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THERMAL INJURY AND INDUCED THERMOTOLERANCE IN RAT LYMPH NODES

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ABSTRACT

Immersion of the rat's hind limb in water at 45° or 46°C for one hour caused a severe burn of the extremity, including the popliteal lymph node. Pretreatment one day in advance at 45° C for 15 minutes or 43° C for one hour prevented most of the damage to lymph node and other tissues. The production of both thermal necrosis and thermotolerance were direct effects of heat on the lymph node. The distribution of necrosis in the lymph node (greatest in the subcapsular region and least in the medulla) probably relates to thermal gradients and not to the microstructure of the nodes, except perhaps for relative resistance of the medulla.

Study of thermal injury of lymph nodes is scant probably because they are so seldom involved in burns. Should a few superficial nodes be lost, nodal local functions are usually fulfilled by connections of regenerated lymphatic vessels to adjacent nodes, while nodal systemic functions are served by other more remote nodes. However, introduction of the thermotherapy for tumors is certain to increase interest in the effects of heat on lymph nodes including revival of Gilchrist's pioneering studies directed at metastatic carcinoma in lymph nodes.

In 1965, Gilchrist, et al destroyed local nodes that had previously taken up finely powdered magnetic oxide of iron by heating them selectively with magnetic fields (1). This demonstration of selective, regional lymph thermonecrosis suggested to us the possibility of selective lymph node thermochemotherapy. Lymph node thermochemotherapy (i.e. the delivery of drugs to heated nodes for an enhanced effect) might be useful not only in tumorous nodes but also with autoimmune diseases characterized by excessive or aberrant lymphoid activity. As a first step in development of lymph nodal thermochemotherapy, it was considered necessary to determine the susceptibility of lymph nodes to elevated temperatures as compared with adjacent tissues. Because thermochemotherapy is likely to involve repeated treatments, we also determined whether subthreshold heating induced tolerance to a subsequent heat treatment, as had been shown to occur in skin and muscle (2-5). In anticipation of the application of these ideas to abnormal and enlarged lymph nodes, we first examined normal lymph nodes or ones artifically enlarged by granulomatous and hyperplastic reaction to inject foreign materials.

MATERIALS AND METHODS

Female Lewis rats, 150-200 g, from Harlan-Sprague Dawley, Walkersville, MD, were maintained in hanging wire cages on Purina Laboratory Chow and tap water for at least one week before use. The rats were anesthetized with pentobarbital, 35mg/kg, intraperitoneally. The popliteal lymph node

was heated by immersing the entire right hind foot, leg and lower thigh in a water bath at 45.0° C or 46.0° C for one hour. The penetration of heat into the popliteal fossa was verified by passing a thermistor probe through a small hole in the skin of the upper thigh and then down into the popliteal space at the moment of immersion; it revealed that the temperature in the popliteal fossa reached water bath temperature within 1 or 2 minutes. Thermotolerance was induced one day in advance by immersing the same region, or only the foot, for a brief period (15 minutes) at 45° C or for one hour at a lower temperature (43° or 44° C). Groups of 4 to 8 rats, always including control animals that had not been pretreated, were heated together.

The right popliteal node of some rats was enlarged by subplantar injection of 0.25 ml of a 20% suspension of powdered metallic tin (type TF-1, Amax Base Metals, Inc., Carteret, NJ) (6) or 10% suspension of carbonyl iron (type SF, GAF Corp., Linden, NJ) (7) in saline, two weeks before the heat treatment.

One day after the last heat treatment, the rats were killed by exsanguination from neck vessels while under ether anesthesia. The burned right foot and the normal left foot were cut off through the ankle joints and weighed fresh. Burn edema was the difference between these weights. The severity of the burn was also estimated by inspection of the foot for vesicles and the leg muscles for hemorrhage and discoloration. The tissues were fixed in Bouin's fluid, decalcified, and cross sections were prepared from the foot and the leg midway between knee and ankle. The popliteal nodes were fixed, bisected if enlarged and embedded in paraffin in entirety. Step sections were prepared and stained with hematoxylin-eosin and sometimes phosphotungstic acid-hematoxylin for fibrin and periodic acid-Schiff-hematoxylin. Lymph node necrosis was scored as severe (+ +) when one-fourth or more of the cortex in the section was necrotic, mild (+) when less than one-

