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Origin and Fate of Cells in the Medulla of Rat Thymus.* (24226)

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Recently, counts were made of the resting and dividing nuclei of the 4 main cell types present in the cortex of rat thymus (reticular cells, large, medium, and small lymphocytes) and a scheme based on these counts was proposed to explain the production of lymphocytes in this tissue (Fig. 1). Briefly, mitoses of reticular cells would yield an equal number of large lymphocytes and of new reticular cells. The large lymphocytes would then begin a series of successive divisions, so that a total of 8 generations of smaller and smaller lymphocytes would be produced and thus, each initial large lymphocyte would yield 128 "mature" small lymphocytes(1). The present work consists of counting cells and mitoses in the medulla of thymic lobules, using procedures devised to study the cortex(1). The immediate aim was to find out whether or not the pattern of lymphocyte formation observed in the cortex also applied to the medulla. Furthermore, since the investigation of the cortex had given no clues as to how lymphocytes leave the thymus, it was hoped that examination of the medulla would clarify this problem.

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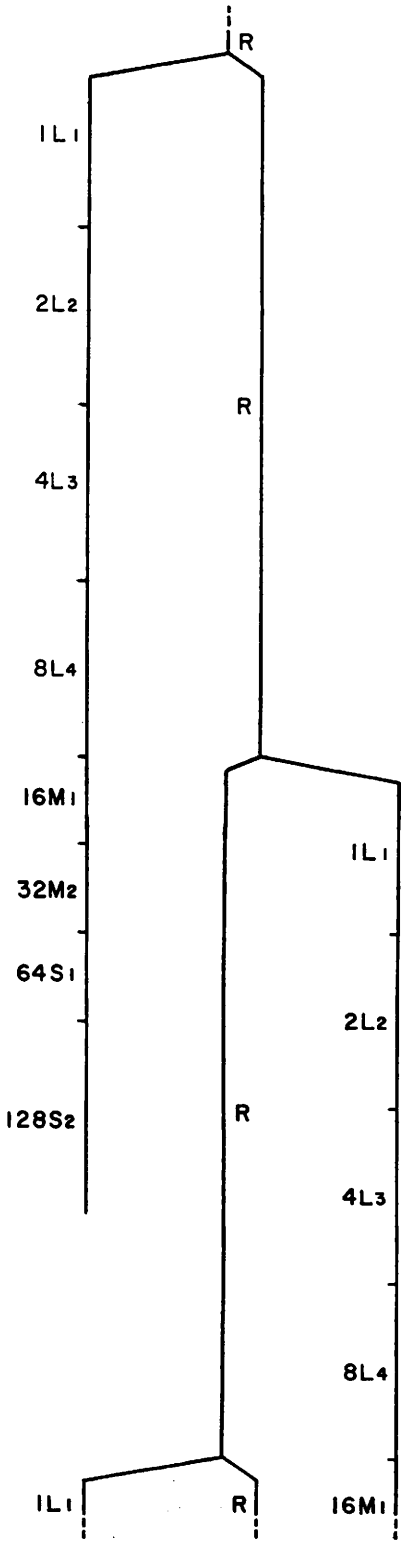
Methods. The same thymus sections as were used in investigating the cortex of young adult rats (Bouin-Hollande fixation, Dominici staining) were utilized in the present study. Resting and dividing cells were counted in 625 μ^2-fields of medulla. It had been previously found that the cortex could be divided into 2 layers: peripheral cortex making up 9%, and deep cortex making up 66% of the parenchymal thymus(1). The medulla accounts for the remaining 25%.

Results. Cytological observations. The 4 cell types seen in the cortex (Fig. 2) were also present in the medulla, but with a few minor differences in their appearance. The cytoplasm of *reticular cells* stained more intensely and was more distinct in medulla (Fig. 7) than in cortex. The nuclei of some cells contained granules of chromatin-like material often present within vacuoles (Fig. 7, lower center)—a picture interpreted as degeneration(2). Moreover, other stages of degeneration up to complete dissolution of nuclear material were seen, particularly in some reticular cells associated with, or making up Hassall's corpuscles (Fig. 7, upper left).

