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SPECIES-DIFFERENCE OF NUCLEAR BODIES IN LYMPHATIC ORGANS

Makoto SUGIMURA

Department of Veterinary Anatomy
Faculty of Veterinary Medicine
Hokkaido University, Sapporo, Japan

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The nuclear bodies of the lymphatic organs were examined in the healthy mice, cats, dogs, sheep, cows, horses and ass with the aim of clarifying species-differences of the bodies. Their structural variation was due to the presence of two kinds of granular elements or vacuoles, and to the distinctness of the separation between the filamentous and granular ones. By the combination of both classifications, 11 types of nuclear bodies were distinguished.

The separation between filamentous and granular components was distinct in the nuclear bodies of the mice, cats and dogs, incomplete in those of the sheep and cows, and both components were so heavily intermingled with each other in those of the horses and ass. There are further specific features in some species; in the cat, the outer filamentous coat of the bodies was thinner than that of other animals. In the dogs, most of the central cores of the bodies were composed of only a spherical mass of dense granules. In the sheep and cows, the nuclear bodies often have a semilunar mass of dense granules in their central cores. In the mice and cats, the number of the nuclear bodies per nucleus was more numerous than that in other animals.

The usual patterns of the bodies were constituted with both filamentous and granular elements, and the bodies which are composed of only fiilaments or include vacuoles were rarely found. The nuclear bodies showed a close relationship with the nucleolus in some cells.

INTRODUCTION

Since nuclear bodies were defined under that name by WEBER & FROMMES, the bodies have been described in various types of cells in several animals. BOUTEILLE et al., DAHL, HENRY & PETTS, HORSTMANN, ISHII & MORI, KRISHAN et al., PAPOFF & STEWART and SUGIMURA et al. classified nuclear bodies into three or several types according to the appearance of their granular and vacuolar elements. One or two types of nuclear bodies seem to appear more numerously in some cells. HORSTMANN & BÜTTNER ascertained the presence of nuclear bodies in cells of many species from the reptile, the bird to the mammal, but the above-mentioned investigators did not pay attention to structural differences in the bodies among animal species.

In this paper, the structures of the nuclear bodies in the lymphatic organs of seven species of animals are reported with the aim of clarifying species-difference of the nuclear bodies.

MATERIALS AND METHODS

The lymph nodes, hemal nodes, patches of Peyer, spleens and/or thymuses of 25 mice, 3 cats, 2 dogs, 2 sheep, 4 cows, 3 horses and an ass were used as materials. These animals had no special diseases. Pieces of the organs were fixed in 1% osmic acid (MILLONIG), or in 2% glutaraldehyde and postfixed in 1% osmic acid, embedded in Epon 812 (LUFT), and cut with glass knives on Porter-Blum MT 1 ultramicrotome in the routine way. Thin sections were stained with uranyl acetate and/or lead citrate and examined on a JEM 7 electron microscope. More than 140 cells with nuclear bodies in an animal species photographed to examine the ultrastructures of the bodies.

RESULTS

1 Types of cells with nuclear bodies and basic structure of the bodies

Almost all types of cells observed in the lymphatic organs had nuclear bodies in their nuclei, but their sizes and frequencies differed among different types of cells; the large bodies seemed to occur more numerously in the small lymphocytes (fig. 3) in the organs other than the thymic cortex, in the cells of the reticular series (fig. 16) and in the endothelial cells (fig. 14). No nuclear bodies were found in the large lymphocytes and mitotic cells.

The structures of the nuclear bodies seemed not to differ among different types of cells in an animal species, but to vary among different species of animals as described later.

The nuclear bodies consisted of filamentous components of about 50 Å in thickness and dense granules of 150 to 360 Å in size basically. A singular body was spherical in shape and generally the filaments concentrically arranged to form an outer layer which surrounded a central core consisting of granular components, except for the horse and ass. In a few cases, a vacuole was found in the area corresponding to the central core of the body (fig. 10). The granular components were divided into two kinds, loose and dense. The loose ones, at a glance, were like granules of 200 to 350 Å in size, but they seemed to consist of winding threads of low density and fine, dense granules of 150 to 200 Å attached on the threads in detailed observation (fig. 15). The granules were clearly distinguished from the chromatins and the perichromatinic granules in the specimens of lead staining, though the size of both types of the loose and perichromatinic granules were similar. On the other hand, the dense granules of 150 to 200 Å appeared as a dense, spherical mass in the nuclear bodies (figs. 5, 8 & 22) and seemed to be that of chromatins at a glance, but were ascertained to be just similar to the granular portion of the nucleolus rather than the chromatin granules in the specimens of lead staining (fig. 11).

A close-relationship between the nuclear bodies and the nucleolus was ascertained in the mice, cats, cows and horses (figs. 4 & 17) and the finding was most prominent in the mice.

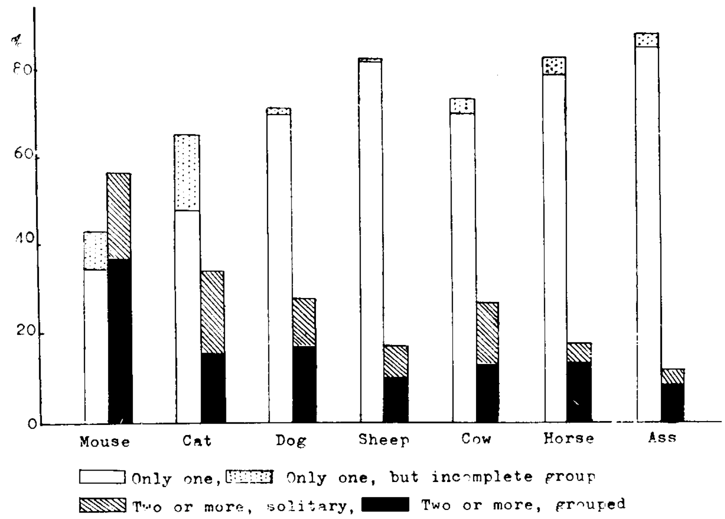
2 Number and size of the nuclear bodies

In this observation, the nuclear bodies of the small lymphocytes, the mesenchymal and epithelial reticular cells were examined without paying attention to cell types, because the structures of the bodies were found to be almost the same among different types of cells of lymphatic organs in an animal species. The size and the number of the nuclear bodies are shown in table 1 and figure 1.

