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Studies on the Lymph Node-Venous Communications

II. The Passage of Labeled Exogenous Erythrocytes*

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Summary

The passage of ^{51}Cr labeled avian erythrocytes has been followed through the medial retropharyngeal lymph node of the dog by injecting labeled avian erythrocytes 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 were consistent with those found when using radioactive iodinated serum albumen. A secondary peak of recovery from the efferent lymphatic channel and adjacent internal jugular vein coincided with timed nodal palpation. The amounts of nodal retention and transnodal passage were quantitated and discussed in terms of passage rates and possible clinical relevance. The transnodal passage of avian erythrocytes suggests the size of the lymph node-venous communication to be on the order of a capillary or larger.

Introduction

Evidence consistent with a pressure dependent lymph node-venous communication has been described and was based upon the rate of recovery of ^{131}I labeled serum albumen injected at elevated, manually induced pressures (1). One afferent lymphatic channel served as the site of injection in these studies which could hence be considered as utilizing the "normal" flow patterns and anatomical pathways through the lymph node. Whereas the lymph node-venous communications may or may not be nonfunctional in the "normal" animal, there is experimental evidence to support their becoming operative when lymphatic pressures are elevated (1, 2, 3).

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The present experiments were conducted to: (1) gain insight into the size of the tracers passing through the lymph node-venous communications at elevated pressures, and (2) compare the results in a quantitative manner.

Materials and Method

The surgical techniques to expose the medial retropharyngeal lymph node and the various sample sites, together with the methods of sample collection, have been previously described (1). In the present study, experiments were performed on 12 adult dogs. Avian nucleated erythrocytes were obtained via venipuncture from a superficial axillary vein in adult chickens for use as tracers. The whole blood was centrifuged, the packed cells washed, tagged with ^{51}Cr, rewashed three times, and following final centrifugation, they were suspended in isotonic saline. The pressures of injection were maintained manually in the range of 200–500 mm Hg (1). In each experiment, intermittent digital pressure was applied to the lymph node during the 6th minute of the experiment and continued for a total of 1.25 minutes. Each timed sample was counted for radioactivity on the basis of a single unit time period with a Radiation Instrument Detection Laboratory deep-well scintillation counter and then converted to percent recovery values by methods previously described (1). These values were then plotted for individual and comparative analysis.

Results

Seven experiments were completed in which the efferent lymphatic channel remained patent. Each experiment consistently showed passage of the tracer into the adjacent internal jugular vein. Three of these experiments (those with the least leakage around the injection site, Table 1) are detailed here (Fig. 1a+c). The percent activity recovered from the efferent lymphatic channel rose immediately upon injection to a level of 10^4–$10^{4.5}$ above the background level. This was followed by a sharp decrease in percent recovered commencing upon cessation of injection pressures to reach a level $10^{2.6}$–$10^{2.8}$ above background. The recovery rates from the adjacent internal jugular vein showed similar rapid rises coinciding with the injection period to levels 10^3–$10^{3.5}$ above the background level. Upon cessation of the injection pressures, the recovery rates steadily declined to levels of 10^2–$10^{2.2}$ above background levels. Increases were noted in the recovery rates above the 6th minute level of $10^{0.48}$–$10^{1.2}$ and $10^{0.16}$–$10^{0.76}$ in the efferent lymphatic channel and the internal jugular vein, respectively. This increase coincided with the beginning of nodal palpation. After a sharp decline coincident to cessation of nodal palpation, the general slope of the decline in recovery rates approximated the decline slope prior to palpation. The slopes of the increases and decreases in rate recovery from the efferent lymphatic channel and the adjacent internal jugular vein were very similar, although the returns from the latter were generally lower than from the former.

The percent recovered from the femoral vein showed considerable variation in amounts, however these values generally approximated a curve showing a steady increase during the injection time to reach a plateau level of 10–$10^{1.2}$ above background levels. This leveling off was reached at, or shortly after, the end of the injection period, and the plateaus did not appear to be markedly affected by palpation of the lymph node.