A few of the *large* and *medium* lymphocytes in medulla had irregularly shaped nuclei. Such irregularities were most pronounced in

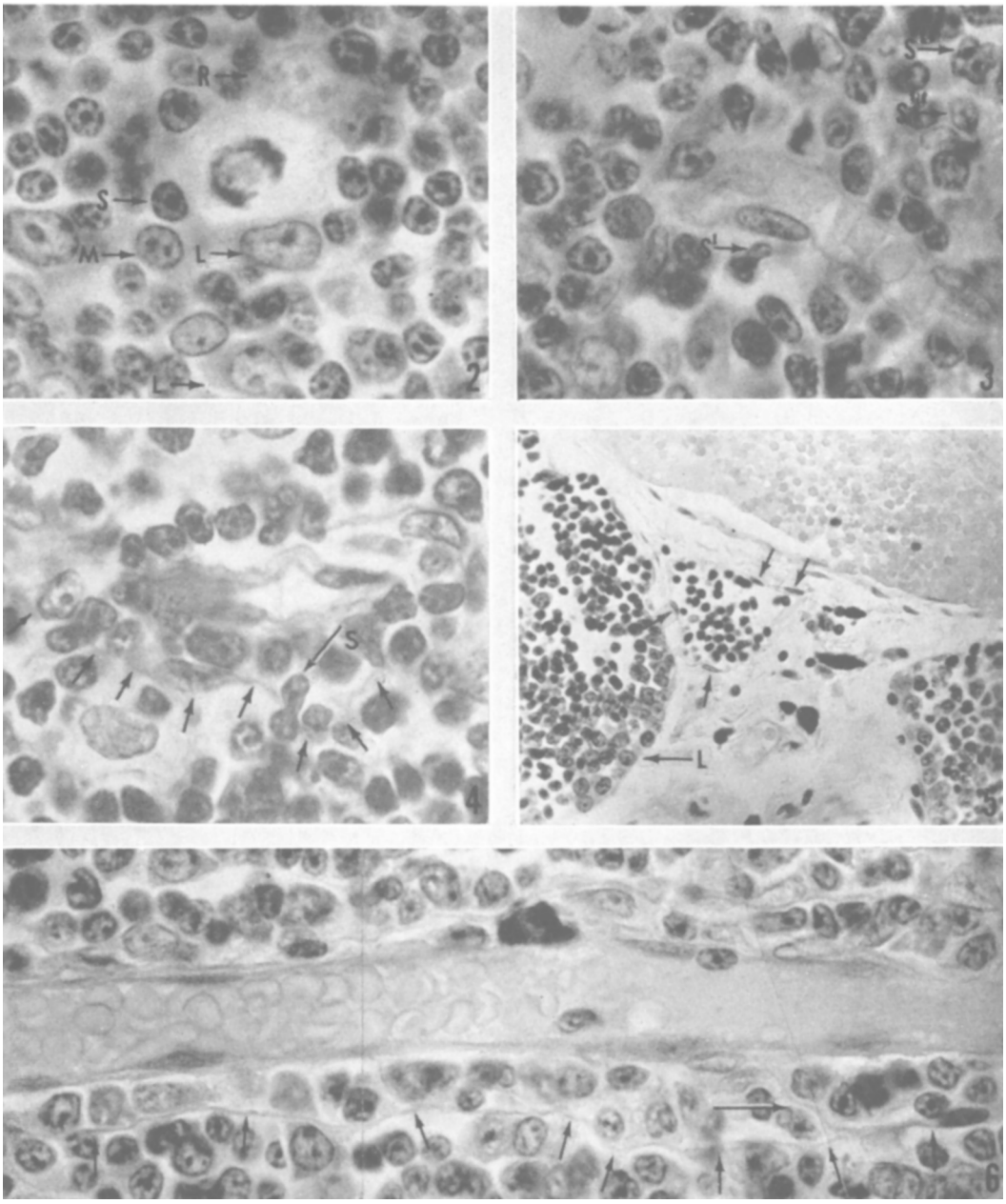


small lymphocytes, which in addition had slightly larger and paler nuclei than in cortex(2,4,5). Pseudopod-like processes (Fig. 3, S′ and S″) and tube-like extensions of the nuclei(3; Fig. 3, S‴), rod-like and hour-glass shaped nuclei (Fig. 4, S) and other nuclear distortions were common in the small lymphocytes of medulla, but were rarely seen in cortex. Such figures were interpreted as indicating that the ameboid activity of the cells is pronounced in medulla.