Table 1.
Thresholds of time and temperature for thermal injury in popliteal lymph nodes and feet*

Size of lymph node	Temperature	Immersion time	Edema of foot, g ± SD	Lymph node necrosis
Normal	45C	15m	0.15 ± 0.07	0
		30m	0.24 ± 0.08	±
		45m	0.60 ± 0.07	± or +
		60m	1.10 ± 0.18	+ +
Large (tin)	45C	15m	0.11 ± 0.02	0
		30m	0.37 ± 0.06	+
		45m	0.78 ± 0.14	+ or + +
		60m	1.00 ± 0.12	+ +
Normal	44C	60m	0.13 ± 0.01	±
Large (tin)	43C	60m	0.23 ± 0.05	0

*Immersion of right hind limb up to lower thigh. Rats killed one day later. Edema obtained from weight of foot on heated side by subtracting weight of unheated foot (usually 1.1-1.3g). Lymph node necrosis estimated histologically as severe (+ +), mild (+), or minimal (±) according to the topographic extent of lymphocyte pyknosis and karyorrhexis (see Methods). Four rats in each group.

fourth was necrotic, or minimal (±) when only a few scattered small groups of cells exhibited pyknosis and karyorrhexis.

In a few rats burned nodes were studied 2 or 3 days later rather than after 24 hours.

RESULTS

A. *Popliteal Lymph Node*

Immersion of the right hind limb produced burns of foot and leg and also yielded necrosis of the popliteal lymph node. Burned popliteal nodes were not enlarged, weighing only 3-6 mg. Prior injection of tin powder induced striking enlargement of the nodes (60-190 mg), whereas iron powder produced only a moderate enlargement. Macroscopically, hemorrhage and necrosis caused by burns were readily visible in the large nodes while congestion was detectable even in tiny, normal-sized nodes.

The threshold for producing burns of the popliteal node was investigated by immersion for various times at the constant temperature of 45° C or at various temperatures for the constant time of 1 hour. These experiments showed that for immersions at 45° C, 15 min was innocuous, while 30 or 45 min produced some necrosis,and 60 min was uniformly devastating (Table 1). Similarly, for 1 hour immersions, temperatures of 43° C or 44° C were tolerable while 45° C caused severe necrosis. Nodal necrosis, however, did not occur when immersion and subsequent

Table 2
Thermal injury and induced thermotolerance in popliteal lymph nodes and feet subjected to two consecutive immersions.*

Size of lymph node	Protective immersion	Thermal injury immersion	Edema of foot, g ± SD	Lymph node necrosis
1. Normal	None	45 C 1h poples	0.93 ± 0.07	+ +
	45 C 15m poples	45 C 1h poples	0.29 ± 0.07	0**
	45 C 15 m poples	None	0.15 ± 0.06	0
2. Normal	None	45 C 1h foot	0.92 ± 0.02	0
	45 C 15m foot	45 C 1h foot	0.10 ± 0.05	0
3. Normal	None	46 C 1h poples	1.14 ± 0.04	+ +
	43 C 1h foot	46 C 1h poples	0.22 ± 0.01	+ +#
	43 C 1h foot	None	0.01 ± 0.00	0
4. Large (tin)	None	45 C 1h poples	1.31 ± 0.10	+ +
	43 C 1h poples	45 C 1h poples	0.64 ± 0.06	+**
	43 C 1h poples	None	0.23 ± 0.05	0
5. Large (tin)	None	45 C 1h poples	1.44 ± 0.03	+ +
	43 C 1h foot	45 C 1h poples	0.53 ± 0.12	+ +#
6. Medium(iron)	None	45 C 1h poples	1.08 ± 0.18	+ +
	45 C 15m poples	45 C 1h poples	0.24 ± 0.03	0**

*Interval between treatments was one day. All treatments on right side. Inasmuch as heating was done by immersion, heating of the poples (posterior surface of knee) necessarily implies heating of the lower thigh, leg and foot as well. Rats killed one day after last treatment. Edema and lymph node necrosis as in Table 1. Three rats in each group.

**Absences or decrease of necrosis in these groups indicates lymph node has developed thermotolerance due to prior protective immersion of the poples.

