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Studies on the Lymph Node-Venous Communications

I. The Passage of Radioactive Serum Albumen

R. F. Dunn, M. V. Burtz, P. H. Ward

Department of Surgery, Division of Head and Neck Surgery (Otolaryngology), UCLA School of Medicine, Los Angeles, California 90024

Summary

The passage of radioactive iodinated serum albumen (RISA) has been followed through the medial retropharyngeal lymph node of the dog by injecting RISA into one afferent lymphatic channel and assaying for radioactivity in samples recovered from the efferent lymphatic channel, the adjacent internal jugular vein, and distally from the femoral vein. Profiles of recovery from the efferent lymphatic and adjacent internal jugular vein showed a rapid, sharp rise in activity to a peak level followed by a rapid decline in activity. These peaks coincided with the injection time. A second recovery peak from the adjacent internal jugular vein also coincided with subsequent timed nodal palpation. Recovery from the femoral vein showed a steady increase to a plateau level which was reached after cessation of injection. The amounts of nodal retention and passage were quantitated to evaluate these parameters. A simplified hypothetical model is presented and discussed. The passage of RISA to the adjacent internal jugular vein indicates a direct lymph node-venous communication.

Introduction

The first experimental evidence for the presence of direct lymph node-venous communications was reported by *Pressman* and his co-workers (1). This evidence was based upon observations following injection of saline and air directly into the substance of the medial retropharyngeal lymph node of the dog and the subsequent observation of air in the communicating vein and the adjacent internal jugular vein without having first passed through the efferent lymphatic channel. Further experiments by this group showed that tracers having a variety of sizes were also transferred (2). The tracers included HeLa cells, autogenous erythrocytes and *B. subtilis* variation golgi. In each instance the tracers were injected directly into the lymph node. Subsequent studies demonstrated that the same phenomenon occurred when the injection was performed either directly into the substance of the node, retrograde via the efferent lymphatic channel, or through the afferent lymphatic channel (3). Due to technical difficulties at that time, the latter injection site was abandoned in favor of the submucosa of the ipsilateral subglottic region from where the tracer passed into the normal afferent lymphatic channels (4, 5). These studies suggested that the direct passage of tracer substances across the lymph node-venous communication was a pressure-related phenomenon (3, 5).

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The present study was designed to ascertain the amounts of radioactive tracer, injected directly into the afferent lymphatic channel, passing through the medial retropharyngeal lymph node, related to time, and to quantitate the amounts recovered.

Materials and Methods

Experiments were completed on ten adult dogs in the following manner. The dogs were premedicated with an intramuscular injection of Tranvet (Diamond Lab., 0.11 cc/kg body weight) and then given nembutal, intravenously, to produce adequate anesthesia (0.4 cc/kg body weight initially). One femoral vein was cannulated with a polyethylene tube for the collection of samples. When not in use for sample collection, this and the other femoral vein were utilized for the infusion of 5% dextrose in saline.

Following a midline neck incision and the evaluation of the skin flaps, the medial retropharyngeal lymph nodes (6) were exposed. Care was exercised to protect the blood supply to the lymph nodes. The efferent lymphatic channel, the three primary afferent lymphatic and the adjacent internal jugular vein were exposed. The animal was given heparin (1 cc of 1 : 10,000) and the efferent lymphatic and the internal jugular vein were cannulated with polyethylene tubing. The size of the polyethylene tubing used for the cannulations was chosen to approximate the diameter of the vessel lumen. It was inserted and the vessel ligated around the tubing, thus allowing free flow but preventing passage past the site of cannulations. One afferent lymphatic was cannulated in close proximity to the node with an N-30 gauge needle with the aid of a micromanipulator under observation with a Zeiss operating microscope. The tracer, radioactive iodinated serum albumen (RISA) with various activities (11 to 34.4 μc/ml), was injected with digital pressures ranging from 200 to 500 mm Hg. The duration of the injection time was approximately one minute. In each experiment, concurrent samples were collected from the cannulated vessels for 15 seconds, four times a minute, for 8 or 10 minutes. The radioactivity of each sample was counted with a Radiation Instrument Detection Laboratory deep-well scintillation counter. The counts per minute (cpm) measured on each sample were then converted to microcuries after subtracting the background counts, and subsequently converted to a percentage recovery figure according to the following:

$$\frac{(\text{Sample cpm} - \text{background cpm})/\text{cpm per } \mu c}{\mu c \text{ amount injected}} \times 100 = \text{percentage recovered}$$

