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Ectasias of the subcapsular sinus in lymph nodes of athymic and euthymic rats: a relation to immunodeficiency

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Summary. This paper describes a morphologically unusual feature occurring in lymph nodes of some aged euthymic animals but mostly athymic animals. It initially consists of small alveole-like excrescences of the cortical wall of the subcapsular sinus. With dilatation, an excrescence becomes an ectasia which expands into the cortex. Observations suggest that ectasias enlarge under the influence of an increased pressure of the afferent lymph of a node. Such condition conceivably results from a greater lymph formation due to inflamation of the drained tissue site, combined with an impairment to the flow of lymph from the subcapsular sinus into medullary sinuses. A probable relation of ectasia formation to immunodeficiency is discussed. This formation results in the atrophy of the affected lymphoid cell populations of a node which likely contributes to aggravate the deficiency of the immune system.

Key words: Lymph node, Sinusal ectasia, Immunodeficiency, Aging, Athymic rat

Introduction

The athymic state induces the formation of morphologically unusual features in lymph nodes (De Sousa et al., 1969; Fossum et al., 1980), in aging animals in particular (Sainte-Marie et al., 1984). These features may arise in one or a few nodes of some aging standard animals as well, but they are less developed (Sainte-Marie and Peng, 1987a). Their occurrence in latter nodes was attributed to a state of partial immunodeficiency. Such a state may indeed emerge with age (Nordin and Makinodan, 1974; Shigemoto et al., 1975; Hayward, 1977). This paper reports an additional unusual feature which consists of ectasias of the subcapsular sinus (or simply ectasias).

The network of lymphatic sinuses in a "compartment" of the normal rat node, as established previously (Sainte-Marie et al., 1982), is shown in Figure 1. A node has as many such compartments as there are openings of afferent lymphatics into its subcapsular sinus, or of terminal branches of these lymphatics in large species (Heath and Brandon, 1983; Aijima et al., 1986). Each compartment is centered on an opening, and the lymph content entering through it influences only the structures of the related compartment. This accounts for the possibly great morphological variations that may occur among various compartments of a same node, because the immunogenic content of its distinct afferent lymphatics may differ. Accordingly, all compartments of an affected node do not necessarily have ectasias. Therefore, the node compartment, rather than the whole node, is considered here.

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Materials and methods

We examined 501 nodes from 44 standard euthymic Sprague-Dawley rats aged 0 to 12 months, 239 nodes from 10 two-month-old germ-free euthymic CD rats, and 230 nodes from 10 gnotobiotic euthymic Sprague-Dawley rats aged 12 or 14 months (ex-breeders from the Research Institute of the National Research Council, Ottawa). Four of the gnotobiotic rats were suddenly placed in a conventional milieu upon reception at the age of 12 months; they were maintained under this condition during two months prior to sacrifice. We also examined 973 nodes from 32 athymic rats aged 2 to 15 months, which were born from heterozygous nude females and homozygous nude males originating from the Veterinary Resource Branch of the National Institutes of Health, Bethesda. The animals were sacrificed with chloroform and cervical, parathymic, mesenteric, brachial, axillary, inguinal and popliteal nodes were removed from each individual. The nodes were fixed in a solution of Bouin-Hollande for 48 hours and paraffin-embedded. Each node was cut serially at 7 μm. One out of every 3 sections was mounted in the case of rats less than one-month-old, and one out of every 15 sections in the case of older rats. The sections were stained with the

technique of Dominici (eosin Y-orange G and toluidin blue).

Results

Ectasias were present in a small minority of animals (Table 1). They were better developed in aged animals, and particularly in athymic animals. Ectasias were little present in germ-free or unexposed gnotobiotic animals; they were less rare in gnotobiotic rats exposed during two months to the contamination of a conventional milieu.

Ectasias were found in a single, or few, nodes of a same animal. The affected nodes were of various locations, but mainly of the cervical site. In cases, a single ectasia occurred in one compartment of a node (Figs. 2, 3); in other cases, a few ectasias were present there. In some nodes, ectasias were spread throughout the organ (Figs. 4-6). With increasing size of ectasia(s) in a compartment, its cortical lymphoid populations and other typical structures were atrophied (Figs. 3-5). In another respect, the medullary cords in nodes with ectasias often unusually hosted polymorphonuclear neutrophils.