#No thermotolerance in these lymph nodes; protective pretreatment limited to the foot.

severe burn were limited to the foot (Table 2, exp. 2). Nonetheless, one day after the foot was burned, these popliteal nodes showed increased mononuclear cells, erythrocytes in sinusoids and edema around hilar vessels, but no necrosis.

Thermotolerance investigated by preliminary immersions for 15 min at 45° C or for 1 hour at 43° C, (treatments known to be innocuous, see above), showed popliteal nodes were partially or completely protected when challenged the next day with an immersion known to cause severe nodal necrosis in non-pretreated rats (Table 2). Thermotolerance (absence or reduction of lymph node necrosis) was demonstrable only when the popliteal fossa was immersed during "preventive" pretreatment (exp. 1, 4, 6). Necrosis was not prevented when prior immersion was limited to the feet (exp. 3, 5).

B. Histopathology of Lymph Nodes

Normal size nodes and nodes slightly enlarged by iron reacted similarly. In a few instances the lymph node cortex was completely destroyed with lymphocytic nuclei uniformly pyknotic, karyorrhectic or agglutinated. The only apparently viable cells were venular and sinusoidal endothelial cells, mononuclear phagocytes in the lumen of subcapsular sinusoids, and fibroblasts in the capsule. The medulla was relatively intact but the mononuclear cells in the cords were replaced by polymorphonuclear leukocytes, while the sinuosids contained polymorphonuclears and fibrin thrombi.

Less severely burned nodes displayed multiple discrete foci of necrosis rather than complete destruction. These were scored ++ or + according to whether the necrosis extended over more or less than one-fourth of the parenchyma in the section. These foci were seen in both the cortex and paracortex but not in the medulla. The superficial cortex adjacent to the subcapsular sinusoid was slightly more affected, and the juxtamedullary cortex slightly less affected than other areas, but there were no

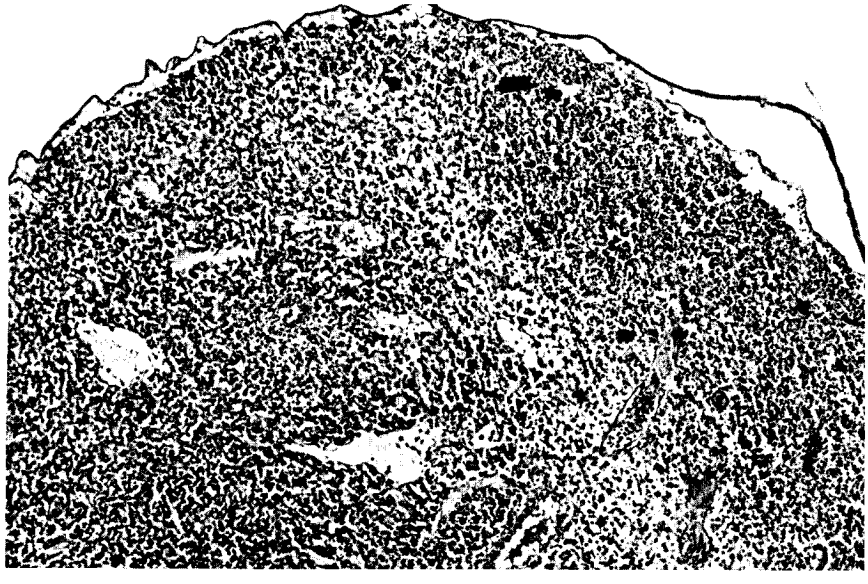

Fig. 1: Thermal necrosis in rat popliteal lymph node. The right side of the photomicrograph demonstrates severe pyknosis, karyorrhexis and clumping of lymphocyte nuclei; hence the very dark and shrunken appearance of the dead cells. Residual viable lymphocytes occupy the left half of the field except for a narrow zone under the subcapsular sinusoid. The junction of necrotic and viable areas is slightly pale due to edema and loss of cells. Hematoxylin and eosin, X70.

