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Age-related Morphological Changes in Hassall's Corpuscles of Different Maturity in Vertebrate Animals and Humans

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Abstract—A comparative morphological study on Hassall's corpuscles of different maturity in vertebrate animals and humans with consideration of the age was carried out by light microscopy. It was found that the number and size of Hassall's corpuscles of different maturity depend on age, as well as the environmental conditions. Conclusions on the functional role of Hassall's corpuscles were drawn based on the present study.

Keywords: thymus, Hassall's corpuscles, vertebrate animals
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INTRODUCTION

Hassall's corpuscles (HCs) are an essential component of the thymus, which is actively involved in providing immune protection [1]. Being a central link of positive and negative selection, HCs are involved in the destruction of autoimmune *T* cell clones through phagocytosis and subsequent lysis [8–10] and are also able to synthesize chemokines, which affect different cell populations of the thymus medulla [12]. Aging of the organism is accompanied by regular changes in the thymus morphology and leads to impaired immunity [4, 7]. HCs are directly involved in these processes, since age-related change in the endocrine activity of the thymus is accompanied by an increase in their number and volume [2, 7]. Because the functions of HCs have not been finally established, the role of these structures in age-specific processes of the thymus involution is also unclear. One of the reasons for this is the lack of comparative morphological studies, which led to a limitation of the research material due to the small group of vertebrates. Meanwhile, the assessment of the nature of age-related changes in HC structure of animals differing in the level of organization, lifestyle, and environmental conditions, makes it possible to understand deeply the principles of morphological and functional HC organization and to evaluate features of age-related changes of the thymus with a new perspective.

The goal of the work was to study changes in the number and area of HCs of different maturity in a comparative morphological series of vertebrates with consideration of age.

MATERIALS AND METHODS

The study was carried out on the thymus of 17 vertebrate species belonging to four classes: amphibians (the class Amphibia)—pool frog (*Rana esculenta*, n = 36), common frog (*R. temporaria*, n = 28), common newt (*Triturus vulgaris*, n = 36); reptiles (the class Reptilia)—sand lizard (*Lacerta agilis*, n = 36), slow worm (*Anguis fragilis*, n = 32), common adder (*Vipera berus*, n = 24), grass snake (*Natrix natrix*, n = 36); birds (the class Aves)—rock pigeon (*Columba livia*, n = 36), jackdaw (*Corvus monedula*, n = 12), spotted flycatcher (*Muscicapa striata*, n = 16); mammals (the class Mammalia)—common shrew (*Sorex araneus*, n = 36), Laxmann's shrew (*Sorex caecutiens*, n = 24), bank vole (*Clethrionomys glareolus*, n = 46), American mink (*Mustela vison*, n = 20), house mouse (*Mus musculus*, n = 24), Ural field mouse (*Apodemus uralensis*, n = 32), human (*Homo sapiens*, n = 65).

The study was carried out on immature individuals and individuals of the second maturity period. The range of corresponding ages for humans was determined according to the classification adopted at the VII All-Union Conference on Age Morphology, Physiology and Biochemistry in 1965. The age of animals was determined by common methods [3, 6]. Immature animals of the following ages were studied: amphibians and reptiles—1–2 years, birds—1–3 years, insectivorous mammals—2–6 months, rodents—1–2 months, American mink—1–1.5 years. At the second stage of maturity, animals of the following ages were examined: amphibians and reptiles—4–6 years, birds—4–5 years, insectivorous mammals—1.5–2 years, rodents—2–3 years, American mink—3–5 years. Trapping of the animals was carried out in ecosystems undisturbed by anthropogenic influence at the territory of the National Park Smolensk Lakes,

