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Presence of multinucleate giant cells in normal thymus of nutria (*Myocastor coypus*)

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Abstract. Collected thymus from three 12-months-old clinically healthy nutria were histologically processed. Multinucleate giant cells have been observed occurring as a response to the existence of an acidophil material in relatively large quantities. This material is result of keratinization of some reticulo-epithelial cells, a process that appears more intense in nutria than in most species of mammals. Multinucleate giant cells are phagocyting this acidophil material and, where it forms large blocks, many multinucleate cells are grouped around them in a similar manner to that in reaction to the foreign body. It is a particular situation in which the body reacts somehow strange, treating some own structures as a foreign material. Note that multinucleate cells are not acting to remove the keratin from Hassall's corpuscles, but only the results from keratinization of reticulo-epithelial cells uncorporate in the organized corpuscles. By eliminating of keratinized reticulo-epithelial cells which could be considered as starting points for apparition of new Hassall's corpuscles, multinucleate giant cells attemper the rhythm of formation of Hassall's corpuscles in nutria.
Keywords: multinucleate giant cells, thymus, nutria.

Résumé. Le thymus collecté des trois ragondins sains cliniquement, âgés de 12 mois, a été transformé histologiquement. Ont été mis en évidence multinucleate cellules géantes, apparu en réponse à l'existence d'un matériau acidophile trouvée dans des quantités relativement importantes. Le matériel acidophile a resulte de keratinisation de cellules epitelio-réticulaires, un processus qui semble porter plus que intens à ragondin que la plupart des espèces de mammifères. Cellules multinucleate fagocitent le matériel acidophil et où il forme de grands blocs, de nombreux multinucleate cellules sont regroupées autour d'eux d'une manière similaire à ce que, en réaction à un corps étranger. Il s'agit d'une situation particulière dans laquelle l'organisme réagit un peu étrange, traitant de certaines structures comme un matériel étranger. Il est à noter que les cellules multinucleate pas à supprimer le keratine existant dans corpuscules Hassal, mais seulement les résultats des keratinisation de cellules epitelio-réticulaires non incorporées dans corpuscules organisées. En éliminant les cellules epitelio-réticulaires keratinisées qui pourraient être dans la points de formation en organisation des nouvelle corpuscules Hassal, cellules multinucleate temperent rythme de formation des corpuscules Hassal à ragondin.
Mots clés: multinucleate cellules géantes, thymus, ragondin.

Rezumat. Timusul recoltat de la trei nutrii clinic sănătoase, în vârstă de 12 luni, a fost prelucrat histologic. Au fost evidenţiate celule gigante multinucleate, apărute ca răspuns la existenţa unui material acidofil aflat în cantitate relativ mare. Materialul acidofil a rezultat din cheratinizarea unor celule epitelio-reticulare, proces ce apare mai intens la nutrie decât la majoritatea speciilor de mamifere. Celulele multinucleate fagocitează acest material acidofil, iar unde el formează blocuri mari, mai multe celule multinucleate se grupează în jurul lor într-o manieră asemănătoare cu cea din reacţia faţă de corpi străini. Este o situaţie particulară în care organismul reacţionează oarecum ciudat, tratând unele structuri proprii ca pe un material străin. De menţionat că celulele multinucleate nu acţionează pentru eliminarea cheratinei existente în interiorul corpusculilor Hassall, ci doar a celei rezultate din cheratinizarea celulelor epitelio-reticulare neîncorporate în corpusculi organizaţi. Prin eliminarea celulelor

epitelio-reticulare cheratinizate care ar putea constitui puncte de plecare în organizarea de noi corpusculi Hassall, celulele multinucleate temperează ritmul de formare a corpusculilor Hassall la nutrie.
Cuvinte cheie: celule multinucleate gigante, timus, nutrie.

Introduction. Multinucleate giant cells are large cells that can contain in the cytoplasm 20-50 nuclei (Papadimitriou & Walters 1979; Diculescu & Onicescu 1987), but sometimes even more than 200 (Mariano & Spector 1974). They are formed by fusion of several macrophages, as a response to the penetration in the organism of a foreign body or in chronic granulomatous inflammation (Chambers 1978; Pintea 2002). Their "behavior" may differ depending on the quantity, shape, size and digestibility of the material to be phagocytizing (Kraus 1982). The multinucleate giant cells have been described in many mammalian species, in different pathological conditions, for example, the systemic granulomatous lesions (Baba et al 2006) in pigs, in mycobacterial infection (Nakamura et al 1984) brucellosis, mucormycosis (Deyoe 1968), and postweaning multisystemic wasting syndrome (PMWS) (Chae 2004). The lesions were observed mainly in the lymph nodes, tonsils, thymus, liver, spleen, Peyer's patches, kidneys, reproductive organs, bone, stomach, and heart. Granulomas in the thymus without pathogen has been reported so far in pigs, such an idiopathic thymic granulomatous lesions (Kawashima et al 2003; Kim et al 2003). Multinucleate giant cells were described in children with AIDS (Joshi et al 1986; Kontny et al 1997). The presence of multinucleate giant cells in animals that were not been affected by lesions mentioned above, was rarely reported. Thus, Rotaru (1977) observed multinucleate giant cells with material in a thymus of a calf asfixied to birth. Their presence was also reported by Mohammad et al (2007) in fish (*Neoceratodus forsteri*), in Australia. Currently, no data or description of multinucleate giant cells in the normal thymus of nutria are available on the study.

