (See Table II)		
14	"	"	5	1.5	1.5	Left in nose 2 hrs. and 55 min. Head examined as in No. 13. Possibly a faint trace of dye found in one olfactory lobe		
15	"	"	5	1.0	1.0	Nos. 15, 16, and 17 unanesthetized rabbits in which dye was dropped into the nose. There was no sneezing, coughing, or any other sign of irritant action of the dye. The animals were killed after 1½ hrs., and the cervical pathway was fully stained		
16	"	"	5	1.0	1.0			
17	"	"	5	1.0	1.0			

18	Monkey	T-1824	5	1.5	1.5	12:45	1:10
19	Cat	Hydrokollag		1.0	1.0	10:37	Not found
20	"	"		1.0	1.0	Hydrokollag left in nasopharynx for 6 hrs. Heads then placed intact in fixative,	
21	"	"		1.0	1.0	and neck dissected out 1 month later. No trace of Hydrokollag in lymph nodes or in cervical duct	
22	"	"		2.0	2.0	Hydrokollag left in nasopharynx for 6 hrs. Neck then dissected, and no trace of Hydrokollag found in cervical node or duct	
23	"	"		1.0	1.0	1:00	Not found

2, and 3.) Here the duct constantly passes through a chain of five or six nodes, and it is noteworthy that the entire duct passes through each node, not merely a tributary.

The Passage through the Mucous Membrane

Practically nothing is known of the mechanism whereby animate or inanimate particles pass through the mucous membrane and enter the lymphatic vessels. There is some evidence, however, that the size of the particles is an important factor. In our experiments trypan blue (molecular weight 960.81) and T-1824 (molecular weight 960.81) passed through readily. Hydrokollag, a fine graphite suspension with particles ranging in size from 0.2 to 2.0 micra, did not reach the lymph. Data concerning experiments with proteins and viruses will be presented in other papers.

The Appearance of Dye in the Lymph

Following the instillation of dye into the nose, the cervical lymph after a short period shows a faint tinge of blue. Subsequently the concentration of dye gradually increases. The time which elapses between the placing of the dye in the nose and its appearance in the cannula is 15 to 30 minutes in the cat and monkey, nearer an hour in the dog (Table I). In either case, however, the time is considerably in excess of that required for the dye to pass through the nasal mucosa. For after its passage through the mucosa the dye has to traverse the large superior deep cervical node, and almost the entire length of the cervical lymph duct, before it appears in the cannula. In other words, the dye-containing lymph has to displace the normal lymph previously present in the node and vessels before its presence in the cannula can be detected. If, however, one dissects high up in the neck, above the superior deep cervical node, one can observe the lymph vessels close to their commencement in the nasopharynx, and the appearance of dye in them may be noted in a very much shorter time. This is illustrated by Experiment 10.

Experiment 10. Dissection of Afferent Vessels to Superior Deep Cervical Node.— Cat, weight 3.5 kg. Feb. 11, 1938. *9:00 a.m.*, general anesthesia by nembutal (5 per cent) 4.0 cc. intraperitoneally.

10:05, dissection of superior deep cervical node and afferent lymph vessels complete on right side. 2.0 cc. of 5 per cent T-1824 in physiological saline placed in right nostril.

10:10, dye observed streaming down in afferents to node. Progressive coloring of node from above downwards, finally reaching efferent lymph in the cervical duct after 20 minutes.

Note.—The passage through the mucosa in so short a time seems to indicate that the question of deterioration of the mucosa does not arise. Further, no tracheal cannula was used in this experiment, nor was the esophagus ligated, so that the condition of the nasal mucosa was presumably normal.

The changing concentration of dye in the cervical lymph is best shown by taking samples of lymph at intervals in fine capillary tubes of equal bore. Fig. 4 depicts a set of such tubes (Experiment 18). Dye was placed in the nose of a monkey at 12:45 p.m. and appeared in the cannula at 1:10 p.m. After an initial period of rapid increase, the concentration of dye becomes much more stable. The last few samples of lymph show very little change.