Ectasias commonly had a rounded shape; some were much irregularly shaped. Their size ranged from tiny to large (Figs. 5,7). While a given compartment generally had just one or a few large ectasias, others had a few to numerous small ectasias as well (Fig. 6). The smallest ectasias showed as tiny alveole-like excrescences on the cortical wall of the subcapsular sinus which thus unusually apppeared crenated (Fig. 7). Somewhat comparably, some large ectasias had small mushrooming excrescences at their periphery.

Most ectasias were contained in the cortex but large ones extended into the medulla (Figs. 4, 6). Furthermore, most ectasias occurred in the peripheral cortex overlying medulla directly, by contrast to the peripheral cortex overlying a deep cortex unit. In addition, the ectasias tended to prevail in areas near the margin of a compartment, and at the perihilar margin of the whole peripheral cortex in particular.

Each ectasia connected with the subca psular sinus; the connection was generally narrow while the

Table 1. Age and numbers of animals studied, number and percentages of animals with sinusal ectasias, numbers of examined lymph nodes, numbers and percentages of lymph nodes with ectasias in normal, germ-free or gnotobiotic euthymic rats, and athymic nude rats.

ANIMALS	AGE IN MONTHS	No. OF ANIMALS	ANIMALS WITH ECTASIAS*		No. OF NODES	NODES WITH ECTASIAS	
			No.	%		No.	%
Euthymic							
Standard	0-12	44	7	16	501	28	6
Germ-free	2	10	0	0	239	0	0
Gnotobiotic	12	6	0	0	134	0	0
Gnotobiotic**	14	4	2	50	96	3	3
Athymic	2-15	32	21	66	973	123	13

*: presence of at least one ectasia in one node of the individual; **: animals of this line were exposed suddenly to a conventional milieu, at the age of 12 months and during two months.

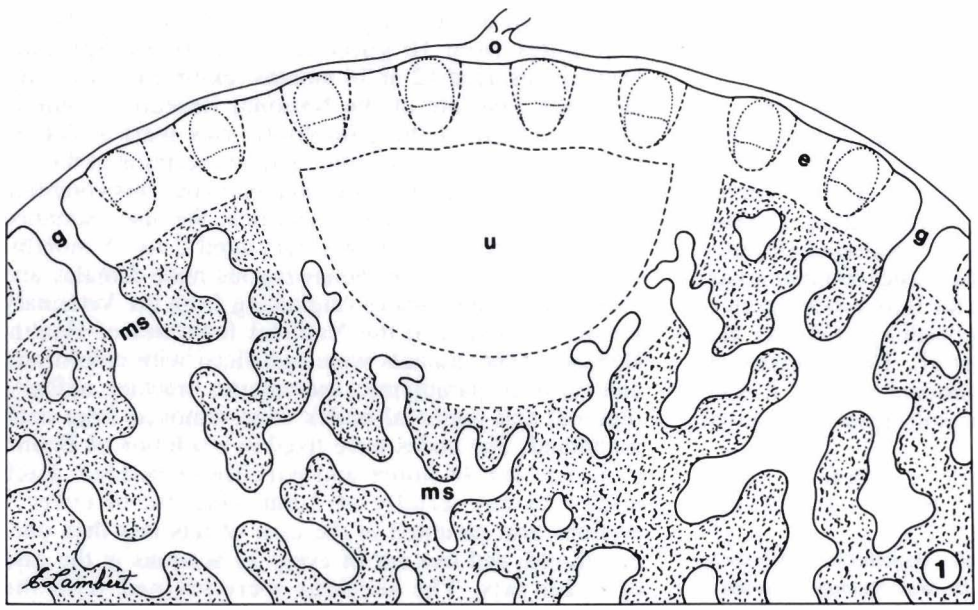


Fig. 1. Schematic illustration of a compartment of the rat lymph node. The drained lymph enters it by the opening (o) of an afferent lymphatic vessel and flows in the portion of subcapsular sinus of the compartment. The lymph reaches medullary sinuses via connecting sinuses present at the margin of the compartment where gaps (g) in the peripheral cortex occur. The peripheral cortex overlies either medulla directly or the semipherical deep cortex unit (u) of the compartment. In the periphery of the unit, lymphatic sinuses arise as culs-de-sac and are extended by medullary sinuses (m s). The variably anastomized medullary sinuses are separated by medullary cords (dark shaded).

peripheral cortex on either side of the area of connection was commonly reduced to a thin layer (Fig. 8). In large ectasias however, this connection was sometimes wide, being possibly almost as wide as an ectasia. On the other hand, the ectasias rarely connected with medullary sinuses which exhibited a usual range of variations of their width. Where a connection of an ectasia with a medullary sinus happened, such connection was narrow.

