CHANGES IN HASSALL'S CORPUSCLES IN REGENERATION OF THE THYMUS AFTER EXPOSURE TO X-RAYS

D. G. Malkina

Department of Histology and Embryology (Head, Corresponding Member AMN SSSR, Professor A. A. Voitkevich) Voronezhskii Medical Institute (Director, Professor N. I. Odnoralov)
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In numerous studies, the participation of the thymus and other lymphoid organs in bringing about adaptive responses has been demonstrated [1, 2, 4, 5, 10, 11]. The thymus is highly sensitive to a number of factors. It has been shown experimentally that trauma, poisons, and infections induce microscopic changes in it [6, 9], and it is particularly sensitive to irradiation [3, 7, 12, 13, 14]. Penetrating radiation affects the structural elements comprising the lymphocytes, epithelial cells and Hassall's corpuscles.

In the work reported here, we have used Hassall's corpuscles as the most typical structures of the thymus, in order to study morphological changes taking place during regeneration after x-irradiation. We were also interested to find out whether the cytological changes in the corpuscles indicated secretory activity.

EXPERIMENTAL METHOD

The work was carried out on 100 young guinea pigs of both sexes weighing 150 - 250 g. In all of them, one third of the right half of the thymus was removed under ether anesthesia. On the second day after the operation, one group was exposed to a single dose of 400 r of x-rays, and the other to a local irradiation of 800 r; the third group was not irradiated, and served as control.

Material for a histological study was taken from 2 to 30 days after the operation, fixed in Zenker, and embedded in paraffin. Sections were stained in Meier's hematoxylin and eosin, by van Gieson's method, and in Heidenhain's azan.

In each animal a study was made of the extirpated portion of the thymus, as representing the initial condition, as well as the part which remained, which in each section was arbitrarily divided into two zones: at periods close to the time of operation (2nd, 5th, 10th, and 20th days) the division was made into a zone bordering on the damaged area and one which was remote from it; long after the operation (on the 30th day) when some regeneration had occurred, the division was made into the regenerated zone, and an area distant from it.

The corpuscles were counted and measured in diameters, in five fields of each zone, and on each of ten sections of a single thymus. Separate measurements were made of the diameter of the small and the large corpuscles. Tables 1 and 2 show the mean values.

EXPERIMENTAL RESULTS

When the irradiation was general, during the first few days after the operation in the zone adjacent to the damaged area the number of Hassall's corpuscles was reduced, and so was their maximum size (Tables 1 and 2); the size of the smallest corpuscles showed a small increase. Characteristically, the corpuscles showed a considerable variety of structure. There were small corpuscles consisting of only one or two cells, which stood out in the medulla as markedly oxyphil, or else as staining very palely (Fig. 1 a). The nuclei of these cells were large, and they contained a very small amount of finely granular chromatin. In the cytoplasm there were frequently small basophil granules of uniform size, which sometimes formed quite large clumps unevenly distributed throughout the cell.

Around the newly formed corpuscles the adjacent cells of the epithelial basis of the parenchyma gradually separated into layers; these cells were flattened and arranged concentrically around the central cells (Fig. 1 b, c).

TABLE 1. Number of Hassall's Corpuscles in Different Zones of the Thymus

Times when killed (days)	Zone of thymus	Conditions of experiment		
		General irradiation	Local irradiation	No irradiation (control)
2	U	6	9	7
	P	5	5	4
	O	9	10	5
5	U	9	8	10
	P	4	2	3
	O	10	9	4
10	U	10	9	10
	P	3	1	4
	O	7	6	7
20	U	9	12	10
	P	2	1	3
	O	7	7	6
30	U	10	15	9
	P	1	2	2
	O	6	5	8

Symbols: U) extirpated portion; P) zone of old tissue adjacent to damaged area; O) zone at some distance from damaged area; R) regenerated tissue.

TABLE 2. Size of Hassall's Corpuscles (minimum and maximum size) in the Different Zones of the Thymus (in μ).

Times when killed (in days)	Zone of thymus	Conditions of experiment		
		General irradiation	Local irradiation	No irradiation (control)
2	U	18,7—81,7	17,5—127,4	17,1—72,2
	P	19,7—67,1	19,9—69,7	19,4—53,6
	O	17,5—59,9	20,7—86,3	17,7—54,2
5	U	18,2—96,9	19,0—218,6	18,2—71,1
	P	22,1—85,8	18,2—62,0	18,8—49,5
	O	19,2—76,5	20,6—93,9	18,7—38,9
10	U	18,3—94,8	17,7—167,4	20,0—71,3
	P	19,4—83,3	36,3—133,8	17,7—36,6
	O	18,3—60,9	25,6—137,4	18,5—52,1
20	U	15,7—143,5	18,9—156,8	17,9—64,1
	P	21,1—55,4	20,7—79,9	17,2—35,9
	O	21,1—103,0	22,0—147,0	17,3—52,8
30	U	15,3—134,2	20,3—159,0	20,5—52,7
	P	22,3—53,4	21,2—68,2	18,4—61,3
	O	20,2—75,1	19,0—89,3	18,4—90,4

Symbols: Indications as in Table 1.