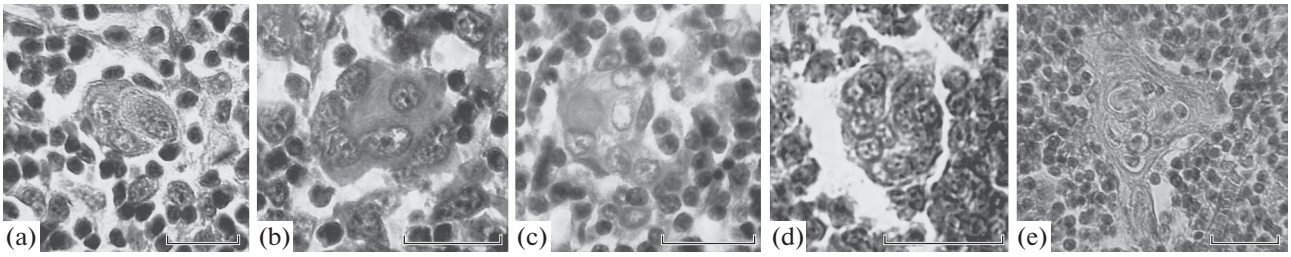

Fig. 1. Structure of Hassall's corpuscles phase I in vertebrates. a—common newt (immature), b—common frog (mature), c—sand lizard (immature), d—sand lizard (mature), e—human (immature); scale bar of 20 μm.

Demidovskii raion, Smolensk oblast. The thymus of American mink was obtained at Gagarinskii zveroplemhoz, Gagarin raion, Smolensk oblast. The thymus of fetuses that died due to asphyxia (24–39 weeks) was studied for humans. To investigate human thymus, material collected on the basis of the Department of Clinical Pathology at Smolensk Regional Institute of Pathology was used.

All of the sectional material was accurately selected according to anamnesis in order to eliminate the causes of death, which could affect or drastically change the structure of the thymus. A total of 265 preparations from immature vertebrates and 274 preparations from mature vertebrates were studied. Euthanasia of the animals was performed by ether anesthesia overdose (Vekton) in accordance with the requirements of the Ministry of Health of the Russian Federation for the work of experimental biological clinics, as well as the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

The thymus, withdrawn immediately after euthanasia, was weighed and measured. A portion of the thymus was fixed with 10% neutral formalin, dehydrated, and embedded in paraffin according to standard procedures. The thymus lobes were fixed with 10% neutral formalin, dehydrated, and embedded into paraffin according to the standard procedure. Thymus sections (5 μm) were made in sagittal and horizontal planes, stained with hematoxylin and eosin, picrofuchsin according to Van Gieson, and with aldehyde fuchsin and Halmi mixture according to Gabe–Dyban. Photomicrographs of the preparations, which were obtained by a Nikon CoolPix 7900 digital device (Nicon, Japan), were exported to a computer. Measurement of the area of thymus histological sections was performed by the program ImageJ 1.38 (National Institutes of Health, Bethesda, United States, free access on the Internet). The total area of the thymus histological preparation was measured at a magnification of 8X, lens 2 (MBS-9, LOMO, USSR). Under the total study of the preparation entire area, identified HCs of different maturity were counted, and their area in μm^2 were determined at a magnification of 15X, lens 40 (MBR-3, LOMO, USSR).

The classification proposed by O.V. Zairat'yants and M. Raica was taken as the basis for the distribution of corpuscles by maturity stages [2, 11]. All available HCs were divided into three groups: immature, mature, and aging HCs. Cell clusters with onset of keratin accumulation and lysis center formation were attributed to immature HCs—phase I (Fig. 1). Clusters in the form of concentric layers were attributed to mature HCs—phase II (Fig. 2). Clusters with necrosis and hyalinosis at the central part were considered aging HCs—phase III (Fig. 3). To compare the thymus of different vertebrate groups, the number of HCs was recalculated per unit of area (1000 μm^2). The average area for each type of HCs was considered; the parameters were represented in absolute (μm^2) and relative values expressed as fractions. The parameters of relative HC area were represented by the average values of the ratio of the total section area to all detected corpuscles on it, as well as corpuscles of each group individually. The significance of differences between compared groups was assessed by parametric and nonparametric statistics Student's *t*-test, Mann-Whitney *U*-test and Kruskal-Wallis test.

RESULTS AND DISCUSSION

The principles of HC structure in all vertebrates and humans are similar. The differences, which are associated with the age and organization level of vertebrates, are found only within single morphological characteristics. Thus, in mature vertebrates the number of cells forming immature HCs is significantly higher than in immature members of the same group. The number of cells involved in the formation of corpuscles is lowest in amphibians and mammals: 3–4 cells in immature and 5–6 cells in mature individuals (Fig. 1, a, b). In reptiles ga reater number of the cells is involved in HC formation. At the same time their number increases with age: 6–8 cells in immature and 8–12 cells in mature reptiles (see Fig. 1, c, d).

