

Resumen por el autor, John Stephens Latta

La histogénesis del tejido linfático denso del intestino (*Lepus*):

Una contribución al conocimiento del desarrollo del tejido
linfático y de la formación de las células sanguínea.

El objeto de la presente investigación es determinar con la mayor exactitud posible el origen y desarrollo de los diversos tipos de células sanguíneas alrededor y dentro de los nódulos linfáticos del intestino y sus proximidades, en la región cecal del conejo. Las primeras células libres que aparecen son los pequeños linfocitos, los cuales se desarrollan in situ a expensas de las células del mesenquima. Unos pocos hemoblastos linfoides se desarrollan también por transformación directa de las células mesenquimatosas. Estos dos tipos son aparentemente estados diferentes del crecimiento de una misma célula; los pequeños linfocitos, mediante crecimiento y ligera transformación, se convierten en hemoblastos linfoides, y estos últimos mediante divisiones repetidas se reducen a pequeños linfocitos. La presencia de grandes macrófagos acidófilos que derivan mediante diferenciación ulterior de los hemoblastos linfoides y la de pequeños linfocitos en vías de degeneración indica que el llamado centro germinal es más bien un centro de degeneración que de proliferación. Los eosinófilos de forma binucleada se desarrollan en el tejido conectivo cerca de los nódulos, especialmente en la túnica propia. La abundante eritropoiesis extravascular se encuentra en el conectivo subnodular o internodular, diferenciándose las células eritroblásticas a expensas de los hemoblastos linfoides. El desarrollo de los tres tipos de células sanguíneas parece estar en cierto modo relacionado con la abundancia de capilares. La formación de tejido linfático nodular puede depender de condiciones nutritivas, suministradas por la sangre. La diferenciación ulterior de los hemoblastos linfoides depende probablemente de la proximidad de asociación con los vasos sanguíneos, la lentitud de la corriente y el espesor de las paredes vasculares; la relación muy íntima de los hemoblastos y la corriente sanguínea produce eritropoiesis, y una conexión más remota da lugar a granulopoiesis.

THE HISTOGENESIS OF DENSE LYMPHATIC TISSUE OF THE INTESTINE (LEPUS): A CONTRIBUTION TO THE KNOWLEDGE OF THE DEVELOPMENT OF LYMPHATIC TISSUE AND BLOOD-CELL FORMATION

JOHN STEPHENS LATTA

Department of Histology and Embryology, Cornell University, Ithaca, New York

FOUR PLATES (SIXTEEN FIGURES)

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INTRODUCTION

The origin, development, and fate of the various cellular elements of the blood, their relation to the loose connective tissue of the body, and the conditions associated with, or causing their production, are still unsettled and debatable questions despite the vast amount of previous investigation of hematological problems.

Some of the most perplexing of these problems arise in discussion of the production of lymphatic tissue, or lymphopoiesis, as found in various places in the body, more particularly in the tonsils of the mouth and intestine. Just what are the conditions which bring about or are associated with the formation of lymphatic tissue? Are these conditions controlling its formation the same, wherever it occurs, e.g., in the tonsils as in the lymphatic nodes? Or are there different conditions, the presence of any one of which may initiate and control lymphopoiesis? Does lymphopoiesis, wherever occurring in the body, always serve the

same purpose? Is the function of the tonsils of the mouth and intestine the same as that of the lymphatic nodes? These questions and others, in view of the evidence set forth in previous investigation, make the interpretation of the lymphatic tissue difficult.

HISTORICAL

This confusion, regarding questions of lymphopoietic processes is added to, in the study of the histogenesis of tonsillar tissue, by the close relationship existing between the lymphatic tissue of the tonsils and the overlying epithelium. This relationship in fact, when the development of the tonsillar lymphatic tissue first became a matter of investigation, led to the conclusion by some authors that the lymphocytes in these regions were of epithelial origin.

This belief was most vigorously defended by Retterer, who, in a series of papers ('91, '92, '93, '09, '13) on the development of the various tonsils, in each case declared the lymphocytes of these structures were derived by downgrowths from the overlying epithelium. Von Davidoff ('86-'87), working on the formation of lymphatic nodules in the jejunum of man and the appendix of the guinea-pig, and Klaatsch ('92), studying the histogenesis of Peyer's patch in the *Echidna*, are among those who, like Retterer, thought the lymphocytes thus formed were of epithelial origin.

The majority of authors concerned with problems relative to the histogenesis of tonsillar lymphatic tissue, however, disagreed with these conclusions. Chief among these was Stöhr, who, in studying practically the same material as Retterer did, arrived at very different conclusions. He found the epithelium remaining inactive in the formation of the tonsillar lymphatic tissue, the elements of which were derived from the mesenchyme of the tunica propria. Others agreeing with Stöhr in ascribing to these cells in tonsillar structures a mesenchymal origin include Flesch ('88), Zwarykin ('89), Tomarkin ('93), Kückenmeister ('95), and, more recently, Mollier ('13) and Hartman ('14).

This diversity of opinion among earlier investigators as to the development of tonsillar lymphatic tissue (i.e., in the intestine) and the practical omission of such study by more recent investigators, together with the great amount of doubt and uncertainty concerning questions of the origin and development of lymphatic tissue in general, seem to warrant further study of the intestinal tonsillar tissue (Peyer's patch and the appendix) in an attempt at some interpretation of these structures.

MATERIALS

As material for the pursuit of such investigation, the rabbit was considered as well suited, chiefly because of the great concentration and remarkable development of lymphatic tissue in Peyer's patch in the lower portion of the ileum, causing an abrupt enlargement in it at its point of junction with the caecum, the sacculus rotundus, or iliac tonsil (Muthmann, '13).

Also, by use of this form, abundant material was easily obtainable in all stages of the development of these intestinal tonsillar structures. It was found after short study that in newborn animals or earlier stages that there were no indications of the formation of lymphatic nodules either in the region of Peyer's patch or the appendix, so no embryonic material was collected. Females were secured, either in late pregnancy or just following the birth of the litter, and the young rabbits removed from the litter at desired intervals. The first indications of nodular lymphatic tissue were found to be at an age of from two to three days, free cells beginning to be massed in the location of future nodules. From this time growth and differentiation proceed at a rapid rate, until at an age varying from five to seven weeks the nodules have assumed a structure like that found in the adult individual (except for size).

During this time of very rapid development, stages were secured at very frequent intervals to be sure to get an uninterrupted developmental picture. In practically all cases two or three individuals of the same age were secured, to correct any deviation which might occur in any single instance. Animals were secured at daily intervals, and sometimes half-day intervals,

ranging from newborn to individuals of fifty-six days postpartum; that is, to a time after the intestinal tonsils had assumed an adult structure. Tissues from older individuals at less frequent intervals, were also obtained, up to an age of about two and one-half months.

Helly's fluid (Zenker-formalin) was used almost exclusively as a fixing reagent, as did most recent investigators of hematological problems. With it, in the writer's experience, is given the best general fixation, without in any way interfering with desired subsequent staining with the compound blood stains. In a few instances a fixer suggested by Downey ('15) was used. This was a 0.9 per cent normal salt solution saturated with HgCl_2 , to which 10 per cent of formalin was added at the time of using. This gave results apparently equally as good as Helly's, but there being no advantages held over the latter, it was seldom used.

The tissues were all imbedded in paraffin and cut in sections from 5 to 7 μ in thickness.

The stain used most extensively in this study was Hasting's modification of the Nochts-Romanowsky blood stain. Wright's blood stain was also used to some extent, but the Hastings-Nochts combination was equally as selective as Wright's for cells of the blood series, and gave a more brilliant, intense and a sharper nuclear stain. The sections were first stained six to ten minutes in the concentrated stain, and then removed, without washing, to a solution of the stain diluted one-half with distilled water for ten to fifteen minutes. The sections were then differentiated and dehydrated in 95 per cent and absolute alcohol, cleared in several changes of neutral xylene, and mounted in neutral xylene damar.

A combination composed of a 1 per cent solution of eosin in methylic alcohol, followed by a weakly alkaline, aqueous solution of methylene blue (Gage, '17), was found to be almost as selective a stain for blood cells as Hastings-Nochts, and to give a still more brilliant, sharp picture. Therefore, this stain was quite extensively used. The sections were carried immediately from 95 per cent alcohol into the 1 per cent methyl alco-

holic eosin, washed in water, then carried into the methylene-blue solution, after which they were differentiated, dehydrated, cleared, and mounted as for the Hastings-Nochts stain.

Another stain, proving of no little value in bringing out certain specific features, was a mixture composed of equal parts of 1 per cent aqueous solutions of methyl green and pyronin (after Pappenheim). After staining in this solution for ten minutes, the sections were washed in water, and carried immediately into a 1 per cent solution of resorcin in absolute alcohol for differentiation and dehydration, cleared in xylene, and mounted in xylene damar.

For the purpose of sharply differentiating the reticular tissue from the free cells of the lymphatic tissue, Mallory's connective-tissue stain was used, after first mordanting the sections for a few minutes in picro-acetoformalin.

Other special methods were used at various times to bring out or make clear certain specific features of these structures. These methods will be discussed as the features made clear by their use are spoken of.

In the study of the histogenesis of the elements of the connective tissue (tunica propria and submucosa) in the region of the formation of the intestinal tonsils, it is noted that, coincident with the formation of lymphatic tissue, other cellular elements of the blood may be developing also.

It has long been a matter of common knowledge that, in the connective tissue of the wall of the digestive tract, varying numbers of cells are found, in the cytoplasm of which are granules of either an eosinophilic or basophilic character. As has been stated, the granules of basophilic character are readily soluble in water and consequently were not found in any of the preparations used, being dissolved out in the course of the preparation. But the cells with eosinophilic granules are found present, often in extraordinary abundance, in the connective tissue in the neighborhood of the lymphatic nodules, more particularly in the tunica propria (figs. 9, 10, 11, 14, 16).

Also, at certain stages in the development of the tonsillar lymphatic tissue, there may be found, in apparent association

with its formation in the subnodular and internodular connective tissue, groups of cells in developmental stage of the formation of erythrocytes (erythroplastids). These erythroblastic cell groups are not of constant appearance, and, when present, vary greatly in number, sometimes there being but a few erythroblastic cells scattered about in the connective tissue and, again, large masses of them, or foci, were found. These erythropoietic foci were found in largest extent in the region of the iliac tonsil (Peyer's patch), but in a few cases erythropoiesis was noted to be occurring to a very limited extent in the connective tissue of the wall of the appendix (figs. 7, 14, 15).

