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LYMPH NODE SIDEROSIS IN TRYPAN BLUE TREATED RATS

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ABSTRACT

The accumulation of iron in the lymph nodes of trypan blue treated rats was examined as a possible experimental counterpart of the lymph node siderosis which occurs in patients with Hodgkin's disease. Lymph nodes removed from the hilus of the liver, retrosternal area, axilla and root of the small bowel mesentery were examined histologically for iron in rats receiving 6-20 subcutaneous injections of trypan blue at biweekly intervals and in control rats. An increase in erythrophagocytosis accompanied by a progressive increase in the amount of stainable iron was found in the RE cells of nodes located in the lymphatic outflow tract of the liver. As in patients with Hodgkin's disease, an increase in erythrophagocytosis together with the prolonged retention of iron by RE cells appears to account for the accumulation of iron in the lymph nodes of trypan blue treated rats.

Histologic and radiographic abnormalities resembling human lymphoma develop in the lymph nodes of rats receiving trypan blue for 6-12 months (1-5). During an earlier attempt to study the sequential development of these abnormalities, an unusual accumulation of stainable iron was noted in some of the lymph nodes of these animals, an abnormality which was subsequently described in patients with Hodgkin's disease (6,7). Based on the consideration that a trypan blue induced lymph node accumulation of iron in rats might represent an experimental counterpart of the Hodgkin's disease associated abnormality, additional observations in these animals seemed warranted and these form the basis for this report.

MATERIALS AND METHODS

All experiments were performed in 18 male Wistar rats (250-300mg). One ml of a 1% solution of trypan blue (Gruber's, Roboz Company) in sterile pyrogen free saline was injected subcutaneously at biweekly intervals into the lower back of 15 rats for periods which varied from 3-10 months. Rats were killed within 7 days of the last injection. Sections of spleen and of lymph nodes located at the hilus of the liver, behind the sternum, root of the small bowel mesentery and in the left axilla were removed, placed in buffered formalin and processed for staining with hematoxylin and eosin and by the Perl's technique for iron. Similar sections were obtained and processed from 3 untreated Wistar rats serving as controls.

The iron content in each section was estimated without prior knowledge of the source of the specimen. An arbitrary 0-4+ estimate was used based

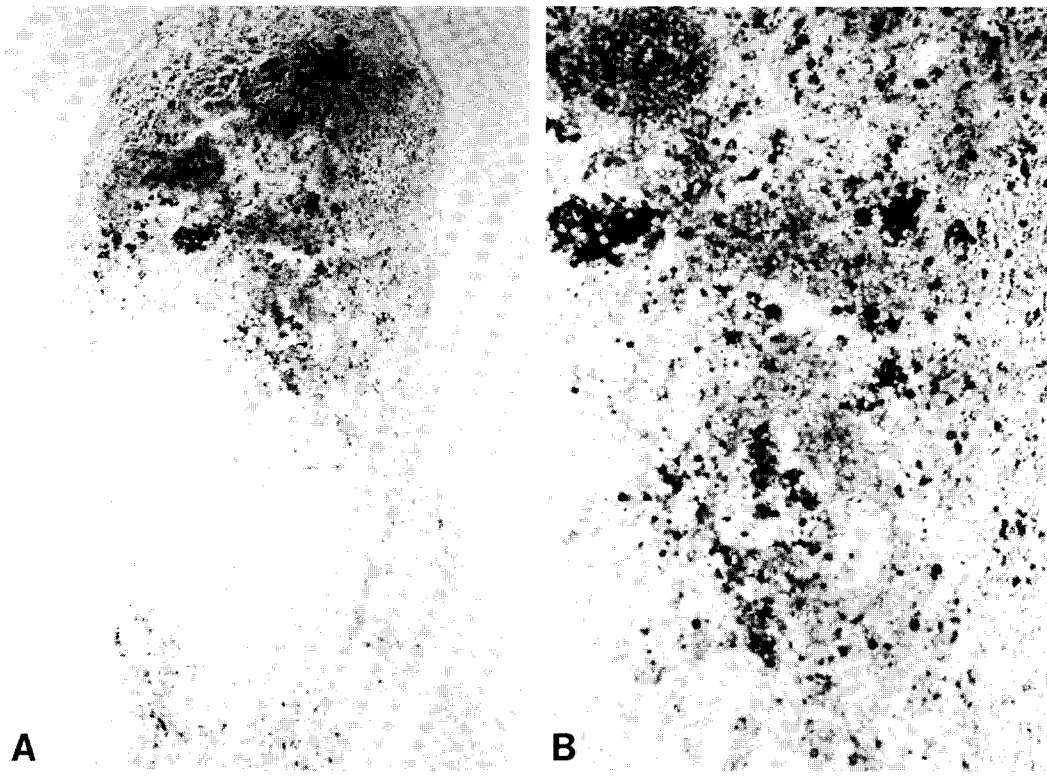

Fig. 1. (A) Low power view of a section of a hepatic hilar lymph node from a rat that received 19 injections of trypan blue at biweekly intervals. The dark areas contain iron stained by Perl's technique (orig. mag. x50). (B) Higher power view of the same section showing a dense accumulation of iron in large reticular cells surrounding the remains of follicles and lining the sinuses (orig. mag. x100).

on the percentage of the area of the specimen which contained iron and on the actual quantity of iron.

RESULTS

After 3-6 months of biweekly injections of trypan blue the hepatic hilar lymph nodes and the spleen contained significantly more iron than comparable nodes in control rats and this accumulation continued to increase in rats that received trypan blue for an additional 4 months *(Fig. 1A and B)*. A similar progressive increase in stainable iron was noted in the retrosternal nodes, although total accumulations were somewhat less than in the hepatic nodes. Axillary nodes, in contrast, rarely contained more than a trace of iron and with, a single exception, mesenteric nodes contained none *(Table 1)*.