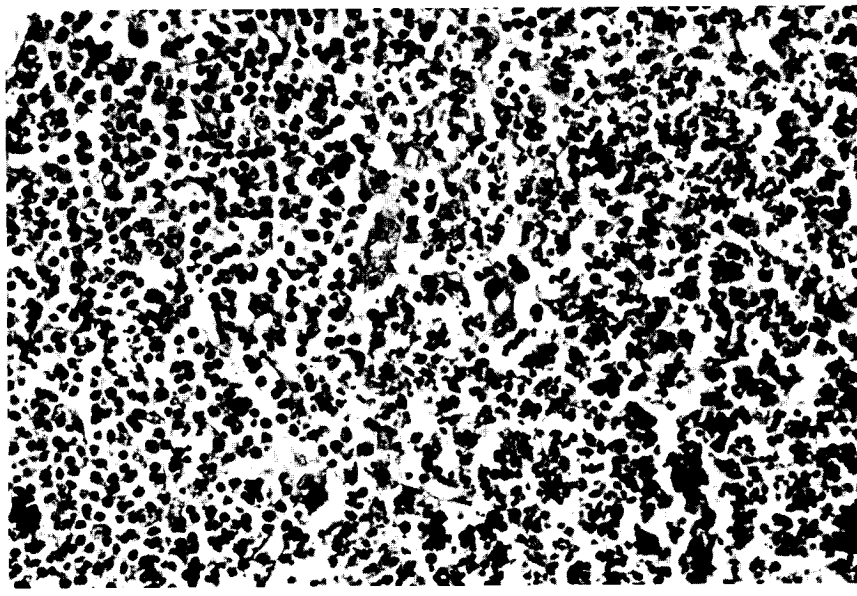

Fig. 2: Higher magnification of the junction between necrotic and viable areas. Pyknosis and karyorrhexis of the dead lymphocytic nuclei are responsible for the small, very dense granules seen on the right. In addition, clumping of necrotic nuclei has produced large irregular globs of dense material. The combination has resulted in a very heterogeneous size distribution in contrast to the uniform appearance of the viable lymphocytes on the left side. (Histologic scores in the Tables were based on the extent and topographic distribution of the necrotic changes illustrated here). Hematoxylin and eosin, X280.

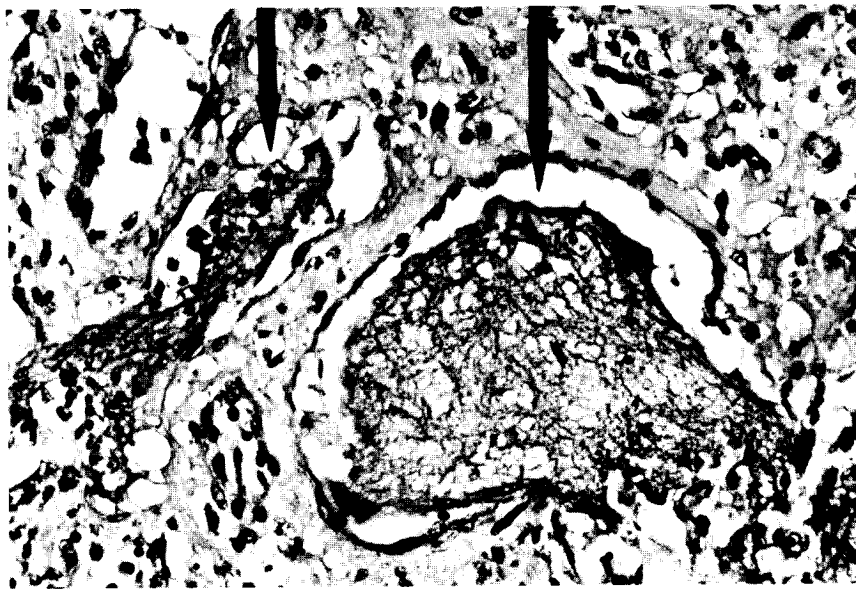

Fig. 3: Thrombosis of 2 lymphatic sinusoids in a burned lymph node. The thrombi (arrows) consist of fibrin clots with karyorrhectic leukocytes. Hematoxylin and eosin, X280.

major sites of predilection. In addition, the cortex between necrotic foci often contained scattered individual or small groups of necrotic cells. High endothelial venules and their contained lymphocytes were sometimes spared even when adjacent lymphoid cells were necrotic. Thrombosed sinusoids and foci of polymorphonuclears were prominent (Fig. 1-3).

Minimal lesions (scored ±) consisted of pyknosis and karyorrhexis of individual cells or small groups of cells. Detection of minimal lesions required careful study with magnification of 400X. More severe lesions were easily detected at magnifications of 40X or 100X but determination of extent required use of higher power.