This phenomenon of erythropoiesis is not confined to any exact, definite period of development, but it does, however, occur most frequently and in greatest amount in animals from two to six weeks after birth. Even though, as before stated, the presence of erythropoietic centers is not of absolute constancy, they are found present so frequently (and in such number) as to render improbable the thought that they arise because of some particular pathological condition in each case.

It is apparent, therefore, that in the study of the histogenesis of elements of the connective tissue in the region of the intestinal tonsils of the rabbit, one is confronted with the problems concerned with the formation of all the types of blood cells, which may be divided into three phases, according to the character of the resulting cells: 1) lymphopoiesis, the development of the components of the lymphatic tissue, the lymphocytes and reticulum; 2) the development of cells containing in their cytoplasm acidophilic granules, or granulopoiesis, and, 3) the development of red blood cells, or erythropoiesis. The origin, development, and the fate of these three different types of cells, and conditions causing and controlling their production, as well as interrelations which might exist between the three phases of development, are questions for which this material gives opportunity for study.

LYMPHOPOIESIS

The most extensive formation of blood cells in the region of the intestinal tonsils is, of course, that of lymphocytes. The free cells, or lymphocytes, which make up the bulk of the nodular tissue of the tonsils have been classed by hematologists into two groups, 1) small, round cells, with a round or slightly oval nucleus, the heavy chromatin particles of which are arranged just within the nuclear membrane so as to appear somewhat as the spokes of a wheel, inside a definite nuclear membrane, covering which is a thin rim of densely basophilic cytoplasm, the small lymphocytes, and, 2) comparatively much larger cells potentially round, but evidently possessing some ameboid tendencies, with a clear vesicular nucleus, round or oval in form, containing a very scanty amount of chromatin, lying for the most part just within the nuclear membrane, and usually a prominent nucleolus. Surrounding the nucleus is a varying amount of densely basophilic cytoplasm. These are the so-called large lymphocytes, or lymphoid hemoblasts.

The relationship between these two types of cells, and their potentialities, distinction as permanent cell types, etc., have long been perplexing and debatable questions. The majority of present-day hematologists seem to consider the small lymphocyte as the more distinct permanent cell type, with no potentialities other than the production of others of its kind, while they ascribe to the other type of cell a more blastic nature, with potentialities of differentiation into cells of any of the three types, given the correct environmental conditions.

The question of the origin of these lymphocytes, especially as occurring in the tonsils, has long been a matter of much controversy. The somewhat spirited debate between Retterer and Stöhr, already mentioned, may be recalled. Retterer insisting upon an epithelial origin for the lymphocytes, and Stöhr equally sure that the epithelium remained passive in their formation, the cells of origin being of the mesenchyme. These and others of the earlier authors did not recognize the two types of lymphocytes. The recognition of these two types complicates the question of their origin still further.

The large (clear-nucleated) cells of the lymphatic nodules, lymphoid hemoblasts, have been described under various names by investigators of problems of blood-cell formation. This type of cell is identical, morphologically at least, with the free wandering cells of the loose connective tissue, the so-called primäre Wanderzellen of Saxer ('96), the primitive large lymphocyte or wandering cell of Maximow and others, hemogonia of Mollier ('13), lymphoblast of Naegeli, mesameboid of Minot, lymphoid hemoblast or hemocytoblast of Danchakoff, etc.

The term, proposed by Danchakoff, seems to be the most descriptive of this type of cell, for, as she pointed out when suggesting this term, it is essentially of a lymphoid nature, and it is quite generally accepted (monophyletic theory) that this type of cell possesses potentialities capable of transforming into any of the many different cellular elements of the blood under favorable environmental conditions. Although environmental conditions are normally such, in lymphatic nodules, that this type of cell remains as a cell of the lymphocyte series, it is believed to retain this potentiality of further differentiation into other types of blood cells (as in one instance, noted by the writer, in which all the lymphoid hemoblasts of a lymphatic nodule of Peyer's patch in a twelve-day-old rabbit had transformed under pathological change of environmental conditions into eosinophilic granular leucocytes). The term, lymphoid hemoblast, will in this article refer to this type of cell.

Hematologists are practically united in ascribing to this cell an origin from the fixed cells of the body mesenchyme, or embryonic connective tissue, Danchakoff maintaining that they may also derived from cells of the vascular endothelium.

But the origin of the small lymphocytes, their potentialities, and relation with the lymphoid hemoblasts are yet much debated questions. Macimow thought the small lymphocytes and lymphoid hemoblasts or large lymphocytes were one and the same cell in different growth stages. Badertscher ('15) found, in the developing thymus, that the small lymphocytes were derived by repeated divisions of 'large lymphocytes,' but is not sure whether the small lymphocytes may again grow into the other type or not.

Downey and Weidenreich ('12) found, in lymphatic nodes, the small lymphocytes were developed from the large reticulum cells of the node. These small lymphocytes, they thought, by growth became 'large lymphocytes.' Danchakoff ('16) found that small lymphocytes in the spleen arose by differentiation of dwarfed lymphoid hemocytoblasts, which had arisen because of intense proliferation and poor nutrition of normal lymphoid hemocytoblasts. The small lymphocyte she considered a distinct, stable cell form, incapable of growth into the lymphoid hemoblast.

Aside from this question of relationship in connection with the study of the formation of lymphatic tissue, in the lymphatic nodes and the tonsils, the question still remains as to whether these lymphocytes arise in situ, wander in from the mesenchyme in other places, or are carried in and dropped by the blood vessels.

Those studying the development of lymphatic nodes are not united on this question. Gulland ('94) thought the first lymphocytes to appear in developing nodes were filtered from the blood stream. Saxer ('96) considered that they arose in situ from the 'primäre Wanderzellen' of the mesenchyme. Sabin ('05) also favored this view, although she considered the evidence at hand insufficient to definitely determine the origin of the lymphocytes. Downey and Weidenreich, as before stated, also thought they arose in situ by differentiation of the reticulum cells of the node.

Hartmann ('14), who studied the development of the intestinal tonsillar tissue of the rabbit, considered that the lymphoid hemoblasts, at least, arose in situ by differentiation of mesenchymal cells, but he did not state any definite conclusions as to the origin of the small lymphocytes.

A study of the connective tissue in the region of the intestinal tonsils of the newborn rabbit, as stated before, revealed no traces of nodular lymphatic tissue. First evidences of future nodules are seen at an age of two to two and one-half days, at which time there is an apparent heaping up or condensation of mesenchymal or embryonic connective-tissue cells of the mucous membrane underneath the epithelium between the bases of the villi. These condensations are due to the appearance of free cells in the meshes of the mesenchymal reticulum. The free cells are, for

the most part, small round cells, with a round nucleus, fairly dense with chromatin, surrounded by a thin rim of densely basophilic cytoplasm. These are considered to be true small lymphocytes or late developing stages of the same, for they are morphologically identical, except that the chromatin particles are not always so heavy in the free mesenchymal cells as in the typical adult small lymphocyte. Many developmental stages are found, showing every possible transition between the fixed mesenchymal cell to the free small lymphocyte (figs. 1 and 2).

The small lymphocytes vary somewhat in size, due, doubtless, to varying sizes of the mesenchymal cells from which they develop. Developmental stages between fixed mesenchymal cells and lymphoid hemoblasts are only occasionally seen. It is exceedingly difficult to classify some of the free cells, as many intermediate stages between typical small lymphocytes and lymphoid hemoblasts are found. Typical adult lymphoid hemoblasts are very rarely found in the lymphatic tissue during the first week of postfetal life.

The small lymphocytes increase in number quite rapidly, both by transformation of mesenchymal cells and by proliferation of those already formed. Mitotic figures in small lymphocytes are numerous. During the first few days of postfetal life, a few mitotic figures can be seen in the ordinary mesenchymal cells also.

The larger lymphoid hemoblasts do not begin to be present in any quantity until some time during the second week of post-fetal life. When they become present to any extent, frequent mitoses may be seen to occur in them. It seems reasonable to assume that, if these divisions are repeated at frequent enough intervals, the size of the resulting cells would be much decreased; i.e., to the size of a small lymphocyte.

The small lymphocytes, rather than the lymphoid hemoblast, seem here to be the first type to develop. Their origin seems threefold; those first appearing developing only by direct transformation from mesenchymal cells and by proliferation of those already formed, and later on appearing also as a result of repeated rapid divisions of lymphoid hemoblasts.

It is also apparent that there are also two sources of origin for the lymphoid hemoblasts; first, by direct transformation of larger mesenchymal cells and, secondly, by growth of small and medium-sized lymphocytes, which have previously been formed from the mesenchyme.

These facts are added evidence to the view held by several authors (Maximow, Weidenreich, and Downey, etc.) that small and large lymphocytes (lymphoid hemoblasts) are not distinct cell forms, but merely different growth stages of the same cell, there being here a growth cycle, the small lymphocytes, by growth, becoming lymphoid hemoblasts, and the latter, by repeated divisions, forming small lymphocytes.

Because of the characteristic wheel-like arrangement of nuclear material in the small lymphocytes (which arrangement is not as characteristic of small lymphocytes in the rabbit as in some other forms), one is inclined to regard them as a definite cell form. The difference in the appearance of the nuclei of the two forms is, however, partially explained by the crowding together of the nuclear material into smaller area, which would also tend to obscure a nucleolus, if such were present.

But in case it is accepted that small lymphocytes and large lymphoid hemoblasts are different growth stages of the same cell form, it must be remembered that the cell as found in the small lymphocyte stage possesses different potentialities than when in the lymphoid hemoblast stage. The small lymphocyte may produce, by division, others of its kind, or by growth, a lymphoid hemoblast, which is the limit of its potentialities. It cannot possess the potentiality of producing other cells of the blood series without first development and growth into a lymphoid hemoblast (i.e., a small lymphocyte will never produce directly a granulocyte or erythroblast without, first, growth into a lymphoid hemoblast).