TABLE 1 *Number and size of nuclear bodies*

ANIMALS	NUMBER OF NUCLEAR BODIES					SIZE
	1	2	3	4	Over 5	Mean value (Range)
Mouse	43.0 %	28.1 %	19.0 %	5.8 %	4.1 %	0.73 (0.25-0.92) μ
Cat	65.9	24.5	6.1	2.6	0.9	0.60 (0.25-1.40)
Dog	71.9	23.2	3.5	1.4	0	0.59 (0.25-0.92)
Sheep	83.0	15.4	1.1	0.5	0	0.77 (0.25-0.90)
Cow	73.1	23.0	2.6	1.3	0	0.93 (0.30-1.32)
Horse	82.7	16.5	0	0.8	0	0.63 (0.25-0.79)
Ass	88.3	10.9	0.8	0	0	0.67 (0.25-0.92)

FIGURE 1 *Appearance and number of nuclear bodies*



The sizes of the nuclear bodies, whose mid-planes were sectioned, were examined. This decision, whether the mid-plane of the bodies should be sectioned or not, was based upon the distinctness of the boundary between a filamentous outer layer and a clear halo surrounding it.

The nuclear bodies were usually larger in the cows than in other animals (figs. 14~17). The size of the bodies in the sheep were next to those in the cows, but those of the other animals were smaller in general.

The number of the nuclear bodies per nucleus varied from one to seven on photographs, and seemed to be different among animals. In some cells, furthermore, two or more nuclear bodies observed in a nucleus appeared in groups (figs. 3 & 23). In other cells, the central granular core of the bodies was incompletely divided by filaments which were continuous to those of the outer layer, even if the body seemed to be singular (figs. 6 & 22).

As shown in table 1 and figure 1, in the mice, 57% of the cells with nuclear bodies contained two or more bodies in their nuclei and the bodies of not less than one-fourth of the cells appeared in groups. In the cats, cells with two or more bodies comprised 31.4% of the total and it was noted that the nuclear bodies showing an incomplete group were numerously found. On the other hand, in the other animal species, most of the cells with nuclear bodies 71.9 to 88.3%, included a singular body in their nuclei and the cells with two or more bodies were very few.

3 Types of the nuclear bodies

The strctures of the nuclear bodies were very variable from cell to cell, though their basic components were similar among animals and among cells. The variation seemed to be based upon the presence of two kinds of granules or vacuoles, and the distinctness of the separation between filaments and granules.

a) Classification according to the granules and vacuoles

According to the appearance of the granules or vacuoles, the nuclear bodies were classified into five types: A; granules were nearly absent (fig. 14), B; only loose granules (fig. 7), C; both loose and dense granules were present (figs. 5, 12 & 16), D; only a mass of dense granules (figs. 8, 9 & 11) and E; vacuoles were contained (fig. 10).

TABLE 2 *Appearance of granular or vacuolar components*

ANIMALS	TYPES				
	A	B	C	D	E
Mouse	19.1 %	58.5 %	9.9 %	11.2 %	1.3 %
Cat	18.5	57.6	20.6	3.3	0
Dog	1.8	18.9	12.6	65.8	0.9
Sheep	1.0	49.0	40.4	9.6	0
Cow	0.8	66.4	23.2	9.6	0
Horse	4.7	59.1	30.5	5.7	0
Ass	0.9	56.5	34.3	8.3	0

A: Absent, B: Loose granules, C: Loose and dense granules,
D: Dense granules, E: Vacuole

As shown in table 2, type B with only loose granules were frequently observed and type C with both loose and dense ones were also numerously found in the animals except for the dogs. It was noted that type D with only a mass of dense granules often occurred in the dogs. The other types, especially type E, were not so often observed. This classification was sometimes difficult to apply to the nuclear bodies of the horse and ass, because their granular elements heavily intermingled with the filamentous one. However, the nuclear bodies of the horse and ass belonged to type B or type C in general.

b) Classification according to the separation between filamentous and granular components

According to this finding, the nuclear bodies were classified into three types, type I; filamentous and granular components of the nuclear bodies sharply constructed an outer layer and a central core respectively (figs. 7 & 8), type II; the bodies had a central core and more granular elements were intermingled in the filaments of the outer layer (figs. 9 & 15) and type III; filaments and granules so heavily intermingled with each other that their central core was not possible to distinguish (figs. 18~21).