The concentration of dye can be estimated with fair accuracy by comparing the set of capillary tubes with another set of tubes of the same internal diameter containing a series of known dilutions of the dye (Fig. 5). This is illustrated by the protocol of the experiment from which the set of tubes in Fig. 4 were obtained (Experiment 18).

Experiment 18.—Monkey (*Macaca mulatta*), weight 3.5 kg. Feb. 15, 1938. *9:20 a.m.*, general anesthesia by nembutal (5 per cent) 3.5 cc. intraperitoneally. *10:20*, trachea cannulated. *11:55*, right cervical lymph duct cannulated. Protein 3.65 per cent.

12:15 p.m., blood sample 1 taken.

12:45–12:48, T-1824 (5 per cent in physiological saline) 1.5 cc. in right nostril. *12:48–12:55*, equal amount in left nostril. *12:50*, lymph flowing spontaneously. Small amount of dry heparin placed in the cannula after withdrawal of each lymph sample, to prevent clotting. *1:05*, lymph flowing well. *1:50*, lymph still flowing spontaneously, but rate of flow slower. *2:45*, rectal temperature 98°C.

2:47, blood sample 2 taken.

2:50, animal killed by bleeding and injection of 5.0 cc. of nembutal (5 per cent) intravenously.

Concentrations of Dye in Lymph

Time	Concentration	Time	Concentration
1:20 p.m.	1:128,000	2:10 p.m.	1:4,000
1:30	1:32,000	2:20	1:3,000
1:40	1:16,000	2:30	1:2,000
1:50	1:8,000	2:45	1:2,000
2:00	1:8,000		

Lymph flowed for 15 minutes after death, and a sample taken at 3.00 p.m. had a dye concentration of 1:1,000.

Concentration of Dye in Blood.—

Blood sample 1.... No dye.

Blood sample 2.... Less than 1:128,000.

Protein in Blood Serum.—5.83 per cent.

Absorption of Dyes Directly into the Blood Stream

Some of the dye placed in the nose is absorbed directly into the blood stream. This can readily be shown by ligaturing the superficial and cannulating the deep lymphatics in the neck, and taking repeated blood samples as has been done in Experiment 9.

Experiment 9.—Cat, weight 3.5 kg. Jan. 20, 1938. *9:25 a.m.*, general anesthesia by nembutal (5 per cent) 3.5 cc. intraperitoneally. *9:50*, trachea cannulated, esophagus ligated. *10:00*, right external jugular lymph vessels ligated. *10:25*, right cervical lymph duct cannulated. Moderate flow. *11:05*, left external jugular lymphatics ligated. Left cervical duct cannulated. Flow slight. *11:25*, lymph now flowing spontaneously on both sides. *11:28*, nembutal 1.0 cc. intraperitoneally.

11:30–11:40, T-1824 (5 per cent in physiological saline) 1.0 cc. in each nostril.

11:42, protein in right cervical lymph, 3.33 per cent. Spontaneous flow. *11:48*, protein in left cervical lymph, 3.26 per cent. Flow spontaneous also. No massage employed as yet.

11:55, right cervical lymph blue in cannula. *11:57*, nembutal 1.0 cc. intraperitoneally.

12:00, left cervical lymph also now visibly blue in cannula.

12:20 p.m., lymph hardly flowing on left side. No massage employed. Still flowing spontaneously on right.

1:30, right cervical lymph still flowing spontaneously, but on left side lymph now obtainable only on duct massage. *2:00*, flow of right cervical lymph almost ceased. *2:40*, animal bled to death.

Post Mortem.—Right. External jugular lymphatics dilated with colorless lymph. Superficial cervical lymph nodes hardly stained. On deep dissection the superior deep cervical node was deeply stained in its upper three-fourths. Only one efferent vessel found, the one cannulated. No sign of any other lymph vessel.

Left. External jugular lymphatics dilated up to site of ligature and contain pale blue lymph. Superficial lymphatics from angle of mouth coursing over masseter also blue. Superficial cervical lymph nodes stained, though not heavily.