The lymph filling an ectasia varied in appearance. At times, it was clear and resembled the usual lymph of the subcapsular sinus (Fig. 2). Most often, it instead contained a variably dense acidophilic material. The latter had a granular aspect, as if it appeared formed of precipitated proteins and/or cytoplasmic debris (Fig. 8).

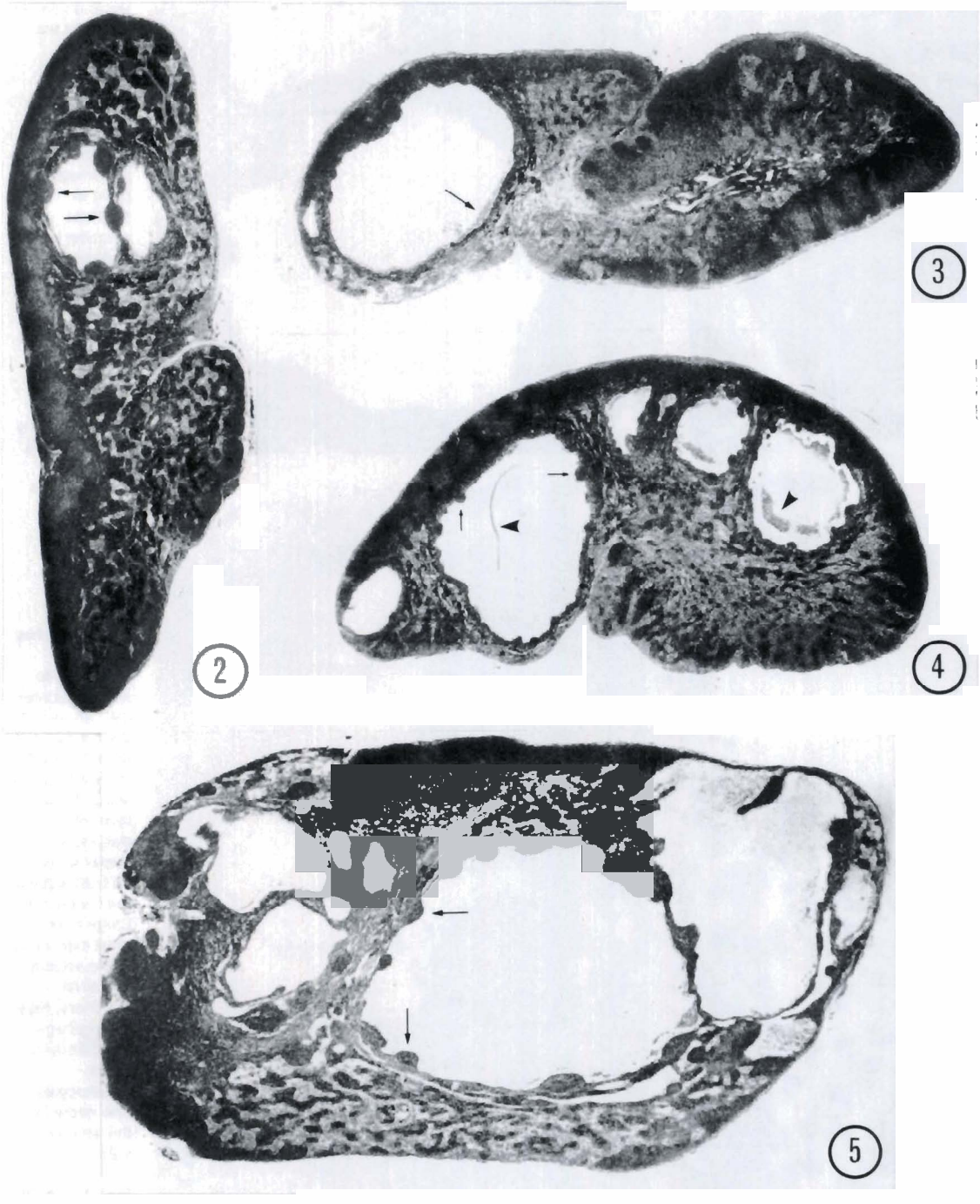


Fig. 2. Cervical node, 11 months. A divided ectasia occurs in a compartment of the node. Ovoid "lymphocyte clusters" (arrows) protrude from the ectasia's wall into its cavity. The lymph, present in the ectasia, appears clear unlike that in the light shaded medullary sinuses. x 10