TABLE 3 *Boundary between fibrillar and granular components*

ANIMALS	TYPES		
	I	II	III
Mouse	92.7 %	4.6 %	2.7 %
Cat	88.0	12.0	0
Dog	66.7	29.7	3.6
Sheep	15.4	79.8	4.8
Cow	12.8	78.4	8.8
Horse	4.6	23.9	71.5
Ass	10.1	23.2	66.7

I: Distinct, II: Partially distinct, III: Indistinct

As shown in table 3, type I was often found in the mice, the cats and the dogs. Type II was numerously observed in the sheep and cows and a large number of type III were observed in the horses and the ass.

c) Types of the nuclear bodies

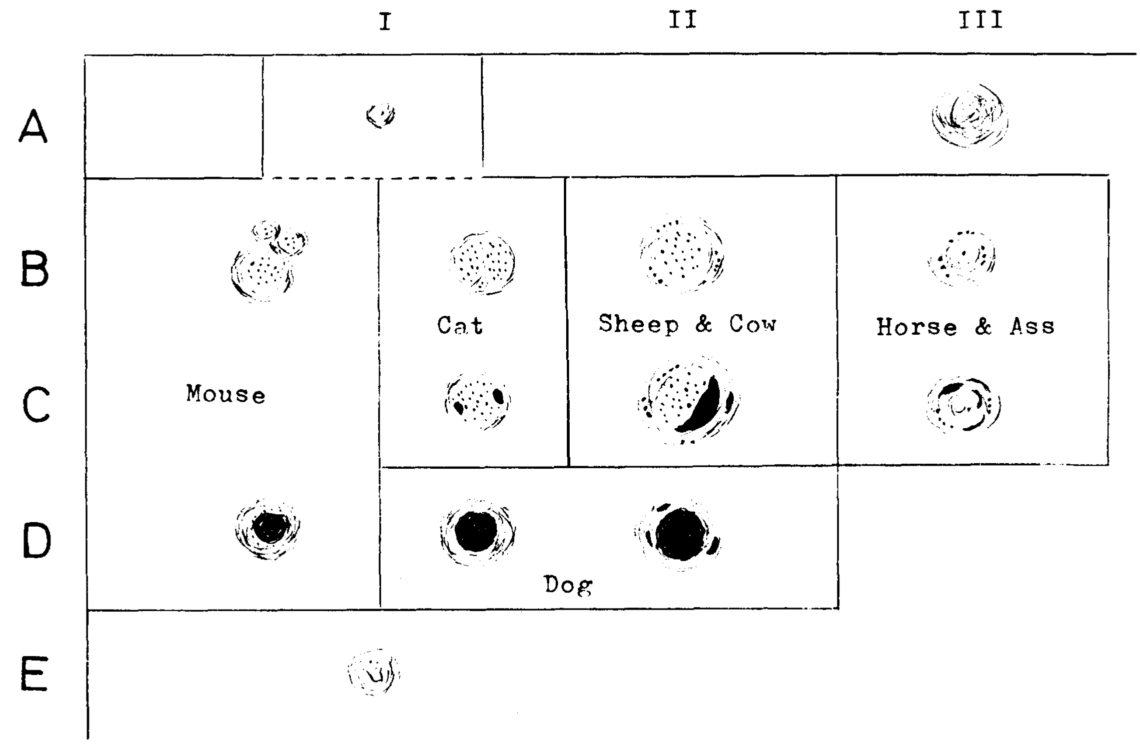
By the combination of both the classifications, 11 types of nuclear bodies were distinguished. As shown in table 4 and figure 2, the appearance of each type was variable, but some types of the bodies seemed to occur more numerously in some animals.

In the mice, type B-I comprised over half of the total and types A-I and D-I ranked next to it (fig. 3). The cats had a similar tendency to the mice, but in addition to types B-I and A-I, type C-I was considerably numerous. In the cat, it was worthy of note that the outer layer of the nuclear bodies was thinner than that in other animals (figs. 6 & 7). In the dogs, types D-I and D-II were often observed (figs. 8 & 9). In the sheep and cows, both of types B-II and C-II occupied over 70 % of total (figs. 12~17). In the ruminants, it may be specific to have a semilunar accumulation of dense granules (fig. 16). In the horses and ass, types B-III and C-III were often observed (figs. 18~21). In general, only a few of types A-III and E-I were observed in the animals used in this observation.

TABLE 4 *Types of nuclear bodies*

ANIMALS	TYPES										
	A		B			C			D		E
	I	III	I	II	III	I	II	III	I	II	I
Mouse	17.1 %	2.0 %	55.2 %	2.6 %	0.7 %	8.6 %	1.3 %	0 %	10.5 %	0.7 %	1.3 %
Cat	18.5	0	52.7	4.9	0	14.6	6.0	0	2.2	1.1	0
Dog	1.8	0	10.8	5.4	2.7	5.4	6.3	0.9	47.8	18.0	0.9
Sheep	0	1.0	5.8	39.4	3.8	5.8	34.6	0	3.8	5.8	0
Cow	0	0.8	6.4	52.0	8.0	3.2	20.0	0	3.2	6.4	0
Horse	0.9	3.8	1.9	12.4	44.8	0.9	2.9	26.7	0.9	4.8	0
Ass	0.9	0	4.6	16.7	35.2	0.9	1.9	31.5	3.7	4.6	0

FIGURE 2 *Types of nuclear bodies and animals*



DISCUSSION

Up to the present time, the ultrastructure of the nuclear bodies has been reported in many types of cells of several organs under normal and pathological conditions, but some unresolved problems seem to remain. The present writer investigated to see what structure is a basic pattern of the bodies, and whether there is or not a structural difference in the bodies among different animals in this report.

The nuclear bodies have been classified into three to seven types according to the appearance of granular and vacuolar components of the bodies by many investigators[1,4,7,8,11,13,15,18]. In this observation, the present writers could also classify the nuclear bodies into five types, A to E, according to the appearance of the granular or vacuolar elements as reported by the above-mentioned investigators. However, this classification could not clearly show the species-difference of the nuclear bodies, so the bodies were furthermore subdivided into three types, I to III, according to the distinctness of separation between filamentous and granular elements. In consequence of the combination of both the classifications, 11 types of nuclear bodies were distinguished in this observation.