Material and Method. In this study were included three 12-months-old male nutria. They came from a private breeder and they were clinically healthy, with a good maintenance. The animals were slaughtered and eviscerated by owner. Thymus fragments were collected in the form of slices with a thickness about 5 mm. Collected samples were fixed in Stieve mixture for 24 hours, were introduced in paraffin and cut on 6 µm. The obtained sections were stained using the Goldner's Trichrome method.

Results and Discussion. Microscopic examination showed that studied thymus had normal structure, characteristic for young and healthy animals. This specification was necessary to be sure that all our findings in the studied thymic lobules, occurred under normal conditions and not after involution or under pathological conditions.

A very peculiar aspect is the presence of multinucleate giant cells in more hypostases. Thus, multinucleate cells are isolated, placed in the medullary zone of the thymic lobules. Some of them do not appear having a direct relationship with neighboring structures (Plate 1). Others are situated in the immediate vicinity of Hassall's corpuscles in process of organization, but without any direct relationship with them (Plate 2). Some multinucleate cells are placed in direct contact with the keratinized and degenerated reticulo-epithelial cells (Plate 3). Some multinucleate cells are available near the keratinized and degenerated reticulo-epithelial cells (Plate 4). Large multinucleate cells were evident in direct contact with lamellar acidophil material in moderate quantities (Plate 5). This acidophil material resulted from reticulo-epithelial cells which appear highly degenerate so that only a few of them can be distinguished having certain structural details. Multinucleate cells issue pseudopode that surround the acidophil material (Plate 6) for the isolation of adjacent structures and their controlled degradation. It will gradually be closed by phagocytosis in a vacuole and the multinucleate cell will work to include the mass of other acidophil material located near of cell (Plate 7). Multinucleate cells were evident and present near Hassall's corpuscles in the early stage of organization, but only in the vicinity of those which had more or less intense processes of keratinization in their peripheral zone and not in the center. Multinucleate cells are placed in contact with the peripheral keratinized zone and leave the impression that they taken the keratinized mass and include other bigger or smaller forming blocks (Plate 8).

Integrated keratin is found in various stages of degradation, and sometimes large differences can be seen from one vacuole to another (Plate 9). There are multinucleate cells in the vicinity of large Hassall's corpuscles phagocyting the keratin resulted from degradation of reticulo-epithelial cells in some extensions of the corpuscules (Plate 10), but not inside them although there are large quantities of keratin and intermediate products resulted from keratin degradation. There are multinucleate cells that have one (Plate 11) or more vacuoles with phagocyted material at different stages of degradation, with no obvious relationship with neighboring structures (Plate 12). When keratin and other products resulting from their degradation form large material blocks, many multinucleate cells are grouped around these blocks (see Plate 13) in a manner very similar to that of the foreign body reaction. Such formations may be observed also in the vicinity of blood vessels (Plate 14) but the arrangement seems random because those vessels do not present changes detectable by optical microscopy. They can also be arranged in the immediate approach of Hassall's corpuscles (Plate 15), but there are not observed any structural or functional relationship between these two types formations either. There are near of Hassall's corpuscles many multinucleate cells phagocyting material in cytoplasm, some of them are grouped and others not, but even here cannot be detected aspects to suggest any direct relationship between this two structures (Plate 16).

Multinucleate cells have a macrophagic origin and they appear in the cases where the normal macrophages are unable to cover and digest a material. In our case, the hardly digestible material, seems to result from keranitization processes comprising some reticulo-epithelial cells. This can be deduced from the arrangement of this material, available quantity and appearance. Tinctorial affinity of this material is acidophil, stained in different shades of red or green. The various tinctorial affinity of such acidophil material shows that it is found in various stages of degeneration.

To eliminate small or moderate amounts of keratin or intermediate products of degradation, multinucleate cells act as individual entities but, in cases of large blocks, several such cells are grouped around the block, sorrunding and isolating it from the neighboring structures. In this situation, multinucleate cells act as in the case of foreign body reaction, but here they isolate and remove the endogenous material by degeneration. It looks rather strange because this process was not reported in other species, although keratinization processes of degenerate reticulo-epithelial cells are present in almost all species of mammals. It was reported the presence of multinucleate giant cells with phagocyted acidophil material in calf thymus (Rotaru 1977), but only as individual cells and not grouped as in foreign body reaction.