Superior deep cervical node deeply stained. No bypassing by any other vessel. No inferior node. Marked extravasation of lymph along course of duct.

Cranium. Cribriform plate exposed from above. No sign of blue.

Concentrations of Dye in Blood and Lymph

Time	Blood	Lymph
11:20 a.m.	Normal	Normal
12:10 p.m.	1:128,000	1:4,000
12:40	1:100,000	1:500
1:10	1:64,000	1:250
1:40	1:32,000	1:250
2:10	1:16,000	1:250
2:40	1:16,000	1:250

The blood at 2:40 p.m. had slightly more dye than at 2:10, but the difference was not sufficiently marked to be measured by our scale.

It will be noted that in both the blood and the lymph the concentration of dye tends to reach a constant level. There is, however, the important difference that the concentration in lymph is very much higher than that in blood.

Communications between the Nose and the Interior of the Cranium

Several observers have described the rapid passage of simple solutions from the nose into the cranial cavity. Clark (4) states that:

"A solution of potassium ferrocyanide and iron ammonium citrate, dropped into the nasal cavities of rabbits, reaches the surface of the brain within one hour." He believed that there was a pathway along "the perineural sheaths of the olfactory nerves," and concluded that "the spaces of these perineural sheaths are continuous above with the subarachnoid spaces and extend peripherally along the peripheral fibres of the olfactory nerves to the olfactory sensory epithelium. The existence of a current running centripetally in these perineural sheath spaces under normal conditions is postulated."

Faber (5) came to similar conclusions as a result of experiments on rabbits. These apparently conclusive findings for simple crystalloids have not been confirmed by our experience with trypan blue and T-1824. In but one instance have we seen the slightest trace of dye in one olfactory lobe, and in that case the appearance was so slight as to be a matter for dispute in the laboratory.

In regard to visible particles, Clark (4) found that particles of carbon failed to reach the inside of the skull after nasal instillation. Our experience with Hydrokollag was similar. On the other hand, Olitsky and Cox (6), in mice, found that Prussian blue granules did pass from the nose into the cranium. Rake (7) made the same observation in mice, and also found that pneumococci and *Salmonella enteritidis* reach the subarachnoid space "with the same rapidity as the pigment," as also does the pantropic virus of equine encephalomyelitis, although neurotropic viruses were not demonstrated in less than 24 hours.

"That the lymphatics of the nasal mucosa are in almost direct communication with the subarachnoid space has been clearly demonstrated" (Peabody, Draper, and Dochez, 8). This statement, however, can only be made with certain reservations. It is true that substances introduced into the subarachnoid space escape into the

TABLE II

Passage of Substances from the Nasal Cavity through the Cribriform Plate

Experiment No. (See Table I)	Animal	Material placed in nose	Time left in nose
5	Cat	3 per cent trypan blue	3 hrs. and 35 min.
7	"	T-1824 (5 per cent)	2 hrs. and 35 min.
9	"	" " " "	3 hrs. and 10 min.
9*a*	"	" " " "	5 hrs. and 50 min.
11	Dog	" " " "	3 hrs.
13	Rabbit	" " " "	2 hrs. and 35 min.
14	"	" " " "	2 hrs. and 55 min.
23	Cat	Hydrokollag	6 hrs.

No dye was found above the cribriform plate in any experiment except possibly Experiment 13. In the rabbit, even where dye is not found above the plate, the plate appears blue on looking down from above. Either the plate is so translucent that one can see the dye in the roof of the nose through it, or the dye may have stained part of it.

lymphatics of the nasal mucosa and then into the cervical lymphatics. Since the first observations of Key and Retzius (9) many workers have confirmed this fact. (For full bibliography see Weed, 10.) The question now at issue is whether the reverse proposition is also true, that substances in and upon the nasopharyngeal mucosa which enter cervical lymph may also pass into the subarachnoid space. In the present series of experiments this was definitely not the case. Our results are of especial interest since by cannulation of the cervical lymph duct it was possible to show that dye was present in the lymph in high concentration over many hours, and yet could never be detected in the interior of the cranium (Table II).