Fig. 3. Brachial node, 5 months. A single large ectasia fills much of a compartment of the node. Its lymph is clear, except for a thin deposit (arrow) along its wall. x 10

Fig. 4. Cervical node, 5 months. Ectasias are scattered in the node, the largest one extending deep into the medulla. Protruding lymphocyte clusters (arrows) occur at their periphery. The ectasias have an amorphous acidophilic content artifactually retracted in the largest one where its outline shows as a dark line (left arrowhead). Two ectasias contain accumulations of lymphocytes (right arrowhead), nearing parts of their wall but separated from it by retraction of their overall content. x 10

Fig. 5. Cervical node, 12 months. Lymphocyte clusters (arrows) protrude from the wall of the large ectasias. The lymph in the ectasias is paler than that in the medullary sinuses where macrophage-like cells are numerous. The cortical lymphoid population, in the right part of the node, has much waned out. x 10

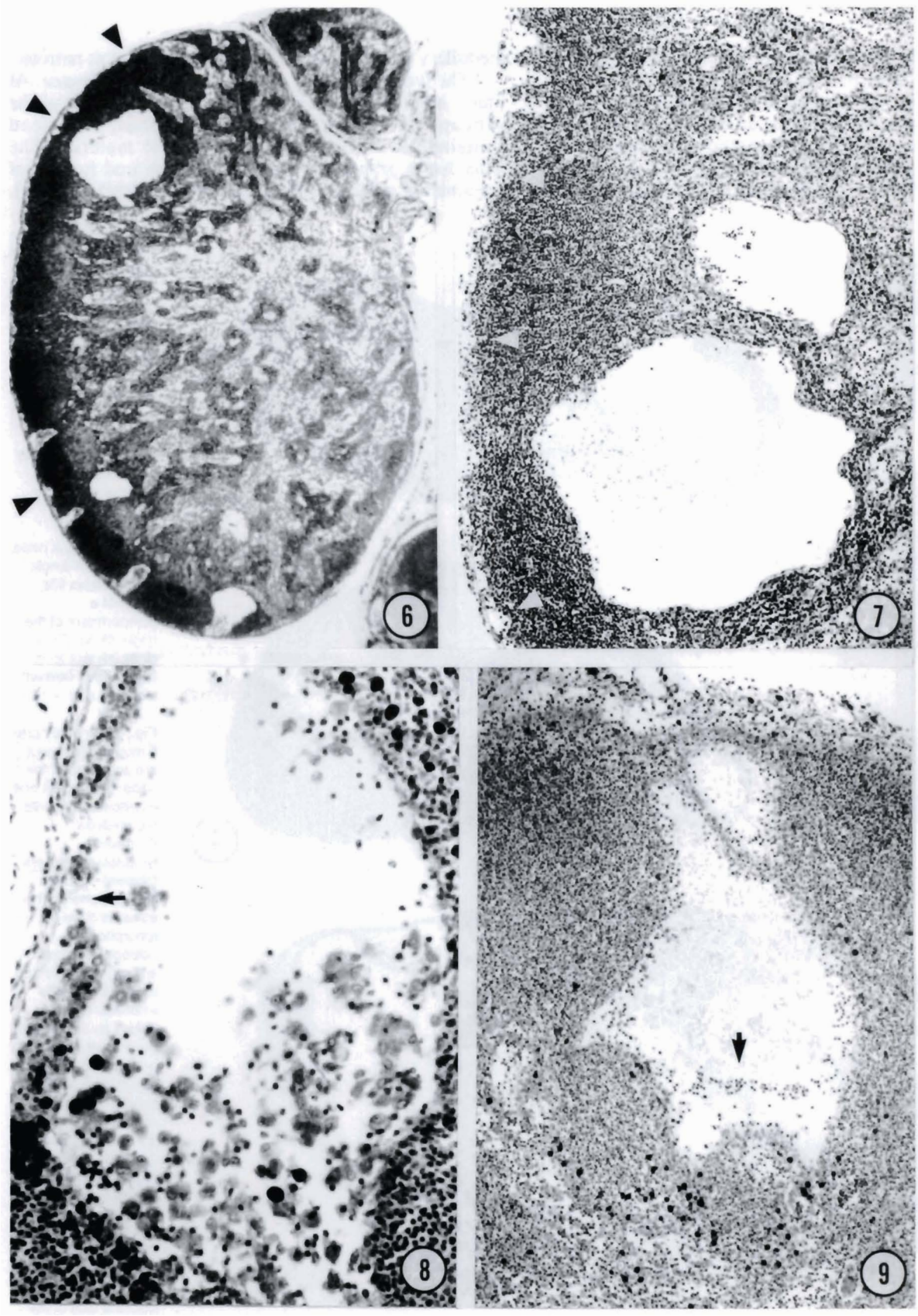


Fig. 6. Cervical node, 2 months. Many tiny alveole-like excrescences (arrowheads) of the inner wall of the subcapsular sinus occur above the peripheral cortex. Small ectasias expand into the cortex at the bottom of Figure. x 10

Fig. 7. Same node, as in Figure 6. The arrowheads point to smallest ectasias which appear as alveole-like excrescences of the cortical wall of the subcapsular sinus. The presence of many such tiny excrescences gives the outline of this wall an unusually crenated appearence. x 100

Fig. 8. Brachial node, 11 months. The arrow indicates the narrow connection of an ectasia with the subcapsular sinus. A thin layer of peripheral cortex remains on both sides of the connection. Large dark mast cells occur in the ectasia and at its periphery. Pale macrophage-like cells and some lymphocytes also occur in the ectasia. x 250

Fig. 9. Cervical node, 3 months. Lymphocytes line the inner side of an ectasia's wall. An artifactual retraction of the ectasia's content separates these lymphocytes from part of its wall (arrow). The lymph of the ectasia also contains an abundant (but hardly dectable) amorphous and acidophilic material. x 100