The *vascular system* was examined in thymus sections in the hope of finding clues as to mode of release of lymphocytes(2,6-9). Typical lymph vessels were rare (Fig. 5), but lymphatic spaces associated with venules or other blood vessels were common in medulla and trabeculae. In cross section, such spaces were seen to surround blood vessels, which were thus enclosed as in a coaxial tube. Hence they will be referred to as "perivascular lymphatic channels." These structures, which were seen by Smith(10,11) (although this author may not have appreciated their significance) were composed of thin, barely visible. endothelium-lined walls, which may be collapsed or enclose rows of lymphocytes (Fig. 6). The walls of these perivascular lymphatic channels often showed cells with constricted nuclei undergoing diapedesis (Fig. 4). Similar observations were made in the walls of the enclosed blood vessels (Fig. 8). The cells in diapedesis usually were small lymphocytes (Figs. 4, 8), occasionally other lymphocytes, but never reticular cells. Diapedesis was not observed in cortex.

Cell counts. The numbers of cells and mitoses counted per field in the medulla (Table I, right) are presented next to those previ-

FIG. 1. Stem Cell Renewal Pattern of Lymphocyte Formation. Letters R, L, M and S refer to reticular cells, large, medium and small lymphocytes respectively. Each lymphocyte generation is indicated by such a letter preceded by a number indicating how many cells are involved and followed by a subscript referring to generation under consideration. Small horizontal bars symbolize mitoses. Vertical dimension is time. The diagram indicates that at regular intervals, each reticular cell yields on the average another reticular cell and a large lymphocyte, which passes through 4 generations and then produces medium lymphocytes (2 generations), which in turn give rise to small lymphocytes (2 generations).



Thymus of young adult rats stained with the Dominici technic. Figures at a magnification of × 1450, except Fig. 5, × 260.

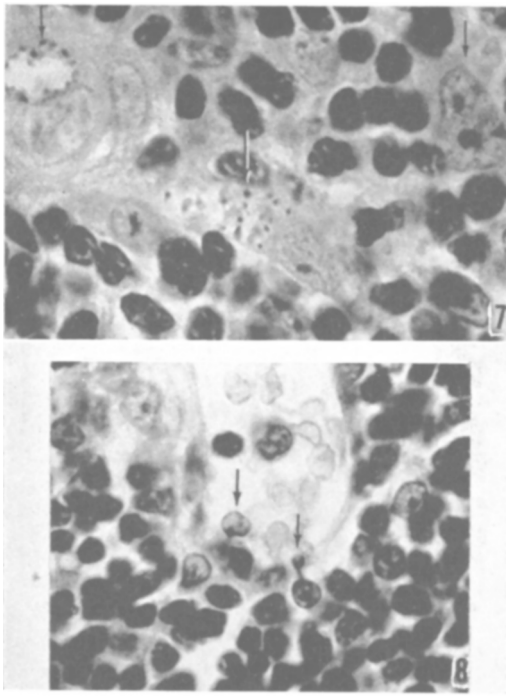
FIG. 2. Cortex. Anaphase of reticular cell may be seen in center. Above, an interphase nucleus of reticular cell (R). Below, arrows point to large lymphocytes (L), a medium (M) and a small (S) lymphocyte.

FIG. 3. Medulla. S′ arrows point to small lymphocyte with its nucleus sending off a large pseudopod-like process. S″ arrow shows crenated nucleus of small lymphocyte. S‴ shows small lymphocyte nucleus sending off a long process in the shape of squirrel tail.

FIG. 4. Medulla. Small arrows line part of wall of a ''perivascular lymphatic channel'' surrounding blood vessel seen above. An hour-glass shaped small lymphocyte nucleus (S) is seen going through this wall.

FIG. 5. Connective tissue trabecula. Small arrows point to endothelial nuclei of wall of typical lymph vessel running close to vein (above). Lymph vessel contains many small and one medium lymphocyte. In nearby cortex the ''L'' arrow shows an island of large lymphocytes.

FIG. 6. Photomontage of vein and associated ''perivascular lymphatic channel'' in medulla. In lower portion of Figure, a series of vertical arrows point to wall of perivascular lymphatic channel. One attachment to wall of vein is shown by horizontal arrow at lower right. In upper portion of Figure, the wall of lymphatic channel stands out on right hand side, but is collapsed and barely visible on the left.



Thymus of young adult rats stained with the Dominici technic. × 1450.

FIG. 7. Reticular cells in medulla. Upper right arrow points to nucleus of healthy reticular cell. Upper left arrow shows degenerating nucleus of reticular cell within Hassall's corpuscle. Note clumping of chromatin material. Central arrow shows nucleus with similar clumping in an isolated reticular cell.