Kraus (1982) asserts that multinucleate giant cells behave differently depending on the quantity, shape, size and digestibility of the phagocyted material. It seems that in the case of the nutria thymus the reaction is comparable to that of foreign bodies due to the presence of more intense keratinization of reticulo-epithelial cells compared to most of mammal species, with the emergence of large blocks of keratin. The reason why these intense keratinization emerge in nutria thymus cannot be accurately determined by morphological investigation, but only assumed. These processes could be related to the particular life of nutria, species adapted to the aquatic environment, which is different in many respects to the terrestrial one, or to the growth in captivity. What can be said with certainty is that the keratinization process of reticulo-epithelial cells evolves significant more rapidly than in most terrestrial mammals. The existence of large amounts of keratin and degradation products resulted from it, lead to apparition of multinucleate giant cells acting to eliminate those materials which because of their structure and large quantities cannot be effectively removed by macrophages. Also, the way they act is very selective, because they include only keratin and degradation products resulted from it after degeneration of reticulo-epithelial cells that are not embedded in Hassall's corpuscles, or include that material situated at the periphery of young corpuscles. It is somewhat strange because although the most of keratin is situated in the central area of the Hassall's corpuscles (see also Miclăuș et al 2009) the macrophages do not enter inside them to form multinucleate cells. It is possible that they do not receive chemical messages from the middle of Hassall's corpuscles. A peripheral crown of corpuscles,

consisting in reticulo-epithelial flattened cells placed on several levels, prevent macrophages to enter into Hassall's corpuscles. Even if they enter inside of corpuscle, they cannot survive in the degraded material existing in the central zone of the corpuscle. What can be said with certainty is that such multinucleate cells are formed outside of Hassall's corpuscles as a response to the presence of keratin and degradation products, which are not isolated from neighboring structures. Contrary, keratin located inside of corpuscles is isolated from neighboring structures and it is not such a target for the multinucleate cells.

The selective behavior of the multinucleate cells is particularly interesting because their apparition is due to degeneration and especially of keratinization of reticulo-epithelial cells. In a similar manner, Hassall's corpuscles emerge from degenerate reticulo-epithelial cells but not necessarily keratinizated (Miclăuș et al 2009). However the process of keratinization of these starting points can begin later, after the corpuscles strengthening. We can say that these two processes: genesis of Hassall's corpuscles and occurrence of multinucleate cells, are based on the same cell type degeneration - the reticulo-epithelial cells - involving different stages of degeneration. Corpuscles emerge from cells found in any stage of degeneration, while multinucleate cells act only against keratinized cells. Thus, these two processes, genesis of Hassall's corpuscles and occurrence of multinucleate cells, take place in parallel and not together. However we are tempted to believe that between these two processes is a direct link but not a morphological, a functional. We believe that by eliminating many degenerated and keratinized reticulo-epithelial cells, which could be starting points of new Hassall's corpuscle, multinucleate cells modify to some extent the genesis of Hassall's corpuscles, which however is large enough in nutria.

Conclusions. This study revealed to the level of medullary lobules of normal nutria thymus, the presence of multinucleated giant cells, isolated or grouped around of acidophil material which appears to be represented by keratin or degradation products resulting from it. Occurrence of multinucleate giant cells is in direct relationship with resistance to digestion and inclusion of acidophil material, which causes a reaction of macrophages rather similar to the foreign body reaction. It is a very particular situation in which the body reacts against its own structures, even if they are degenerated, in the same manner as it reacts against foreign bodies. By eliminating a large number of keratinized reticulo-epithelial cells, the multinucleated giant cells modify the course of genesis of Hassall's corpuscles in nutria.

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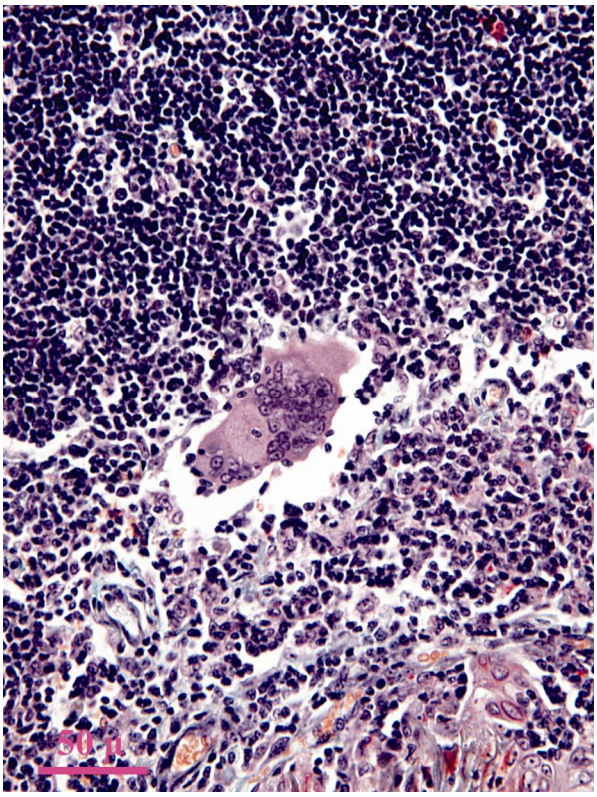

