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THE TOPOGRAPHY OF MERCURIAL LYMPHADENOPATHY IN BROWN NORWAY RATS

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ABSTRACT

Injection of mercuric chloride caused a progressive systemic lymphadenopathy and splenomegaly in Brown Norway (BN) but not in Lewis rats, as reported by others. We studied the number, duration and route of $HgCl_2$ treatments and the topography of the resultant lymphadenopathy, as well as its age and strain dependency. Five injections of $HgCl_2$ increased three-fold the weight of the lymph nodes which became considerably heavier than the spleen. Weanling rats were less susceptible than adults. A regional lymphadenopathy could not be produced. However, a synergistic interaction was observed when the systemic effects of $HgCl_2$ were added to the regional lymphadenopathy produced by injections of metal powders, such that the lymph node mass approached 2% of body weight.

BN (Brown Norway) rats are uniquely susceptible to subcutaneous injection of mercuric chloride ($HgCl_2$) (1). They develop a two-phase glomerulonephritis, autoantibodies, increased IgE in serum, and enlargement of spleen and lymph nodes attributed to polyclonal B-lymphocyte activation (1-9). Most of the investigations of lymphoid tissue have emphasized the spleen. Although lymph node enlargement is well known (5,7), there are few quantitative data on lymph node responses to $HgCl_2$. It has not been determined whether $HgCl_2$ can produce a regional B-cell activation in lymph nodes, as we have done with metallic tin injections (10). The present work concerns the effects of local injections of $HgCl_2$ on regional and distant lymph nodes.

MATERIALS AND METHODS

Female BN and Lewis rats from Harlan Sprague Dawley, Indianapolis, IN, or Charles River Laboratories, Wilmington, MA, were maintained in hanging wire mesh cages on Purina Rodent Chow 5001 and tap water ad libitum. $HgCl_2$ (Mallinckrodt Chemical Works, St. Louis) was dissolved in water or saline at a concentration of 1mg/ml (0.0037 molar) and injected three times a week in a volume of 1ml/kg except as specified otherwise. Because of the known resistance of baby rats to mercury, double the usual volume of $HgCl_2$ was injected into each foot, yielding a dose of 2mg/kg, repeated for 6 doses. Ether anesthesia was used only for injections in the feet or intravenously. Rats were killed 2 or 3 days after the last of a series of 5 or 6 injections by exsanguination from the aorta while under ether anesthesia. Lymph nodes, spleen and other tissues were weighed fresh, fixed in Bouin's fluid, embedded in paraffin, sectioned, and stained with hematoxylin-eosin-phloxine.

Table 1

Lymphadenopathy in BN Rats After Three to Twelve Injections of $HgCl_2$

No. of injections	Days of treatment*	Day of necropsy	2 Popliteals weight, mg.#	Spleen weight, mg	Body weight, g
3	0-4	7	27-40	420	170
		14	28-55	520	181
5	0-9	12	80-87	601	198
6	0-11	14	77-80	764	179
		21	52-62	633	170
		28	15-27	514	160
9	0-18	21	44-75	688	161
12	0-25	28	64-69	617	151

*$HgCl_2$, 1mg/kg subcutaneously, divided between two hindfeet, repeated three times weekly in groups of 2 or 4 BN rats.
#Lymph node weights given as range because of well-known variability in size of nodes; spleen and body weights are averages.

In order to study interactions between $HgCl_2$ and other lymphadenopathies, adult BN were given subplantar injections in the feet of of 1.0ml of 20% saline suspension of metallic tin powder (Alcan Powders and Chemicals, Elizabeth, NJ, type 115) (10), or cadmium light red pigment (Harshaw/Filtrol partnership, Louisville, KY), or 5% iron powder (carbonyl iron, GAF Corp., New York, NY, type SF) (11), or 1% silica (Syloid 72, Grace Davison Chemical Co., Baltimore, MD). The $HgCl_2$ treatments in the feet were started 2 weeks later when the regional draining nodes were enlarged by the metal powders.

