

Resumen por el autor, John S. Latta.

La interpretación de los llamados centros germinales del tejido linfático del bazo.

Desde la publicación de los resultados de la investigaciones de Flemming ('85) en los cuales dicho autor concluye que las áreas centrales más claras de los nódulos linfáticos densos son centros de proliferación, o "centros germinales," los investigadores que han estudiado el problema del tejido linfático han estado acordes invariablemente con este concepto de su significación. En un estudio reciente ('21) de estas estructuras en el tejido tonsilar intestinal, no he encontrado prueba alguna de la actividad proliferativa de estas áreas, sino que he llegado más bien a la conclusión de que representan centros de degeneración. Un cuidadoso estudio histogenético ha sido llevado a cabo por el autor en los centros de los nódulos linfáticos esplénicos. En estas regiones no he podido hallar prueba alguna de actividad proliferativa o transformativa. El aspecto más claro de estos centros, cuando existen, se debe a una menor densidad de los linfocitos libres en este sitio, asociada con mayor prominencia del tejido reticular. El autor ha encontrado estados de la degeneración de los pequeños linfocitos y hemoblastos linfoides, los cuales se deben, sin duda alguna, a la disminución de la nutrición de los centros. El autor indica, por consiguiente, que el término "centro germinal" no describe las condiciones existentes en estos puntos, y que deben considerarse más bien como centros en los cuales tienen lugar cambios degenerativos.

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## THE INTERPRETATION OF THE SO-CALLED GERMINAL CENTERS IN THE LYMPHATIC TISSUE OF THE SPLEEN

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### THREE FIGURES

In the histological examination of dense lymphatic tissue as found in the lymphatic nodes, the various tonsils, and in the spleen of mammalian forms, it is noted that often the central portions of the lymphatic nodules are of lighter appearance than their peripheral portions. This fact was first studied in detail by Flemming ('85), who designated these lighter central portions of nodules, 'Keimzentrum,' or germinal centers, for he considered these areas of the nodules as centers of proliferation of lymphocytes. Since that time investigators have invariably followed Flemming in his interpretation of these light colored areas. There has been, however, apparently some confusion as to exactly what is meant by the term, germinal center, for several authors have included in this term an entire lymphatic nodule (Baum u. Hille, '08; Bunting, '05), instead of their lighter colored central portions, as evidently intended by Flemming and most investigators following him.

As I have indicated in a previous paper ('21), in the study of the lymphatic tissue of the intestinal tonsils, it seemed that the term, germinal center, was non-descriptive of conditions existing in those nodules of the intestinal wall which contained these light colored central areas. Results of this study seemed to indicate that the so-called 'germinal center' was not a place of special lymphocytic formation and proliferation, but rather one of degenerative changes.

Whether these centers are proliferative or degenerative in character, their interpretation is very closely associated with the nodular blood supply (i.e., the question of nutrition). In this respect, the Malpighian bodies (lymphatic nodules) of the spleen are of special interest because of the fact that they here develop as an hypertrophy of portions of the lymphoidal arterial sheaths, thereby changing, to some extent, the usual vascular relationships of nodules in lymphatic tissue. Then, too, the histological appearance of splenic nodules which possess the so-called 'germinal centers' seems to differ somewhat from nodules in other varieties of lymphatic tissue. Therefore, it is thought, that some study of the splenic lymphatic tissue, with special reference to the presence of the lighter colored central areas, their relation to the vascular supply, and their histological structure and development, might aid in more clearly interpreting their significance.

Human spleens, collected from autopsies performed at the University Hospital, University of Nebraska, in which the causes of death were, in no apparent way, associated with the spleen, were used chiefly in this investigation. These were from individuals of ages varying from still-born infants, in which the splenic nodules were ill-defined and few in number, to adults of somewhat advanced age. This human material was supplemented by spleens of dogs, cats, guinea-pigs, and rabbits, which were used for comparative purposes.

The fixing fluid found to give the best results with the stains which followed, and hence that used most extensively, was Helly's fluid or Zenker-formalin, prepared by adding 10 per cent of formalin to Zenker's stock solution. Picro-aceto-formalin (Bouin's fluid) was also used to some extent with very good results.

All of the material was imbedded in paraffin, the sections cut from 6 to 8  $\mu$  thick, and portions mounted serially, to be sure that correct relationships between the nodules and the surrounding parts could be determined.

The most commonly used stains were the blood stains, Hastings-Nochts modification of the Romanowski blood stain, and a

$\frac{1}{2}$  per cent solution of alcoholic eosin in methyl alcohol, followed by an aqueous solution of methylene blue, made slightly alkaline with KOH (as used by Gage). A solution composed of equal parts of 1 per cent aqueous solutions of methyl green and pyronin gave good results (after Pappenheim). Finally Mallory's aniline-blue connective-tissue stain was used, mainly upon tissue which had been previously fixed in picro-aceto-formalin, to show clearly the extent and amount of the reticular tissue in its relation to the splenic nodules.