A similar situation is typical for birds: 7–10 and 10–15 cells in birds of the same age groups. In the human thymus, 5–12 cells participate in HC formation, regardless of age (see Fig. 1, e). In amphibians and mammals, regardless of age, the peripheral zone

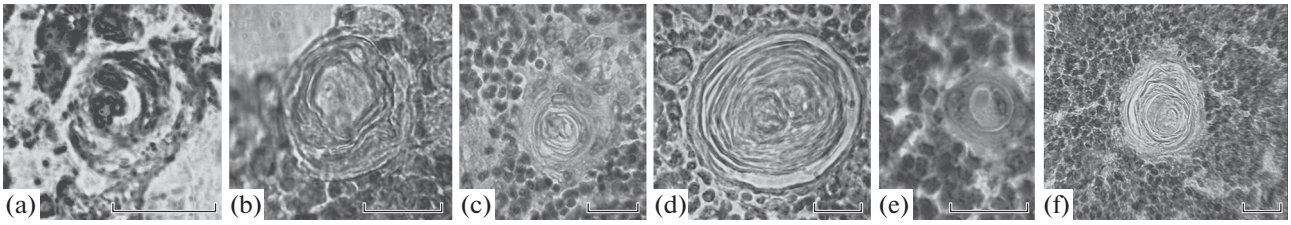

Fig. 2. Structure of Hassall's corpuscles phase II in vertebrates. a—common frog (immature), b—slow worm (mature), c—rock pigeon (immature), d—rock pigeon (mature), e—bank vole (mature), f—human (immature); scale bar of 20 μm.

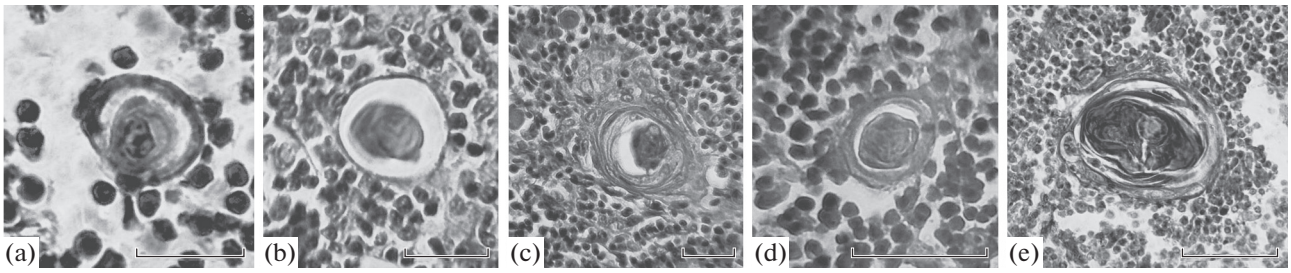

Fig. 3. Structure of Hassall's corpuscles phase III in vertebrates. a—pool frog (immature), b—slow worm (mature), c—rock pigeon (immature), d—bank vole (mature), f—human (mature); scale bar of 20 μm.

of HC II consists of 2–3 concentric layers surrounding a cavity with cellular infiltrate (see Fig. 2, a, e).

In reptiles age-related differences of HC II structure are absent, but 3–4 concentric layers of flattened cells are noted around a cavity filled with cellular infiltrate (see Fig. 2, b). HC IIs of birds are similar in structure to HC IIs of reptiles, but it is typical for only immature stage of the life cycle (see Fig. 2, c), while in the thymus of mature birds the number of concentric layers of the cells in HC IIs is twice as much: 6–7 (see Fig. 2, d). In humans, regardless of age, the number of such layers in HC IIs ranges from 4 to 6 (see Fig. 2, f).

In all vertebrates, regardless of age, HC IIIs have similar structures: 1–2 layers of tightly packed flattened cells surrounding a cavity filled with calcified content (see Fig. 3, a, b, d). In a mature human, as well as in birds, the number of such layers in HC IIIs can reach 3–4 (see Fig. 3, c, e).