RESULTS

Maximum lymph node enlargement in BN rats was obtained by 5 or 6 injections of $HgCl_2$ during 2 weeks, and this schedule was used in subsequent experiments. The nodes shrank rapidly if necropsy was delayed. Additional treatments maintained but did not increase the lymphadenopathy *(Table 1)*.

Lewis rats did not develop much enlargement of the popliteal nodes after $HgCl_2$ treatment, regardless of the route of inoculation and despite the use of double the dose given to BN rats *(Table 2)*, as anticipated from the literature (1,4,5,8,9). Both strains developed larger mediastinal nodes after intraperitoneal inoculation than after any other route, and microscopic study showed considerable hyperplasia in both strains. However, this was probably a nonspecific reaction because the treatments had caused a chemical peritonitis with resultant fibrosis of visceral surfaces and with a small amount of bloody ascites.

In this experiment *(Table 2)*, $HgCl_2$ in the feet enlarged the popliteal nodes of BN rats more than the same treatment given in the flanks. However, a more detailed experiment did not confirm the occurrence of a regional component in the lymphadenopathy *(Table 3)*. After five $HgCl_2$ treatments either in feet or in flanks, the lymph nodes were dissected and weighed in groups according to drainage patterns (12,13):

1. Draining feet but not injection sites on trunk:
   Popliteal nodes, retroperitoneal nodes: pelvic (caudal), lumbar (iliac, paraaortic), renal, celiac (gastric, splenic)
2. Potentially draining either feet or trunk:
   Superficial nodes: inguinal, axillary, elbow (brachial)

Table 2

Lymphadenopathy in BN Compared to Lewis Rats After Six Injections of $HgCl_2$ by Various Routes*

Route of $HgCl_2$	2 Popliteals, mg		Mediastinal, mg#		Body weight, g, avg.	
	BN	Lewis	BN	Lewis	BN	Lewis
SC, feet	32-37	18-36	22-81	42-54	218	182
SC, flanks	17-27	14-26	42-60	28-35	209	178
IV	26-30	11-20	54-91	32-49	208	180
IP	24-36	8-13	63-152	42-103	220	174

*$HgCl_2$, 1mg/kg, injected subcutaneously (SC) divided between the two hindfeet or the two flanks, or given into the jugular veins (IV) or the peritoneal cavity (IP), repeated three times weekly for 6 doses, in groups of 2 or 4 rats. Lewis rats were given twice this dose each time. Lymph node weights are ranges; body weights are averages.
#All parathymic, paratracheal and posterior mediastinal nodes.

Table 3

Lymphadenopathy in Various Regions of BN Rats After Five Injections of $HgCl_2$ in Feet or Trunk

Weights of Lymph Node Groups and Spleen, Average ± SD, mg#

	TREATMENT SITE*		
	$HgCl_2$		Saline
	Feet	Trunk	Feet or Trunk
2 Popliteals	52 ± 15	36 ± 14	18 ± 3
Retroperitoneal	133 ± 48	162 ± 70	38 ± 9
Superficial	347 ± 77	372 ± 124	103 ± 26
Cervical	404 ± 132	369 ± 105	127 ± 17
Mediastinal	97 ± 22	91 ± 33	57 ± 23
Mesenteric	324 ± 50	275 ± 89	225 ± 72
Total	1360 ± 198	1303 ± 394	567 ± 125
Spleen	599 ± 118	574 ± 90	342 ± 22
Body Weight, g	171 ± 6	181 ± 22	168 ± 3

*$HgCl_2$, 1mg/ml in saline, subcutaneous injections of 1ml/kg divided between the two hindfeet or two symmetrical trunk sites; or $HgCl_2$, 0.2mg/ml, subcutaneous injections of 5ml/kg divided between the two hindfeet or five sites on the trunk. Results are combined because changes in concentration and volume had no effect. Repeated for a total of 5 doses during 9 days in groups of 4 or 6 BN rats. Saline controls combined because route of saline injection did not affect the weights.
#See text for specific nodes included in each regional group.