FIG. 8. Two small lymphocytes undergoing diapedesis through walls of vein located at limit between cortex and medulla. On right, small lymphocyte is outside the vein, and sends off into the wall a process with claw-like arrangement of chromatin. On left, small lymphocyte is within vein, but a nuclear process (not quite in focus) is held within the wall.

ously reported for the cortex (Table I, left). The medulla contained nearly 4 times more *reticular cells,* but 25 times less *large lymphocytes* and 10 times less *medium lymphocytes* per field than the cortex. The numbers of mitoses varied in the same direction, so that mitotic indices for each of these 3 cell types were comparable in both regions. (No standard errors are given for the numbers of large lymphocytes and their mitoses, since these cells were rare in the medulla, where only 32 were counted.) The number of *small lymphocytes* in medulla was 1.7 times less than in cortex, while the number of mitoses was decreased by a factor of 4, so that the mitotic index of these cells was lower than in cortex.

Discussion. Except for small lymphocytes, mitotic indices were comparable in cortex and medulla (Table I); and therefore, reticular cells, as well as large and medium lymphocytes, have a comparable life span in the 2 regions(1). Furthermore, mitoses were abundant enough to indicate a continuous renewal of the 4 cell types(1). Thus, it was felt likely that lymphocytes evolve in a similar manner in cortex and medulla. To test this hypothesis, the relation of each cell type to its precursor was examined in detail in the medulla. Whenever the relation differed from that observed in cortex, an explanation was sought in the cytological and architectural features characteristic of the medulla.

Relation of reticular cells to large lymphocytes. The number of large lymphocytes in medulla was only .025 per field. If reticular cells yielded large lymphocytes at the same rate as in cortex, this number would be 2.61 per field (Table II), *i.e.* about 100 times more. Thus, only about 1% of reticular cell mitoses may produce large lymphocytes, the other 99% yielding new reticular cells instead.

The production of reticular cells as a result of mitotic activity must be balanced by an equivalent loss to maintain a steady state.

TABLE I. Number of Cells and Mitoses per Field, and Mitotic Indices in Cortex and Medulla of the Thymus of 10-Week-Old Male Rats.

	Total cortex*			Medulla		
	No. of cells	No. of mitoses	Mitotic index	No. of cells	No. of mitoses	Mitotic index
Reticular cells	.20 ± .03†	.003 ± .001†	.015	.798 ± .06†	.007 ± .003†	.009‡
Large lymphocytes	.66 ± .08	.046 ± .004	.065	.025	.0014	.053
Medium "	1.15 ± .05	.172 ± .011	.131	.114 ± .04	.019 ± .002	.143
Small "	15.75 ± .39	.240 ± .011	.015	9.040 ± .40	.065 ± .008	.007

* From reference 1. † ± S.E. ‡ A recount of 1000 reticular cells in medulla yielded a mitotic index of .010.

TABLE II. Numbers of Cells Found in Medulla of Thymus of 10-Week-Old Male Rats as Compared with Numbers Expected from Population of Immediate Precursor.

	Experimental	Expected	Difference
Reticular cells	.790		
Large lymphocytes	.025	2.61	−2.56
Medium "	.114	.045	+ .069
Small "	9.04	.840	+8.20

Indeed, the signs of nuclear degeneration up to complete disintegration found in some reticular cells (2; Fig. 7) indicate that at all times some of these cells are dying. Accordingly, Hassall's corpuscles may be considered to be structures into which reticular cells become aggregated and eventually degenerate.

Relation of medium to large lymphocytes and of small to medium lymphocytes. In the medulla, comparison of cell counts for medium and small lymphocytes with the values expected from the numbers of their immediate precursors (Table II) revealed that medium lymphocytes were more than twice, and small lymphocytes more than 10 times as numerous as expected. Where do the extra cells come from? It was suggested that lymphocytes migrate from cortex to medulla(2, 4) and then enter the circulation(2,8). Indeed, since diapedesis of lymphocytes occurs in medulla (Fig. 4, 8) but not in cortex, lymphocytes must pass into the medulla before entering blood and lymph vessels. From Table II, it may be calculated that, if about 60% of the medium and 91% of the small lymphocytes present in medulla were to come from cortex, the counts obtained for these 2 cell types would be explained satisfactorily.‡

Examination of the cells undergoing diapedesis and those present within lymph channels revealed that medium lymphocytes and even the odd large lymphocyte may enter the circulation. However, the larger the lymphocyte, the less it migrates from cortex to medulla to circulation. Those medium and large lymphocytes present in blood and lymph

TABLE III. Behavior of Small Lymphocytes of First (S_1) and Second (S_2) Generation in the 3 Zones of the Thymus of 10-Week-Old Male Rats.