In 1854, Brücke, in a study of lymphatic tissue, noticed that occasionally the dense nodules of this tissue had lighter colored central areas. He said, "One sees occasionally in the cortical nodules and still more often in Peyer's patches a central lighter portion." His ('62) also had observed lighter areas in the center of lymphatic nodular masses, which he called 'Vacuolen.' Frey ('74) said, "In the large follicular masses are seen lighter colored translucent centers." These centers, he considered, were the true follicles, the darker periphery being something peculiar to these follicles.

Flemming ('85) made a more exhaustive study of lymphatic tissue than previous investigators. He used as objects of study mesenteric lymphatic nodes from the ox and rabbit, Peyer's patches from the caecum of rabbits, and human lingual tonsils. He observed these lighter central areas in all of these different tissues, and considered them to be of the same significance in the tonsils, lymphatic nodes, and the spleen. In the center were cells with a large amount of cytoplasm and large nuclei, while cells at the periphery were smaller and stained more deeply, thus giving a lighter appearance to the central portion. In the center mitotic figures were prominent and at the periphery were scarce. Consequently he called this lighter central area the 'germinal center.'

Subsequently, as has been before intimated, there has been some confusion in regard to the 'germinal center,' and to what the term should be applied. Leading text-books have invariably followed Flemming in their interpretation of this structure, but in the study of the results of investigators of problems relating

to lymphatic tissue, one finds that the term 'germinal center' is often used in an indefinite way. The use of the term by Bunting ('04) and Baum and Hille ('08) has already been referred to. Gulland ('94) also seems to have had a rather vague idea of the significance of this term. He says the germinal centers (in lymphatic tissue) are known as light circles with dark circumferences. Sabin ('05) considers the second part in the development of lymph nodes to be the formation of germ centers, which latter she described as condensations of cells where lymphocytes are actively proliferating (also Ribbert, '89; Richter, '02).

These descriptions seem unsatisfactory as concerning the so-called 'germinal centers,' but correspond rather with current conceptions of complete dense lymphatic nodules.

Czermack ('93) followed Flemming in believing the 'germ centers' of lymphatic nodules to be composed of cells which continually produced lymphocytes. These cells he called 'Keimzellen.'

Saxer ('96), in speaking of 'germ centers,' said that the presence of more numerous divisions of fixed cells in the germ centers than in reticular and areolar tissue in other places is understandable because of the increased physiological function and the importation of nutritive elements. Just what he included under the term 'Keimzentrum' could not be determined.

All recent investigators who have studied the 'germinal centers' of lymphatic tissue agree with Flemming in the general interpretation of their significance (Schridde, '07; Maximow, '09; Downey and Weidenreich, '12; Hartmann, '14; Thiel and Downey, '21; Nakahara and Murphy, '21). The lymphoblastic nature of cells in the 'germinal center' has been quite generally accepted. Some authors have followed Czermack in giving these cells the specific name of 'germinal center cells' (as Hartmann, '14). Thiel and Downey, in discussing the germinal centers of the spleen, considered these areas as regions of special activity of the reticular tissue, as manifested by the hypertrophy, increase in number, isolation, and transformation of its cells.

In addition to cells of a lymphoblastic character, there have been demonstrated in the light colored 'germinal centers' of lymphatic tissue in various places certain large phagocytic cells (those containing the 'tingible Körper' of Flemming), considered by some to be derived from cells of the reticular tissue (Weidenreich and Downey, '12; Hartmann, '14), and, as studied by myself in intestinal tonsillar tissue ('21), thought to be derived by further differentiation and degeneration of lymphoid hemoblasts.

As the special proliferative activity of the 'germinal centers' is accounted for by many as a result of increased physiological function and unusual possibilities of nutrition (Saxer, Thiel and Downey, et al.), and as my previous explanation of them as degenerative centers is based upon an insufficient blood supply at these points, the relation of the lymphatic nodules to the vascular supply was a question of special interest in the present investigation.

Although, as before stated, splenic tissue from the dog, cat, and other forms were studied for comparison with the structure of the human spleen, in all respects in which comparison was made there was little or no variation, so the conclusions arrived at in this paper are based upon the study of the human spleen.

It becomes very evident, when sections of the spleen from individuals of varying ages are studied, that arteries of the spleen very early (before birth) acquire lymphoidal sheaths (which, according to Danchakoff, Downey, and others, is preceded by a mesenchymatous sheath). At the time of birth, however, there is little distinction to be drawn between the red and the white pulp (lymphatic tissue). The lymphoidal arterial sheaths, however, seem to increase markedly in density, and, in young adults, are a very distinctive splenic structure. Lymphatic tissue was very abundant in all of the adult human spleens studied, more particularly in their central portions.