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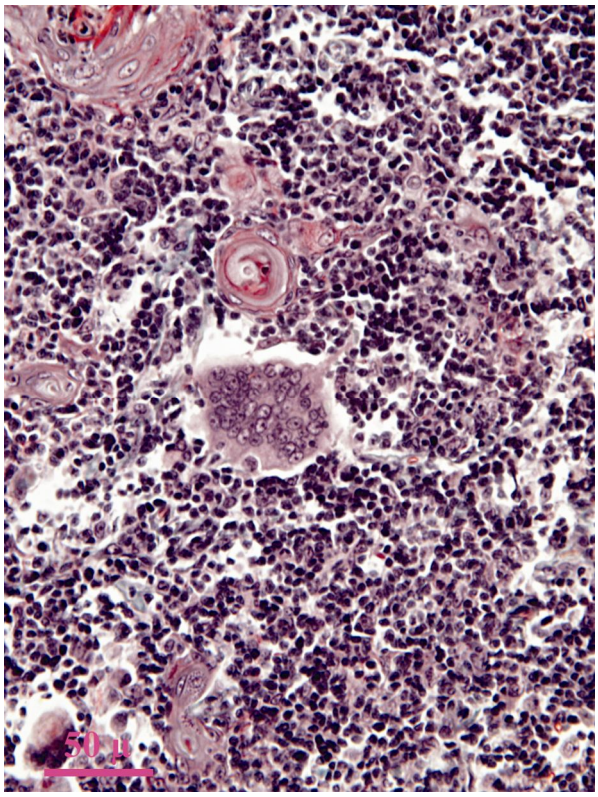

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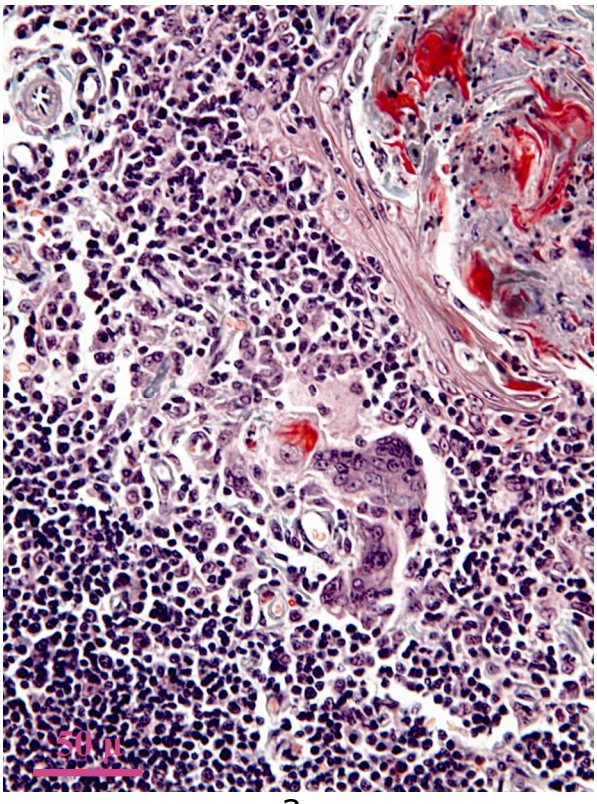

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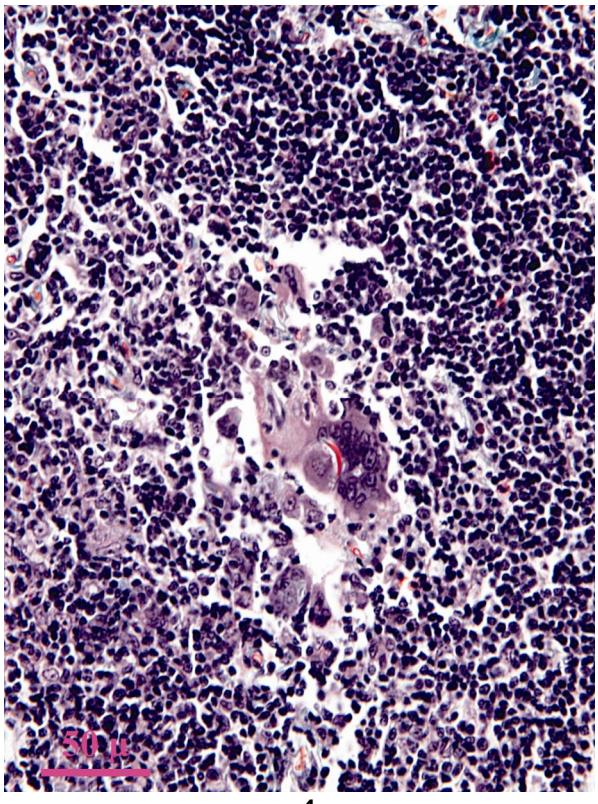