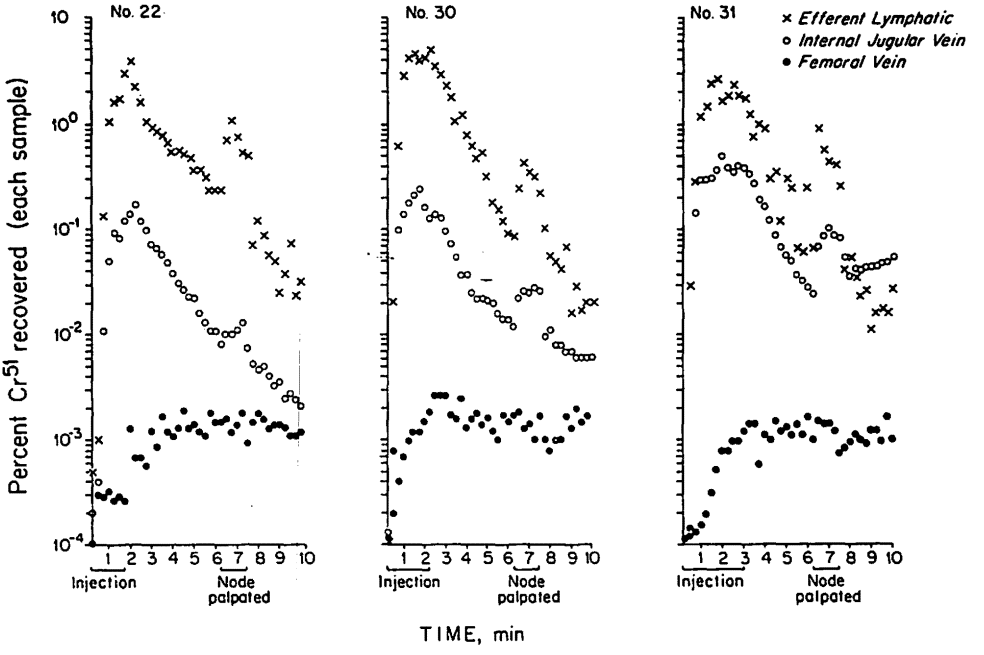

Fig. 1

Fig. 1 The results of three individual experiments each show a rapid rise to a peak and decline in the percent recovery values in both the efferent channel and the internal jugular vein. This initial peak corresponds directly to the injection period (I.P.). A secondary peak of recovery is evident and corresponds to the period of nodal palpation (N.P.). The rates of recovery from the femoral vein, while rather variable, do show a steady rise to approximate a plateau level.

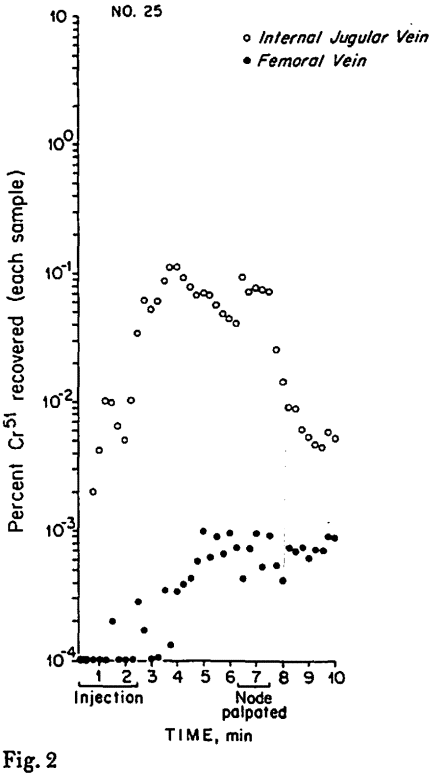

Fig. 2

Fig. 2 The results of an individual experiment in which the efferent channel was ligated show a broader initial peak of recovery in the internal jugular vein than is evident in Fig. 1. This peak did relate to the injection period (I.P.). A secondary peak is evident and corresponds to the period of nodal palpation (N.P.). Recovery from the femoral vein, while initially variable, does rise to approximate a plateau level.