Table 4

Lymphadenopathy in BN Rats After Injections of $HgCl_2$ in Right Foot

Weights of lymph node groups and spleen, average ± SD, mg

		TREATMENT SITE*	
		$HgCl_2$ Right Foot	Saline Right Foot
Popliteals:	Right	23 ± 4	5 ± 1
	Left	13 ± 6	6 ± 2
Superficials:	Right	70 ± 13	34 ± 8
	Left	64 ± 8	35 ± 9
Cervical nodes		190 ± 20	110 ± 14
Mesenteric nodes		220 ± 33	181 ± 34
Spleen		442 ± 18	372 ± 32
Body weight, g		185 ± 17	173 ± 12

*$HgCl_2$, 1mg/ml, or saline, subcutaneous injection of 0.5ml/kg in right foot, repeated for a total of 6 doses during 2 weeks in groups of 5 BN rats.

3. Potentially draining trunk but not feet:
   Cervical nodes: superficial, deep
4. Draining neither feet nor trunk:
   Mediastinal nodes: parathymic, paratracheal, posterior mediastinal
   Mesenteric node

The total weight of all these nodes in control (saline treated) rats was somewhat lower than data reported for BN rats of a different substrain (14). All nodal groups weighed considerably more in $HgCl_2$-treated rats than in saline-treated controls without overlap of values except for the mesenteric nodes where there was only slight enlargement. Enlargement of paratracheal nodes due to mild chronic pneumonitis may have influenced the weights of the mediastinal nodes in some rats. The total yield of nodes was doubled or almost tripled by $HgCl_2$. The popliteal and retroperitoneal nodes, draining the feet, were not consistently enlarged to a greater extent by $HgCl_2$ in the feet than by $HgCl_2$ in the trunk even though the trunk inoculation sites do not drain into these nodes at all. The cervical nodes were actually enlarged slightly more by treatment in the feet than by treatment in the trunk (including the neck) even though the lymphatic drainage from the feet does not reach the cervical nodes.

The absence of a regional effect in this experiment was confirmed in another experiment in which $HgCl_2$ or saline was injected only into the right foot. The right and left popliteal and superficial nodes were weighed separately in order to detect any lateralization of the lymphadenopathy *(Table 4)*. No lateralization was found in the superficial nodes even though lymphatic drainage from the right foot should reach the right inguinal node; in fact, the left superficial nodes were larger than the right in two of the five rats studied. The left popliteal node was enlarged despite the fact that it receives no drainage from the right foot and despite the lower dose of $HgCl_2$ which reduced the weights of all lymphoid tissues compared to *Table 3* (where both feet were given $HgCl_2$). The right popliteal node was slightly larger than the left but this was not considered significant because it was not uniform (the left popliteal was larger in one of the five rats) and because some or all of this enlargement might have been due to nonspecific effects of the repeated inocu-

Table 5

Lymphadenopathy in Response to $HgCl_2$ in Weanling BN Rats

Treatment*	Age, weeks		Weights, average ± SD, mg			Body
	Start	Necropsy	2 Popliteals	Spleen	Thymus	weight, g
Saline	4	6	12 ± 3	335 ± 59	351 ± 49	113 ± 16
$HgCl_2$	4	6	46 ± 44#	431 ± 110	155 ± 43	81 ± 9
$HgCl_2$	5	7	80 ± 36	485 ± 96	173 ± 55	100 ± 25
$HgCl_2$	6	8	152 ± 42	565 ± 33	215 ± 17	125 ± 4

*Six treatments during 2 weeks with saline or $HgCl_2$, 1mg/ml, 1ml/kg in each foot sole, subcutaneously, in groups of 3 or 4 rats.
#One of the popliteal nodes in one rat was four times as large as the largest of all the other nodes in this group. Without that node, the average weight of 2 popliteal nodes was only 30mg ± 10.

lation into that foot (edema, focal necrosis).

Histologic study of the enlarged lymph nodes revealed expansion of the medullary cords due to an increase of plasma cells, a less conspicuous hyperplasia of cortical follicles with germinal centers and a relative diminution of the paracortical (T-cell) zones. The same changes were present in all the regional groups. Mercurial nephrosis was mild with damage only in the pars recta of the proximal convoluted tubules.