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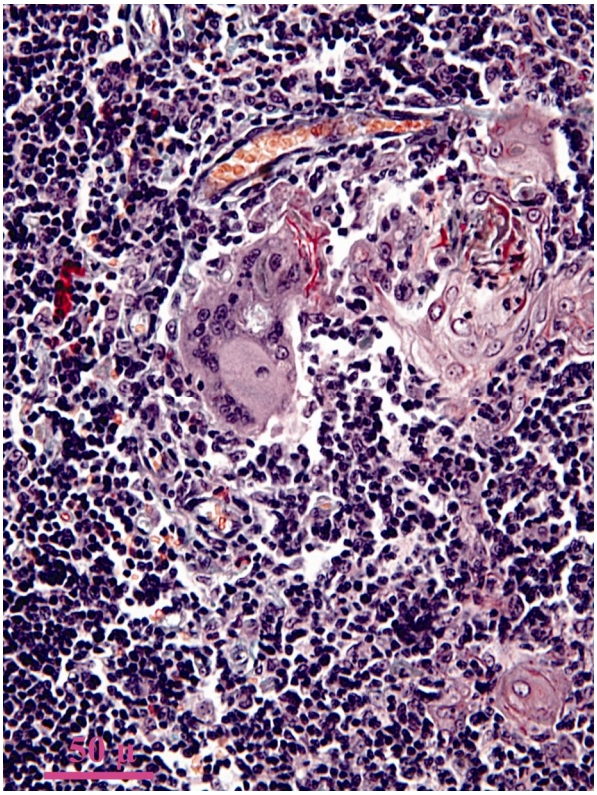
Plates: 1-2 isolated multinucleate giant cells; 3-4 multinucleate giant cells in contact with keratinized reticulo-epithelial cells (1-4 Goldner's Trichrome stain, Ob. 40X).



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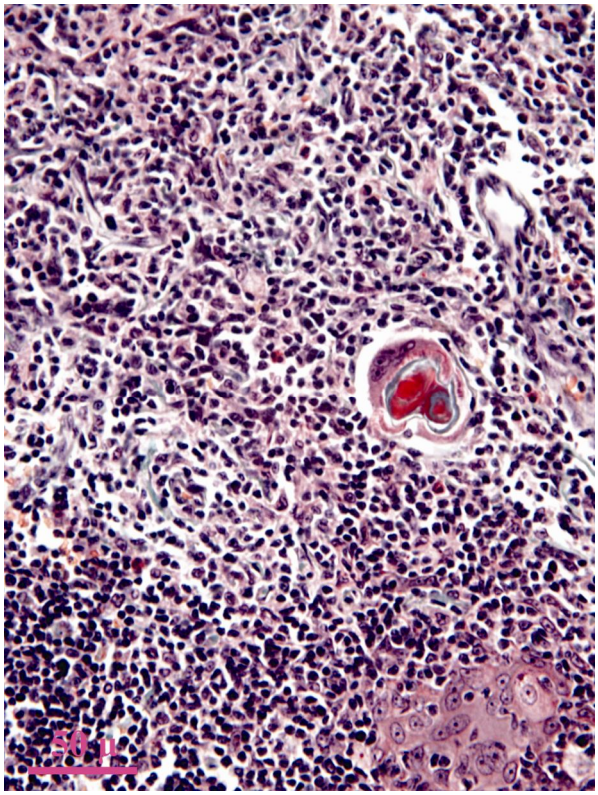

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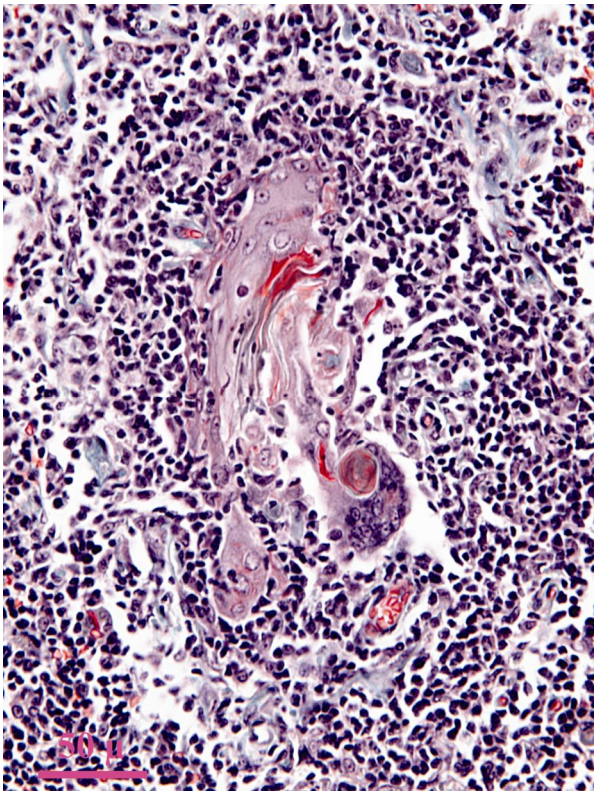

