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The Systemic Distribution of Soluble Antigen Injected into the Footpad of the Laboratory Rat

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Summary. Injection into the hind footpad of the rat combines intradermal and subcutaneous routes. Material injected into the dermis of the sole is rapidly distributed by the lymphatics to several lymph node groups and enters the bloodstream principally with the thoracic duct lymph. The diffuse character of the lymphatic drainage of this site is emphasized. Material in the subcutaneous tissues gradually enters the systemic circulation and is filtered through various organs. Antigen injected into the footpad can elicit both effective delayed hypersensitivity and humoral host responses.

INTRODUCTION

The hind footpad of the laboratory rat is a convenient and commonly used injection site in bacteriological, immunological and tumour investigations. During the course of other studies, it became apparent that the uptake of injected material by regional lymphatics from this area is a rapid and diffuse process involving several lymph node groups. Egress to the systemic circulation occurs predominantly with thoracic duct lymph. As the results of experiments employing footpad injections may be misinterpreted unless its entire lymphatic drainage pattern is appreciated, this study is presented.

MATERIALS AND METHODS

Thirty-six normal adult rats of three strains were examined. Inbred strains of hooded, albino and 'stock' animals from a closed outbred albino colony were used, as no strain differences had been demonstrated in other studies of lymphatic anatomy (Tilney, to be published).

The injected substances were of two types. Colloidal carbon (Pelikan Ink, Gunther Wagner, Germany) was injected in volumes of 0·05–0·10 ml into either footpad of animals anaesthetized with ether. This particulate material clearly stains regional lymph nodes and accurately defines lymphatic channels. The rate of uptake and relative distribution of soluble antigen in lymphoid tissues was assessed by footpad injections of 0·05 ml of [^{131}I]human serum albumin (Radiochemical Centre, Amersham, Bucks.). This protein rapidly enters lymphatics at the injection site and streams to the draining nodes. Regional and distant lymph nodes were removed from a series of animals at different times following injection and counted in a gamma scintillation spectrometer (Packard Series, 410A). Blood samples, liver and spleen, were counted concomitantly.

Thoracic duct cannulations were performed using the technique of Gowans and Knight (1964). The hind footpad of rats with freely flowing lymph fistulae was injected with [^{131}I]albumin. Concentrations of labelled protein in regional lymphoid tissue, blood and lymph were determined serially.

RESULTS

Lymphatic anatomy was constant among the animals examined. Lymph channels draining the footpad, foot and hind leg below the knee follow the greater and lesser saphenous veins to the popliteal node. This node lies near the superficial muscular vein in the lateral aspect of each popliteal space. It receives a small lymphatic radicle from the gluteal lymph node and acts as a minor secondary drainage site for the tail. Efferent channels from the popliteal node run with the femoral vein to a retroperitoneal lymphatic plexus beneath the iliac vein. Subsequent drainage may follow two routes. The main efferent trunk continues centrally with the iliac vein to the lumbar nodes at the aortic bifurcation. These large nodes receive direct lymphatic channels from pelvic viscera, and secondary drainage from hind limbs and tail. They drain along large para-aortic channels through the renal nodes and into the cisterna chyli. The cisterna continues as the thoracic duct which courses cephalad with the great vessels and empties into the left subclavian vein.

Smaller lymphatics from the retroperitoneal plexus follow the superficial epigastric vein peripherally to the inguinal nodes. These lie embedded in subcutaneous fat in the flanks and drain to the axillary nodes through a major efferent channel along each nipple line. Both axillary chains empty into the posterior aspect of the subclavian veins through the large subclavian lymph ducts.