Our results therefore appear to conflict with those of previous workers. There are two factors to consider: (*a*) the nature of the solution used, and (*b*) the size of the animal. Clark (4) and Faber

(5) both used in their successful experiments simple solutions, potassium ferrocyanide and iron ammonium citrate, whereas in our own experiments colloidal dyes were used. It is to be noted, however, that Faber, when using thorotrast (a solution of thorium dioxide in a protective colloid), obtained results as completely negative as our own. "The sections from the rabbit into the nostrils of which thorotrast had been instilled were completely negative for the passage of thorotrast through the nasal mucosa." Similarly Clark's results when using trypan blue are in agreement with those now presented. Clark left a 0.5 per cent solution of trypan blue in the nose of a rabbit for 24 hours, and at the end of that time found no sign of dye within the cranium. It seems evident that for anything but solutions of simple crystalloids the cribriform plate offers an effective barrier to the passage of substances (non-living) from the nose to the interior of the skull.

Another factor which has been insufficiently considered is the size of the animal. In a small animal such as the mouse the cribriform plate is an exceedingly tenuous structure, and might conceivably permit a passage of dyes to which the thicker cribriform plate of larger animals would be more resistant. The rabbit, on the other hand, is much nearer in size to the cat, in which our findings were all negative. In 4 rabbits we instilled T-1824 (5 per cent) into the nose and examined the region of the cribriform plate 3 hours later. In none was there any real staining above the cribriform plate though in one there may have been a very faint trace of blue in one olfactory bulb. Our results suggest, therefore, the advisability of interpreting with great caution the results of experiments on small animals. They may be very suggestive, but at the same time not applicable with certainty to man.

SUMMARY

1. In the monkey, dog, cat, and rabbit the cervical lymph duct was cannulated, and then a solution of T-1824, or trypan blue, or a fine graphite suspension, all in physiological saline, was dropped into the nose. T-1824 was used in all four animals, trypan blue in the cat and dog, the graphite suspension (Hydrokollag) in the cat alone.

2. No Hydrokollag was ever found in the cervical lymph.

3. Trypan blue and T-1824 appear in the cervical lymph 15 to 30 minutes after being placed in the nose of the cat and monkey, 51 to 53 minutes in the dog, and 14 minutes in the rabbit.

4. T-1824 and trypan blue were also absorbed from the nose directly into the blood.

5. Neither the dyes nor the Hydrokollag, though left in the nose for as long as 6 hours, were found to pass through the cribriform plate and reach the interior of the cranium.

6. In the monkey cervical lymph passes through a chain of five or more lymph nodes, in the rabbit frequently through two nodes, in the cat through one node except in rare instances, and in the dog through one node.