The five experiments in which the efferent lymphatic channel was ligated also consistently showed passage of erythrocytes into the adjacent internal jugular vein (Table 1). One of these is detailed in Fig. 2. In this study, the initial recovery peak from the adjacent internal jugular vein was much less well defined in relation to the injection period. The peak in recovery was reached well after cessation of injection, which may be accounted for in part by the length of the collection catheters. There was an increase of 10^3 in the recovery rates which then declined at rates similar to those seen in Fig. 1 and 2. A secondary increase and decline coincident to nodal palpation was seen as an obvious deflection in the primary decline. The recovery rates of the early femoral vein samples were rather erratic, and they continued to be so throughout the experiment. These rates approximated a curve of recovery similar to those in the patent efferent channel experiments and reached an average level of $10^{0.07}$ above the background level.

Quantitatively, the experiments indicated a relatively good accountability for the total activity recovered which ranged from about 66% to 90% (Table 1). The unrecovered amounts of the tracer are accounted for on the basis of that remaining in the animal and the inability to accurately measure the activity of the node, catheters and sponges due to technical limitations. Marked fragility of the afferent lymphatic was encountered when using the avian erythrocyte tracers as compared to the use of RISA tracers (1). The leakage from the injection site is reflected in the percent recovered from the catheters and sponges (Table 1). The nodal retention in the patent efferent channel experiments ranged from approximately 18% to 37% with a mean of 27.4% (Table 1). A trend toward higher nodal retention was indicated in the ligated efferent

Table 1 Total Percent ^{51}Cr Recovered.

Dog. No.	Internal Jugular Vein (%)	Efferent Channel (%)	Femoral Vein (%)	Catheter and Sponges (%)	Node (%)	Total Recovered (%)
Efferent Patent						
21	0.25	0.59	0.02	62.01	27.35	90.22
20	0.49	1.26	0.01	58.16	18.14	78.06
24	3.24	1.14	0.02	52.27	20.16	76.83
27	0.01	17.74	0.03	30.87	36.87	85.03
31*	5.68	25.32	0.04	20.15	33.35	84.54
30*	2.09	43.50	0.06	10.94	22.05	78.64
22*	1.42	27.35	0.04	3.36	34.10	66.27
Mean	1.9	16.7	0.03	34.0	27.3	79.9
Efferent Ligated						
34	0.69		0.04	68.17	15.87	84.77
35	0.01		0.01	60.85	10.47	71.34
32	0.59		0.09	42.56	25.85	69.09
33	0.21		0.02	36.68	48.18	85.09
25*	1.61		0.02	20.06	63.29	84.98
Mean	0.62		0.04	45.7	32.7	79.0

* Detailed as Figures 1a–c and 2, respectively.

channel experiments where the mean nodal retention was 32.7%. This higher level may be associated with the ligation involved since, as might be expected, the percent recovered from the efferent channel in the patent situation was quite high overall, but varied from animal to animal.

Considering the four detailed experiments, the percent recovered from the adjacent internal jugular vein ranged from 1.4% to 5.7% in the patent efferent channel experiments. The recovered percentage from the same site in the ligated efferent experiment was also within the above range which may indicate the lymph node-venous communications reach a 'saturation' point in the passage of cells above which no additional nucleated erythrocytes pass upon increase of pressure.

The amounts recovered from the femoral vein in both sets of experiments remained remarkably similar, 0.01–0.06% and 0.01–0.09%. This might be interpreted as indicative of a consistency in the collateral communications to the circulatory system via pathways other than those to the internal jugular vein.

Discussion

The most striking variability in this series of experiments was the fragility of the afferent lymphatic channels which served as the site of injection. This parameter is reflected in the percent recovered in the "Catheters and Sponges" (Table 1). In only four of the 12 experiments did this value approach 20% or less. Since we were interested in analyzing a closed system for quantitative purposes, this parameter served in judging the success or failure of the experiment. As seen in Table 1, passage into the internal jugular vein occurred, likewise, in the experiments not illustrated.