Burns in lymph nodes enlarged by tin powder varied from almost total necrosis to involvement of large eccentric segments with a tendency to subcapsular predilection, much as in normal-sized nodes (see above). Enlarged lymph nodes were protected by pretreatment at lower temperature, but not to the same degree as normal-sized nodes (exp. 4,scores reduced from + + to + in all the pretreated and protected rats, rather than to zero). However, these partially protected nodes showed a clear predisposition to undergo necrosis in the subcapsular region. Further, this surface effect was never completely circumferential and did not involve the hilum.

Node study 2 to 3 days after thermal injury showed foci of advanced necrosis or fibrin thrombi in sinusoids. Some showed no lesions.

C. *Nonlymphoid Tissues*

One hour of immersion at 45° or 46° C caused a severe burn of foot and leg with necrosis and vesiculation of skin and with necrosis and hemorrhage of muscle and bone marrow. Blood vessels, nerves and bone cortex were relatively spared. Edema of the foot was so severe that the weight of the foot was sometimes doubled (Tables). In contrast, a short period of immersion at 45° C (15 minutes) or one hour of immersion at 43° or 44° C produced only slight edema and minimal vesiculation, or sometimes no abnormality.

When the hour-long immersion at 45° or 46° C was preceded 24 hours earlier by a brief (15 minutes) 45° C pretreatment, or by an hour-long exposure at the lower temperature of 43° or 44° C, the changes were strikingly ameliorated. Swelling and vesiculation of the foot and hemorrhage and necrosis of the muscles were much reduced by macroscopic inspection and by weight determinations (Table). Microscopically, however, muscle necrosis was still present, albeit less extensive. Pretreatment restricted to the foot had a beneficial effect only on the foot and not on the leg. Pretreatment by immersion at 37° C had no influence on the subsequent burn procedure. Pretreatment for 15 minutes at 45° C restricted to the *opposite* foot and leg had no preventive effect.

Discussion

We have described a simple, reproducible experimental model for thermal injury of a lymph node by immersion of an extremity in thermoregulated water. The interesting aspects of this study concern the distribution of injury and induction of thermotolerance. This phenomenon was clearly the direct effect of heat on nodes and not related to absorption of "toxic" material from the lower limb portion because it was observed only after immersion of the popliteal space containing the nodes and not when immersion was restricted to the foot or contralateral hind limb. The predilection of necrosis for the surface of nodes, but not for the hilum, likely relates to the intensity and direction of the temperature gradient from the water bath to the interior of the lymph node, perhaps ameliorated by blood flow (8) or even lymph flow in the hilar region. There was no selective effects on preferred habitats of T-lymphocytes (paracortex) or B-lymphocytes (cortex) in contrast to in vitro thermal sensitivity of lymphocyte populations from spleen and peripheral blood (9, 10). Clearly, however, nonhomogeneous distribution of thermal injury in our in vivo system makes it unsuitable

for study of lymphocyte subpopulation thermoselectivity. Variability of nodes studied 2 to 3 days after thermal injury may reflect repair of minor injuries with earlier thermotolerance.

Induction of thermotolerance by prior heat has also been studied extensively in vitro (11). In vivo, tolerance to thermal injury has been induced in the mouse ear (3), mouse foot (2, 4), and porcine adipose tissue and muscle (5). Whereas our data on the rat foot confirm these studies, we also show that thermotolerance can be induced in lymph nodes. Although the pathogenesis of thermotolerance has not been determined, it may involve intrinsic changes in the lymphocytes as shown by in vitro studies of spleen (12) and other cells (11). Alternatively, it may involve adaptive changes unique to in vivo studies (e.g. blood flow) (8), or involve intrinsic or extrinsic vascular, neurogenic or biochemical factors to the lymph node.

The future of this line of investigation relates to regional or local thermotherapy or thermochemotherapy directed at lymph nodes. We have established the approximate thresholds for thermal injury to lymph nodes which is either desired or avoided according to protocol. The untreated extremity provides a useful internal control. Most important for repetitive treatments involving heat is the demonstration of thermotolerance. Whereas it has not been fully established that effects of heat on lymph nodes other than necrosis are also subject to tolerance, it seems reasonable to assume similar tolerance after thermotherapy and thermochemotherapy.

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