Malpighian bodies, or splenic lymphatic nodules, are not recognized until some time after the formation of the lymphoidal arterial sheaths. They are formed, according to Downey and Weidenreich ('12), by the rapid increase of the number of lymphocytes, derived from fixed cells of the reticulum, in certain

regions of the lymphoidal arterial sheaths. These regions of increased proliferative activity are supposedly at points where there has been formed a dense capillary network which is absent in other places in these sheaths (Thiel and Downey, '21). By the study of serial sections of spleens, in some cases of which the arteries had been injected with a carmine gelatin mass and others which had become highly congested with blood, it was demonstrable that the splenic nodule (Malpighian follicles) were always very well supplied with capillaries, which branches from the central artery, so that the first development of these nodules is undoubtedly due to an increase in nutrition and metabolic rate at these particular points.

The lighter colored central areas, which according to Thiel and Downey may also be peripherally located in the nodules, are not present in the nodules at first formed. In fact, the so-called 'germinal centers' are infrequently found at any time in splenic nodules as compared to nodules of the various tonsils and of the lymphatic nodes.

In splenic nodules, in which the lighter colored central areas are apparent, the accompanying artery is always seen to be extremely excentric to the nodular mass, and never in the lighter colored portion. Nodules which possess no lighter colored 'germinal center' are relatively smaller and the artery is usually located centrally in the nodule or nearly so. Neither is the so-called 'germinal center' a region of extraordinarily rich vascularity, as was found in the nodules at the time of their formation. In most cases no capillaries or blood could be demonstrated in these regions, the increase in size of the nodules and their increase in density at the periphery apparently inhibiting the vascular supply to the central portions. In some nodules the vascular supply was found to be very extensive throughout the entire nodule, as at the time of their formation, but these nodules never exhibited a 'germinal center.'

The histological appearance of these 'germinal centers' was first discussed by Flemming ('85), who found their lighter appearance to be due to the preponderance here of cells with large, vesicular nuclei, which were also relatively rich in cytoplasm

(lymphoid hemoblasts), so that the nuclei were farther separated from one another here than at the nodular periphery. This phenomenon was observed by later authors, and the large cells apparently peculiar to the areas were called by some the 'germinal center' cells (Czermack, '93; Hartmann, '14).

Thiel and Downey, in their study of the development of the mammalian spleen, found that the 'germinal centers' were areas in which the reticular tissue, particularly the reticular cells, were hypertrophied and were specially active in the reproduction and transformation into lymphocytes, thus disagreeing with Saxer ('96), who, in the study of the development of lymphatic nodes, said, "The presence of a fixed mother tissue for the production of lymphocytes is neither practically demonstrable nor a theoretical postulate."

In the present study of the structure of the so-called 'germinal centers' as they appear in lymphatic nodules of the human spleen, it was found that, in these locations, the lymphocytes were much less closely packed together than at the periphery of the nodules. The cells in these centers were surely not predominately of the lymphoid hemoblast (lymphoblast, germinal center cell) type, as found by Flemming, Czermack, Hartmann, et al., the proportion between the small lymphocytes and the lymphoid hemoblasts remaining the same as at the periphery. Fibers of the reticular tissue are possibly more prominent in the 'germinal centers' than elsewhere in the nodules; this fact being due, it is thought, rather to the lesser density of the free lymphocytes and an increase of space between them, than to an hypertrophied condition of the reticulum. Nuclei of reticular cells were seen in the loose central portions, but not in unusual numbers, certainly not to a great enough extent to warrant a statement that the reticular cells are here specially active as a mother tissue productive of lymphocytes. It would seem that such prominence as the reticular tissue and cells may have in the so-called 'germinal centers' can be accounted for solely by the lesser number and density of the free lymphocytes in these places.

Evidences of proliferation of cells as indicated by the presence of mitotic figures are not as numerous, apparently, in splenic



lymphatic tissue as in other forms of lymphatic tissue. The regions where mitotic figures are most numerous are, therefore, not points of such special interest and significance as in other cases. Whenever mitotic figures were found, however, they were never located in the central areas, but in the denser peripheral portions of the nodules. There seemed to be no special points, at which the production of lymphocytes by mitosis was very active, and absolutely no evidence of such activity in the lighter central portions, so that the statement of Maximow ('09) and others, "that it is now a commonly accepted fact that small lymphocytes are formed by division of large lymphocytes in the germ centers," certainly cannot be applied to these areas in the lymphatic nodules of the spleen.