In studying the relations causing or associated with the formation of lymphatic tissue, it is found that this tissue always develops in places where there are rich lymphatic plexuses, and also in close relationship with the blood capillaries. These relationships were noted by Gulland ('94), Saxer ('95), and Sabin ('04) in developing lymphatic nodes. Hartmann ('14) decided

that the submucosal lymphatics of the intestine do not serve primarily in the transportation of chyle, but are, in a certain sense, related to the formation of follicles. As evidence of this he finds the first large groups of lymphocytes arranged about vessels, "as in lymph glands, and the tonsils of the mouth."

In the present study of the region of the intestinal tonsils, it was found that in the newborn animals and late fetuses before there were any evidences of lymphatic tissue and before absorption of food had started, that lymph vessels had formed quite extensively in the submucosa, making quite a rich submucosal plexus, such as the plexuses Gulland, Sabin, and Saxen found in regions of developing lymphatic nodes. This plexus connects with the retroperitoneal sac and through it with the thoracic duct and the venous system by means of lymphatics of the mesentery. (Heuer, '09; Sabin, '14). When the lacteals and the mucosal plexus form, they become connected with the submucosal plexus, the latter then becoming a part of the system for the transportation of chyle. None of the lymphatic vessels, then, which are found in the wall of the intestine are connected with any entering vessels, the lymph flow being all directed away from the intestine. Consequently, any lymphocytes which may be found in the lymph vessels in the intestine wall have probably entered them from the surrounding lymphatic tissue rather than have been carried in by the lymph stream. Thus the theory that lymphocytes of the intestinal tonsillar lymphatic tissue have been carried in and left by the lymph stream seems highly improbable.

It is noted, however, that there is also a close relationship between the blood capillaries of the mucosa and submucosa and the developing lymphatic tissue of the intestine. The first lymphocytes to appear, however, bear very little or no definite relation to the blood vessels, and only after they become present in considerable numbers do they appear to gather in clumps about the blood capillaries. This seems to the writer to indicate that the part played by the blood vessels should be considered as a nutritive one rather than a source of lymphocytes, as was thought by Gulland.

One factor apparently common to the formation of lymphatic tissue anywhere is that of an extensive supply of lymph vessels or lymph plexuses. It seems probable, therefore, that there is some influence exerted by the lymph or lymph vessels on the surrounding mesenchymal or loose connective tissue to induce lymphopoiesis. These lymph plexuses are always found in places where the blood supply is also very extensive. This very rich blood supply affords excellent nutritive conditions for the growth and multiplication of lymphocytes after the lymphocytopoietic reaction has been initiated by the lymph plasma or the lymph vessels.

Coincident with the differentiation of mesenchymal cells of the mucosa into free lymphocytes, there occurs a formation by other cells of the mesenchymal reticulum of connective tissue. This is at first a richly cellular, embryonic connective tissue. As the formation and proliferation of lymphocytes proceeds, the free cells separate the fibers of the connective tissue so that a branching, reticular network of fibers, reticular tissue, is formed, a skeletal framework, in the meshes of which the lymphocytes proliferate.

The first masses of cells formed in the region of the future Peyer's patch and of the nodular tissue of the appendix, composed mainly of small lymphocytes and transitional stages between them and mesenchymal cells or those of the embryonic connective tissue, bear little resemblance to adult lymphatic nodules (fig. 1). Lymphoid hemoblasts appear first to any extent at an age of eight days or later. Until this time the formation of most of the free cells has been by differentiation from mesenchymal cells. Many transitional stages between mesenchymal cells and free lymphocytes were seen in all preparations of tissues studied up to this time in development. At about the ninth or tenth day after birth mitotic figures begin to appear with some frequency, especially in the small lymphocytes, and the masses of cells at the same time begin to assume a more definite nodular shape. Lymphoid hemoblasts constantly increase in number, both by differentiation from mesenchymal cells and by growth from small lymphocytes. These lymphoid hemoblasts

assume no definite position in the nodules, but are found scattered throughout (figs. 3 and 4).

As the number of lymphocytes in a nodular mass increases, the connective tissue surrounding it tends to become pushed back and piled up or condensed apparently by outward pressure of the increasing number of lymphocytes, and the fibers and connective-tissue cells (fibroblasts) tend to assume a circular or concentric arrangement about the mass of lymphocytes, thus giving rise to a fairly dense sheath or capsule, which gives the nodule more definite limits and outline.

This sheath or capsule is by no means as complete and definite a structure as is found, for example, in the capsule of lymphatic nodes, but is rather a physical expression of the proliferation of lymphocytes in regions of unusually rich vascularity. Lymphocytes are always found to some extent among and outside of the connective-tissue fibers of the sheath, being by no means strictly confined within it.

In the process of the proliferation of lymphocytes and the formation of the connective-tissue sheath, reticular fibers and cells are gradually forced outward from the center of the mass, so that in later stages of nodular development few or no fibers or reticular cells can be demonstrated except near the periphery of the nodule.

Although the nodular sheath does not form an impassable barrier for the passage of lymphocytes, its presence and the fact that the reticulum is so much closer meshed at the periphery tend to inhibit the spreading of lymphocytes which are rapidly increasing in number. This inhibition causes the lymphocytes to become more closely packed at the periphery of the nodule than in the center. Thus the center of the nodule, because of the lesser number of cells there, appears lighter than at the periphery. This lighter appearance of the center of the nodule was noted by Flemming ('85) and named by him the 'Keimzentrum,' or 'germinal center,' for he considered this lighter central area a center of proliferation of lymphocytes. Later investigators have followed Flemming in calling this area the germinal center of the

nodule, a term which, as will be shown later, must certainly be considered as non-descriptive of existing conditions.

Flemming, in studying the structure of nodular lymphatic tissue found the lighter appearance in the center of the nodules was due largely to the fact that the cells making up that center were mostly those containing comparatively large, rather lightly staining nuclei with a relatively large amount of cytoplasm about them (germinal center cells, lymphoid hemoblasts), so that the nuclei were farther apart, while, on the other hand, those cells at the periphery were those with much smaller, more dense nuclei, with a smaller amount of cytoplasm surrounding them (small lymphocytes), so that when seen en masse the more closely packed, dense nuclei gave a darker appearance to the outer portions of the nodule than that of the central portion.

Although the evidences of proliferation, mitotic figures, were not confined strictly to the lighter colored central area, he found them occurring most frequently there, and not all in the dark, peripheral zone. Therefore, he called the lighter central area of the nodule, the germinal center. Other authors holding to this theory advanced by Flemming include Baum u. Hille, Baumgartner and Ribbert, Saxer, etc.

Others believed the germinal center was an expression of the rapid proliferation of lymphocytes, which proliferation, however, was scattered throughout the lymphatic tissue (Weidenreich and Downey, '12, also Maximow, Mollier, et al.).

Several authors noted the preponderance of cells of the lymphoid hemoblast variety in the lighter colored center, and some, consequently, named them 'germinal center' cells. Hartman ('14) found the germinal centers of the nodules of the intestinal tonsils to be due to this fact.

In the present investigation of the tonsillar lymphatic tissue of the rabbit it was found that the lightly staining central area in the nodules appeared at no very definite time, this depending, apparently, somewhat upon conditions which varied according to the individual. However, there is usually some little evidence of the lighter colored center at about the age of two weeks, but

it does not become well marked till an age of twenty-one days or older. Invariably the nodules of the appendix are further advanced in this respect than are those of Peyer's patch. At an age of twenty-four days, however, the lighter area is very definite both in nodules of the appendix and of Peyer's patch.

A study of all developmental stages and of adult nodules clearly indicates that these so-called 'germinal centers' are not centers of proliferation. In young animals mitotic figures are numerous in both the lymphoid hemoblasts and the small lymphocytes, but these mitotic figures are not confined to the center of the nodule, being apparently scattered throughout the nodule. In fact, in older stages, if anything, they seem more abundant out near the periphery of the nodule than in its center (figs. 3 and 4).

Mitotic figures are surely not confined to large or medium-sized lymphoid hemoblasts, for mitoses are very frequent in the small lymphocytes.

The grouping of lymphoid hemoblasts or germinal center cells in the center of the nodule to produce its lighter appearance could not be seen. In nodules in which the so-called 'germinal center' was most prominent, the lymphoid hemoblasts and the small lymphocytes were scattered equally throughout the nodule. At no time was there noted a special grouping of lymphoid hemoblasts in the center of the nodule or of small lymphocytes about the periphery. The proportion of small lymphocytes to lymphoid hemoblasts is great, but that proportion is the same throughout the nodule.

The so-called germinal center, then, should not, as that name would indicate, be considered a center of proliferation of the lymphocytes. Its lighter appearance is due merely to a different concentration of lymphocytes here than at the periphery of the nodule. The reason for the concentration of lymphocytes at the periphery is the purely mechanical one of the lymphocytes being held there in greater concentration by the denser reticular network and the surrounding capsule of connective tissue. In any mass of proliferating cells the natural tendency is to spread outward, which tendency is, in this case, checked somewhat by the

surrounding connective tissue causing the cells to pile up at the periphery.

Careful examination of the central portions of the nodules reveals the fact that the different concentration of cells is not alone responsible for the lighter appearance of the center. There are found among the elements making up a nodule, certain large cells, whose abundant cytoplasm is acidophilic in character, surrounding a fairly large nucleus, which also has a tendency to react to acid stains or stains only weakly basophilic. Some of these large acidophile cells were observed to contain in their cytoplasm bodies of various sizes and reacting variously to basis stains (fig. 5).

These cells have been quite differently described and interpreted by authors who studied them. Flemming ('85) described cells in the germinal center of lymphatic nodules, the nuclei of which were identical with the 'large lymphocytes,' containing in their cytoplasm various deeply staining bodies, which he designated stainable bodies. The nature of these bodies he did not know.

Downey and Weidenreich ('12) also found in the germinal centers large free cells, derived from cells of the reticulum which possessed phagocytic powers. The phagocytized elements they thought comparable to the stainable bodies of Flemming, which were possibly cellular, particularly nuclear remnants.

Hartmann ('14) also saw large acidophile cells in the nodules of the intestinal tonsils, which did not occur in lymphatic tissue elsewhere in the body. These were large, free, irregular shaped cells, which were derived from cells of the reticulum, with cytoplasmic inclusions. The inclusions he found to be always round or oval, and concluded they must be of a fluid or gelatinous nature. Because they stained a 'braunrosa' color with sudan III he thought them to be of a lipid nature.