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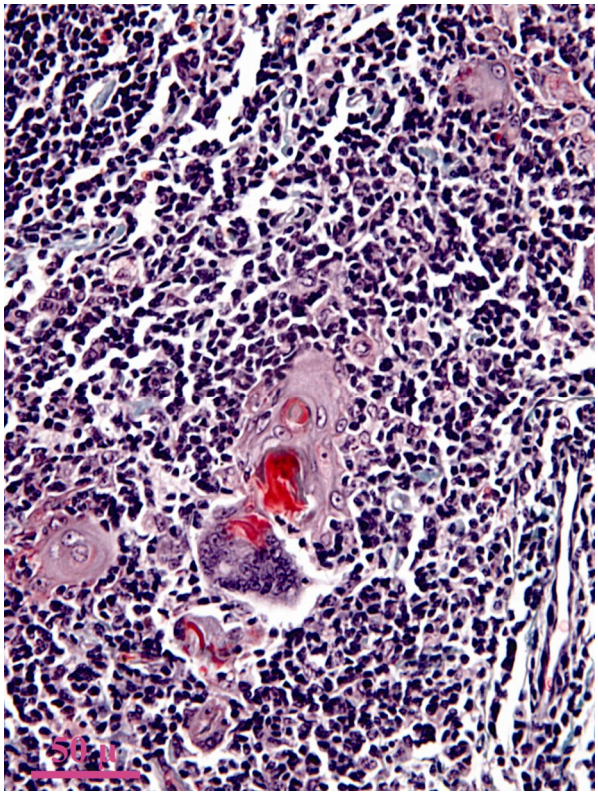

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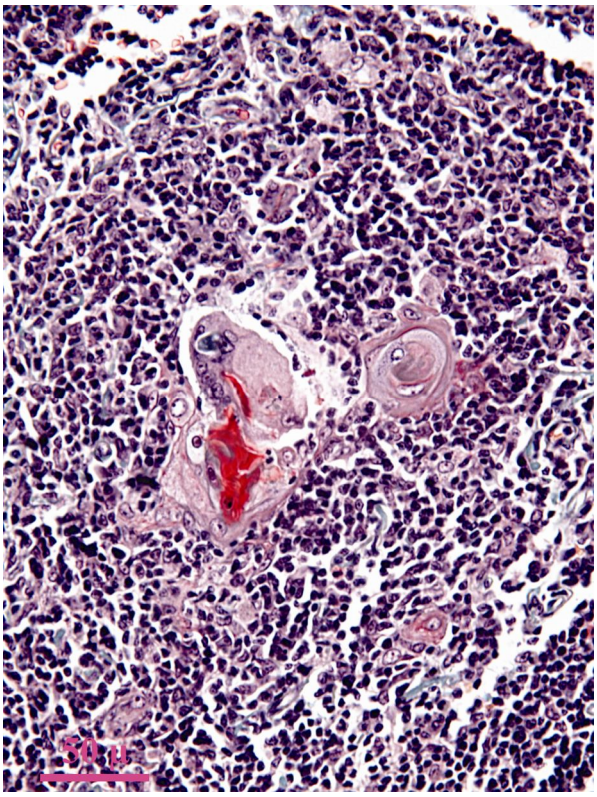
Plates: 5- multinucleate giant cells in contact with tickened lamellary acidophil material; 6- multinucleate cells issue pseudopode that surround the acidophil material; 7-8 multinucleate giant cells phagocyting acidophil material from the periphery of ongoing organization of Hassall's corpuscles (5-8 Goldner's Trichrome stain, Ob. 40X).



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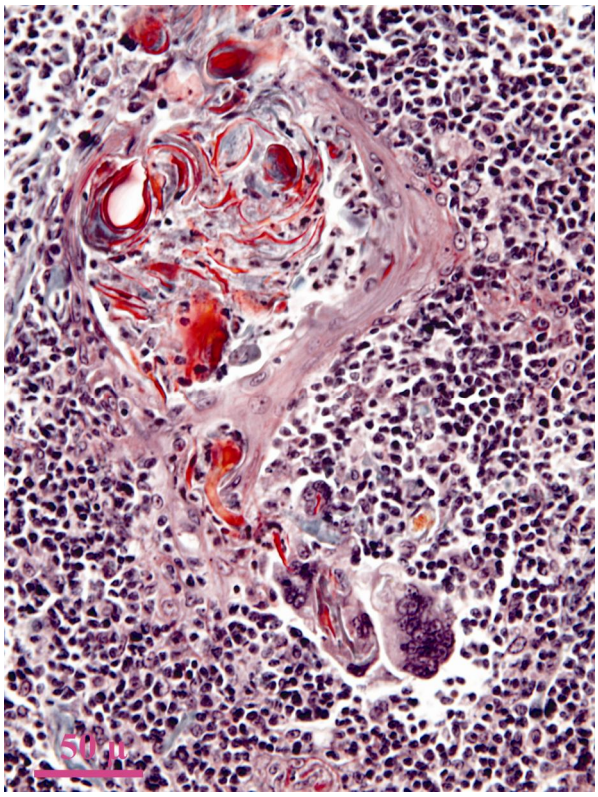