Distinctive features of HC structure were found in the study of their relative size and number. In the vast majority of vertebrates, an increase in relative size of corpuscles of all maturity stages occurs with age. Only two species (American mink and human) differ by an age-related decrease in these parameters (Fig. 4). Comparison of relative CH areas of different maturity makes it possible to divide all of the studied vertebrates into two groups. The first group includes animals in which the value of the relative HC I area is greater than that of HC II and HC III regardless of age; these are amphibians, lizards, birds, insectivores, mammals, and rodents (see Fig. 4). In the thymus of all these animals, the relative size of HC II and HC III is similar. Snakes and humans can be attributed to the second group; their HC III exceeds other groups of corpuscles in relative size both at the immature stage and mature stage of life cycle (see Fig. 4). No statistically significant differences in the sizes of HC II and HC III were found in members of the second group. Only in the American mink at the first and the second age compared with other vertebrates is the relative size of corpuscles greatly reduced, and statistically significant differences in the relative area of corpuscles of different maturity are absent (see Fig. 4).

Comparison of absolute values of the HC area (μm^2) in contrast to the relative data leads to different results. In all vertebrates, regardless of age, the absolute (μm^2) sizes of mature HC groups are higher than the corresponding values for HC I. At the same time, during age-specific involution, the sizes of HCs II and HC IIIs are increased only in cold-blooded vertebrates (except snakes). In turn, in all warm-blooded vertebrates and snakes is the size of mature corpuscles reduced with age (Table). Birds, and especially humans, have enlarged HCs of all maturity stages, which is typical for one or the other age (see Table).

On the contrary, small mammals (rodents and insectivores) have the smallest HC sizes of all maturity stages, which can be observed in both immature and mature individuals (see Table). Among cold blooded vertebrates, snakes have HCs of the maximal size. For example, the areas of HC IIIs in immature and HC IIs in mature snakes are 1.5 and 2 times higher than in other cold-blooded animals, respectively (see Table).