The large acidophile cells found present in the intestinal lymphatic tissue are, morphologically, very similar to the cells described by Flemming, Weidenreich and Downey, and Hartmann. The cytoplasmic inclusions certainly do not, however, react as lipoids, as according to Hartmann, but in appearance

and staining reactions are identical with the 'tingible Körper' as described by Flemming.

The nature of the inclusions, if they are such, and the origin, fate, etc., of these acid-staining cells have not been satisfactorily determined. If these deeply staining bodies are foreign bodies, phagocytized by the non-granular acidophile cells, what, then, are the foreign bodies derived from?

If these bodies are inclusions and the cells containing them phagocytic, the cells should then take up particles of colloidal dyes if solutions of the dye are injected into the animal's body.

In order to determine whether the acid-staining cells possessed any phagocytic power or not, solutions of the acid azo colloidal dye, trypan blue, were used. Large quantities of a 5 per cent aqueous solution of the dye were injected into the peritoneal cavity of rabbits. In these instances all the dye was phagocytized by the large free cells in the peritoneal cavity and cells of the subperitoneal connective tissue. In order, then, to get the dye in a position where it could be acted upon by cells in the intestinal lymphatic nodules, operations were performed in which the peritoneal cavity was opened and a 5 per cent aqueous solution of the dye injected directly into the lymphatic tissue of Peyer's patch and the appendix, and the wound sewed up. After twenty-four to forty-eight hours the animals were killed and the tissue fixed in the usual way.

Examination of this tissue revealed the fact that large quantities of the dye had been phagocytized by the acid-staining cells and by the connective-tissue cells outside the nodules, the lymphocytes being free of it, except immediately about the point of injection, where they were diffusely stained, the cells at this point being apparently killed by the shock of injection. This shows clearly that these cells possess phagocytic powers and belong to the group of cells called macrophages (Evans) (fig. 6).

As to the nature of the inclusions, or 'tingible Körper,' found in these cells, it might be assumed from their appearance and staining reaction that they were nuclei of degenerated small lymphocytes in this region, and such, indeed, is the case. The 'tingible Körper' of Flemming, then, are remnants of nuclei

of small lymphocytes which have been phagocytized by the acid-staining macrophages (fig. 5).

These macrophages, when first making their appearance in the nodules (ten to twelve days after birth), seem scattered about throughout the mass of lymphocytes, but as the nodule assumes a more definite shape and as the light central area becomes more prominent, it is seen that the macrophages tend to become confined to the central area. The so-called 'germinal center,' then, is an expression of two things: first, the lesser number of cells found here as compared to the peripheral portion of the nodule, and, secondly, the presence of acid-staining cells in greater abundance in this central portion (figs. 3, 4, 5).

As to the origin of these macrophages, no transitions between them and cells of the reticulum could be found (as by Weidenreich and Downey, and Hartmann). But, on the other hand, many transitional forms between cells of the lymphoid hemoblast type, with a basophilic cytoplasm and a large clear distinct nucleus, and the macrophages with an acid-staining cytoplasm and a nucleus somewhat indistinct because of its weak basophilic or almost acidophilic character. This change in nuclear and cytoplasmic staining reaction indicates, I believe, beginning degeneration of the cell. It is well known that many kinds of cells during a degenerative process acquire phagocytic powers. Maximow has proved, in study of tissue cultures of lymphatic nodes, that large lymphocytes (lymphoid hemoblasts) upon further differentiation acquire phagocytic powers. Therefore, it is considered that acid-staining macrophages in the light staining nodular center are the results of further differentiation and degeneration of lymphoid hemoblasts in that region.

Rather than being a place of special cell proliferation, then, the so-called germinal center seems to be a place of degenerative change. It is here where the degenerating small lymphocytes are found, the nuclear remnants of which are phagocytized by the macrophages, which themselves are apparently further differentiated, degenerating lymphoid hemoblasts.

The reasons that seem to the writer most plausible for this degenerative change are those of nutrition. The nodule arises

in a place of abundant blood supply, but as the nodule increases in size, due to differentiation and proliferation, the capillaries traversing the nodule become insufficient to nourish the larger amount of lymphatic tissue. The cells nearer the periphery, then, are better nourished than those more centrally located, because they receive nourishment also from blood vessels of the tunica propria and the submucosa. Therefore, degenerations are more numerous in the more centrally lying cells in a completely formed nodule.

The relationship between the system of lymph vessels and the nodules of lymphatic tissue in the intestinal tonsils is much the same as the relationship between the nodules of lymphatic and hemolymph nodes and the surrounding secondary sinuses. But in the lymphatic nodes the lymph in the sinuses bathes the lymphatic tissue directly, while there are no lymphatic sinuses in connection with the intestinal nodes. Injection of the lymphatic vessels (Berlin blue gelatin mass) shows, however, that there is a dense network of lymphatic capillaries just outside the connective-tissue sheath of the nodule. This is, of course, part of the submucosal plexus, which is, in turn, part of the system for transportation of the chyle (Heuer, Sabin). No lymphatics could be found penetrating the nodule in any case.

Among the things which must be considered in an interpretation of intestinal tonsillar tissue is the relationship existing between the lymphatic tissue and the overlying epithelium. Lymphatic nodules of the intestine do not remain restricted to definite areas, but spread in all directions from the point of formation. Thus the tunica propria in villi directly over lymphatic nodules become infiltrated with lymphocytes to such an extent as to form enlarged 'lymphatic villi.' After a certain stage in development (about fourteen days) has been reached, the lymphocytes begin to wander into the epithelium covering these lymphatic villi. It is, doubtless, this close association between the epithelium and the lymphatic tissue which led some of the earlier authors (as Retterer and von Davidoff) to the belief in an epithelial origin of these lymphocytes. Most authors, however, consider the relationship between the lymphocytes and the

epithelium to be secondarily acquired, due to the rapid growth of the lymphatic tissue. Jolly ('11), who noted the extraordinary infiltration of the epithelium over the intestinal tonsils, included them (i.e., the infiltrated epithelium) with the tonsils of the mouth and the thymus in one group, the 'lympho-epithelial organs,' in which he believes there exists a symbiotic relationship between the epithelial cells and the lymphocytes.

The lymphocytes, as stated above, first began to invade the epithelium to any extent about fourteen days after birth. Of course, from the very first appearance of lymphocytes in the tunica propria, some may be found in the epithelium but they do not invade it in great numbers till an age of two weeks or more has been reached. Until that time the basement membrane of the epithelium is quite complete, making a definite boundary between the epithelium and the underlying tissue, clearly indicating the impossibility of any of the elements of the lymphatic tissue taking origin from the cells of the epithelium. As more and more lymphocytes crowd into the epithelium the basement membrane becomes gradually less distinct, and the epithelium, at first was of a simple columnar type, gradually acquires the appearances of a heavily infiltrated, stratified epithelium, because of the displacement of the epithelial cells by the invading lymphocytes. Its thickness from cuticular border to basement membrane is greatly increased, but it really resembles an epithelium very little because of the huge number of contained lymphocytes. The invading cells, for unknown reasons, are all of the small lymphocyte variety. About each lymphocyte soon after it enters the epithelium is found a clear area, indicating some chemical activity by the lymphocytes upon the epithelial cells immediately surrounding them (fig. 12).

Not all of the lymphocytes found in the epithelium have wandered in from the underlying tissue, as there is evidence of proliferation of those which have already invaded it (fig. 12). Mitotic figures are occasionally seen in lymphocytes in this location. The spaces among the epithelial cells in which the lymphocytes rest are seen to often contain in later stages several small lymphocytes which have doubtless been derived by succes-

sive divisions of one which had invaded the epithelium. The lymphocytes in the intestinal tonsils, as opposed to those in the tonsils of the mouth and pharynx, rarely, if ever, wander through the epithelium into the intestinal lumen.

An interesting feature of the epithelium covering the lymphatic villi is the lack of goblet cells, which are so plentifully found elsewhere in the intestinal epithelium (Muthmann, Hartmann). In a study of the early developmental stages, it is found that goblet cells are numerous in the epithelial covering of all the villi. Glands (crypts of Lieberkühn) are not present as such until later (showing that goblet cells are not, necessarily at least, derived from the intestinal glands), but the epithelium between the bases of the villi is of a different character. In these regions no goblet cells were found. The places of nodular formation are in the tunica propria directly below places where there is an epithelium of this nature, and from these places the lymphatic villi arise usually, the epithelium being gradually forced upward between the ordinary villi as the lymphatic tissue increases in amount, the original character of the epithelium between the bases of the villi being maintained (i.e., without goblet cells). In a few instances, goblet cells were found in the epithelium of some of the lymphatic villi before its infiltration with lymphocytes occurred. The lack of goblet cells in the epithelium covering the lymphatic tissue is doubtless due to change in function of the epithelium, brought about by its relation to the lymphocytes (fig. 12).

The fundamental reasons for the development of lymphatic tissue, especially of the tonsillar lymphatic tissue, and the complete function performed by it are perplexing problems.

The only definite function which has been ascribed to lymphatic nodes is the production of lymphocytes. This surely is a function, but whether it is their only one is doubtful. Then the question arises as to whether tonsillar lymphatic tissue has other function than does the lymphatic tissue in other places. Some authors consider that, in the light of our present knowledge of tonsillar tissue, the only function that can be accurately ascribed to it is that similar to the function of lymphatic nodes; i.e.,

the production of lymphocytes (Brücke, '51; Hartmann, '14). In view of the fact that lymphatic tissue develops in places of degenerating body material, such as degenerating glands, rudimentary organs, etc., many authors thought the tissue as developing in these places assisted in absorbing and doing away with this degenerating material (Gulland, '91; Stöhr, etc.). Still others noted some relationship in the intestinal tonsils between the intestinal glands and the formation of lymphatic tissue, but were not sure of the nature of this relation (Flesch, '88; Klaatsch, '92).