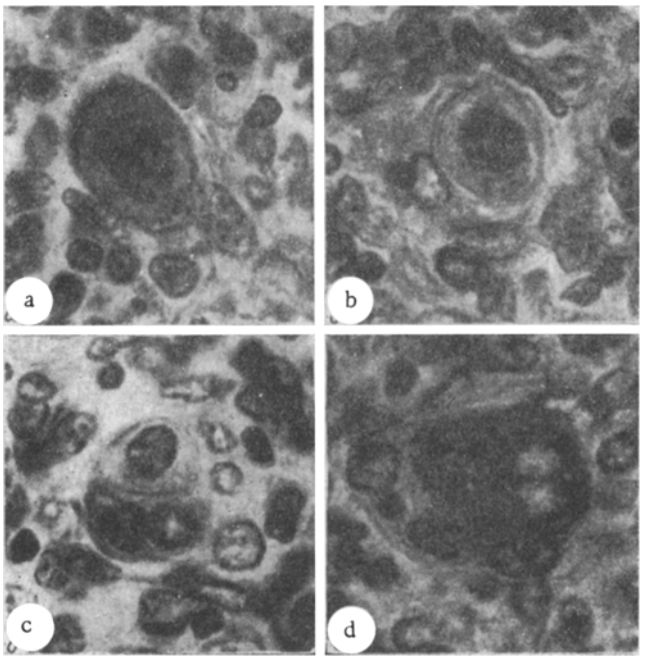

Fig. 1. Hassall's corpuscles after total x-irradiation a, b, c) newly formed corpuscles; d) vacuoles in the cytoplasm of a central cell. Magnification 1200.

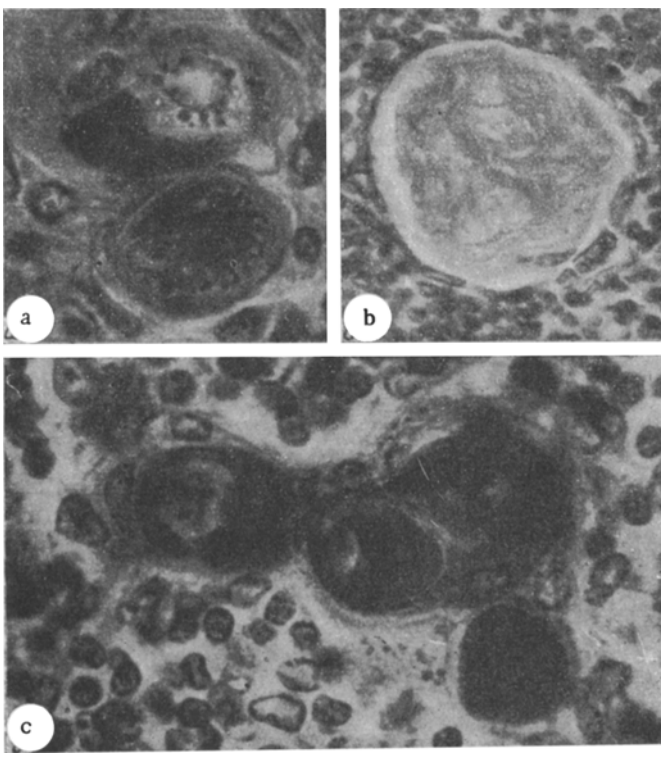

Fig. 2. Hassall's corpuscles (general irradiation). a) Extrusion of drops of chromatin from the nucleus; b) corpuscles with mucous detritus in the center, and flattened epithelium at the periphery; c) merging of corpuscles. Magnification 1200.

As a result of this layering of the cells, large and more complex bodies were formed. In the cytoplasm of the central cells there were sometimes pale, clearly outlined vacuoles, arranged either singly, or in groups (Fig. 1 d). In some of the central cells, grains of chromatin were formed in the cytoplasm, which indicated the breakdown of the nucleus (Fig. 2 a).

In many corpuscles, the central cells had become cornified, the nuclei had disintegrated, and the cytoplasm had either become homogeneous, or else had a lamellar structure; the glistening appearance was due to the strong refraction of the light rays. On this account, in the center of the corpuscle a detritus was formed, which stained orange or bright red with Heidenhain's azan. The peripheral cells of the corpuscle were swollen to various extents. Their nuclei were pale and vesicular. In some of the cells there were vacuoles, and in others, basophil granules; in some cases fibrous structures were present in the cytoplasm.