Lymphocytes were the most common cells in the lymph of an ectasia, occurring in a variable concentration there (Fig. 10). Where lymphocytes were in a small number in an ectasia, they generally adhered to, or were close to, its wall (Fig. 9). In many ectasias, neutrophils were present as well; in some ectasias, they were almost the prevailing cell type. Moreover, ectasias had occasionally small numbers of degenerating leucocytes, macrophage-like cells, and mast cells (Fig. 8). Let us note that the lymph of an ectasia resembled that in the subcapsular sinus at site of connection with this sinus. Comparably, the lymph in a connected portion of medullary sinus generally exhibited the characteristics of that in the related ectasia; these characteristics waned in this sinus with distance from the ectasia.

The ectasias had a thin wall which looked like that of a sinus (Fig. 11). Many ovoid "lymphocyte clusters", each consisting of an accumulation of lymphocytes, occurred along the wall of some ectasias, in athymic animals in particular (Figs. 2, 4, 5). The clusters protruded in the lumen of the ectasias and they were distributed rather regularly along their wall in a manner resembling the distribution of follicles along the cortical wall of the subcapsular sinus. Such clusters occurred along the portion of the wall of ectasias in contact with the medulla, just as well as along portions in contact with the cortex (Figs. 2-6). It furthermore occurred along thin layers of tissue separating two adjacent ectasias or subdividing an ectasia (Fig. 2). Mast cells were often numerous at the wall of an ectasia; they were in fact generally more abundant in the cortex of an affected node, in athymic animals predominantly.

Some nodes of athymic, or aged euthymic, animals bore one or a few "compartment replicas", a previously described unusual feature developing mostly in athymic animals (Sainte-Marie and Peng, 1987b). A replica is a gross mirror-image duplication of a partially atrophied compartment of a node; in cases, a replica may have become larger than the bearer-node. Replicas often exhibited ectasias (Figs. 12, 13).