It is well known that, in the presence of regressive structures, mesenchymal, or embryonic connective tissue shows a 'lymphocytopoietic' reaction. Accumulations of lymphocytes are encountered in glands of various kinds, as kidney, thyroid, salivary glands, minor digestive glands, etc., these accumulations being accompanied by degeneration and infiltration of certain of the glandular acini (Kingsbury, '15). Stöhr also calls attention to the fact that 'leucocytes' collect in places where organs are degenerating, as the pronephros of lower forms, gills of anura, thymus, processus vermiformis.

In the case of the rabbit, it is quite clear that the degeneration of any structures in the region of the intestinal tonsils does not initiate the formation of lymphatic tissue there. For no crypts of Lieberkühn are formed until lymphopoiesis is well underway. However, as the nodules of lymphatic tissue increase rapidly in size, they often include in their midst portions of intestinal glands, which are usually degenerating, this degeneration probably due to the infiltration of the lymphocytes, rather than the opposite. Any association observed here between the crypts of Lieberkühn and the lymphatic nodules is accidental, being due to the very rapid growth of the lymphatic tissue.

If the lymphatic tissue were to be interpreted as a lymphocytopoietic reaction of mesenchyme to regressive or degenerating structures, these structures must have been present at some time in the ancestral history, which in the animal, as existing now, do not appear at any time in its development.

It certainly seems as though the presence of a lymphatic plexus in the submucosa is insufficient to account for the extraordinary development of lymphatic tissue in Peyer's patch and the appendix, for this plexus is found throughout the small and large intestine and the stomach. It is noted that tonsillar tissue is greatly concentrated in and about the caecum. There may be some relationship existing between fecal matter in the caecum and appendix and the great development of lymphatic tissue here.

In discussing the functional possibilities of the intestinal tonsils, it may be recalled that the lymph vessels of the submucosal plexus form a fairly dense network of capillaries about the nodules. Lymphocytes are found quite abundantly in these vessels. The only source of these lymphocytes could be the lymphatic nodules of the tonsils, for there are no entering lymphatics which could carry them on. As the submucosal plexus is a part of the system for transportation of the chyle, it is evident, then, that the tonsillar tissue here serves, in part at least, in forming elements of the chyle.

GRANULOPOIESIS

It has already been pointed out that sometimes certain cells with granular content are abundantly present in the connective tissue of the wall of the intestine, especially in the region of the intestinal tonsils in and about the caecum. We are here able to study only the significance of those with acidophilic granules, as the basophilic granules of the mast cells have been dissolved out by the methods of tissue preparation used. Alcoholic fixation, which was found to preserve the basophilic granules, did not furnish material upon which their histogenesis could be determined.

The free eosinophile cells occur in varying abundance in the connective tissue of the submucosa and the tunica propria in the region of the intestinal tonsils, being found, however, most extensively in the deeper portions of the tunica propria, above and between the nodules of lymphatic tissue. They are often found in quite large numbers in the villi, closely associated with the intestinal epithelium, to a less extent in the connective-tissue sheath of the nodules and in the submucosa, and very rarely

in the midst of the nodules. They are usually found in quite intimate association with the blood vessels of the mucosa. In the appendix they are found in greater abundance than in the iliac tonsil of the same individual.

Modern hematologists are practically united in declaring for eosinophile leucocytes (granulocytes) an origin, either directly or indirectly, from cells of a lymphoid character. But, in respect to eosinophile cells of the intestinal mucosa, it must yet be decided whether they arise by local differentiation of lymphoid cells or have been carried in and dropped there by the blood stream.

Hartmann ('14) observed the large groups of granulocytes in the region of the intestinal tonsils, and he considered the majority of them to be true eosinophile leucocytes, with somewhat rodlike granules, and a polymorphous nucleus. He found, rarely, mononuclear forms with a light staining nucleus. He considered the eosinophiles found here as having been carried in by the blood stream.

Weill ('19), on the other hand, in studying the formation of the eosinophiles of the intestinal mucosa, favored the view that they were differentiated in situ from cells of a lymphoid character (lymphoid hemoblasts). He found transitional stages between the non-granular lymphocytes, through mononuclear granular forms, the 'eosinophilic myelocytes,' to a polymorphonuclear eosinophilic leucocyte. He declared that the young mononuclear forms, at least, could not have been carried in by the blood stream, for no cells of that type were found in the blood, all there being of the polynuclear or polymorphonuclear type.

Although hematologists are practically agreed as to the lymphoid origin of granulocytes, the question of the origin and significance of the acidophilic granules of the eosinophiles is still a much debated one. Several authors are of the opinion that eosinophiles are derived from neutrophiles or other special cells by change in the granular character (van der Stricht, Gulland, Thayer, Arnold, Jolly, et al.). Others considered that neutrophiles might transform into eosinophiles after taking up exoge-

nous material such as hemoglobin from extravasated red blood corpuscles (Klein) or products of degenerating muscle (Brown).

That the eosinophile granules of eosinophile leucocytes are phagocytized exogenous material of a hemoglobin nature is a belief held by many. This theory was followed by Schott, Gütig, Th. Lewis, Badertscher, and many others, but has been most vigorously defended by Weidenreich ('01, '08, '11, etc.). His conclusions were based on observations in hemolymph nodes and the spleen, and also on a series of experiments in which quantities of erythrocytes were injected into the peritoneal cavity of animals, where they undergo degeneration, breaking up into small particles which are phagocytized by large lymphocytes (lymphoid hemoblasts) of the peritoneal cavity and of the *tâches laiteuses* of the omentum (Weidenreich, '08; Schott, '09). In case there is no evidence of erythrocyte fragmentation, which Weidenreich admits is possible, he assumes the hemoglobin is released in solution, absorbed by the lymphocytes, and deposited in them in the form of granules. Badertscher ('13) found also that fragments of degenerating muscle may be phagocytized by lymphocytes, which then undergo a series of changes to become identical with blood eosinophiles. Some authors consider eosinophilic granules as related to hemoglobin in their nature, but they think are formed endogenously, and not introduced from without (Marwedel, '97; Pappenheim, '05).

Many of the recent investigators of this problem disagree with Weidenreich in his belief that the eosinophilic granules were exogenous material, but consider them true endogenous formations. Even here there is considerable difference of opinion as to the method of formation of the granules. Some (Danchakoff, Weill) thought the eosinophile granules were formed directly as such, appearing in the midst of the basophilic cytoplasm of lymphoid hemoblasts (according to Danchakoff, about the periphery of a slightly acidophilic centrosphere), the cytoplasm gradually losing its basophilic character as the granules increase in number. Maximow ('10) thought that the eosinophile leucocytes were not formed directly from lymphoid hemoblasts, but that first cells with 'pseudeosinophil' granules were formed

(‘Promyelozyten’ of Pappenheim), the granules of which later transformed into true eosinophile granules. Still others found the granules in these cells when first formed to be of a basophilic character, direct transformation and change later taking place, the staining reaction of the granules changing from basophilic to eosinophilic character, at the same time as other changes necessary to produce the adult eosinophile leucocytes are occurring (Downey, '15; Ringoen, '15).

The eosinophiles, occurring in the connective tissue of the tunica propria and submucosa of the digestive tract, in the region of the intestinal tonsils, occur in varying numbers, sometimes there being but a very few widely scattered, and again being found as large, concentrated masses, or granulopoietic foci. There is no definite time in development at which they appear in greatest abundance, this being apparently controlled by local conditions, varying according to the individual. They are, however, never present in great numbers until after the first week of postfetal life. Also, as stated before, they are almost invariably more abundant in the wall of the caecum and the appendix than in the iliac tonsil.

Their distribution in the connective tissue has already been spoken of. They are usually in greatest abundance in the tunica propria, especially around the bases of the typical, ordinary villi (figs. 10, 11, 16) (except isolated cases where large granulopoietic foci were found in the subnodular connective tissue).

A careful study of the eosinophile granulocytes as found in these places makes evident the fact that they are morphologically of two types, 1) the blood eosinophile and, 2) the connective-tissue eosinophile.

The first type, as the name given indicates, closely resembles the eosinophile leucocytes found in the blood stream. The cell is of a rounded form and contains a very polymorphous richly chromatic nucleus. The contained granules are rounded or slightly elongated (as those of the blood stream), and are packed quite closely together within the cytoplasm of the cell. With blood stains (as Hastings-Nochts) these granules stain a bright red color.

The other type of eosinophile found here is quite variable in shape according to its immediate surroundings, being sometimes rounded and again quite elongate, spindle-shaped, or irregular in contour. The nucleus is of an entirely different appearance than that of the first type, never being of an extreme polymorphous shape. It does, however, present variations in shape, sometimes appearing as one dense, round nuclear mass, and again appearing as two round masses, lying either side by side in the cell or widely separated at opposite sides of the cell. The cell body of these cells is very closely packed with particles, which are definitely rod-like and elongated. These rod-like particles stain an intense dark red when compound blood stains (Nochts Hastings, eosin-methylene blue) are used.

The first type, the blood eosinophile, is very evidently the same as Hartmann found in the neighborhood of the intestinal tonsils (of the rabbit). It is the first type to appear in the connective tissue at this place, the connective-tissue eosinophile not being found until between the first and second weeks of postfetal life. The blood type of eosinophile is never found in large numbers, except under pathological conditions (such as presence of parasites, etc.). It seems very evident that they, as Hartmann thought, are carried into the connective tissue and dropped there by the blood stream. No developmental forms are found to indicate a possible local formation of this type of cell.

On the other hand, there is evidence to indicate that the connective-tissue eosinophiles, found so abundantly in close relationship with the intestinal tonsils, are formed *in situ* at the expense of lymphoid hemoblasts also developing there (figs. 9, 10, 14, 16).

This process of development was found to be exceedingly difficult to follow because of the scarcity of developmental stages. This is accounted for by the great rapidity of the formation of the eosinophile granules after they have begun to differentiate out. Quite a few cells were found, however, the cytoplasm of which was only slightly basophilic, or colorless, which contained a few scattered, round eosinophilic granules (dark red). The nuclei of these cells were identical with those of lymphoid hemo-

blasts or slightly smaller (fig. 9). The mononuclear forms of rodded eosinophiles present the next step in nuclear differentiation, being similar to the 'hemoblast' type, but smaller and with more condensed chromatin material. By division of this nuclear material into two parts the binucleated rodded eosinophile leucocyte is produced. All transitional stages between mononuclear and binuclear forms can easily be found. First the round nucleus assumes an oval form, then an indentation appears in one side, and soon the two parts thus divided begin to pull apart, forming two lobes with a connecting strand. This connection seems gradually to disappear, so that there are apparently two distinct nuclear masses in the cell (figs. 10, 16).