The amount injected was determined by measuring the cpm of the syringe filled with RISA, subtracting the cpm of the injection equipment after the experiment, and converting this figure to microcuries utilizing an appropriate geometry factor value. The percent recovered was plotted against time for comparative analysis. For analysis of individual experiments the cpm were plotted against time.

Two series of experiments were performed. In the first series, the efferent lymphatic channel was cannulated and remained patent with concurrent samples collected from both the efferent channel and the adjacent internal jugular vein. In the second series, the efferent channel was ligated and samples collected from the adjacent internal

jugular vein. Concurrent samples were collected from the femoral vein in each animal of both series to ascertain the general body level of activity during the course of each experiment.

The pressure of injection range (200–500 mm Hg) was established on a separate series of animals to prevent radioactive contamination of the pressure transducers. In this series of animals the same procedure as outlined above was used with the exception that saline was injected and pressures were recorded during injection.

Results and Discussion

The lymphatic supply to the medial retropharyngeal lymph node consists of three primary afferent lymphatics (superior, medial and inferior), however this may vary slightly as can be demonstrated by the use of dyes injected into the submucosa of the larynx and sublgottic region. Exit of the lymph from the medical retropharyngeal node is via a single efferent lymphatic channel. This has been true in each dog thus far studied during the course of this and similar projects. Similar observations have been reported by *Sabiston, et al.* who generally observed a single efferent lymphatic but occasionally found two (7). Variation of the efferent channel in our experience appears to be limited to variability in diameter of the vessels, not in the number of efferent channels present.

During the injection period of the patent efferent experiments, there was a rapid and immediate rise in the counts recovered in the efferent lymphatic channel (Fig. 1 a). This value rose to a peak, 10^4 times the background, which then declined sharply at the cessation of injection. A steady decline followed thereafter throughout the remainder of the experiment to reach approximately 10^{-1} less then the peak value. The resultant type of curve can be accounted for on the basis of a pressure-related phenomenon. The initial rise in recovery rates may result from the initial filling of the lymph node to a level of pressure consistent with the injection pressure. The initial sharp decline and subsequent steady decline in recovery is directly related to cessation of the injection pressures followed by further steady emptying of the lymph node. The recoveries from the adjacent internal jugular vein showed a similar sharp rapid increase in the counts recovered, an increase of 10^3. Upon cessation of the injection pressures, there followed in the internal jugular vein a sharp, rapid decrease in the recovered counts per minute (a decrease of slightly more than 10^2). This decline occurred within one minute of the cessation of the injection. The subsequent samples showed a gradual decline to approximate the general body level as represented by the values of the samples collected from the femoral vein.

In the second group of experiments, the efferent lymphatic channel was ligated and samples were collected only from the adjacent internal jugular and femoral veins. The initial profile of recovery from the internal jugular vein was entirely similar to the results of the experiments just described (Fig. 1 b). During the 7th minute of the occlusion experiments, when the recovery rates began to level off, the lymph node was subjected to moderate increased digital pressures, similar to those used upon clinical nodal palpation, for 1.25 minutes. The increase in recovered counts showed a rapid increase to a peak followed by a rapid decline. These latter events corresponded directly to the digital nodal palpations.