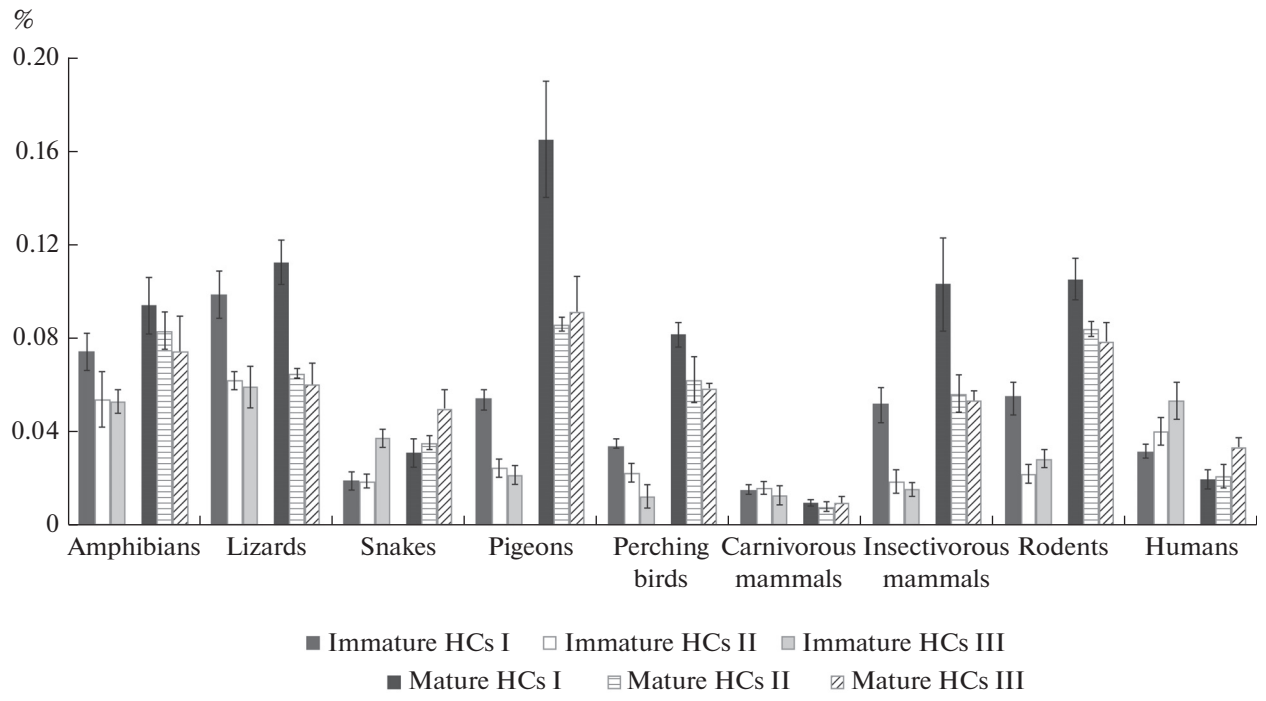

Fig. 4. Relative area of Hassall's corpuscles (HC) of different maturity (relative to the area of the slice).

In all vertebrates, regardless of age, the relative HC I number exceeds the corresponding values for HC IIs and HC IIIs. Only in the thymus of a mature human are the numbers of HC IIs and HC similar (see Table). Moreover, no significant changes in the number of HC Is was observed in amphibians and lizards with age. On the contrary, in warm-blooded vertebrates and snakes, the number of HC Is in the thymus of mature representatives, as compared to immature representatives, significantly increases (see Table). During aging in the thymus of humans and American mink, unlike other mammals, the number of HC Is decreases. The study of age-related changes in the number of mature HCs showed that an increase in the number of HC IIs and HC IIIs occurs in the thymus of the majority of vertebrates. The most potent increase is found in birds and small mammals. Moreover, in line with other parameters, a decrease in the number of mature HC groups was observed in American mink and humans with age. In cold-blooded vertebrates, age-related changes of these parameters do not have definite trend because of the primitive organization. An increase in the number of HC IIs was observed in amphibians and lizards with age. The number of HC IIIs increases only in snakes. No age-related changes in the number of HC IIs in snakes and HC IIIs in lizards were registered, while the number of HC IIIs in mature amphibians is totally reduced (see Table). It is remarkable that the representatives of the legless life form turned out to be more similar in the size and HC numbers of different maturity to mammals and birds than cold-blooded tetrapods (see Table).

Analysis of the results makes it possible to argue that the reasons leading to HC formation and the mechanisms controlling this process slightly depend on the organization level and therefore are determined to a greater extent by the similarity of the functions of these clusters. The similar principles of HC structure in all vertebrates serve as an evidence of this. It becomes obvious that HCs and their functions have been already formed at the very early stages of phylogeny in the first groups of land vertebrates. However, in ontogenesis, during aging of the organism, the role of HCs increases significantly, which is manifested by an increase in their number and area in all vertebrates.

The results of this study make it possible to interpret Hassall's corpuscles as indicators of the degree of age-specific thymus involution, not only in mammals and humans [7] but in all vertebrates. Thus, regardless of the organization level of chordates, the laws of age-specific involution turn out to be immovable and lead to a decrease in the endocrine activity of the thymus and to impaired immunity [2, 11]. In turn, the metabolism level is also an important factor that can affect the immune status of the organism [5]. It is no coincidence that more scale age-related changes in HC morphology are observed in warm-blooded vertebrates, whereas in cold-blooded representatives such changes are minimal.

Environmental factors play an important role in affecting the immune system [2, 5], which is evidenced by significant differences in the HC morphology and the dynamics of their age-related changes in

Morphometric parameters of Hassall's corpuscles of different maturity stages in vertebrate thymus ($x \pm S_x$)