BIBLIOGRAPHY

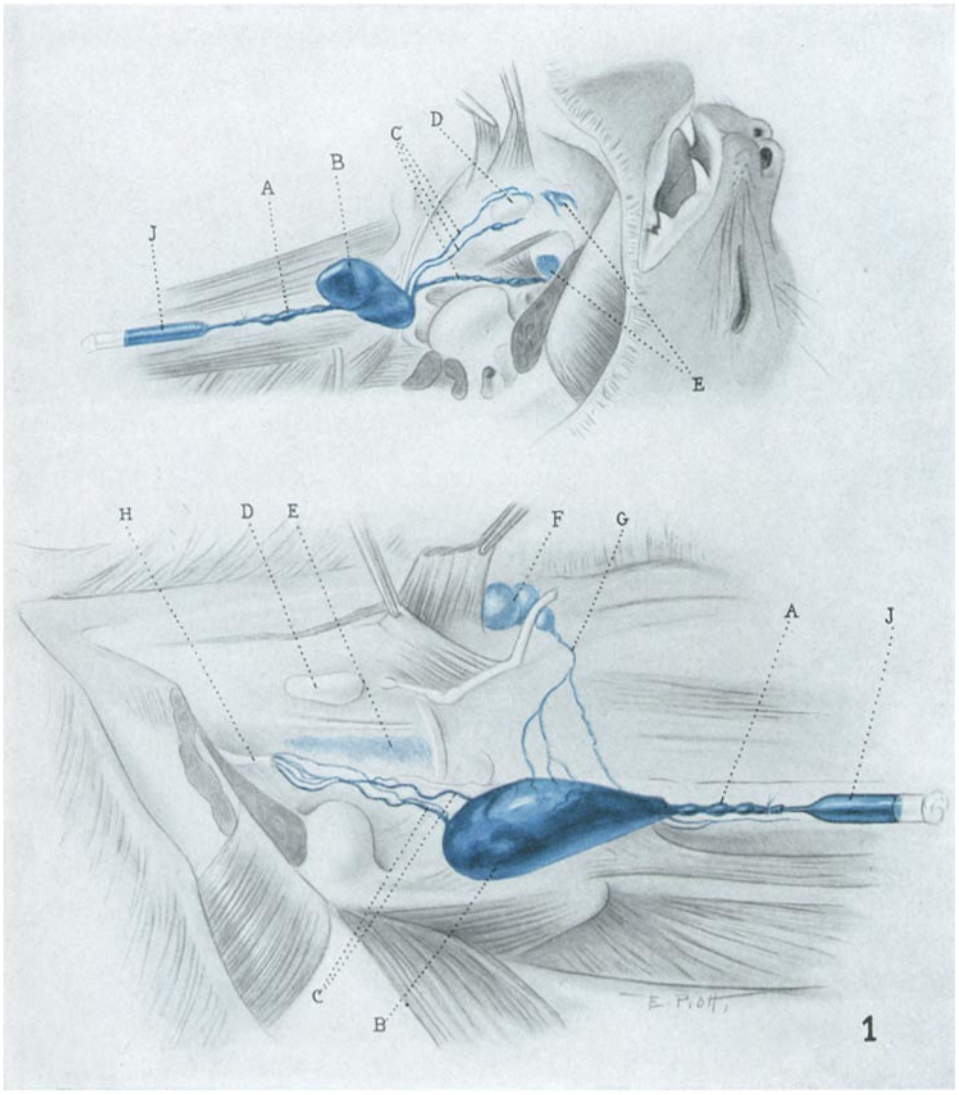
1. Schulz, R. Z., Warren, M. F., and Drinker, C. K., *J. Exp. Med.*, 1938, **68**, 251.
2. Gregersen, M. I., and Gibson, J. G., 2nd, *Am. J. Physiol.*, 1937, **120**, 494.
3. Drinker, C. K., Field, M. E., and Ward, H. K., *J. Exp. Med.*, 1934, **59**, 393.
4. Clark, W. E. Le G., *Great Britain Rep. Pub. Health and Med. Subj., Ministry of Health, No. 54*, 1929.
5. Faber, W. M., *Am. J. Anat.*, 1937, **62**, 121.
6. Olitsky, P. K., and Cox, H. R., *Science*, 1934, **80**, 566.
7. Rake, G., *J. Exp. Med.*, 1937, **65**, 303.
8. Peabody, F. W., Draper, G., and Dochez, A. R., A clinical study of acute poliomyelitis, Monograph of The Rockefeller Institute for Medical Research, No. 4, New York, 1912.
9. Key, A., and Retzius, G., Anatomie des Nervensystems und des Bindegewebes, Stockholm, 1876.
10. Weed, L., *Physiol. Rev.*, 1922, **2**, 171.

EXPLANATION OF PLATES

PLATE 25

FIG. 1. Cervical lymphatic pathway in the cat (upper drawing), and in the dog (lower drawing).

Key to lettering in Figs. 1, 2, and 3: *A*, cervical lymph duct; *B*, superior deep cervical node; *C*, afferent vessels to superior deep cervical node; *D*, tonsil; *E*, patches of mucous membrane through which dye can be seen in mouth and pharynx; *F*, superficial cervical node or nodes; *G*, lymph vessels draining from superficial to deep cervical nodes; *H*, chain of deep cervical nodes; *I*, superficial lymph vessel running from cheek and angle of mouth, over surface of masseter, into superficial cervical node; *J*, cannula in cervical lymph duct.