Frequently corpuscles lying close together merged to form complex structures more than 100μ in diameter (Fig. 2c). Some large corpuscles consisted of a mass of lamellar detritus which stains to various depths, and there was one layer of greatly flattened cells at the periphery (Fig. 2b). There were also bodies with a detritus containing leukocytes (neutrophils, lymphocytes, and less frequently eosinophils), which phagocytoze breakdown products.

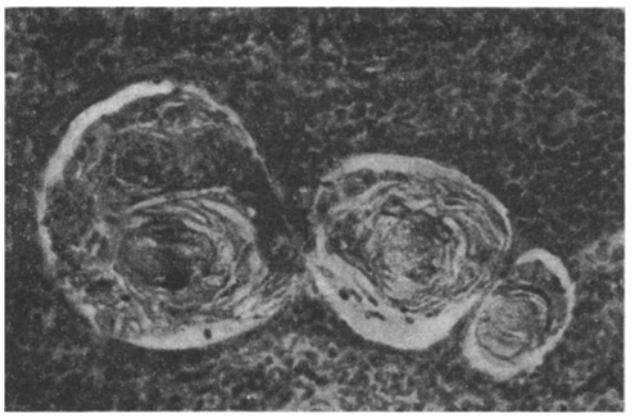

Fig. 3. Four corpuscles combined to form one giant corpuscle (local irradiation). Magnification 240.

In the zone remote from the damage, as can be seen from the Tables, during the 2-5th days after the operation, the number of corpuscles increased to above the initial amount, and the corpuscles reduced in size. Here too the corpuscles were of many shapes, and in the medullary portion of the lobules all the different modifications described above could be found. However, it should be noted that in this zone the corpuscles were usually large and contained detritus in the center; newly formed corpuscles made up of many cells were seldom encountered.

Later, on the 10th, 20th and 30th days after the damage, the number of corpuscles in the thymus fell, both in the zone adjacent to the damage and in the area remote from it (see Table 1); this change was best shown on the 30th day in the regenerated tissue. The size of the corpuscles in both the zones had changed (see Table 2). The maximum dimensions were smaller, particularly in the area adjacent to the cut portion, and in the regenerated tissue; on the other hand, the size of the smallest corpuscles had increased somewhat. There were practically no structural features of the corpuscles related to the zones. Only certain individual differences could be perceived. In some animals, small nuclei consisting of 214 cells preponderated, and they had pale nuclei and an oxyphil protoplasm. In others, large corpuscles were in the majority, and they contained a glistening detritus in the center, surrounded by swollen or flattened cells.

After local irradiation (see Tables 1 and 2) at all stages of the repair, the number of corpuscles and their dimensions changed in the same way as after general irradiation. During the first days after the operation, particularly in the zone remote from the damage, large corpuscles were most commonly encountered, and they consisted of swollen cells with large nuclei which quite frequently extruded drops of chromatin. In the cytoplasm of the cells there were numerous vauoles or clumps of basophil material. At the periphery the cells were swollen, but towards the center they became increasingly flattened and cornified, and merged with the detritus present in this region.

With local irradiation, at a late stage of regeneration, only large corpuscles were found; often they were complex structures derived from several corpuscles, and sometimes attained a great size (Fig. 3). These corpuscles consisted essentially of a lamellar detritus, stained various shades, and containing fragments of cells and nuclei. Around the detritus there are one or two layers of greatly flattened cells with elongated nuclei.

It can be seen therefore that the morphological changes developing in the corpuscles of a regenerating thymus under the influence of local irradiation were more uniform than when the radiation was general. In the latter case, there was a great variety in the shape of the corpuscles, chiefly in the zone adjacent to the damage and in the area remote from it; in the regenerating portion there was little variation in structure.

The results indicate that general and localized x-irradiation of the thymus during regeneration produced marked morphological changes in Hassall's corpuscles. The changes were more marked in the early stages of regeneration, i.e. in the first days after irradiating. Later, from the 20th to the 30th day, the reactive changes in the corpuscles were much less well shown.

The changes which we have observed in the cells of Hassall's corpuscles, which consisted of a swelling of the cells, extrusion of drops of chromatin from the nuclei into the surrounding cytoplasm, and the development of granularity and vacuoles in the cytoplasm led to cornification of the cells, their death, and to the formation of detritus in the center of the corpuscle. The changes resemble those occurring in the cells of an epidermis. There is no good reason to interpret these changes as representing a secretory process, as do some investigators [8, 9]. On the contrary the morphological changes in the cells of Hassall's corpuscles clearly represent a breakdown process, and as Sh. D. Galustyan [3] urges, they indicate a specific differentiation of a stratified epithelium.

SUMMARY

Morphological changes in Hassall's corpuscles were studied in guinea pigs during regeneration of the thymus, after local and general x-irradiation. The structural changes in the cells, i.e. swelling, extrusion of chromatin droplets from the nucleus, vacuolization of the cytoplasm, cornification, and other signs, represented an ordered reconstruction of the stratified epithelium. They were destructive in character, and should not be regarded as representing a secretory process.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