Vertebrates	Immature vertebrates					
	immature CHs (phase I)		mature CHs, concentric layers (phase II)		aging CHs, necrotic cavities (phase III)	
	number, in 1 mm^2	S, μm^2	number, in 1 mm^2	S, μm^2	number, in 1 mm^2	S, μm^2
Amphibians	1.46 ± 0.4 * ° c, d, e, f, n, g, h	393 ± 48.6 d, e, h * °	0.17 ± 0.06 * ' c, d, e, g, h °	662 ± 89 ' b, d, n, g, h	0.26 ± 0.04 * * c, d, e, f, n, g, h '	577 ± 93 * ' c, d, e, f, n, h
Lizards	1.23 ± 0.3 * ° c, d, e, f, n, g, h	326 ± 65.2 * ° c, d, e, f, h	0.15 ± 0.04 * c, e, d, g, h ' °	393 ± 49.7 * ° a, c, d, e, f, h	0.26 ± 0.06 * c, d, e, f, n, g, h '	673 ± 68.9 * * c, d, e, n, g, h '
Snakes	0.19 ± 0.04 * * ° a, b, d, f, n, g	429 ± 75.8 « ° b, d, e, h	0.06 ± 0.01 ' a, b, d, f, n	649 ± 87.3 * b, d, n, g, h ' °	0.05 ± 0.01* a, b, e, g '	1313 ± 119 * ' a, b, f, n, g, h *
Pigeons	0.62 ± 0.15 * * ° a, b, c, e, f, g, h	876 ± 112 * * ° a, b, c, e, f, n, g, h	0.10 ± 0.01 * ' ° a, b, c, e, f, n	1202 ± 180* ' a, b, c, d, f, n, g, h	0.05 ± 0.01 * ' a, b, e, g *	1154 ± 103 ' a, b, f, n, g, h
Perching birds	0.19 ± 0.03 * * ° a, b, d, f, n, g	604 ± 93.4 * ° a, b, c, d, f, n, h	0.04 ± 0.01* ' ° a, b, d, f, n, g, h	610 ± 90.6 * ° b, d, n, g, h	0.01 ± 0.002 * a, b, c, d, f, n, g, h	1009 ± 95* * a, b, f, n, g, h '
Carnivorous mammals	0.31 ± 0.06 * * ° a, b, c, d, e, n, h	459 ± 70.3 ° b, c, d, e, h	0.15 ± 0.05 * ' ° c, d, e, g, h	589 ± 75.9 ° b, d, n, g, h	0.05 ± 0.01 * a, b, e, g ' *	795 ± 73.6 * * a, c, d, e, n, g, h '
Insectivorous mammals	0.53 ± 0.10 * * ° a, b, c, e, f, g, h	390 ± 61.9 * d, e, h °	0.16 ± 0.05 * ' ° c, d, e, g, h	334 ± 30.5 * ° a, c, d, e, f, h	0.06 ± 0.01 * a, b, e, g ' *	273 ± 33.7 * a, b, c, d, e, f, g, h
Rodents	0.37 ± 0.08 * * ° a, b, c ,d, e, n, h	419 ± 59.4 d, e, h	0.13 ± 0.03 * a, b, e, n '	353 ± 38.3 ° a, c, d, e, f, h	0.10 ± 0.04 * a, b, c, d, e, f, n, h '	455 ± 42.6 * b, c, d, e, f, n, h
Humans	0.15 ± 0.02 * * ° a, b, d, f, n, g	1486 ± 238 * * a, b, c, d, e, f, n, g °	0.10 ± 0.03 * a, b, e, f, n ' °	2834 ± 313 * ' ° a, b, c, d, e, f, n, g	0.06 ± 0.01 * a, b, e, g ' *	5128 ± 400* * a, b, c, d, e, f, n, g '
Vertebrates	Mature vertebrates					
	immature CHs (phase I)		mature CHs, concentric layers (phase II)		aging CHs, necrotic cavities (phase III)	
	number, in 1 mm^2	S, μm^2	number, in 1 mm^2	S, μm^2	number, in 1 mm^2	S, μm^2
Amphibians	1.21 ± 0.3 * ° c, d, e, f, h	481 ± 51.2 d, e, n, h * °	0.41 ± 0.08 * c, e, f, g, h ' °	655 ± 61.2 ' ° c, d, e, n, g, h	0.11 ± 0.03 * b, d, e, f, n, g, h ' *	951 ± 112.6 * c, e, f, n, g, h ' *
Lizards	1.31 ± 0.4 * ° c, d, e, f, n, h	563 ± 43.8 * e, n, g, h °	0.31 ± 0.09 * c, d, f, n, g, h '	751 ± 59.5 * ' c, d, e, n, g, h °	0.27 ± 0.07 a, c, d, f, n, g, h '	1015 ± 94.2 * a, c, e, f, n, g, h ' *
Snakes	0.41 ± 0.1 * * ° a, b, d, e, f, n, g, h	488 ± 40.6 d, e, n, h * °	0.05 ± 0.01 ' ° a, b, d, e, n, g	1023 ± 88.6 * a, b, e, f, n, g, h ' °	0.10 ± 0.02 * ' a, b, d, e, f, n, g, h *	632 ± 50.3 * a, b, d, e, n, g, h ' *
Pigeons	2.30 ± 0.6 * * ° a, b, c, e, f, n, g, h	682 ± 57.1 * * a, c, f, n, g, h °	0.47 ± 0.1 * ' b, c, e, f, g, h	941 ± 89.1 * ' a, b, c, e, f, n, g, h	0.59 ± 0.1 * ' a, b, c, e, f, n, h	1027 ± 71.5 ' c, e, f, n, g, h
Perching birds	0.80 ± 0.2 * * ° a, b, c, d, f, g, h	777 ± 64.4 * * ° a, b, c, f, n, g, h	0.23 ± 0.09 * ' a, c, d, f, n, g, h	1357 ± 200 * ' a, b, c, e, f, n, g, h °	0.27 ± 0.06 * ' a, c, d, f, n, g, h	2196 ± 228 * ' a, b, c, d, f, n, g, h *
Carnivorous mammals	0.11 ± 0.03 * ° a, b, c, d, e, n, g, h	524 ± 43.1 d, e, n, h *	0.07 ± 0.01 * ' a, b, d, e, n, g, h °	661 ± 48.2 c, d, e, n, g, h	0.02 ± 0.004 * a, b, c, d, e, n, g	555 ± 39.4 * a, b, d, e, n, g, h
Insectivorous mammals	0.93 ± 0.22 * * b, c, d, f, g, h °	292 ± 24.6 * * a, b, c, d, e, f, g, h	0.48 ± 0.1 * b, c, e, f, n, g, h	452 ± 40.1 * ' a, b, c, d, e, f, h °	0.35 ± 0.1 * ' a, b, c, d, e, f, g, h	245 ± 23.8 * a, b, c, d, e, f, g, h
Rodents	1.54 ± 0.4 * * c, d, e, f, n, h °	413 ± 34.8 b, d, e, n, h	1.11 ± 0.3 * ' ° a, b, c, d, e, f, n, h	418 ± 34.6 a, b, c, d, e, f, h	0.68 ± 0.2 * ' * a, b, c, e, f, n, h	377 ± 26.1 a, b, c, d, e, f, n, h
Humans	0.03 ± 0.008* a, b, c, d, e, f, n, g	2168 ± 294 * * a, b, c, d, e, f, n, g °	0.04 ± 0.01 * a, b, d, e, f, n, g °	5357 ± 436 * ' a, b, c, d, e, f, n, g °	0.02 ± 0.003 * a, b, c, d, e, n, g '	3697 ± 386 * * a, b, c, d, e, f, n, g '