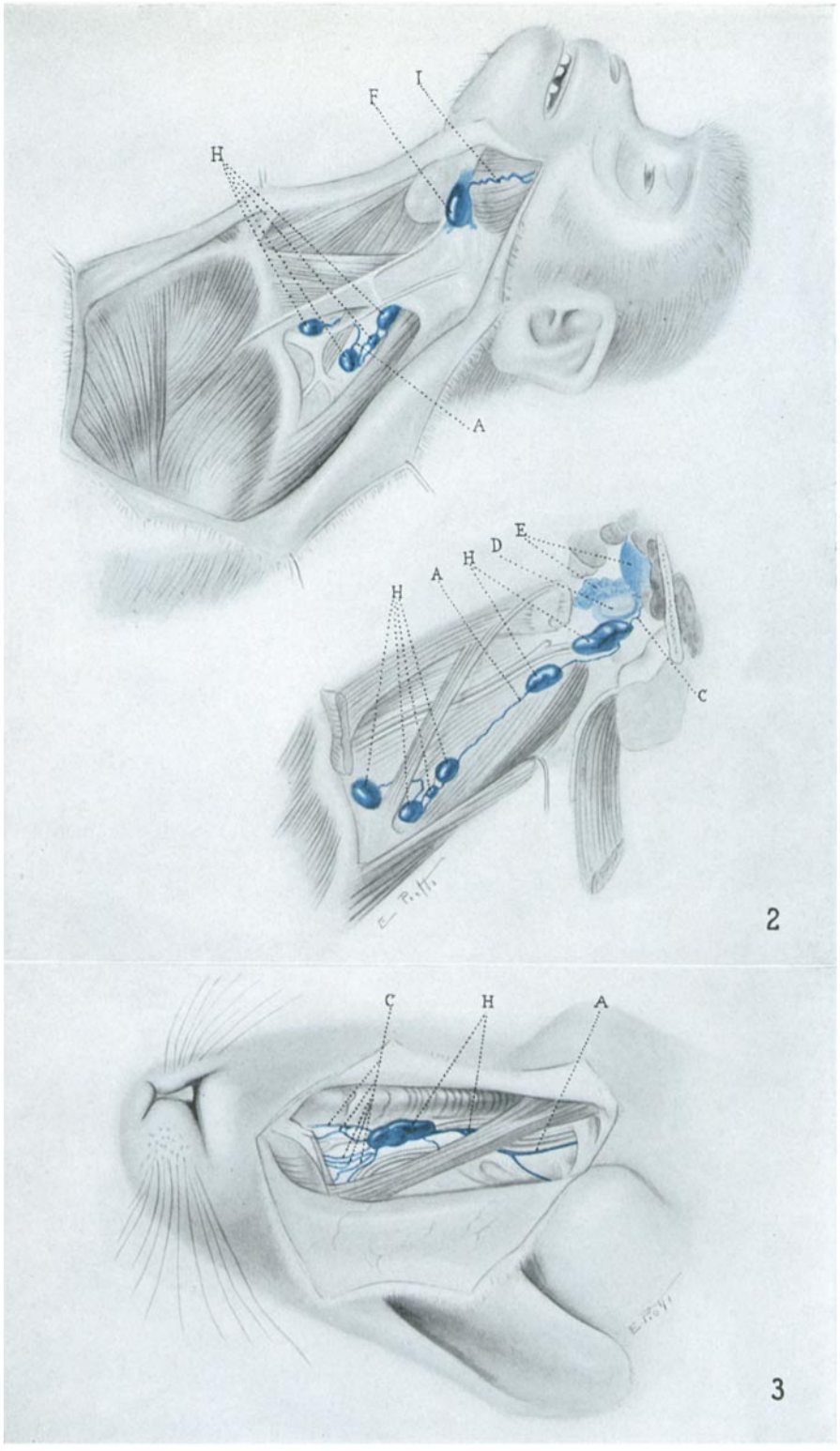


(Yoffey and Drinker: Lymphatic pathway from nose and pharynx)

PLATE 26

FIG. 2. Cervical lymphatic pathway in the monkey (*Macaca mulatta*). Upper drawing, superficial dissection; lower drawing, deep dissection.