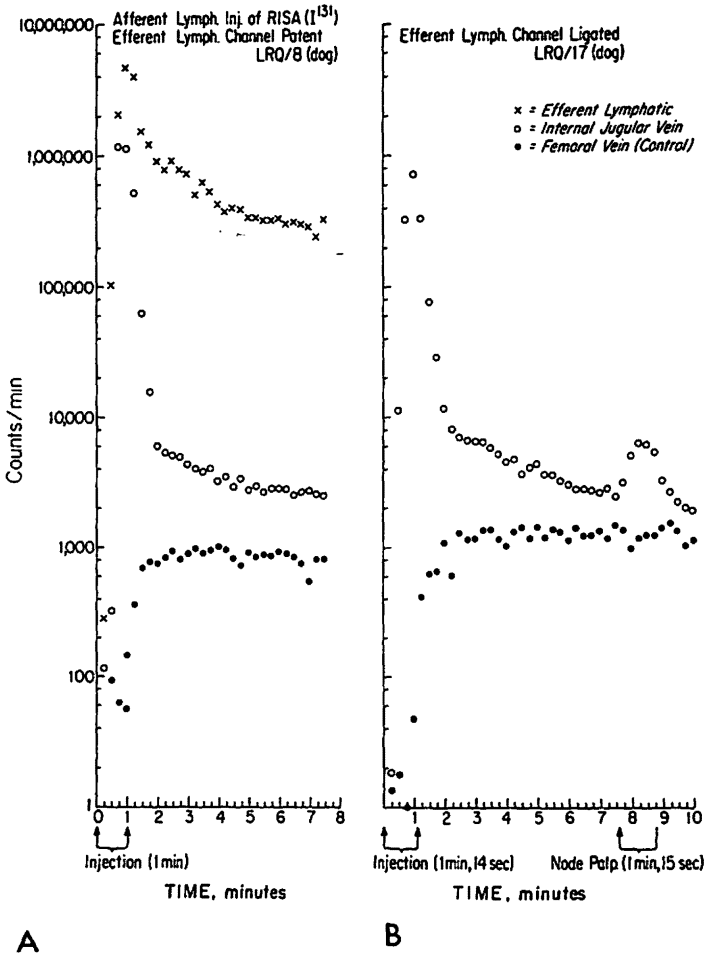

Fig. 1 Comparison of two individual experiments. The efferent channel (patent in a) and the internal jugular vein show a rapid rise and decline from a peak in the cpm recovered which coincided with a steady rise to a constant level of return from the femoral vein. With the efferent channel ligated (b), there was a similar recovery curve in the internal jugular vein with a subsequent rise and decline resulting from nodal palpation.

Since the amount of RISA injected was known, the counts per minute on each sample after conversion to microcuries was converted to a percent recovered figure. This negated the variability in the amounts of RISA injected in each different experiment. The average of these values from each sample group of the two series of experiments was determined and plotted as a summary of the two series of experiments (Fig. 2). A graphic summary of the six patent efferent lymphatic experiments is shown in Fig. 2 a. The amounts recovered from the efferent lymphatic channels and the adjacent internal jugular veins show similar rapid increase to a peak. The rise and peak level corresponded directly to the injection time. Following the peak, there was a rapid decrease in the