The profiles of recovery, both the initial and secondary peak recoveries from the efferent lymphatic channel and the internal jugular vein, coincided well with increases of the intranodal pressures, and suggest a pressure dependent mode of transfer. Marked fluctuations were noted in the amounts recovered from the efferent lymphatic channel (Fig. 1 c) and in each of the femoral vein recovery percentages. These variations may be accounted for by the differences in the amounts of fluids recovered in each sample. However, the average profiles of recovery show similar rates of increase and decrease of the initial peaks in both the efferent lymphatic channel and the adjacent internal jugular vein. The levels of recovery from the latter were consistently less than from the former. This might be expected since the primary exit channel is via the efferent lymphatic channel and is consistent with the idea that lymph nodes are aggregates of lymphoid and reticular tissues surrounding independent pathways or channels (4).

The presence of functional lymphaticovenous communications is well documented, and they have been found on the basis of corrosion-cast experiments (5) and lymphangiography (see 6, 7). Such communications generally appear to become functional as by-pass mechanisms in situations of lymphatic duct obstruction as a result of either induced or pathologic obstructions (2, 3, 8, 9, 10, 11). Questions concerning the presence of intranodal lymphaticovenous communications, referred to here as lymph node-venous communications, have been raised when direct intranodal injections were employed (12). Such resulting communications were felt to have been due to the formation of injury areas surrounding the tip of the injection needle, regions of disruption which would allow passage to occur. Such abnormal disruptions may very well account for the great

variability of recovered tracers as reflected in counts when injection is directly into the substance of the lymph node (13). These conclusions served as the basis for utilizing the present experimental system whereby one afferent lymphatic channel was the injection site with the remaining lymphatic and circulatory systems subserving the medial retropharyngeal lymph node retained as much intact as possible within the limits of the anatomical dissections. Under these circumstances, recovery of the tracer from the adjacent internal jugular vein is a consistent finding in each experiment. While there is variation in the percent of recovery with this system, the recovery range is quite similar when using ^{131}I labeled serum albumen, 0.1–5.2% (1), or avian erythrocytes, > 0.1–5.7% (Table 1). Further, the peaks of recovery coincide with the increases in the lymphatic pressures.

Should normally potential lymphaticovenous or lymph node-venous communications become functional only following "up-stream" obstructions and the resultant increase in "down-stream" pressures, then the elevated pressures of injection would be expected to produce a similar transfer and hence mimic an obstruction. Our experimental system allows the potential lymph node-venous communications to become operative as a pressure release mechanism. A simple hypothetical model system has been proposed (1) in which functional passage would occur only when the pressures within the lymphatic chambers exceed that of the venous system. This model presupposes the presence of the appropriate anatomical pathways necessary for such a transfer. Evidence for the existence of these morphological pathways is currently being collected in our laboratory.

The presence of the tagged nucleated erythrocytes in the adjacent internal jugular vein not only indicates direct lymph node-venous communications, it also suggests that, due to the dimensions of the tracer, the minimal size of the communication must be on the order of a capillary or larger, as already indicated by *Sabiston* et al (14), although they did not demonstrate lymph node-venous communications. A direct intra-nodal communication is further suggested from observations on histological sections of these lymph nodes in which no evidence of emigration was seen from the sinuses by the nucleated erythrocytes across the sinus endothelial cells (*Dunn,* unpublished observations). The rapid rates of recovery, less than one minute, are not consistent with an emigration method of transfer and they obviate an endothelial circulation back to the site of internal jugular vein sampling. This is supported by *Burn* (15) who found the normal transfer time to the circulation system is 10–20 minutes. Since the efferent lymphatic channel is cannulated or ligated in our experimental system, hence preventing passage past the collection site, there is not transfer to the general circulation via this pathway.