FIG. 3. Cervical lymphatic pathway in the rabbit.

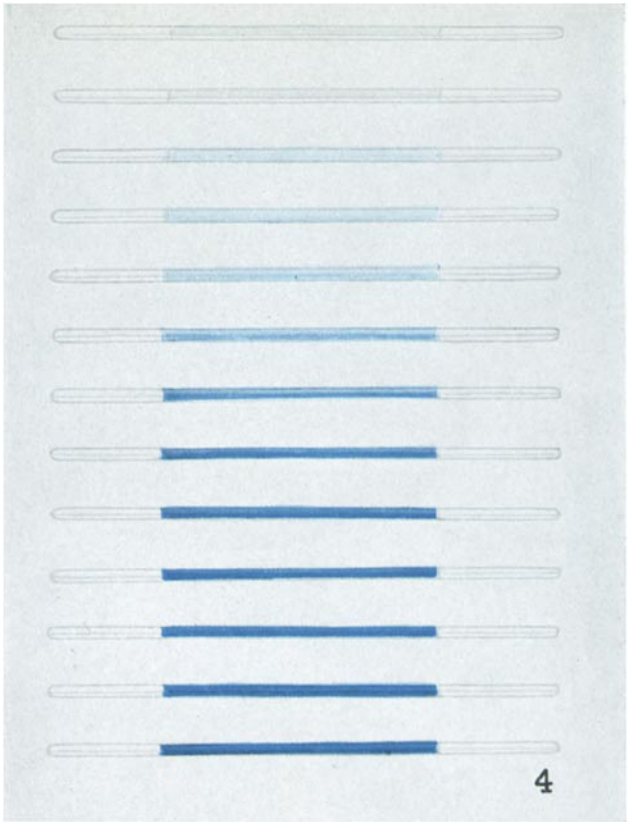


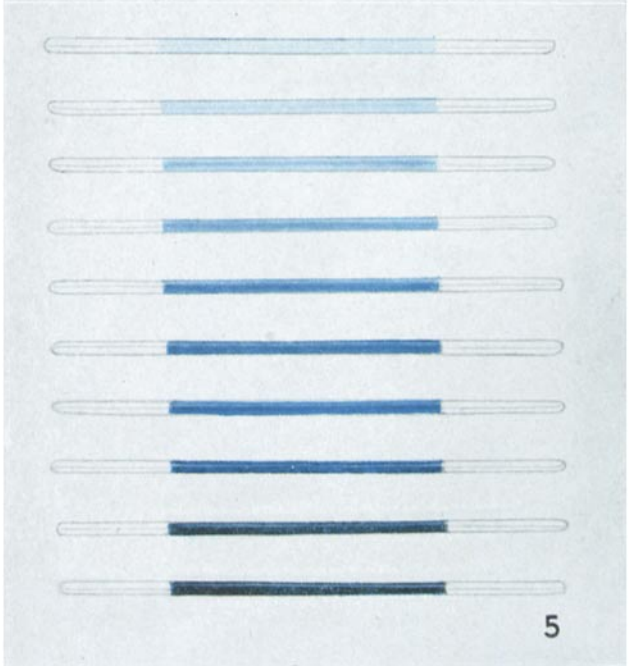
(Yoffey and Drinker: Lymphatic pathway from nose and pharynx)

PLATE 27

FIG. 4. Set of capillary tubes containing cervical lymph taken at intervals. 1.5 cc. of T-1824 (5 per cent) were placed in the right nostril at 12:45 p.m. It will be observed on looking at the chart that the first appearance of blue in the lymph is at 1:10 p.m. The time at which each sample was taken is given at the side of the tube

FIG. 5. Set of capillary tubes with known dilutions of T-1824.





(Yoffey and Drinker: Lymphatic pathway from nose and pharynx)