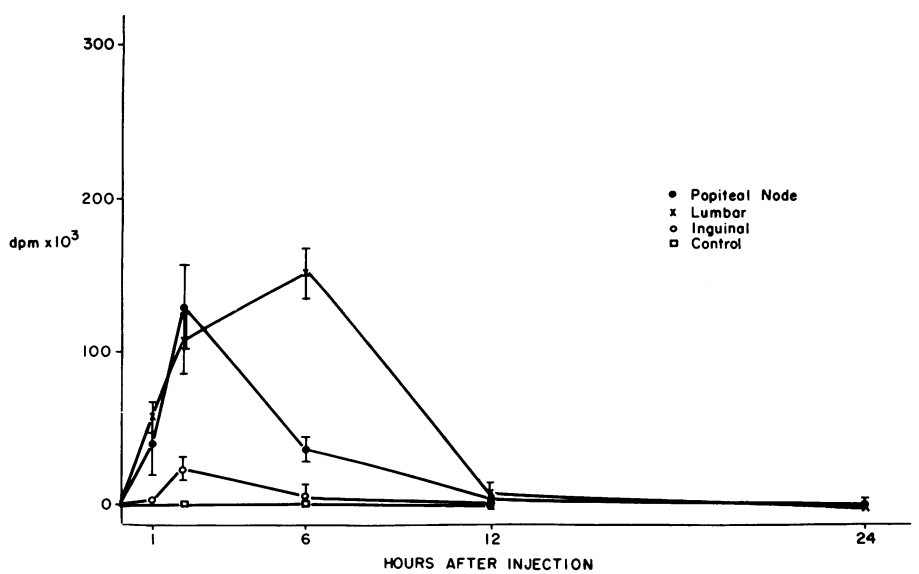

FIG. 1. Concentration in regional lymph nodes of [^{131}I]HSA. The rapid uptake and diffuse pattern of lymphatic drainage of soluble antigen injected into the footpad is illustrated. Most of the labelled albumin enters the central lymph pathway, while only small amounts drain to the inguinal nodes. The popliteal nodes from the uninjected legs serve as controls. Four animals were tested at each time interval.

[^{131}I]albumin injected into the footpad rapidly enters all regional lymphoid groups (Fig. 1). The high concentrations appearing rapidly in the popliteal node decline within a few hours. The lumbar nodes collect larger concentrations of the material, but more slowly because of their central position. Minor amounts of antigen enter the peripheral inguinal–axillary pathway, which may function as a collateral channel.