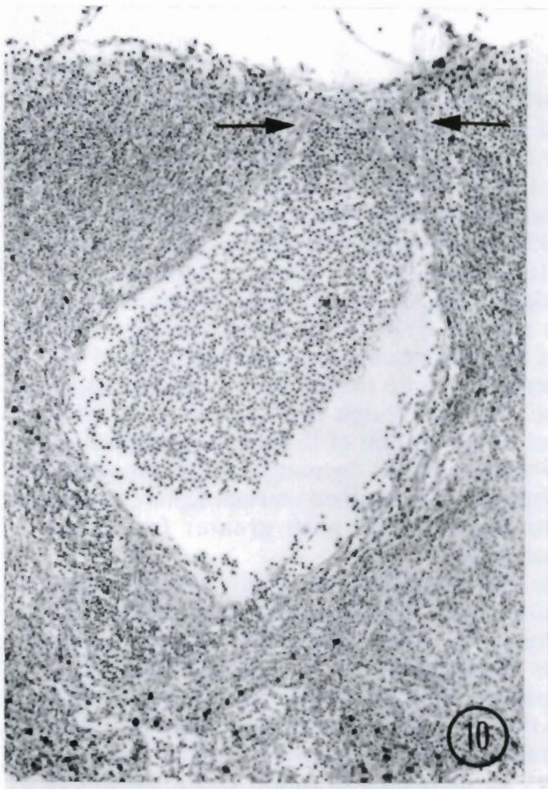
Discussion

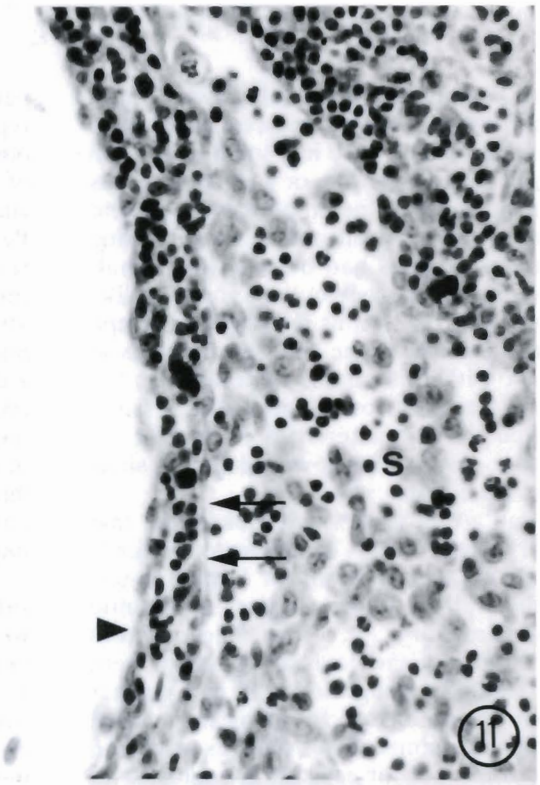
The simplest and likely best interpretation of the present observations is that a sinusal ectasia arises as a tiny excrescence on the cortical wall of the subcapsular sinus. As lymph is pushed in it, the excrescence progressively blows out into an ectasia. The ressemblance of the lymph of an ectasia with that of the subcapsular sinus, at their site of connection, indicated indeed that the ectasia's content comes from this sinus. With a pronounced enlargement, some ectasias may eventually come into contact with a medullary sinus and develop a connection with it. Comparably, the similarity of the lymph in this sinus with that in the connected ectasia indicated a lymph flow there from the ectasia into this sinus.

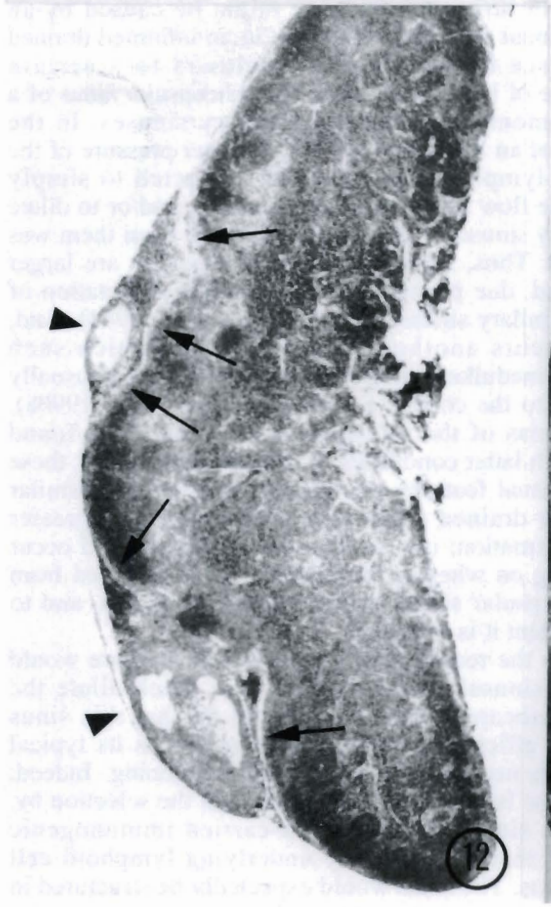
Why do sinusal excrescences and ectasias prevail at the vicinity of the margin of compartments, or at the perihilar margin of the whole peripheral cortex in particular? Actually, this preferential location is also typical of some other morphologically unusual features observed in immunodeficient animals: an accumulation of cortical mast cells or a fibrosis of the subsinus layer and the peripheral cortex, for instance (Sainte-Marie and Peng, 1990c). We proposed that sinusal phenomena related to unsuccessful immune responses due to immunodeficiency, some of which phenomena might alter the sinusal wall, occur preferentially near the margin of a compartment: i.e. beyond the central area of a compartment where events of successful responses take place. Because lymph flow appears to be of more importance at the margin of the whole peripheral cortex of a node than in gaps at the margin of its compartments, the development of concerned unusual features moreover correspondingly occur with greater frequency or importance there.