* Significance of age-specific differences ($p \le 0.05$); '—significance of differences ($p \le 0.05$) compared with CHs I; «—significance of differences ($p \le 0.05$) compared with CHs II; °—significance of differences ($p \le 0.05$) compared with CHs III; significance of differences ($p \le 0.05$) compared with: a—amphibians; b—reptiles; c—snakes; d—pigeons; e—perching birds; f—carnivorous mammals; n—insectivorous mammals; g—rodents; h—humans.

two unrelated species (human and American mink—the number of the cells). This is associated with the influence of similar factors on immunity induced by anthropogenic environment: lack of exercise and the use of antibiotics and vaccines.

Also, a certain influence on the HC structure results in morphological and functional characteristics of the organism arising within the one or another life form. It has been found that the transition of reptiles to the legless life form led to a significant reduction in the number and a decrease in the relative volume of HCs. Regardless of age, snakes, according to many parameters of the HC morphology, are more similar to warm-blooded vertebrates than to reptiles, the group to which they belong. The specific toponymy and features of bird and human mobility determine the different natures for the contact of the organism with antigenic environmental factors affecting the HC structure, especially by increasing the number of the cells constituting the corpuscle and increasing their absolute size (in μm^2).

CONCLUSIONS

The results of the study make it possible to argue that the morphology of Hassall's corpuscles depends on a number of factors: age, environmental conditions, belonging to the taxon, intensity of metabolic processes, and, in part, organization level. They perform a number of functions, the value of which increases during the aging of the thymus. Most likely, such functions include regulation of the processes associated with the destruction of cellular material.

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Translated by D. Novikova

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