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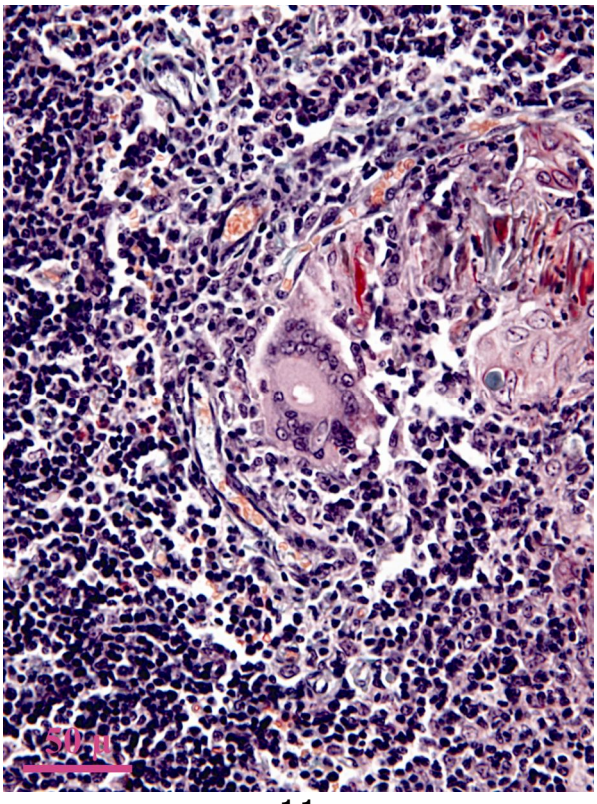

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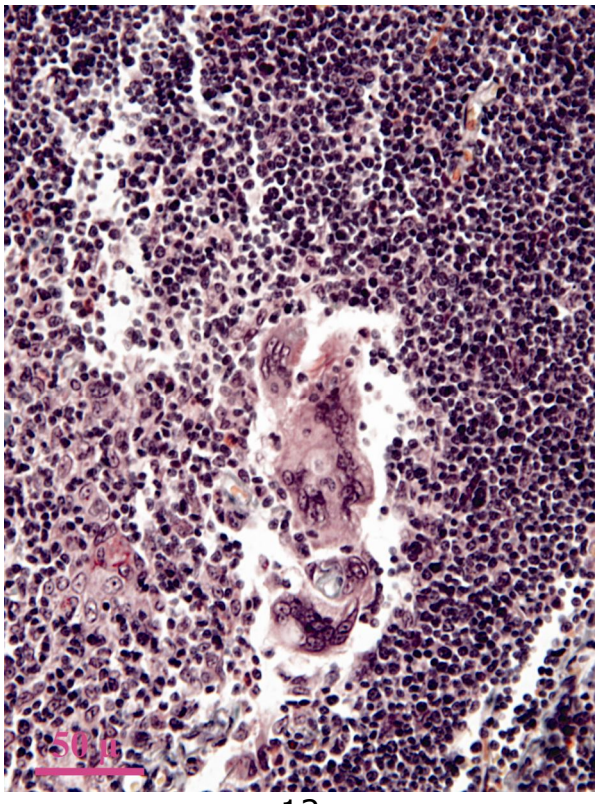

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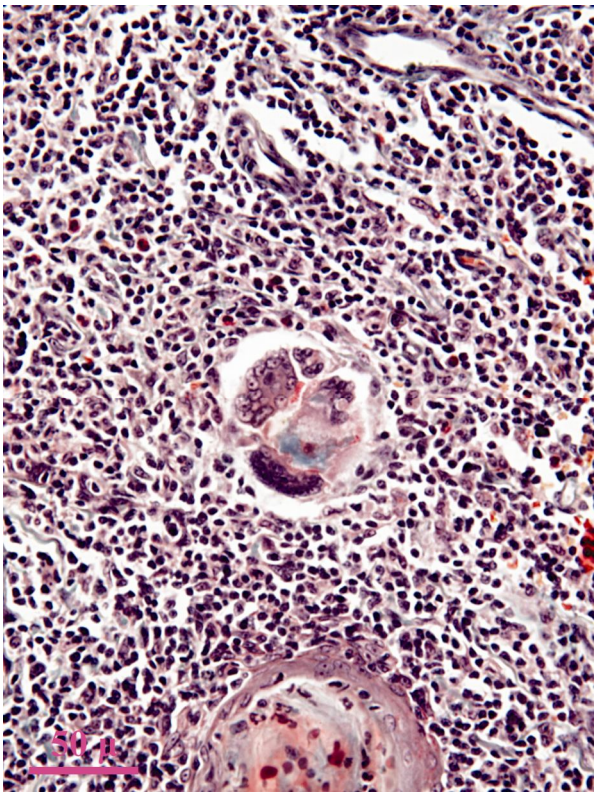
Plates: 9-12 Acidophil material phagocyted by multinucleate giant cells, in various stages of degradation (9-12 Goldner's Trichrome stain, Ob. 40X)