While the formation of a tiny excrescence of a subcapsular sinus might conceivably reflect a focal weakening of the inner wall of the sinus by unknown factors, its development into an ectasia probably requires a concomittant increase in pressure of drained lymph. Indeed, the development is accompanied by the disappearance of the local cortical tissue, which likely implicates an increased pressure of the lymph in an ectasia. In turn, such increase might be caused by an enhancement of lymph formation in an inflamed drained tissue (see below), likely combined to a certain hindrance of lymph flow from the subcapsular sinus of a compartment into related medullary sinuses. In the absence of an hindrance of flow, a higher pressure of the afferent lymph would indeed be expected to simply accelerate flow in the concerned sinuses, and/or to dilate medullary sinuses if the escape of lymph from them was impaired. Thus, some nodes of athymic rats are larger than usual, due primarily to a pronounced dilatation of their medullary sinuses (Sainte-Marie et al., 1984). And, there occurs another condition under which such widened medullary sinuses dilate further and unusually extend into the cortex (Sainte-Marie and Peng, 1990a). But ectasias of the subcapsular sinus were not found under both latter conditions. It is likely that, in fact, these three unusual features are variants induced by a similar state in a drained tissue site which results in greater lymph formation; one or the other variant would occur depending on whether the lymph flow is impeded from the subcapsular sinus, or the medullary sinuses, and to which extent it is impeded.

As to the reason why a high lymph pressure would produce sinusal ectasias rather than much dilate the overall subcapsular sinus, it could be that this sinus offers an efficient resistance to dilatation as its typical thinness is necessary to its normal functioning. Indeed, its thinness favors the contact with, and the selection by, the inner sinus wall of lymph-carried immunogenic elements that stimulate the underlying lymphoid cell populations. The sinus would expectedly be structured in such a manner as to permit the preservation of its essential narrowness, in spite of an occasionally pronounced increase in the pressure of the afferent







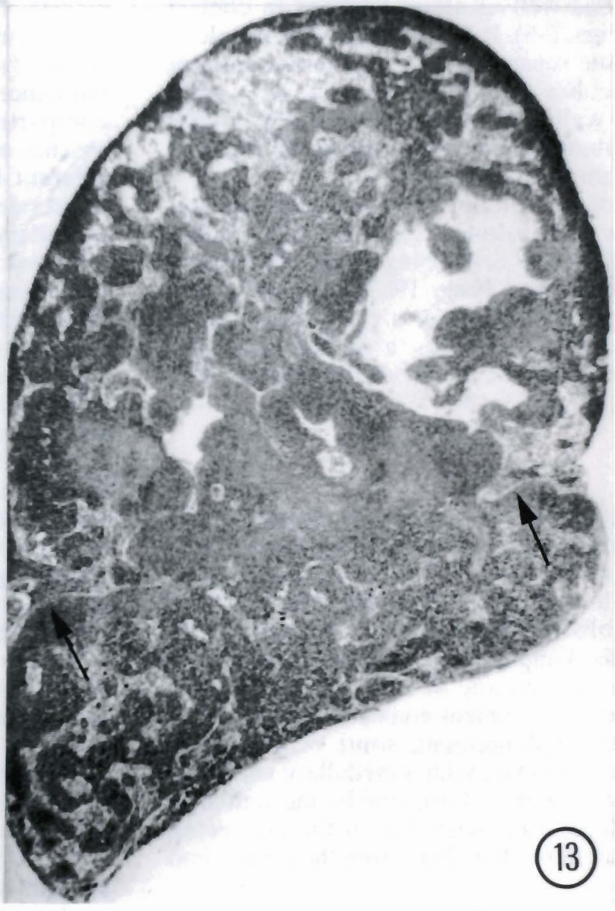


Fig. 10. Axillary node, 12 months. An ectasia, connected to the subcapsular sinus, contains abundant lymphocytes. Artifactual retraction of its content accounts for the pale area at lower part of the structure. The arrows indicate the upper margin of the ectasia. x 100