In the production of granulocytes, then, the cytoplasm of involved lymphoid hemoblasts, the cells of origin, partially (as Maximow states) or entirely lose their basophilic character. After the basophilic character of the cytoplasm is lost, rounded eosinophile granules are formed endogenously in it, the nuclei at the same time decreasing in size. These granules increase rapidly in size and number, at the same time changing into a rod-like shape (in the rabbit). Soon the eosinophile particles fill all available space in the cytoplasm of the cells. Coincident with this is noted a continued decrease in the size of the nuclei with correspondingly increased density of the chromatin material. The change from the mononuclear to the binuclear form, as described above, apparently occurs after the cytoplasmic changes have been completed. This binucleated form is, as far as can be determined, the adult form of the connective-tissue eosinophile as occurring here.

Concerning the possibilities of homoplastic formation of eosinophiles, it certainly does not occur in the blood eosinophiles found in the connective tissue. No mitotic figures were seen in this type, as might be expected, for they are only present here in the adult condition. Mitotic figures in adult connective-tissue eosinophiles are never seen. They have been reported (by Dan-chakoff and Weill) in developing granulocytes or granuloblasts. The writer found no eosinophiles showing evidences of mitosis after granules had begun to be formed. This, then, as a source

of eosinophiles must be considered negligible; the main sources being by the heteroplastic differentiation of lymphocytes and from the blood stream.

The conditions causing or related to the phenomenon of granulopoiesis have not as yet been clearly brought out. The close association of granulopoietic foci with the blood vessels, which can usually be seen, indicates the probability that the blood in some way plays an active part in the formation of the eosinophiles. It is noted also that when parasites (as *Coccidia*) are present in any abundance that eosinophiles tend to become very numerous, especially in the tunica propria in the immediate vicinity of the parasites. The *Coccidia* may be found in groups near the bases of the villi, either imbedded in the tunica propria, just under the lining epithelium, in among the epithelial cells, or in the intestinal lumen, just outside the epithelium. The eosinophiles being formed locally or migrating from the blood vessels may invade the epithelium, or in case the parasites are found in the intestinal lumen, may break through the epithelium into the lumen where they gather about the parasites. The eosinophiles often invade the epithelium in such numbers that it is entirely broken up and loses its identity as such. The eosinophiles of both types always gather about and in the midst of the groups of *Coccidia* in an apparent effort to destroy them or combat their influence.

Other inflammatory or irritating conditions other than the presence of parasites may also be associated with extensive development of granulocytes. The fact that eosinophiles are more abundant in the appendix and caecum than in other parts of the intestinal tract may possibly be accounted for by their irritation caused by the presence of fecal matter in the intestinal lumen.

There is, therefore, surely some relationship existing between the presence of irritating substances, pathogenic organisms, etc., and the production of eosinophile cells. It is well known that eosinophiles from the blood collect in and about places of inflammation, but, in addition to that, inflammation or irritation of some kinds in the intestine seems to initiate granulopoiesis locally or the production of connective tissue eosinophiles. The more ex-

tensive the inflammation, the greater seems the production of eosinophiles.

The close association of blood vessels of the connective tissue with the groups of eosinophiles has already been mentioned. This is partially explained when it is recalled that some of these eosinophiles in the connective tissue are of the type found in the blood stream, which have wandered out through the capillary endothelium into the surrounding tissue. But there is an equally close association between the blood vessels and the granulopoietic foci of connective-tissue eosinophiles. Groups of granuloblasts (eosinophilic myelocytes) are always in the immediate neighborhood of some of the blood vessels of the mucosa. When the blood supply is increased as under inflammatory conditions of any nature, as caused by some irritant, such as presence of pathogenic organisms, the number of eosinophiles increases correspondingly. The greater the blood supply, the greater becomes the granulopoietic activity. It is very probable, therefore, that some agent in the blood stream (possibly in the plasma) plays an important rôle in the process of granulopoiesis. It may be suggested, therefore, that the underlying cause for excessive granulopoiesis is, then, the presence of some irritation bringing about essentially inflammatory conditions, the direct cause being some constituent of the blood stream (plasma?), the exact nature and action of which influence is unknown.

ERYTHROPOIESIS

The possibility of erythropoiesis occurring in the mesenchyme in various regions in the embryonic body has been noted by many hematologists.

Some of the earlier authors found in the subcutaneous connective tissue in some animals what they considered as intracellular development of red corpuscles. (Schafer, '74; Ranvier, '74; Le Boucq, '75.) This apparent intracellular development is now usually considered as an instance of the reverse process, atrophy of already formed vessels and breaking down of the contained erythrocytes by phagocytosis.

Typical erythropoiesis, however, has been observed by many authors in the mesenchyme or embryonic connective tissue in various places in the embryonic body. Saxer ('96) found it occurring in the mesenchyme in and about the musculature of the neck, in subcutaneous tissue, and various other places. Maximow found in rabbit embryos, it might be found in practically any place in the body mesenchyme, especially of the head region. Badertscher ('15), in study of pig embryos, found developing red blood cells in portions of the neck and upper thorax, as well as in the cortex and medulla, and interlobular septa of the thymus. Danchakoff also described erythropoiesis in the mesenchyme of the head in younger embryos, and in older stages also in the mesenchyme in other parts of the body.

Erythropoiesis, as occurring in these places, is mainly extravascular, although Danchakoff found it occurring both intra- and extravascularly. This, however, she does not consider as true, typical erythropoiesis for few or none of the thus formed erythrocytes get into the blood stream.

Aside from erythropoiesis occurring in the body mesenchyme, it is well known that it occurs in, or at least among, the mesenchymal or reticular cells in the embryonic liver and also in the embryonic spleen to some extent (in a few animals also in the adult spleen).

But the process of erythropoiesis, according to our present knowledge, is in postfetal life, limited to the red bone-marrow, with the exception of the first few days after birth, before the erythropoietic activity of the liver and of the spleen has ceased, and of the spleen, which may under pathological conditions (and in some animals, as the skunk, normally) reassume or continue its embryonic erythropoietic activity.

With this fact in mind, that, normally, after the second week of postfetal life, the sole seat of erythropoiesis known is the red bone-marrow (except in the spleen as stated), it is interesting to observe that, in studying sections from the appendix and the iliac tonsil of rabbits of an age varying from two to six weeks postpartum, there may be found in the connective tissue of the submucosa of these regions groups of cells in different stages of

the formation of erythrocytes. These erythropoietic foci are found mainly in subnodular and internodular connective tissue of the submucosa, just the opposite of the principal granulopoietic foci which are, in large part, found in the tunica propria above and between the lymphatic nodules. Erythropoietic foci are also of more frequent occurrence, and more extensive when present, in the region of Peyer's patch than in the appendix, again just the opposite of the granulopoietic activity.

Scattered erythroblast cells were also found in the subnodular and internodular connective tissue in stages both younger than two weeks and older than six weeks, but only during this period were erythropoietic foci of any extent found.

This close association between erythropoiesis and lymphoiesis is of exceptional interest because of the exceeding rarity of red-cell formation in connection with lymphatic tissue. One case of erythropoiesis occurring in lymphatic nodes was described by Pappenheim. This was found under pathological conditions, however, being associated with an acute case of hemorrhagic macrolymphocytic leukemia. Scattered erythroblasts have also been noted from time to time in hemolymph nodes. In the ileum and occasionally in the appendix of the rabbit one finds erythropoiesis occurring in no uncertain manner under apparently normal conditions in close relationship with the lymphatic tissue there present (figs. 7, 14, 15).

The developmental history of the three types of blood cells and possible relations between them has long been a perplexing question. The relation in origin between cells of the erythrocyte and granulocyte series has been particularly a matter of spirited controversy. On this question hematologists are divided in opinion into two groups.

The first group, believing in a monophyletic origin of blood cells, consider the erythrocytes and granulocytes are derived from a common stem cell, which, under certain environment, will develop in to the other type of blood cell.

The second group, on the other hand, believing in a polyphyletic origin of blood cells, consider that all types of cell of the blood series arise from stem cells of different character, which,

under any conditions of environment, will give rise to one type of cell only.

The monophyletic theory, first advanced by Saxer ('96) and Bryce, is, in some form, accepted by most of the more recent hematological workers.

Among those who most vigorously defend this monophyletic theory are Maximow, who made careful study of hematopoiesis as occurring in various places in the mammalian embryo, and Danchakoff who made investigations similar to those of Maximow in chick embryos, and also, more recently, studied experimentally produced erythro- and granulopoiesis in the spleen of the chick. Weidenreich, Pappenheim, in part, and many others also uphold this view.

With the histogenesis of erythroblastic tissue so clearly established by Saxer, Weidenreich, Pappenheim, and especially by Maximow and Danchakoff, it is unnecessary to discuss this in detail. A study of the regions in the connective tissue about the intestinal tonsils, where erythropoietic foci are forming, clearly indicates that the stem cells from which the cells of an erythroblastic nature develop, are identical, morphologically, with the lymphoid hemoblasts, or so-called germinal center cells of the lymphatic nodules, and, therefore, morphologically identical with the stem cell producing connective-tissue eosinophiles; i.e., a cell with a large, rounded or oval clear vesicular nucleus, with scanty chromatin, and one or more prominent nucleoli, and a variable amount of basophilic cytoplasm.

Subsequent changes of this type of cell to form an adult erythrocyte involve both the nucleus and cytoplasm. These changes of nucleus and cytoplasm are possibly not interrelated in any way, but they do occur simultaneously, and are brought about by conditions affecting the cell as a whole. Cytoplasmic changes are the gradual change from a basophilic to a brilliant acidophilic character, due to the laying down of hemoglobin in it. Nuclear changes are a gradual shrinkage in size, assumption of a pycnotic condition, and finally its extrusion from the cell. Many of the non-nucleated adult erythrocytes (erythroplastids) are found free in the connective-tissue spaces as well as developmental stages.