HENRY & PETTS considered that the basic pattern of nuclear bodies is composed of finely fibrous materials which in its well-developed form have a whorled appearance, or is arranged as concenteric rings of microfibrils in the human thymuses. HORSTMANN et al. seemed to have a similar opinion; they reported that the body composed of fine filamets was observed in the basal cells in the observation of the human epididymis. DUPUY-COIN et al. also stated that conceivably, the distribution of differentiated types III and IV, which have granular components, is different from that nondifferentiated types I and II, which are composed of only fibrous elements and only the type I would be the constant one, though this point cannot be cleared up since types I and II can be confused with tangentially sectioned types III and IV.

The present writer observed the nuclear bodies in seven species of animals. In this observation, the bodies, whose mid-plane could not be sectioned, were omitted from the classification. The decision, as to whether their mid-plane should be sectioned or not, was based upon the distinctness of the boundary between a filamentous outer layer and a clear halo surrounding it. Actually, most of the omitted bodies were seen to belong, at a glance, to "basic patterns" by HENRY & PETTS, and they exceeded over about 35 % of total bodies observed. In this observation, it was ascertained that a few of the bodies were type A corresponding to the "basic pattern" or "nondifferentiated type", and a large number of them belonged to types B, C and D. Type E, containing vacuoles corresponding to the "complex form", was rarely found in this observation. As pointed out by BOUTEILLE et al., DAHL, HENRY & PETTS, HORSTMANN and WEBER et al.[23], the complex form seems to appear under only some hormonal stimulated or pathological conditions. Accordingly the usual pattern seems to be composed both of filaments and granules in all of the animals species observed.

The names of the "basic pattern" and "nondifferentiated type" would include a developmental or evolutional significance. SUGIMURA et al.[19] observed the nuclear bodies of various lymphatic organs in the mice and ascertained that the large nuclear bodies are most often found in the small lymphocytes of the lymph node, spleen and patch of peyer, but in the small lymphocytes of the thymic cortex, the small nuclear bodies are very rarely found. Furthermore, they showed a clear morphological evidence that the nuclear bodies have a close-relationship with the nucleoli. On the base of these findings and the recent reviews regarding migration-route of the thymic lymphocytes, they suggested that the nuclear bodies in the small lymphocytes are formed from a nucleolus in the thymic lympocytes at the first time and after migrating to the thymic medulla and to the other lymphatic organs, the small lymphocytes come to contain the large nuclear bodies. If this suggestion is justifiable, the basic pattern of the nuclear bodies should be small bodies with a filamentous outer coat and a small number of granular elements in a developmental meaning. This tendency could also ascertain in the nuclear bodies of the cow's lymphatic organs[20]. However, EVERETT et al. reported that short-life span lymphocytes are most numerous in the thymus and bone marrow, so it is possible that the tendency may show a difference between short- and long-life span lymphocytes.

Until the present time, the structures and types of the nuclear bodies were reported in many animal species; in the chickens[4], mice[11,18,19], hamsters[13], dogs[1,5,8], sheep[18], cows[20~23], monkeys[4], humans[2,7,10,13,17] and some plants[14]. Especially, BÜTTNER & HORSTMANN and HORSTMANN & BÜTTNER ascertained the presence of nuclear bodies, "sphaeridies", and described their structures in humans and 24 species of the mammals, birds and reptiles. However, nobody paid any attention to the species-difference of the nuclear bodies.

The present writer found that the structural types of the bodies differ among animal species, although this tendency may by limited in the lymphatic tissues. This fact is mainly due to distinctness of separation between filamentous and granular components in the body. In general, the separation is distinct in the mice, cats and dogs, it is incomplete in the sheep and cows, and both elements so heavily intermingle with each other in the horses and ass. There are further specific features in some species, though some of them may show a difference on an altered or special metabolic state in the cell nucleus as pointed out by BOUTEILLE et al., DAHL and KRISHAN et al.; in the cats, it is specific to have a thinner filamentous coat. In the dogs, it is noted that most of the central core of the bodies are composed of only a dense, spherical mass of granules. In the ruminants, it is specific that a semilunar mass of dense granules appear in the central core of the bodies. In the mice and cats, the number of the nuclear bodies per nucleus is more numerous than that of the other animals.

At the present time, it seems to remain an unresolved question concerning the relationship between the nuclear bodies and the nucleolus, and the chemical components of the bodies. WEBER et al.[23] considered that the bodies may serve to act as "nucleolar organizers", as the bodies are in close proximity to the location of the nucleolus. On the other hand, DUPUY-COIN et al. denied such a relationship by statistical observations and stated that this finding is not in agreement with the hypothesis that the nuclear body could be a "nucleolar organizer."

The present writer and colleague[18,19] showed actual evidence of a close relationship between the bodies and the nucleolus in the mice. Recently, KIERSZENBAUM also reported that the bodies are present in close proximity to the nucleolus and the dense central zone is extractable with cold perchloric acid in the same way as the nucleolus. In this observation, the present writer also ascertained a close relationship between the nuclear body and the nucleolus in the mice, cats, cows and horses. These findings do not decide whether the nuclear bodies are to act as "nucleolar organizers" or are originated from the nucleolus, but it is clear that they have a close relationship with the nucleolus. KRISHAN et al. reported that the fibrous nuclear bodies do not contain any DNA or RNA but may have proteins in their structure on the basis of enzyme extraction. From the present writer's findings, however, it seems that the chemical components of the bodies ought to be examined in relation to that the nucleolus. Actually, the photographs of single lead staining show that the dense granules of the nuclear bodies are just similar to the nucleolus rather than the chromatins, as pointed out by ISHII & MORII.