Fig. 11. Cervical node, 2 months. The wall (arrowhead) of a pale ectasia (at left) is thin and ressembles that (arrows) of a medullary sinus (s) containing many macrophage-like cells, lymphocytes and some polymorphonuclear neutrophils. x 250

Fig. 12. Cervical node, 11 months. Ectasias occur in both "compartment replicas" (arrowheads) bore by the node. Inner outlines of the replicas, corresponding to the pale subcapsular sinus of the original node, are pointed to by arrows. A replica is a gross mirror-image duplication of a partly atrophied compartment of a node. x 10

Fig. 13. Parathymic node, 13 months. The node (bottom part of Figure) bears a replica (upper part of Figure) larger than itself. The outline of the replica is pointed to. The subcapsular sinus of the original node is much disorganized in such situation so that the outline between the replica and the original node is vague. The replica contains a few ectasias. x 10

lymph. In fact indeed, it is delimited on one side by a firm capsule and, on the opposite side, by a peculiar "subsinus layer" having a network of reticular fibers running in a direction parallel to the sinus (Sainte-Marie and Peng, 1985a); moreover, both constituents are linked by numerous coarse fibers.

The aforecited dilatation of medullary sinuses, observed in athymic animals, was concluded to relate to immunodeficiency: representing a potential accessory effect of the athymic state. The fact that ectasias predominated in athymic and aged euthymic animals indicated that their development there is related to immunodeficiency as well. This relation is also suggested by the rapid formation of ectasias in gnotobiotic animals suddenly exposed to abundant antigens, which results in the emergence of a certain state of immunodeficiency and in the formation of morphologically unusual features of the node associated with it (Sainte-Marie et al., 1987b; Sainte-Marie and Peng, 1990b). The same relation is moreover suggested by the frequent presence of neutrophils in the afferent lymph and medullary cords of nodes with ectasias, an abnormal presence often occurring under immunodeficiency and reflecting an inadequate control of antigens in inflamed drained sites. Another probable indicator of the same relation is the abnormal occurrence of an abundant acidophilic material in the afferent lymph of most ectasias which likely represents extravasated proteins and/or cytoplasmic débris from such inflammed sites. Lastly, a state of immunodeficiency can account for a higher pressure of an afferent lymph since injury of a drained tissue, as caused by uncontrolled antigens, increases local lymph formation (Yoffey and Courtice, 1970).

Lymphocyte clusters occurred along the wall of ectasias in nodes from athymic animals mostly. Similar clusters are a common unusual feature in nodes of nude animals without ectasias, but then forming in association with deep cortex units or medullary cords (Sainte-Marie and Peng, 1983). Such clusters consist of IgM^+ cells; they represent additional follicles which arise abnormally deep in a node (Sainte-Marie and Peng, 1985b). Their formation, and the concomitant hypertrophy of the usual follicles in nodes of nude animals, were interpreted as a compensatory increase of B cell populations induced by the failure of most humoral immune responses in the absence of T-helper cells. In another respect, the formation of additional follicles at the periphery of ectasias is in concordance with the fact that the ectasias appear as excrescences of the subcapsular sinus along which follicles normally form. This raises the possibility that lymphocytes of the afferent lymph, observed to adhere to the wall of ectasias, contribute to form these clusters. The latter assumption would seem to receive support from the rather regular distribution of clusters along an ectasia's wall, as is the case of usual follicles along the wall of the subcapsular sinus. It would seem to be supported also by the bulging of the clusters into an ectasia, as if their lymphocytes were added there from within this structure.

To conclude, the formation of sinusal ectasias in nodes of immunodeficient animals leads to the destruction of their lymphoid populations and, thus, likely contributes to accelerate the lethal failure of the immune system as do also others affections of nodes accompanying immunodeficiency (Sainte-Marie and Peng, 1990b).

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