Lymphadenopathy in weanlings

The occurrence of lymphadenopathy depended on the age of the rat. Treatment of weanling rats with $HgCl_2$ between 4 and 6 weeks of age produced only a moderate increase in the weight of the popliteal nodes *(Table 5)* despite the use of 2mg/kg doses, double the adult level. Histologically, however, there was already clear evidence of hyperplasia of plasma cell cords in the medulla and of follicles with germinal centers in the cortex. When the pups were treated with $HgCl_2$ between 6 and 8 weeks of age, the weight increased and the histology of the popliteal nodes were fully comparable to that which occurred in adult rats. The enlargement of lymph nodes, and, to a lesser extent, of spleen, contrasted with the decrease in thymus weight and the retardation of body growth, compared to saline-treated controls *(Table 5)*. Mercurial nephrosis in these immature rats was minimal, less than that seen in the adults despite the relatively high dose. Nevertheless, it might have contributed to the retardation of body growth.

Interaction of $HgCl_2$ with regional lymphadenopathies

Metallic tin powder produces a spectacular regional lymphadenopathy in Lewis rats (10). In addition to granulomas in response to the foreign particles, tin induces a severe hyperplasia of the medullary plasma cells in Lewis rats, much more than that produced by $HgCl_2$ in BN rats. BN rats respond to tin much less than Lewis rats (10). Nevertheless, the popliteal nodes of BN rats given tin in each foot were considerably enlarged after 2 weeks at which time $HgCl_2$ or saline was instituted.

Necropsies after 2 weeks of mercury treatment revealed a synergistic interaction: BN rats that received both tin and $HgCl_2$ had much larger nodes than could be ascribed to a summation of the effects of tin alone and $HgCl_2$ alone *(Table 6)*. Microscopically, tin alone produced the expected granulomas and plasmacytosis. The very large nodes in BN rats that were treated with both tin and with $HgCl_2$ did not have granulomas despite abundant tin particles, but there were extensive sheets of plasma cells and plas-

Table 6

Synergistic Lymphadenopathy in BN Rats from $HgCl_2$ and Metal Powders

Metal Powder	Weight of lymphoid tissues, mg, avg. 2 Popliteals		All nodes		Spleen		Body Weight, g	
	Saline	$HgCl_2$	Saline	$HgCl_2$	Saline	$HgCl_2$	Saline	$HgCl_2$
None	26	24	632	989	371	462	180	156
Tin	219	564	993	2486	356	624	157	142
Cadmium	94	246	1080	3149	398	820	170	170
Silica	43	265	674	1502	277	508	130	140
None	13	93			414	730	176	153
Tin	276	543			381	782	133	131
Iron	45	275			341	744	152	145

Metal powders or saline injected only once, distributed between the hindpaws (bottom 3 lines) or among all four paws (top 4 lines). Two weeks later, 1mg/kg $HgCl_2$ or saline was injected into the hindpaws (bottom 3 lines) or on the trunk (top 4 lines) and this was repeated for a total of 6 treatments during 12 days. Groups of 4 or 2 rats.

mablasts and a lesser increase of cortical follicles with germinal centers.

Carbonyl iron by itself produced a relatively mild regional lymphoid hyperplasia which seemed to involve all elements of the node. Again, the combination with $HgCl_2$ produced striking enlargement due mostly to increase of medullary plasma cells. Similar results were obtained when $HgCl_2$ was administered after injections of cadmium light red pigment or silica powder. The data on spleen *(Table 6)* contrasted with the lymph node data. Neither of the metal powders enlarged the spleen because their effects were limited to the nodes draining inoculation sites. On the other hand, $HgCl_2$ increased the size of the spleen to approximately the same degree whether or not the metal powders were injected.

The interaction of $HgCl_2$ and tin offered another opportunity to search for a regional component in the mercurial lymphadenopathy. To this end, the same procedures used on normal rats for *Tables 3* and *4* were applied to rats 2 weeks after injection of tin into both feet. The dose of $HgCl_2$ was halved by injecting it only into the right foot and the effect on the ipsilateral popliteal node was compared to the effect on the contralateral node as well as on the corresponding nodes in other rats given $HgCl_2$ on the trunk. Although the combined treatment did not enlarge the popliteal nodes to the degree recorded in *Table 6* (because a lower dose of $HgCl_2$ was used), the important result was the similar size of right and left popliteal nodes in rats given $HgCl_2$ at either local (right foot) or distant (trunk) sites *(Table 7)*. Thus, $HgCl_2$ acted systemically even in its influence on the regional lymphadenopathy produced by tin.