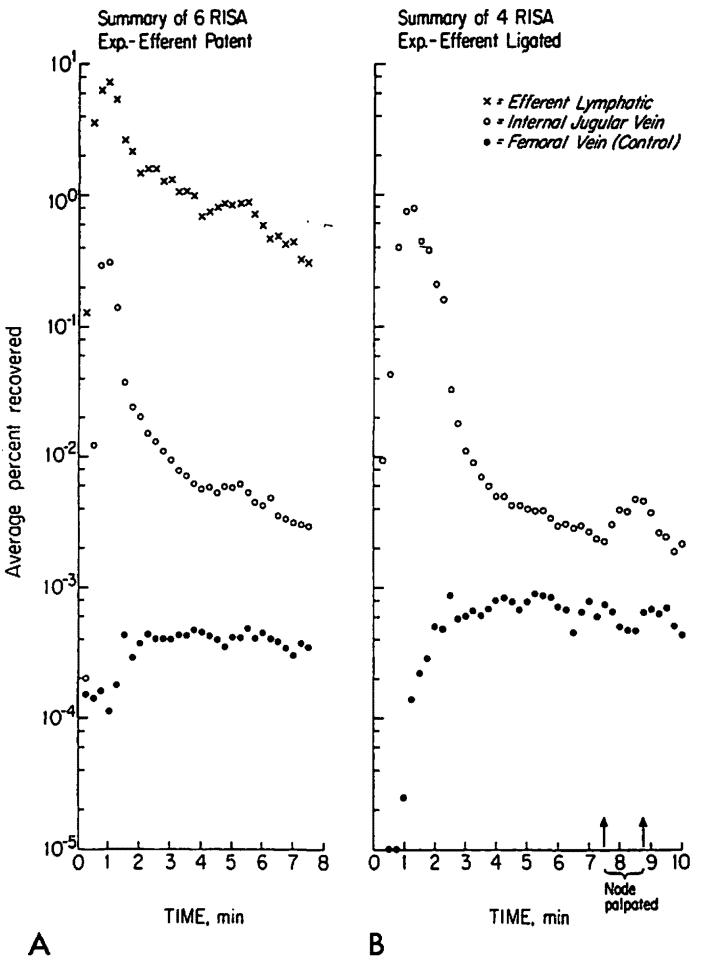

Fig. 2 Comparison of two series of experiments in which the average percent recovery value for each timed sample is plotted. With the efferent channel patent (a), the profiles of the recovery curves are similar to those in Fig. 1 a. With the efferent ligated (b), the recovery profiles are similar to those in Fig. 1 b.

percent recovered, followed by a steady, less sharp decline to the end of the experiments. The amounts of RISA recovered from the femoral veins showed a rapid rise 1 to 1.5 minutes after cessation of the injection, to approximate a steady body level.

A graphic representation of the four ligated efferent lymphatic channel experiments is shown in Fig. 2 b. In these experiments, the rapid increase in the percent RISA recovered also corresponded directly to the times of injection. After a rapid decline and leveling off of the recovery percents, the lymph nodes were palpated digitally for 1.25 minutes which produced a second peak rise in recovery which then rapidly declined after cessation of palpation. These profiles of recovery are consistent with a pressure dependent mechanism of transfer.

The profiles of the recovery rates from the adjacent internal jugular vein are interpreted as reflecting a pressure dependent lymph node-venous transfer of the RISA tracer. In such a system it would be necessary that the lymphatic pressures be higher than the venous pressures for the transfer to occur, as idealized in Fig. 3. The hypothetical system would also require the proper anatomical communications. While the passage of the tracer is assured by the increased pressures in our experimental system, such increases, although smaller, could in fact occur as a result of edema, tumor blockage, or simple digital palpation of the lymph node. This latter effect is clearly seen in the recovery rates from the internal jugular vein in the ligated efferent channel experiments.

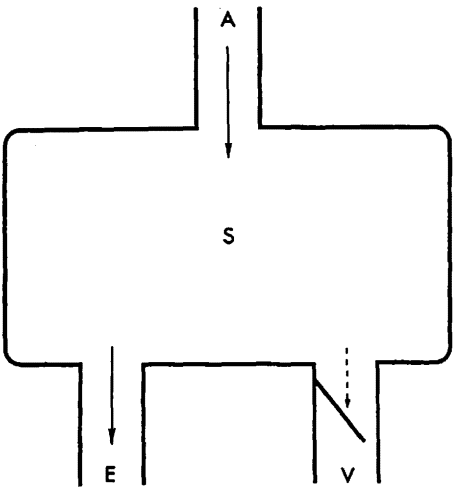

Fig. 3 A schematic representation of a hypothetical model to explain the rapid pressure dependent transfer through the lymph node-venous communication. The model is based upon the presence of a mixing chamber (S), an entrance (A) via the afferent channel, and two exits (E, V). When $S_{pressure} < V_{pressure}$, then exit is only through the efferent channel (E). This assumes a simple valve at V. When $S_{pressure} \geq V_{pressure}$, then exit is possible both via the efferent channel (E) and the vein (V).