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EXPLANATION OF PLATES

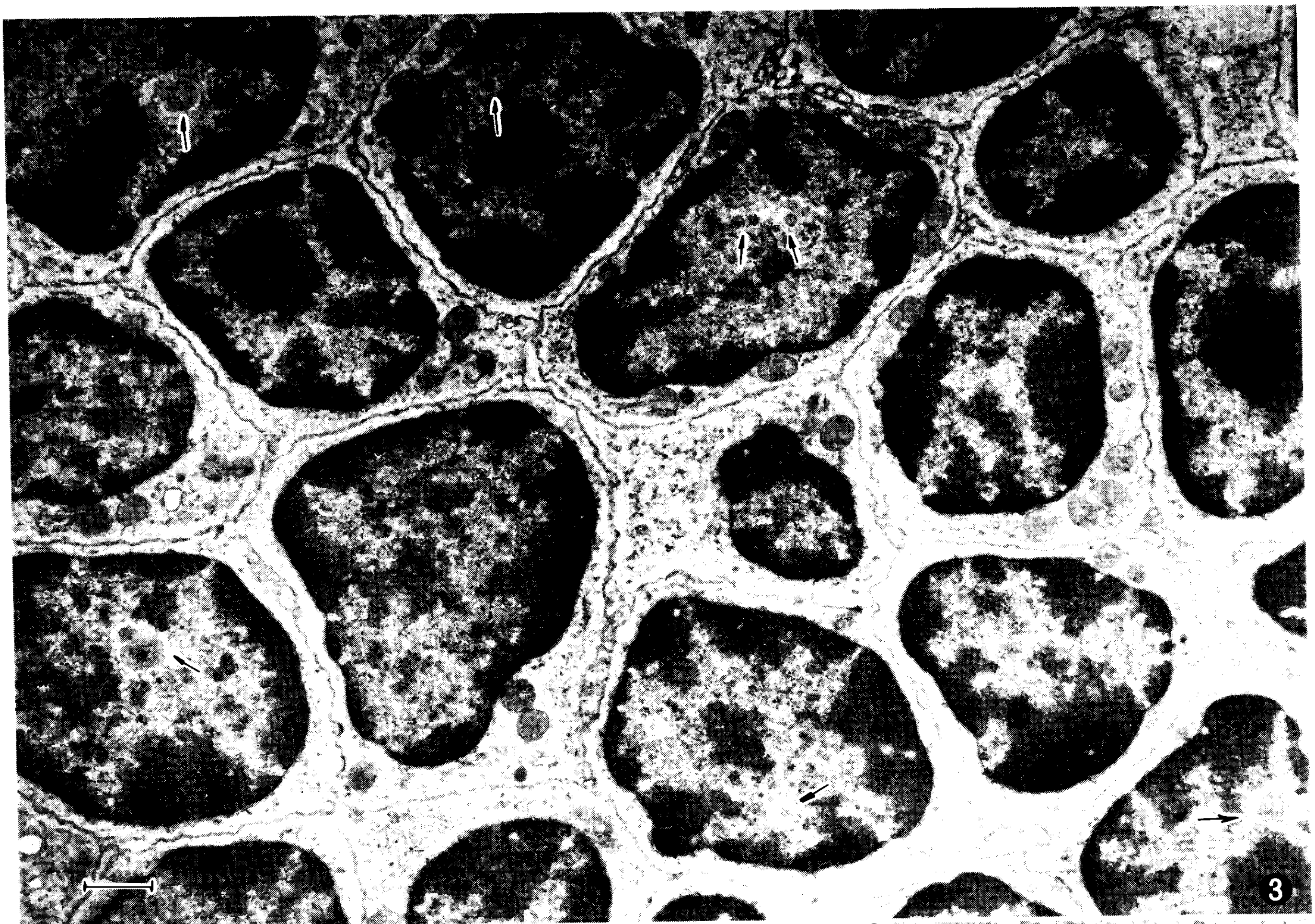
All figures are electron microscope photographs of sections stained with uranyl acetate (U) and/or lead citrate (L). All scales indicate 1 μ.

PLATE I

Fig. 3 Lymph node of the mouse: Six small lymphocytes include the nuclear bodies in their nuclei (arrows). U+L

Fig. 4 Lymphocyte in the mouse lymph node: Filaments (arrows), which are of the same structure as the outer coat of the nuclear bodies, cover the nucleolus (N). U+L

Fig. 5 Lymphocyte in the patch of Peyer of the mouse: Two nuclear bodies are found (arrows). A larger body is located in close proximity to the nucleolus (N). U+L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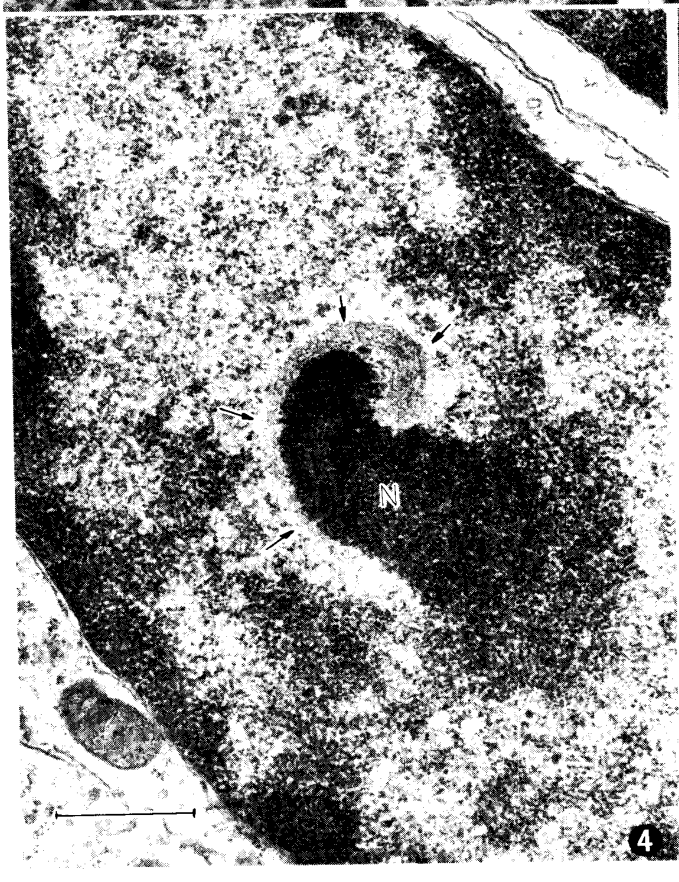
N
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N
5