Presupposing the stem cells of the erythroblastic line of development to be elements of a lymphocytic nature (lymphoid hemoblasts) which are morphologically the same as the stem cells of the granuloblastic line of development, the question is raised as to what the factors are causing morphological similar cells to differentiate in such entirely different ways. Do the stem cells, as some polyphyletists say, possess only morphological similarity, with some inherent differences in them, by reason of which each must develop along a certain line? It seems more feasible to attempt to explain this difference in development by changes in environmental conditions, which would affect the metabolic activity of the cell.

In forms below the mammals a decided difference in environmental conditions is noted. Maximow has shown that erythrocytes develop intravascularly and granulocytes extravascularly in amphibia and selachians. It has been observed in studies of blood-cell development in birds and reptiles that almost invariably the erythropoietic foci are located intravascularly, the granulopoietic foci extravascularly (van der Stricht, Bizzozero, Danchakoff, and others). This relationship has been most carefully studied by Danchakoff. She did not, however, as van der Stricht, think the presence of an endothelial wall as sufficient evidence of the separate origin of these two types of cell.

If this were invariably true that erythropoiesis occurs intravascularly and granulopoiesis occurs extravascularly, that difference in environmental conditions would surely be sufficient to account for the different development of these cells; but, unfortunately, there seems to be an exception to this rule in the case of mammals, for it is a commonly accepted fact now among hematologists (except van der Stricht and a few others) that erythropoiesis occurs in large part extravascularly in mammals.

But if one finds similar cells under exactly the same environmental conditions developing into totally different types of cells, it strengthens the belief that there must be some inherent differences between these apparently similar stem cells. Stockard ('15) arrived at this conclusion after noting the extravascular formation of both types of blood cells in mammals. He found

also, in *Fundulus*, that erythropoiesis occurred only extravascularly, and that as soon as the erythropoietic tissue became included in a vessel, the process shifted to other regions of the embryo. The intravascular conditions he considered inadequate and inhibitory, rather than active factors for erythropoiesis.

As a partial solution of this apparent discrepancy in mammals might be advanced the results of Mollier ('13) in his study of erythropoiesis in the human embryonic liver. He found the endothelial walls of the sinusoidal vessels to be reticulated so that communication was made between the lumina of blood vessels and the immediately surrounding mesenchymal spaces in which the erythrocytes develop. Thus conditions in these spaces would be essentially intra- rather than extravascular.

Secondary extravascular erythropoiesis was also found to occur in birds (chick) by Danchakoff in the allantois following splenic grafts upon it. Some of the walls of allantoic blood vessels degenerated allowing the contents of the vessel, early stages of erythroblasts, to wander out into the mesenchyme forming extravascular erythropoietic foci. The cells thus liberated did not revert to lymphoid hemoblasts, but continued their development as begun intravascularly.

Using Maximow's experiments on the formation of bone-marrow in the kidney of the rabbit following ligation of the renal vessels, she applies this theory to mammals. Maximow found large groups of lymphoid hemoblasts collecting in the vessels due to the slower current caused by ligation which began erythropoietic differentiation intravascularly, and wandered out into the surrounding tissue at the normoblast stage. With this evidence at hand she thinks it probable that all extravascular erythropoiesis, whenever found, is only secondarily so, the process outside of the vessels being a homoplastic differentiation of specific cells (erythroblasts), irreversible in their development. Erythropoiesis, as occurring in the submucosa in the region of Peyer's patch, in the rabbit, is definitely extravascular. Erythropoietic foci of very large proportions are often found at certain developmental stages of the tonsillar tissue, sometimes apparently filling most of the available space in the subnodular

and internodular connective tissue. Most of these foci do, it is true, contain cells in the later stage of erythroblastic development (normoblasts). But megaloblastic, extravascular foci may also be found. Cells in the same group are often at various stages of erythroblastic development (figs. 8, 14, 15).

If this extravascular erythropoiesis were secondarily so, one would expect to find at some developmental stage intravascular megaloblastic foci, or at least intravascular groups of lymphoid hemoblasts, but at no time were such found. The entire process of erythropoiesis as it occurs here is apparently extravascular.

No evidences were found to indicate that the walls of the submucosal blood vessels were reticulated so as to make the mesenchymal spaces connected with the lumina of the blood vessels. This is true extravascular erythropoiesis, and not secondarily so, nor occurring under conditions of intravascularity (as Mollier described).

Though this erythropoiesis is extravascular, erythropoietic foci are always seen to be in very close relationship with the blood vessels of the submucosa. There is little doubt but that some action of the plasma or some element contained in it upon lymphoid hemoblasts locally developed incites them to further differentiation along the erythroblastic line of development. This seems to be very similar to the granulopoietic relations before described, but it is found that this relationship between erythropoietic foci and the blood vessels is much closer than between the granulopoietic foci and the blood vessels. There certainly is, however, very great similarity between granulopoiesis and erythropoiesis, and only a slight difference in environmental conditions must exist.

Both granulopoiesis and erythropoiesis are found to occur in largest extent when the blood vessels are gorged with erythrocytes (i.e., when the current in the blood stream is slow). If one considers the various places in the body of the embryo and adult where these two phenomena are known to occur, as in the liver, spleen, bone-marrow, etc., these are found to be invariable places where the blood current is slow, the slowing of the current usually being due to the sinusoidal nature of the vessels. This slowing

of the current, together with the thinness of the vascular walls, usually only a lining endothelium, affords excellent opportunity for the transudation of substances from the blood stream to the outlying near-by lymphoid hemoblasts.

It seems very probable, though, that the complete explanations for these erythropoietic tendencies are deeply seated.

Elements are present in the blood stream in certain definite proportions. A disturbance of this balance between elements in any way, as by change of conditions, destruction of some of the elements, etc., causes initiation of erythropoiesis to restore that balance. The submucosal connective tissue in these regions affords an excellent place for this to occur, for here stem cells (lymphoid hemoblasts) are being produced, the current of the blood stream is slow, the blood vessels are numerous, and the vascular walls, to a great extent, quite thin. Then, too, the connective tissue is here performing no very active function, and ample space is provided between the bases of the nodules and the muscle coats (especially in Peyer's patch) for the development of erythropoietic foci.

DISCUSSION OF THE RELATIONS CONCERNED WITH THE FORMATION OF THE BLOOD CELLULAR ELEMENTS

The formation and development of the three different types of blood cells, then, and relations causing or connected with the appearance of each type evidently are very closely associated.

Lymphopoiesis, granulopoiesis, and erythropoiesis are all closely associated with the vascular system. Lymphopoiesis, of course, must be the first process to occur, for it is from stem cells of a lymphoid nature that the cells of the granuloblastic and erythroblastic series are developed. This lymphopoietic process is, apparently, in some way, initiated by the lymphatic vessels, free lymphoid cells (lymphoid hemoblasts and small lymphocytes) forming by differentiation of mesenchymal cells. The subsequent fate of these cells, thus formed, is dependent upon the blood supply and the closeness of their relation with the blood vessels. If there seems to be no particular association with the blood vascular system, these free cells continue to

develop in a somewhat random fashion, and the result is a diffuse lymphatic tissue. If there is a very good blood supply giving excellent nutritive conditions for growth and proliferation, there is a rapid increase in the number of free cells in regions immediately about the blood vessels, so that dense, nodular lymphatic tissue is formed.

After cells of the lymphocyte series are formed (lymphoid hemoblasts and small lymphocytes), some of them may become more intimately associated with some of the large thin-walled blood vessels of the mucosa or submucosa, in which the current of the blood stream is quite slow, and differentiate further into either granular leucocytes (eosinophilic) or erythrocytes, according to the closeness of this relationship, and the degree to which the conditions of the vascular wall and the current of the blood stream make the transudation of the necessary materials from the blood stream possible. This is also governed by the distance of the hemoblasts from the source of materials, the blood vessels, those farthest from the vessels having granuloblastic tendencies and those nearer to them erythroblastic tendencies. No small lymphocytes, however, develop directly into either granuloblasts or erythroblasts, always first by growth, developing into cells of the lymphoid hemoblast type.

These factors influencing further development and differentiation of lymphoid hemoblasts may account for the greater concentration of granuloblastic cells in the tunica propria and the greater concentration of erythroblastic cells in the submucosa in the region of the intestinal tonsils. For the submucosal vessels are larger and more sinusoidal and comparatively much thinner walled than those in the tunica propria. The slower current, due to the more sinusoidal character of the vessels, coupled with the comparative thinness of the walls, affords better opportunities for transudation of materials from the blood stream, so that conditions are better for erythropoiesis in the submucosa. Similar conditions, to a lesser degree, exist in the tunica propria, so that here the process most usually taking place is granulopoiesis.

SUMMARY

1. The first free cells appearing in the process of the formation of lymphatic tissue in the intestinal tonsils are mainly small lymphocytes, which arise by differentiation of fixed mesenchymal cells. A few of the large mesenchymal cells, however, transform directly into large lymphoid hemoblasts. These two types are apparently different growth stages of the same cells, the small lymphocytes by growth and slight differentiation becoming lymphoid hemoblasts, and the latter by repeated divisions becoming reduced to small lymphocytes. Further indication of their close relation is the fact that either type may arise by transformation of mesenchymal cells, according to the size of the transforming cell.

2. The so-called 'germinal center' is not really a center of proliferation of lymphocytes as that name would indicate. The lighter appearance of the central portion is partially due to the looser arrangement of cells there than at the periphery where the cells pile up as a result of their outward spread being limited by the surrounding connective tissue. The other factor producing this lighter appearance is the greater number of large acid-staining cells in this position.

3. The acidophilic cells are found to arise by further differentiation and degeneration of lymphoid hemoblasts. Experimentation proves that they possess phagocytic powers. Inclusions found in their cytoplasm ('tingible Körper' of Flemming) are considered as nuclear remnants of degenerating small lymphocytes which have phagocytized by the degenerating lymphoid hemoblasts, the acid-staining macrophages. Indications are, then, that this lighter central area is essentially a degenerative center, brought about possibly by poorer nutritive conditions in the center of the nodule.

4. The intestinal epithelium overlying the lymphatic tissue begins to be infiltrated with lymphocytes at an age of about fourteen days. The number of invading cells rapidly increases both by proliferation of those already in the epithelium and by continued invasion from the lymphatic tissue. The nature of

the relation between the epithelial cells and the lymphocytes is not definitely known. The lymphocytes rarely, if ever, pass through the epithelium into the intestinal lumen.