A trace of stainable iron was found in the hepatic hilar lymph nodes in two of the three control rats while the other lymph nodes contained none.

As described in earlier reports (2,8), hematoxylin and eosin stained sections of the lymph nodes of trypan blue treated animals showed a progressive loss of normal architecture, disappearance of follicles, accumulations of plasma cells and binucleate cells, RE (reticuloendothelial) cell hyperplasia with sinus histiocytosis and evidence of erythrophagocytosis. These alterations were found in nodes from each of the four regions examined but occurred earlier and more prominently in hepatic

Table 1

Iron Content of Lymph Nodes and Spleen in Trypan Blue Treated Rats

Rat	Axillary	Mesenteric	Hepatic	Retrosternal	Spleen	Month
1	0	0	2+	0	2+	3
2	-	Tr.	2+	2+	-	5
3	0	0	3+	1+	3+	5
4	Tr.	0	3+	2+	3+	5
5	0	0	2+	1+	3+	6
6	Tr.	0	2+	1+	3+	6
7	Tr.	0	3+	1+	3+	6
8	1+	0	3+	1+	-	6
9	0	0	2+	1+	-	6
10	Tr.	0	4+	-	3+	7
11	-	0	4+	4+	4+	9
12	Tr.	0	-	4+	-	9
13	Tr.	0	-	2+	-	9
14	Tr.	0	4+	-	-	10
15	-	-	4+	-	4+	10

Note: In 2 of 3 control rats of the Wistar strain the hepatic lymph nodes contained only a trace (Tr.) of stainable iron while the spleen of all 3 contained an amount which was evaluated as 2+.

hilar and retrosternal nodes. None of the nodes examined contained tumor but the liver of four animals receiving trypan blue for 9-10 months did contain collections of abnormal appearing histiocytic cells resembling a human lymphoma. When sections of lymph node stained for iron were compared with those stained with hematoxylin and eosin, it became evident that iron was located predominantly in large reticular cells surrounding follicular remnants and lining the sinuses. In the spleen, which was regularly enlarged, most of the excess iron was seen in large reticular cells located in abnormally prominent perifollicular marginal zones.

Atomic absorption spectroscopic analysis of the trypan blue used in these experiments disclosed an iron concentration of 500 micrograms %, ruling out injected dye as an exogenous source of excess iron.

DISCUSSION

These results demonstrate that in trypan blue treated rats excess iron (hemosiderin) is retained selectively in hepatic hilar and retrosternal lymph nodes. Axillary nodes retain little, and apart from a single exception, mesenteric nodes none. This pattern suggests that the hepatic and retrosternal lymph nodes are exposed to higher concentrations of the dye than other nodes and that this selective exposure is due to dye transported in lymph rather than in blood. These lymph nodes receive lymph directly from the liver and in the case of the hepatic lymph nodes practically all of the afferent lymph derives from this source (9), while the retrosternal nodes receive lymph from other areas as well (10).

Liver capillaries are completely and uniquely permeable to plasma protein and, as a result, liver lymph has a protein content practically identical to that of plasma and higher than lymph from any other part of the body. Since trypan blue circulates in blood bound to albumin (11), it can be assumed that liver lymph contains a higher concentration of the dye than lymph from any other organ or region. Viewed in these terms, it is not surprising that the effects of the dye are most conspicuous in nodes draining the liver.

Lymph formed in the intestine has approximately half the protein content of liver lymph but significantly more protein than peripheral lymph. If mesenteric nodes were perfused with higher concentrations of the dye than the axillary nodes, as seems likely, it is curious that they usually contained no iron while nodes in the axilla consistently demonstrated a trace or more. The mesenteric nodes are unique, however, in that they are directly exposed to absorbed lipid (10), an exposure which causes their phagocytic cells to detach from sinuses and transform into mobile macrophages (12). Since the rats in our study received a diet containing about 4% fat, the absence of even small amounts of iron in these nodes may have been due to the continuous detachment of iron retaining reticular cells into efferent lymph and the thoracic duct.

Information available from studies in patients with Hodgkin's disease and from observations in trypan blue treated rats, suggest that the mechanism responsible for excess lymph node accumulation of iron may be the same in both. An increase in erythrophagocytosis in the spleen and the lymph nodes together with a severe anemia are striking features of the trypan blue treated rat (2,8). Red blood cells are also sequestered and destroyed abnormally rapidly in patients with Hodgkin's disease and this derangement together with failure of RE cells to release iron accounts for the sideropenic anemia and ferritinemia (13) associated with the disease (14). Since the accumulation of iron in RE cells results from their retention of iron released from catabolized red cells at their site of destruction (15), an increase in the local catabolism of red blood cells by these cells is likely to be the direct and principal source of the excess iron in the spleen and lymph nodes of trypan blue treated rats and of patients with Hodgkin's disease. A possible contribution of iron from lymph borne ferritin (16) or transferrin (17-19) has however not been excluded.

Iron is stored in the secondary lysosomes of RE cells as either ferritin or hemosiderin and the release of iron from these macromolecules and, in turn from the cell, is regulated by the activity of lysosomal enzymes (20). This activity is significantly depressed in the RE cells of trypan blue treated rats (21-23) as well as in the RE cells (Reed-Sternberg cells) which are characteristic of Hodgkin's disease (24). That this dysfunction is responsible for the retention of iron in the spleen and lymph nodes of both trypan blue treated rats and patients with Hodgkin's disease seems likely. A specific histologic similarity between these animals and patients with Hodgkin's disease has probably been overdrawn in the past (25), but with respect to the accumulation and retention of iron in lymph nodes, the trypan blue treated rat appears to be an unusually close experimental counterpart of the disease in man.

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