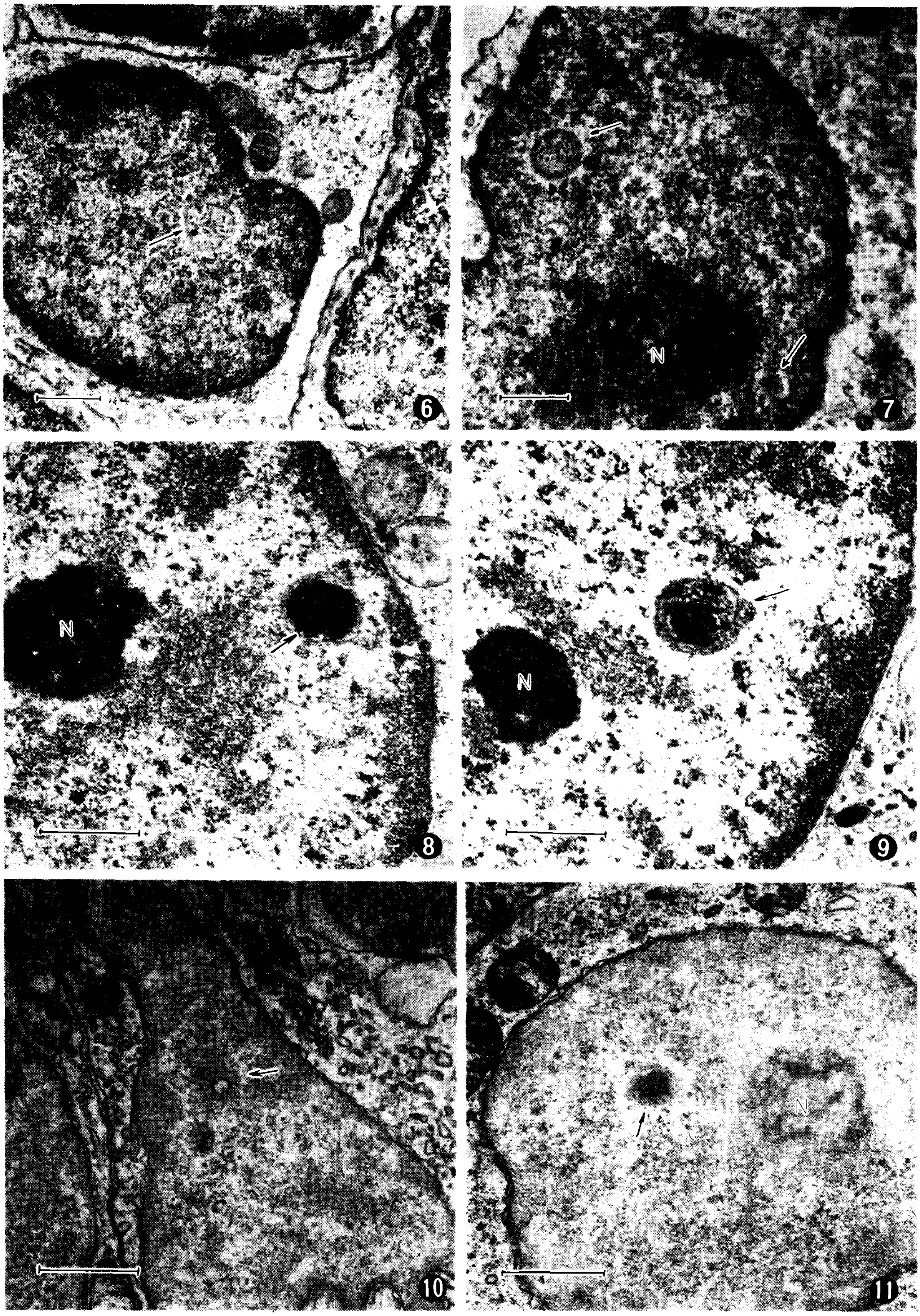
Plate II

Figs. 6 and 7 Lymphocytes in the cat lymph node: Nuclear bodies with a thinner coat are seen (arrow). In figure 6, the central core includes a small dense body and loose granules, and is trisected by filaments (type C–I). In figure 7, two nuclear bodies are observed. The upper one belongs to type B–I and the type of the lower one cannot be decided, because of sectioning tangentially. U+L

Figs. 8 and 9 Lymphocytes of the dog lymph node: The central cores of the bodies are formed with a mass of dense granules. In figure 9, the dense granules intermingle in the outer filaments. Those bodies belong to types D–I and D–II respectively. U+L

Fig. 10 Reticular cell in the dog lymph node: A nuclear body with a vacuole are seen (type E). L

Fig. 11 Lymphocyte in the dog lymph node: A nuclear body (type D–I) and a nucleolus (N) are seen. The granules of the central core are similar to those of the nucleolus rather than that of the chromatins. L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N
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Plate III