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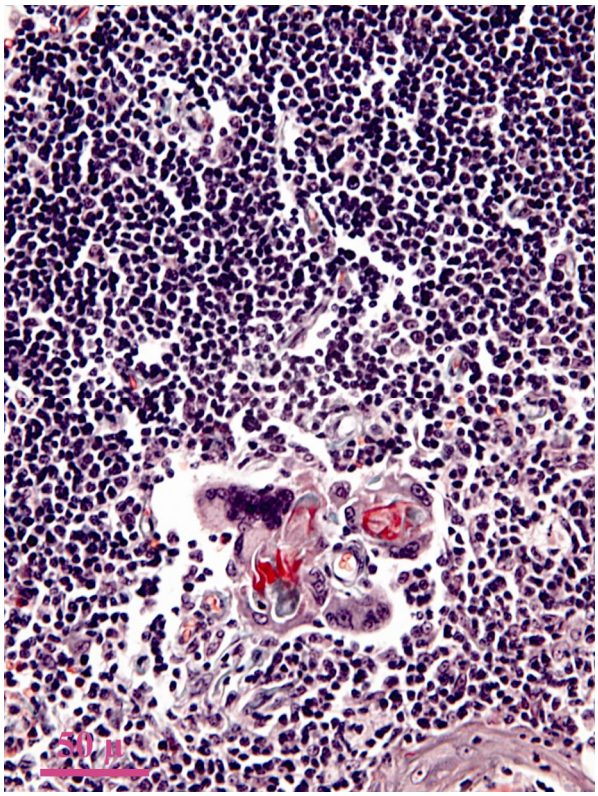

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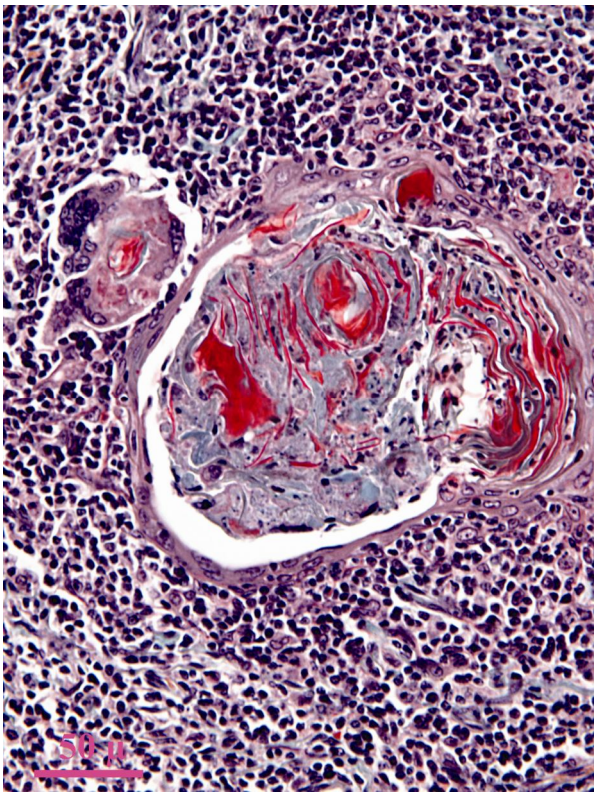

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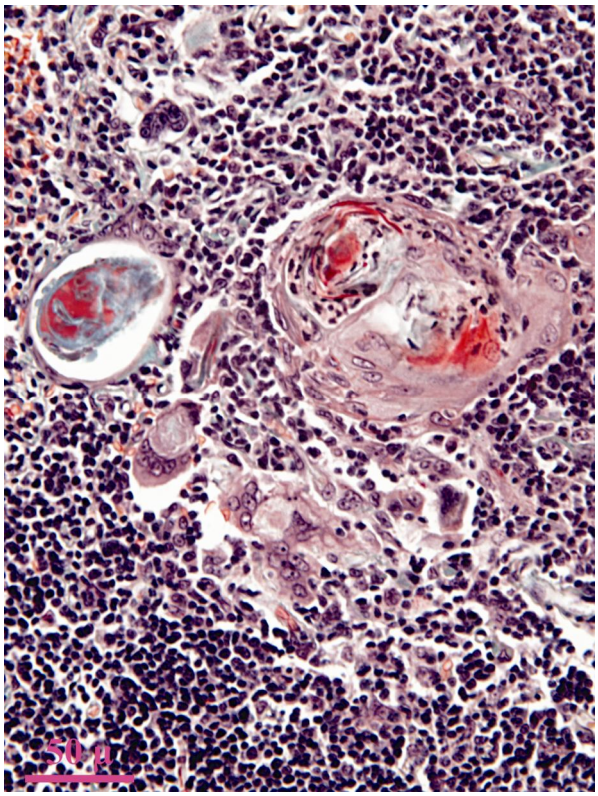

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Plates: 13-15 multinucleate giant cells surrounding the acidophil material;
16- multinucleate giant cells with high phagocytic activity (13-16 Goldner's Trichrome stain, Ob. 40X).

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