The variation between animals becomes apparent when considering the total amounts collected from each sample site, that retained in the lymph node and that recovered from the catheters and sponges. These values (Table 1) indicate the nodal retention is fairly constant in the patent efferent channel experiment with the exception of number six. The lower nodal retention is accompanied by an increased amount collected from the efferent channel. The percent recovery from the efferent channel shows more variability which might be accounted for by variation in the size of the lymph nodes in the different animals as well as variations in the internal structure of the nodes. A considerable variation was noted in the percentages recovered from the adjacent internal jugular vein as well as in the general body levels as reflected in the femoral vein samples. In Experiment 3, the efferent channel recovery was the smallest whereas the internal jugular vein recovery was the greatest. This was the only experiment in which a superior afferent lymphatic channel served as the injection site. In the other experiments of this group, the injection was via a medial afferent channel.

Since the cannulation of the vessels was such that there was no flow past the catheters and only a single efferent lymphatic appears to be present, the recovery of RISA from the femoral vein indicates routes of passage into the systemic circulation other than via

Table 1 Total Percentage of RISA Recovered.

Experiment Number	Lymph Node (%)	Efferent Channel (%)	Internal Jugular Vein (%)	Femoral Vein (%)	Catheter and Sponges (%)	Loss from System (%)
Efferent Lymphatic Channel Patent						
1	28.6	37.0	0.2	0.01	17.9	16.3
2	28.0	50.1	0.2	0.01	9.9	11.8
3	35.4	32.0	3.9	0.03	8.5	20.2
4	23.4	43.4	0.02	0.01	19.3	13.9
5	16.6	43.2	1.6	0.01	18.2	20.4
6	9.0	54.8	0.1	0.01	13.1	23.0
Mean	23.5	43.4	1.0	0.01	14.5	17.6
Efferent Lymphatic Channel Ligated						
7	40.8		4.0	0.03	28.6	26.6
8	54.3		2.7	0.02	20.5	22.5
9	51.7		1.6	0.04	28.5	18.2
10	62.1		5.2	0.01	13.0	19.7
Mean	52.2		3.4	0.02	22.6	21.8

either the internal jugular vein or the efferent lymphatic. This could conceivably occur through small veins leading to the external jugular vein which ultimately drains this region.

The experiments in which the efferent lymphatic channel was ligated generally showed greater nodal retention (mean of 52% versus 24%) as well as greater transfer into the systemic circulation (Table 1). This latter is reflected as increased percentages of recovery from the internal jugular and femoral veins. Experiment 10 showed the greatest nodal retention, together with the greatest internal jugular vein passage and the smallest femoral vein returns. In this experiment, the injection was via an inferior afferent lymphatic channel, whereas in the remaining three experiments the injection was via a medial afferent lymphatic channel.

The amount of RISA lost from the system (the difference between the amount injected and that recovered) is partially accounted for in the catheters and sponges. The remainder, indicated as "Loss from the System", can be accounted for by the amounts retained in the general circulation. Another possible contributing factor may be the inability to critically measure the amounts of radioactivity in the injection equipment, the sponges, and the catheters due to geometric limitations.

The relative amounts of RISA retained in the node and recovered from the efferent lymphatic channel and internal jugular vein are shown in Table 2. It is apparent in Experiments 1–6 that the preferred exit path from the node is via the efferent lymphatic channel and that there is considerable variation in the amounts recovered from the internal jugular vein (0.03 to 5.5%). When the efferent channel is ligated (Experiments 7–10, Table 2), the nodal retention values increase considerably, as do the relative percentages of RISA passage into the internal jugular vein. This increase probably results from compounding the effective pressures within the lymph node due to the

Table 2 Relative Amounts Recovered.