DISCUSSION

Previous evidence suggests that T-cell dependent polyclonal B-lymphocyte activation is the basis for some of the immunological effects caused by $HgCl_2$ in BN rats (1,5,7). T-cells of helper/inducer phenotype (W3/-25 positive), after exposure to $HgCl_2$, caused lymphoproliferation (15). The relative contributions of lymph nodes and spleen have not been elucidated previously. Our data on tissue weights provide only one of many possible views of the lymphoid system, but it is interesting that the large size of the lymph node compartment compared to the spleen, and the relatively greater response of the lymph nodes to $HgCl_2$, suggest that the nodes may be more

Table 7

Synergistic Lymphadenopathy in BN Rats After Injection of Tin in Both Hindfeet and $HgCl_2$ in Right Hindfoot or Trunk

Weights of lymph nodes and spleen, avg. ± SD, mg

	TREATMENTS*					
	I Tin Both Feet	II $HgCl_2$ Rt. Foot	I Tin Both Feet	II $HgCl_2$ Trunk	I Tin Both Feet	II Saline Rt. Foot
Popliteal: Right	213 ± 83		296 ± 80		146 ± 21	
Left	265 ± 49		327 ± 65		153 ± 15	
Retroperitoneal	410 ± 67		422 ± 35		181 ± 14	
Superficial	194 ± 84		187 ± 69		74 ± 7	
Cervical	228 ± 48		222 ± 53		108 ± 25	
Mediastinal	78 ± 7		52 ± 10		46 ± 29	
Mesenteric	235 ± 32		228 ± 45		183 ± 20	
Spleen	408 ± 38		422 ± 62		340 ± 51	
Body Wt.	132 ± 4		125 ± 7		132 ± 4	

*I: single subplantar injection in the hindfeet. II: six subcutaneous injections in right hindfoot or trunk, starting 2 weeks after I, and lasting for 2 weeks. The dose of $HgCl_2$, 0.5mg/kg, was half of that used for *Tables 1,2,3, and 6*, and the same as that used for *Table 4*. Groups of four rats.

important than the spleen in the B-cell activation. Furthermore, we did not dissect all lymph nodes in the body, so that our "total" node weights must be underestimates, whereas the spleen weights were true totals. The progressive enlargement of lymph nodes and spleen *(Table 1)* and the resistance of Lewis rats *(Table 2)* are in harmony with earlier findings. However, continued injections of $HgCl_2$ past the first 2 weeks maintains the lymphosplenomegaly whereas some other effects disappear despite continued treatments (1-3,5,6,8).

Lymphoid cells and metallic powders inoculated into the feet are abundantly absorbed into the popliteal nodes where they may produce a regional graft-versus-host disease (13) or a regional polyclonal B-cell hyperplasia (10). Although a regional enlargement of the popliteal node has been reported in mice following $HgCl_2$ injection into one foot (16), we did not find a regional lymphadenopathy in BN rats. The simplest explanation is that $HgCl_2$, in contrast to cells and particulates, may be absorbed directly into the bloodstream rather than into lymphatics.

The degree of lymphadenopathy produced by $HgCl_2$ depended on the pre-existing state of the lymph nodes. The relatively underdeveloped nodes of weanling rats supported only a modest lymphadenopathy, whereas nodes made hyperplastic in advance by metal powders became greatly enlarged after $HgCl_2$ treatment, reaching close to 2% of body weight. Lymphadenopathy also depended on genetic factors, as exemplified by the reciprocal relation of the Lewis and BN strains, the former responding much more vigorously to metallic tin and only the latter responding to $HgCl_2$. It will be interesting to determine if the reduced reactivity of immature animals and the strikingly expanded mass of lymphoid tissue after synergistic interaction with metal powders will cause a decrease and increase, respectively, in the manifestations of autoimmunity in mercury-treated BN rats.

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