	Peripheral cortex	Deep cortex	Medulla
Total No. of small lymphocytes*	128	1053	226
No. of Type S_1†	23	99	11
No. of Type S_2—Total No.‡	105	954	215
No. arising *in situ*	105	774	18
from peripheral cortex		180	37
from deep cortex§	0		160
Mean time spent by S_2 cells in each zone§ (using life span of S_1 as unity)	2.25	3.19	.81

* Relative numbers obtained as follows: Counts of small lymphocytes/field for the 3 regions were multiplied respectively by 9, 66 and 25 (% of volume of parenchymal thymus which these regions occupy).

† Calculated from No. of small lymphocyte mitoses (all attributed to S_1), assuming that life span of S_1 in any region is 2.25 times as small as the mean time spent by S_2 cells in peripheral cortex(1).

‡ Obtained by difference between the above two figures.

§ Calculated from contributions made to each S_2 pool by S_1 cells from the various zones, assuming steady state conditions. (Detail of calculations will be sent on request.)

‡ Number of medium lymphocytes which would migrate under these conditions may be estimated, if it is remembered that the ratio of volumes of medulla to cortex was exactly 1 to 3, i.e., 25 fields of medulla for 75 of cortex. Data on the right in Table II indicate that in each field a mean of 0.069 medium lymphocyte would come from the cortex, that is 1.7 for 25 fields. Number of medium lymphocytes/field in cortex is 1.15 ± .05 (Table I), i.e., 86.25 ± 3.8 for 75 fields. Number of these cells leaving the cortex (1.7) to enter the medulla is therefore relatively small, smaller even than standard error of the number of these cells in cortex (3.8), so that the loss of medium lymphocytes to the medulla is too small to affect the reasoning in reference 1.

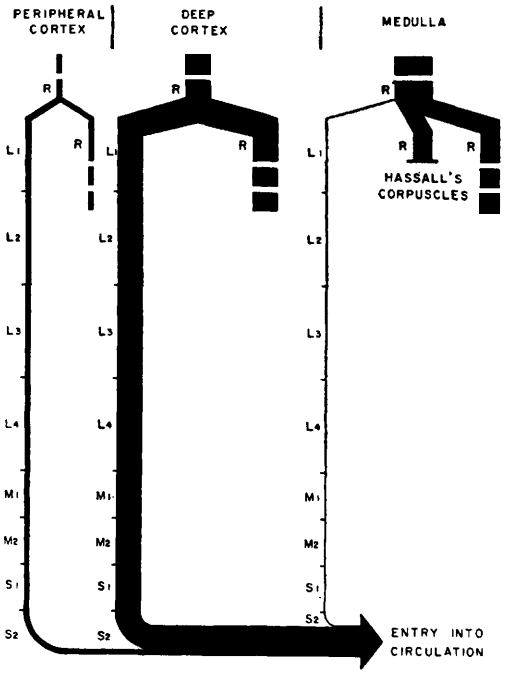


FIG. 9. Formation and migration of lymphocytes in peripheral and deep cortex and in medulla of rat thymus (lettering as in Fig. 1). Basic pattern in Fig. 1 is repeated for each of the 3 zones, with thickness of long vertical portion made proportional to amount of local lymphocyte production. At top of diagram, reticular cells are dividing. In peripheral and deep cortex, each such division yields on the average a large lymphocyte (L_1) and another reticular cell (R). In medulla, few large lymphocytes are produced from reticular cells, most divisions yielding reticular cells, of which on the average half die, frequently in Hassall's corpuscles. At base of diagram, the lengths of horizontal portions for the 3 zones were made proportional to mean time spent by S_2 cells in each. Vertical line standing for peripheral cortex is curved to the right to join line standing for deep cortex; then both together join line standing for medulla, thus indicating pathway of S_2 migration to medulla and hence to circulation.

channels were seen to undergo mitosis occasionally, just as they do in the parenchyma. It appears that their life span is the same not only in cortex and medulla (Table I), but perhaps even in the circulation, as indicated by unpublished studies of "medium" lymphocytes labelled after thymidine-H^3 injection (B. J. Bryant and Lola S. Kelly). Thus, medium lymphocytes—and presumably the odd large lymphocyte—entering the circulation would continue their evolution towards the "mature" small lymphocyte stage (Fig. 1).