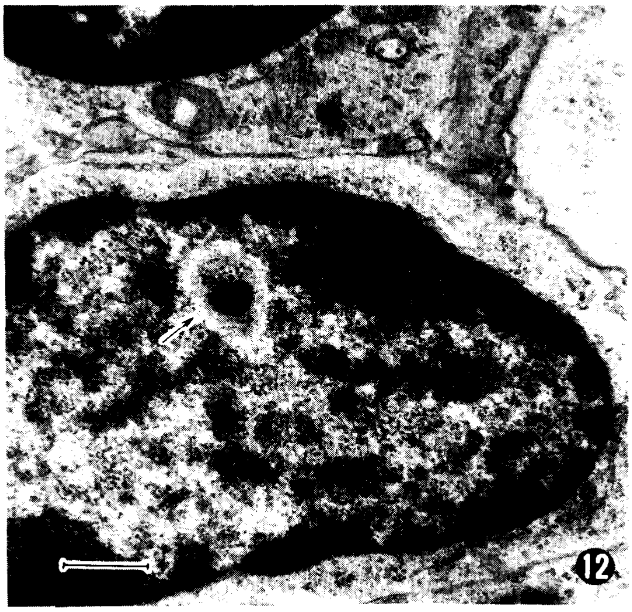
Figs. 12 and 13 Lymphocytes in the sheep hemal node: The central cores of the nuclear bodies include the dense and loose granules. In figure 12, the granules are also seen in the outer filamentous coat (type C–II). Figure 13 gives similar findings to figure 12, but there are no granules in the outer coat of the body (type C–I). U+L

Fig. 14 Endothelial cell in the cow hemal node: The body does not include granular elements (type A–III). U+L

Fig. 15 Lymphocyte in the cow hemal node: A large nuclear body is seen. The central core of the body consists of winding threads and fine granules attached on the threads. The granules are also seen in the outer filamentous coat (type B–II). U+L

Fig. 16 Reticular cell in the cow lymph node: The body includes the loose and dense granules in the central core. A mass of dense granules is semilunar in shape. The granules are also seen in the outer coat (type C–II). U+L