Experiment Number	Lymph Node		Efferent Channel		Internal Jugular Vein		Total	
	μc	%	μc	%	μc	%	μc	%
Efferent Lymphatic Channel Patent								
1	20.0	43.5	25.9	56.2	0.1	0.3	46.0	100
2	9.2	35.8	16.5	64.2	0.01	0.03	25.7	100
3	26.4	49.6	23.9	44.9	2.9	5.5	53.2	100
4	11.5	35.0	21.3	65.0	0.01	0.03	32.8	100
5	10.5	27.1	27.4	70.4	1.0	2.5	38.9	100
6	5.3	14.0	32.5	85.8	0.08	0.2	37.9	100
Efferent Lymphatic Channel Ligated								
7	29.1	91.8			2.6	8.2	31.7	100
8	72.8	95.3			3.6	4.7	76.4	100
9	53.4	97.1			1.6	2.9	55.0	100
10	51.9	92.3			4.3	7.7	56.2	100

efferent channel ligation. Since the pressures of injection could not be dissipated by exit through the efferent lymphatic channel, the increased recovery from the internal jugular vein may reflect an expanded capacity for transfer via this path due to the compounded increase in effective pressures.

Several authors have indicated the possible presence of lymph node-venous communications (7, 8, 9, 10), however presence of these communications have been demonstrated both experimentally and clinically by a number of methods (11, 12, 13, 14, 15). It is generally accepted that lymphaticovenous communications become functional in response to increased pressures resulting from obstructive changes to the lymphatic system. Experimentally induced localized high pressure, as used in the present studies, might be expected to mimic an obstructive increased pressure. The pressure of injection range as measured was that pressure recorded from the injection needle which represents a single vessel with a given cross sectional area. Upon entrance into the intranodal sinuses, the initial injection pressure would be expected to drop due to the total larger cross sectional area presented by the sinuses. Since no information is readily available concerning these latter variable volumes, the injection site was chosen for recording and reporting of pressures. The sinus pressure will thus be lower than the injection pressure when the efferent lymphatic remains patent. When ligated, the velocity is effectively halted, except through any existing lymph node-venous communications, and the intranodal pressure should approximate the injection pressure. In this regard, the total percentage of RISA recovered from the adjacent internal jugular vein with efferent ligated tended to be higher than with the efferent patent (Tables 1 and 2) which may have resulted from a maximum diversion through lymph node-venous communications with the efferent channel ligated.

Both the amounts of recovery from the internal jugular vein, the very short times necessary to reach the peak recovery values, as well as the interruption of the efferent channel preclude passage of the tracer through the efferent channel and the entire

systemic circulation back to the internal jugular sample site as the route of passage. Hence, direct lymph node-venous communication remains as a likely alternative. The general profiles of recovery rates are consistent with a pressure dependent transfer. The coincidence of the peak rise with the increased pressure as well as the peak decline with the cessation of pressure also indicates a pressure dependent mechanism of transfer which is further substantiated by the secondary peaks of recovery produced by the increased pressures of nodal palpation during the occlusion experiments. A possible idealized model system to account for these observations is a mixing chamber (corresponding to the nodal sinuses) with one entrance (the afferent system) and two possible exits (the efferent and venous sytems), as shown in Fig. 3. The path of exit from the mixing chamber would then be determined by the fluid pressure within the mixing chamber relative to that in the venous system. This model system is currently being tested in a series of experiments by means of tracer injections at various pressure including those within the "normal" physiological range in which the pressures are maintained at a constant level and recorded simultaneously to the injection. It is anticipated that these studies will answer such questions as: At what pressure do these communications begin functioning?

While the absolute amounts of RISA being transferred through this communication is small and variable, it nonetheless appears to be a real and possibly important pathway when considering the spread of tumors, bacteria, and their toxins.

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R. F. Dunn, Ph.D., M. V. Burtz, R.N., H. Ward, M.D., Dept. of Surgery/Reh 31-19, UCLA School of Medicine, Los Angeles, Calif. 90024, USA