Origin and migration of cells in thymus. An analysis of cell and mitotic counts in cortex had led to the elaboration of a Stem Cell Renewal Pattern of Lymphocyte Formation (1). The same method of analysis did not yield a clearcut pattern for the medulla. This may be explained by the failure of reticular cells to yield large lymphocytes as in cortex, and also by the complication introduced by migration of medium and small lymphocytes, which are thus added to the cells formed in the medulla.

Our tentative conclusions regarding the role of cortex and medulla in formation and migration of lymphocytes are illustrated in a diagram (Fig. 9), which was constructed as if only second generation small lymphocytes (S_2) were involved in migration (Table III). This assumption was adopted for the sake of simplicity, since it was known that small lymphocytes play a major role in migratory processes, and precise data concerning the part played by other lymphocytes were lacking.

The *peripheral cortex* (Fig. 9. left) shows the typical pattern pictured in Fig. 1, but with the base of the diagram curved to the right to indicate that small lymphocytes (S_2) arising in this zone migrate across the deep cortex where they join with more S_2 cells and together migrate towards and into the medulla.

Again, lymphocytes are formed in *deep cortex* according to the basic pattern but in greater numbers than in peripheral cortex, as indicated by the thicker line for the central diagram in Fig. 9. That small lymphocytes spend more time in deep than in peripheral cortex (Table III, last line), is represented in the diagram by the greater length of the horizontal portion of the diagram for deep than for peripheral cortex.

In *medulla,* the pattern is changed as far as reticular cells are concerned, since the mitoses of these cells yield reticular cells almost exclusively, instead of large lymphocytes as in cortex; and the new reticular cells either divide again or die by degeneration, an occurrence observed in scattered cells as well as in some of those grouped in Hassall's cor-

puscles. The various types of lymphocytes seem to reproduce in the same manner as in cortex, but the sizable population of small lymphocytes found in medulla comes largely from cortex. The concept of the medulla as a passageway for these cells is consistent with the observations of ameboid activity and of diapedesis into the vessels of this region (Figs. 4 and 8). Lymphocytes which have entered the perivascular lymphatic channels (Fig. 6) may reach the main circulation in 2 ways: 1) by diapedesis from these channels into the enclosed blood vessels and 2) by travelling along these channels to the main lymphatic circulation.

In conclusion, it seems that an important function of the thymus is to produce lymphocytes(12,13) to be delivered to the circulation through the medulla.

Summary. 1) Counts of resting and dividing cells in the thymic medulla of 10-week-old male rats revealed that reticular cells are more abundant than in cortex, but the 3 types of lymphocytes (large, medium, small) are less abundant than in cortex. However, except for small lymphocytes, each cell type has approximately the same mitotic index and, therefore, approximately the same life span in the 2 regions. 2) In view of the small number of large lymphocytes present, it appears that the evolution of reticular cells into lymphocytes is the exception in medulla, instead of being the rule as in cortex. Mitoses of reticular cells would mainly give rise to other reticular cells. Since these cells frequently show signs of degeneration, it is suggested that their loss through degeneration balances the overproduction indicated by their high mitotic activity. Clumps of reticular cells, many of which are in various stages of degeneration, make up the structures known as Hassall's corpuscles. 3) That the small lymphocyte is the end product of a series of 8 successive generations from large lymphocytes through smaller and smaller cells, seems to hold in medulla as in cortex. However, the presence of many more small lymphocytes in medulla than expected from the number of medium lymphocytes raised the possibility that small lymphocytes immigrate from cortex. Indeed, since diapedesis of small lymphocytes was commonly seen across the walls of the perivascular lymphatic channels and blood vessels in medulla but not in cortex, a migration of cortex-formed small lymphocytes through the medulla must be postulated to allow them to reach the circulation. To a lesser extent, medium lymphocytes (and perhaps even the odd large lymphocyte) may also migrate from cortex to medulla and from there to the circulation. It is concluded that only a small degree of lymphocyte formation occurs in medulla. This region is mainly a passageway allowing the lymphocytes arising in cortex to reach the circulation.

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