Fig. 17 Lymphocyte in the cow lymph node: A nuclear body is seen in close proximity to the nuclear envelope. A part of the nucleolus (N) is surrounded by a clear halo in which filamentous structures are observed (arrows). U+L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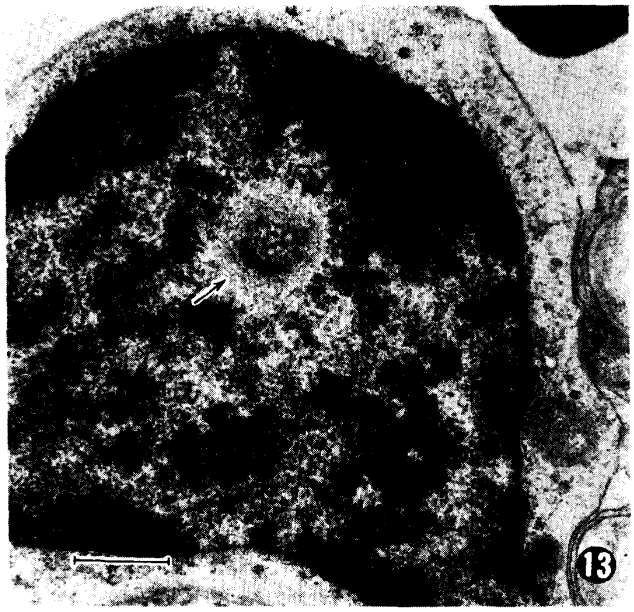
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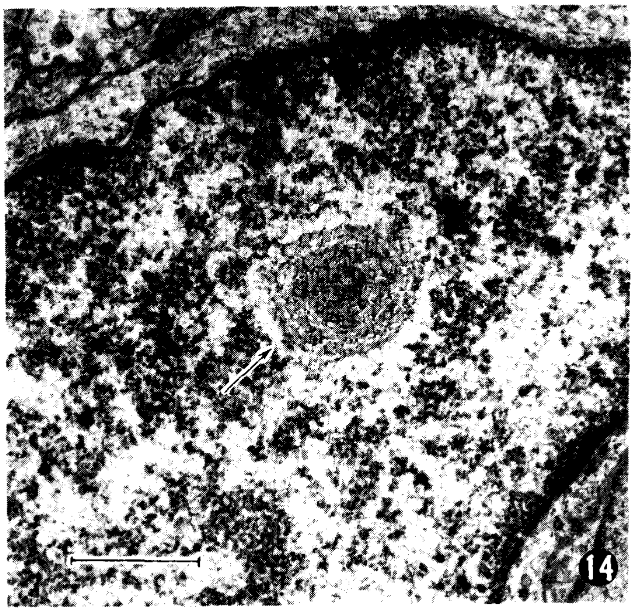
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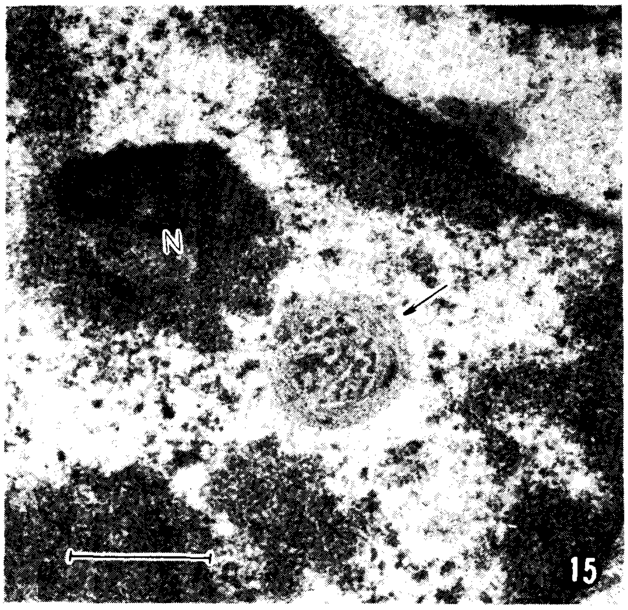
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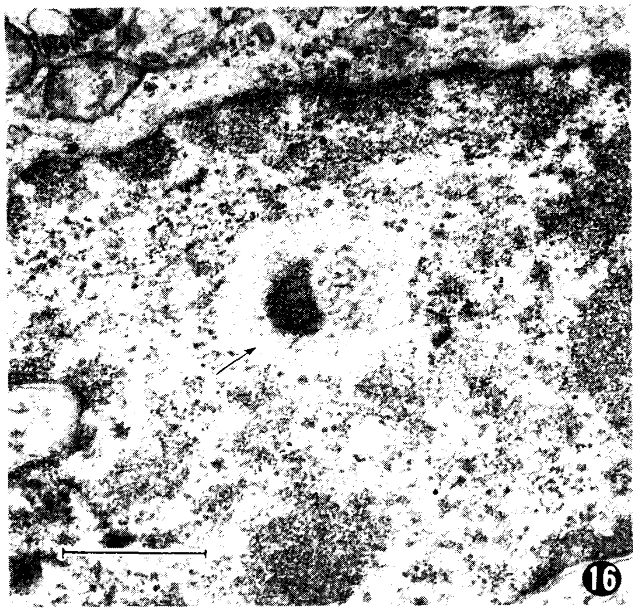
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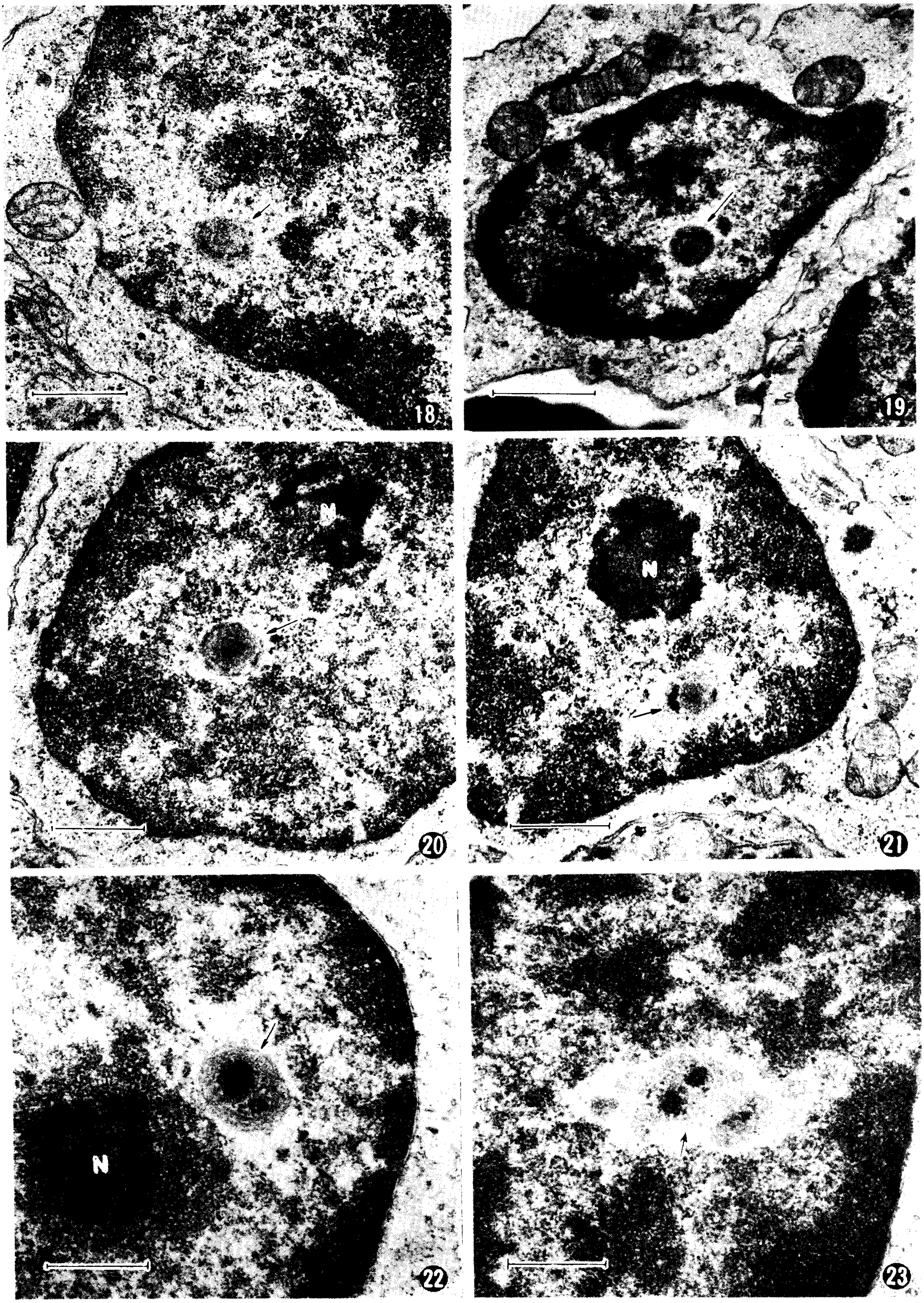
N
17

Plate IV

Figs. 18 and 19 Lymphocytes in the horse lymph node: A nuclear body of figure 18 has no central core, but granules are intermingled in the filamentous element (type B-III). Figure 19 gives similar findings to figure 18, but homogenous dense materials are arranged circularly. The body corresponds to type C-III. U+L

Figs. 20 and 21 Lymphocytes in the ass lymph node: The nuclear bodies are similar to those of the horse and belong to types B-III and C-III respectively. U+L

Figs. 22 and 23 Lymphocytes in the patch of Peyer of the mouse: The bodies are in incomplete and complete groups respectively. In the body of figure 22, the central core is bisected by filaments. In figure 23, the body consists of a group of five or six. U+L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