Fisher and *Fisher* (16, 17) have presented quantitative evidence that nodal retention of erythrocytes by the normal popliteal lymph node is approximately 90%, while that of tumor cells is only about 40%, from which they conclude that nodal retention and transmission of erythrocytes cannot be equated to similar processes involving tumor cells. These same authors further found no significant change in nodal retention to tumors in post-radiation or post-inflammation nodes (17, 18). The use of nucleated erythrocytes in the present study does give an indication of the size requirement necessary for the continuity (the order of a capillary or larger) with a much lower value of nodal retention. The passage of HeLa cells through the lymph node-venous communication

has also been reported (19) and calculated to be as many as a total of 7.3×10^5 cells transferred during the collection time. The strong challenge to the nodal barrier concept based upon quantitative data (16, 17), as well as the proposed free intranodal liquid communication between the lymphatic and venous systems (20) correlated with the clinical patterns of tumor dissemination would indicate some fundamental changes must be imposed upon the transmitted tumor cells. *Bron* et al. (2) and *Retik* et al. (21) have remarked that rapid alterations in the progress of some neoplastic diseases, particularly lymphomas which disseminate primarily via the lymphatics, may be attributable to lymphaticovenous communications becoming operative. With a 40% tumor cell nodal retention rate in a single node system, one would perhaps expect a higher incidence of distant metastatic foci without those cells transmitted having undergone fundamental biochemical, physical or physiological changes. The filtration efficiency may have added evolutionary pressures favoring the development of multiple lymph node chains. A chain of six nodes, each unobstructed with 40% retention, should have an additive effect and would be approximately 94% effective in prevention of tumor cell passage. Such chains are common in the human head and neck region (22) where the common spread of tumors encountered is that metastases are primarily confined to the regional lymph nodes. It is further possible that tumors within chains must proliferate locally within a given node to create an obstructive increase in pressure for the lymph node-venous communications to become functional, thereby allowing passage of cells in quantities sufficient to increase the possibility of distant metastases by circumventing any changes imposed by the lymph node.

The suggestion of a direct lymph node-venous communication is a plausible alternative to account for the presence of the whole cell tracers in the internal jugular vein. Further, it is a pressure-related transfer which could account for the profiles of recovery found in relation to the pressures of injection.

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BOOK REVIEW AND ABSTRACT

BLOOM, B. R., R. GLADE, editors (Dept. of Microbiol. and Immunol. Albert Einstein Coll. of Med., Bronx, N. Y., and Dept. of Ped., Div. of Infectious Dis., Mt. Sinai School of Med., New York): **In vitro Methods in Cell-Mediated Immunity.** Academic Press, New York/London, 1971

While the important role cell-mediated immune reactions most likely play in the pathogenesis of various diseases is now being widely recognized, basic understanding of immune phenomena mediated by lymphoid elements and other cells is still rudimentary. A series of *in vitro* model systems for the study of cell-mediated immune reactions has been established in recent years. In a conference/workshop, sponsered by the National Institute of Allergy and Infectious Diseases and held at the end of May, 1970, in New York, most of these models were presented by experienced investigators currently working in laboratories that have originated the technique. The present volume is the product of this meeting, and the editors, B. R. BLOOM and P. R. GLADE are to be commended for an excellent and rather unorthodox presentation. The first part of the book contains a word-for-word transcript of presentations and frank discussion remarks by the various participants. In an introductory session the major types of assays used for studying mediators were discussed. Factors and activities produced by lymphocytes *in vitro*, the biological implications of these *in vitro* phenomena as well as prospects for improved methodology were the topics covered in the other sessions. The second section contains detailed descriptions and protocols for performing a total of 38 of the major assays used throughout the world in *in vitro* studies of cell-mediated immunity. The possibility to reproduce these techniques anywhere with the aid of these methodological recipes and to analyze the results in the light of the critical discussion as presented in the first part of volume may provide a unique stimulus for further work in this important area of immunology. The book will prove to be of value to immunologists, while to the uninitiated the lack of a keyword register impedes access to the wealth of information contained in text, graphs and many tables. Because of the short "half-life" of some of the newly developed methods, this book suffers, perhaps more than other conference proceedings, from a 2-year time lapse until it was published. M. W. HESS

PEIRCE, J. C., G. E. MADGE, H. M. LEE, D. M. HUME (Depts. of Surg. and Pathol. Strauss Surg. Res. Labs. Med. Coll. of Virginia, Health Sciences, Virginia Commonwealth Univ., Richmond, Virg.): **Lymphoma, A Complication of Renal**