In examination of the lighter colored central areas in lymphatic tissue in other locations it has been found that the different concentration or character of the cells is not alone responsible for their appearance. Flemming noted large free cells in these places, similar to large lymphocytes, containing various deeply staining bodies in their cytoplasm, the 'tingible Körper.' Downey and Weidenreich ('12) also found here large cells, derived from reticular cells, which possessed phagocytic powers. Hartmann ('14) saw in the centers of intestinal lymphatic nodules large acidophilic cells with inclusions, which he thought were of a lipoid nature. These cells I also observed ('21) in similar locations, and termed them acidophilic macrophages. They were considered as further differentiated, degenerating lymphoid hemoblasts, which had acquired phagocytic powers. The inclusions in the cytoplasm were thought to be remnants of nuclei of degenerating small lymphocytes.

Very few cells of such description were found in the nodules of splenic lymphatic tissue. A few large acid-staining cells which resembled the acidophilic macrophages of intestinal tonsils were found. Their nuclei were very similar to those found in the lymphoid hemoblasts, but did not give a typical staining reaction, staining rather with the acid stain. The basic cytoplasm staining reaction of the lymphoid hemoblasts was also changed to an acid reaction. Some intermediate stages could be found. No evidences

of their origin from reticular cells were found, although it might be considered as possible. In a very few instances deeply staining bodies were found in these cells. In some cases evidences of degeneration of small lymphocytes, such as their pycnotic nuclei and partial fragmentation, were found in these centers, and so, as before concluded in the case of inclusions of the macrophages of the intestinal tonsils, the inclusions of these macrophages were thought to be nuclear remnants of degenerating small lymphocytes.

The same significance is given to the large acid-staining macrophages in the so-called 'germinal centers' of the splenic lymphatic nodules, as to those similarly located in intestinal tonsillar tissue. The change in nuclear and cytoplasmic staining reaction from basic to acid, together with the acquisition of phagocytic powers is considered evidence of the further differentiation and degeneration of the lymphoid hemoblasts of these regions.

From these indications it would seem that the so-called 'germinal center' is not a region of special cell proliferation or transformation, but rather a center of degeneration. The fact that the lighter colored centers are present only in quite large nodules, which is associated with a decrease in the blood supply to these centers, the absence of mitotic figures, or a special mother tissue transforming into lymphocytes, together with the evidences of degeneration of small lymphocytes and of lymphoid hemoblasts, all point to an interpretation of these areas as degenerative rather than proliferative centers.

This interpretation certainly seems to be borne out in a study of pathological conditions. As is well recognized, pathological nodular growths, such as tubercular or sarcomatous nodules, when they become very large, have areas of caseous degeneration in the center. Extreme hypertrophy of lymphatic nodules as occurring in various blood diseases is coincident with degenerative changes in their central areas. In the history of infectious diseases involving the palatine or intestinal tonsils, it has been observed that the central portions of the nodules are the first areas to become affected, indicating that these points are areas of

lowered metabolic rate and resistance. It would seem somewhat illogical to consider these centers as areas of unusual proliferation due to excellent nutritive conditions and high metabolic rate and, at the same time, to admit them as possible foci of infection, associated with points of lowered resistance.

The lighter colored central areas, the so-called 'germinal centers,' when found in nodules of the lymphatic tissue of the spleen, are to be considered, then, as areas, in which the size of the nodules has caused a deficiency in the nutritive supply, as shown by the poor vascularity, which has lowered the metabolic rate to such an extent that evidences of degeneration are found, as shown by the presence of degenerative stages of small lymphocytes and lymphoid hemoblasts, and by the lesser density of lymphocytes there. These centers would consequently be points of lowered resistance and become possible foci of infection in the case of bacterial diseases invading the spleen.

The term 'germinal center,' therefore, seems non-descriptive of existing conditions, as it implies that it is a center of proliferation of lymphocytes, or transformation of other lymphoblastic tissue into lymphocytes, rather than a center of degeneration, due to poor nutritive conditions, as might be concluded from a study of these structures as found in the human spleen.

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## PLATE 1

### EXPLANATION OF FIGURES

1 Spleen, Homo, adult, showing a small lymphatic nodule, possessing no lighter colored center, with its accompanying artery located near the center of the nodule.

2 Spleen, Homo, adult, showing the character and density of cells in the lighter colored center of a lymphatic nodule. The reticular tissue is prominent here because of the lesser density of the free cells. Degenerative stages of lymphoid hemoblasts and of small lymphocytes (pycnotic nuclei) may be seen.

3 Spleen, Homo, adult, showing the comparative density of cells in the lighter colored center and in the peripheral portion of a lymphatic nodule. Several cells in various stages of degeneration of lymphoid hemoblasts (lightly staining nuclei) and of small lymphocytes (pycnotic nuclei) are found.