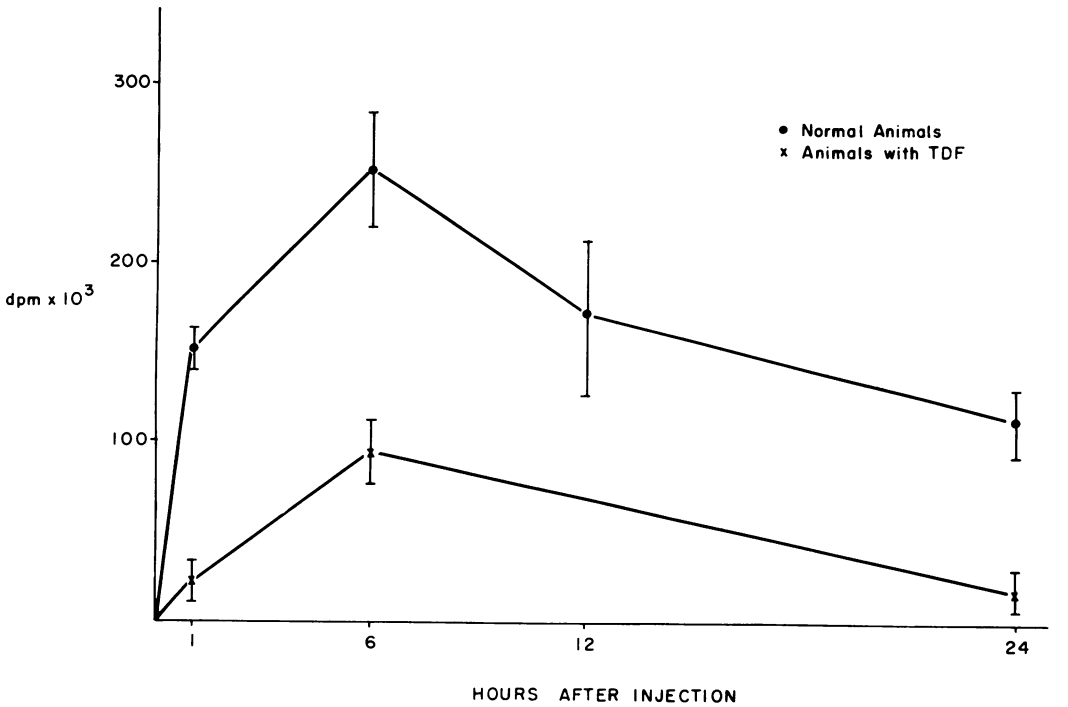

FIG. 2. Concentration of [^{131}I] HSA per ml blood following footpad injection. Much injected albumin entering the lymphatics reaches the circulation with the thoracic duct lymph. The concentration of the material in the blood is decreased significantly in animals with freely flowing thoracic duct fistulae (TDF). Three animals are included at each time period.

Much of the labelled protein leaving the injection site traverses regional lymph nodes to the cisterna chyli and reaches the systemic circulation with the thoracic duct lymph. The soluble antigen is picked up principally by liver and spleen, although it gradually appears in lymph nodes distant from the depot, in time following injection. There is significant decline in concentration of the material in the bloodstream of animals with external division of the thoracic duct ($P<0{\cdot}001$ at 1 hour, $P<0{\cdot}01$ subsequently) (Fig. 2). The albumin remains solely in the lymph fraction and is not present in thoracic duct lymphocytes. The injected material which gains the circulation of animals with lymph fistulae, presumably enters the capillaries directly from the subcutaneous depot. Minor amounts may flow through the subclavian ducts from the axillary lymph nodes.

DISCUSSION

Areas especially subject to environmental trauma possess a richer lymphatic supply and more diffuse lymphoid drainage than surrounding skin (Tilney, to be published). Although the popliteal node specifically drains the footpad, foot and lower leg, material from

footpad injections rapidly enters other lymphoid groups, both peripheral and deep. This generalized pattern has been readily demonstrated by the lack of effect on delayed hypersensitivity response in animals whose popliteal nodes were removed immediately following footpad injections of antigen (Waksman, Pearson and Sharp, 1960).

The importance of the route of injection in the response of the animal to antigen has been clearly defined (Leskowitz and Waksman, 1960). Hudack and McMaster (1932) have shown that any mild cutaneous stimulation may cause a marked increase in lymphatic permeability. The cutaneous capillary bed remains relatively impermeable to large molecules following mild trauma, although simple chemical compounds may enter these vessels directly (Macher and Chase, 1969). Protein antigens injected intradermally, enter the lymphatic channels selectively and drain to regional lymph nodes where the events of cellular immunity are initiated. This route promotes effective delayed hypersensitivity response but low production of antibody. Materials entering the systemic circulation, either directly, with the lymph, or released gradually from intramuscular, subcutaneous or intraperitoneal depots, are filtered by the lungs, spleen, and bone marrow. Such organs, with the injection site itself, are important sources of antibody production (Askonas and Humphrey, 1958). High levels of circulating antibody, but poor delayed hypersensitivity reponses are produced by this route. The liver forms little antibody, as antigen filtered from the circulation accumulates in fixed macrophages within its substance.

Injections into the footpad include intradermal and subcutaneous depots. Material in the intradermal plane readily enters the lymphatic plexus in the sole, the larger lymph channels and regional lymph nodes. Material in the subcutaneous compartment is released slowly into the capillary bed and the systemic circulation. The appearance of high concentrations of labelled antigen in the bloodstream and parenchymatous organs following local footpad injection may increase generalized host response to soluble antigen beyond that caused by the purely regional effect. This combination of routes elicits both effective cellular and humoral host responses.

Although some injected material entering intradermal lymphatics is retained in lymph nodes, the remainder gains the systemic circulation through major lymphatic channels. With diversion of the thoracic duct lymph, concentrations of labelled protein in the blood fall significantly. The subclavian lymph ducts may provide other direct routes to the bloodstream, but are relatively unimportant if the central pathway is patent. Although lymphatico-venous communications between lumbar lymphatics and the inferior vena cava or renal veins have been demonstrated in wild rats (Job, 1918), more recent studies using newer techniques have failed to confirm this (Engeset, 1959). It is unclear whether local communications exist within the lymph nodes themselves through which soluble antigen can gain the bloodstream directly.

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