5. The granulocytes (eosinophilic) as found in the region of the intestinal tonsils are of two types, 1) blood eosinophiles, wandering into the connective tissue from the blood stream, and, 2) connective-tissue eosinophiles, being derived in situ by differentiation of lymphoid hemoblasts.

The granules of the connective-tissue eosinophile are endogenous formations. They appear first as round bodies, later changing to a rod-like form. The formation of granules is preceded slightly by loss in the basophilic character of the cytoplasm. No change in staining reaction of the granules during the course of their development was noted. Nuclear changes follow those of the cytoplasm. The nucleus first becomes smaller and finally divides into two, the binucleated condition being, apparently, the adult condition.

6. Granulopoiesis is most active in animals infected with parasites, as *Coccidia*. It also occurs most abundantly in and about the caecum, where there may be assumed to be a constant irritation due to the presence of fecal matter. It may be suggested, then, that inflammatory conditions may be associated with a granulopoietic reaction.

7. Extravascular erythropoietic foci occur in abundance at certain stages in the development of the lymphatic tissue. These are extravascular throughout their entire development, at no time being within the blood vessels. No groups of lymphoid hemoblasts or megaloblasts were found intravascularly at any time of development. These erythroblastic cells also arise by differentiation of lymphoid hemoblasts.

8. It is evident that conditions associated with the development of the three types of blood cells are closely interrelated, all being doubtless in some manner associated with the vascular supply. The initiation of lymphopoiesis is apparently brought about by some influence of the abundant lymph vessels or the lymph upon the mesenchyme. The formation of nodular lymphatic tissue may possibly be controlled by the nutritive conditions furnished by the blood supply. The further differentiation

of lymphoid hemoblasts into cells of the granuloblastic or erythroblastic line of development may be conceived as dependent upon the closeness of association between the blood vessels and the lymphoid hemoblasts, the slowness of the current of the blood stream, and upon the thinness of the vascular walls. If the association between the lymphoid hemoblasts and the blood vessels is very close and the conditions necessary for the transudation of materials from the blood stream (plasma) are very good, erythropoiesis occurs. If the association is not so close or the transudation conditions present to a lesser degree, granulopoiesis results.

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PLATE 1

EXPLANATION OF FIGURES

1 Rabbit, age five days. To show the position of the first masses of free cells appearing in the region of Peyer's patch. Photo $\times 180$.

2 Rabbit, age nine days. To show the consistency of these masses. With the exception of one lymphoid hemoblast, all shown are of the small lymphocyte type. Photo $\times 750$.

3 Rabbit, age thirty-two days. A portion from the center of an intestinal lymphatic nodule of Peyer's patch. Note how loosely packed the cells are here as compared with the peripheral portion of the nodule (fig. 4), also the scarcity of mitotic figures.

4 The same as figure 3. From the peripheral portion of the nodule. The lymphocytes of both types are very densely packed here. Mitotic figures are numerous.

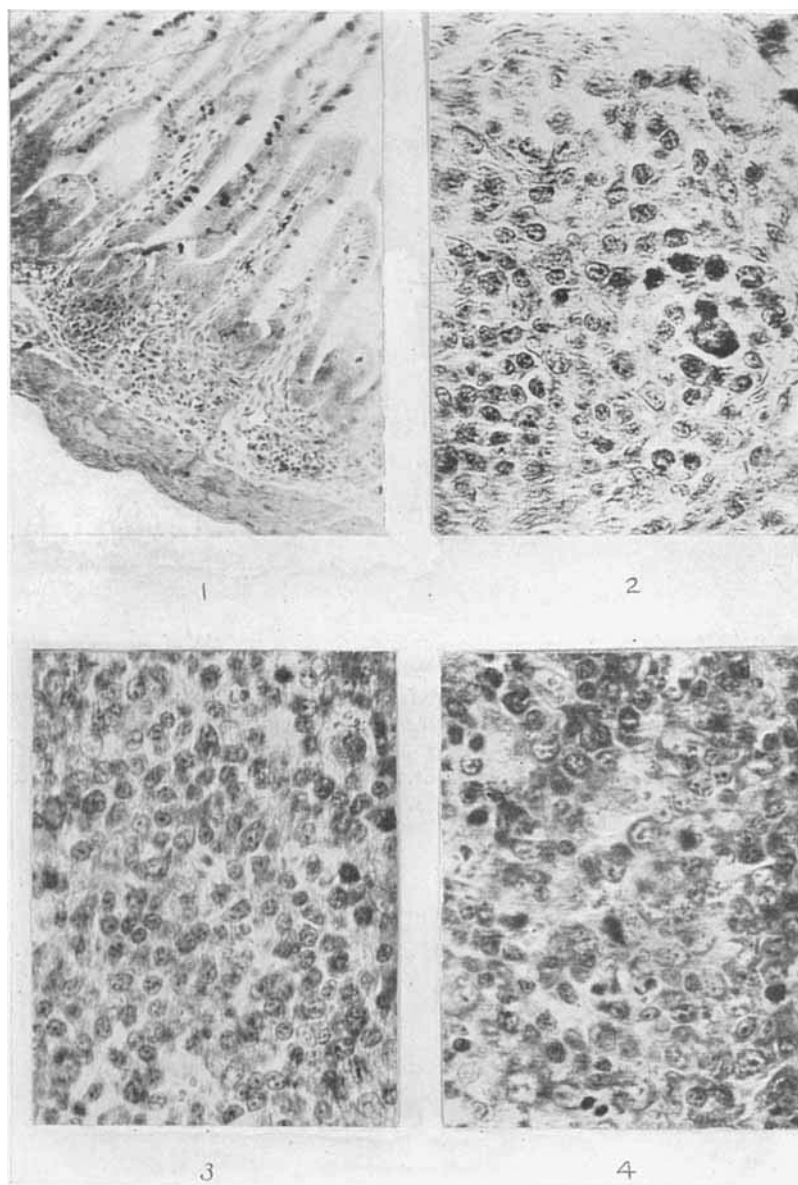


PLATE 2

EXPLANATION OF FIGURES

5 Rabbit, age forty-six days. A portion from the center of a nodule of Peyer's patch, showing the abundance of acidophilic macrophages (degenerating lymphoid hemoblasts), some with nuclear remnants of small lymphocytes as inclusions. Other degenerating small lymphocytes are also found. Photo $\times 750$.

6 Rabbit, adult. To show the appearance of a nodule of Peyer's patch, following injection of a 5 per cent trypan blue solution into the intestinal wall. The acidophilic macrophages which are found chiefly in the central portion have phagocytized large quantities of the dye. Photo $\times 180$.

7 Rabbit, age twenty-eight days. Showing very extensive erythropoietic foci in the sub- and internodular connective tissue in the region of Peyer's patch. These foci appear as dark masses of cells. Photo $\times 180$.

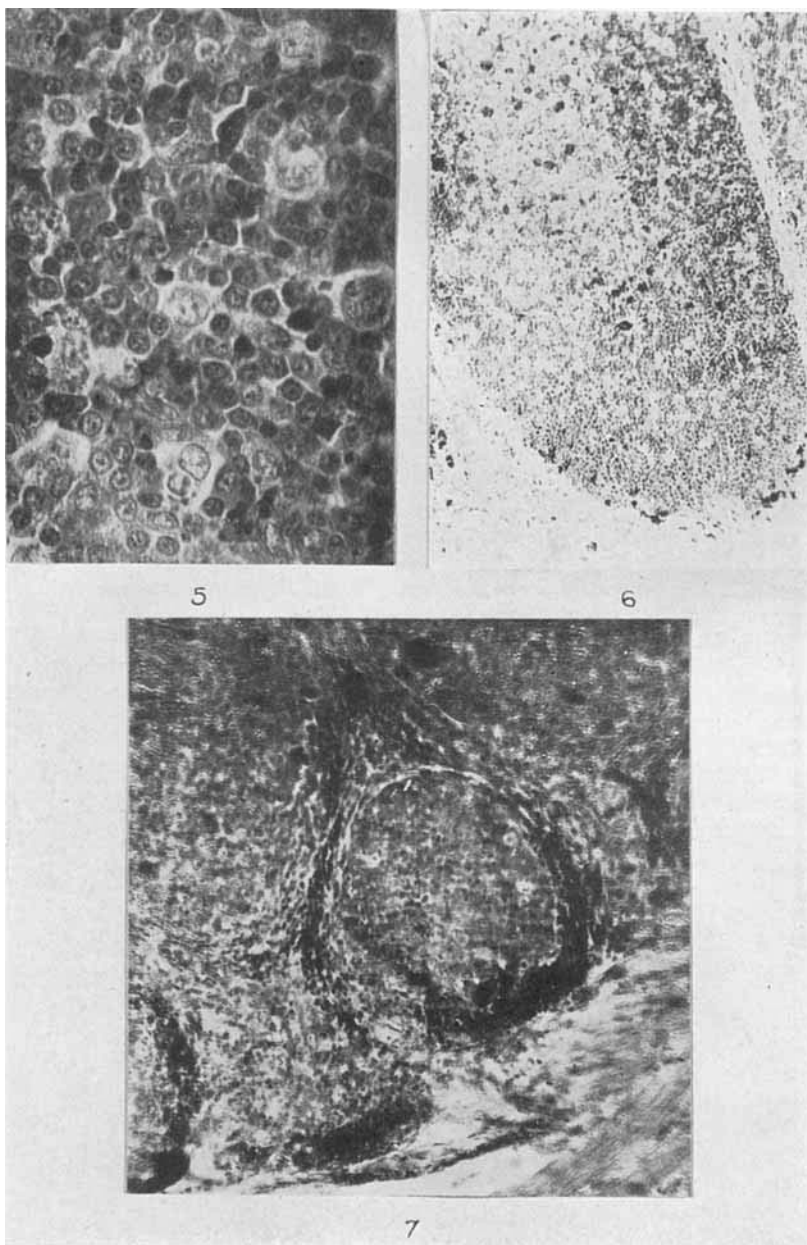


PLATE 3

EXPLANATION OF FIGURES

8 Rabbit, age forty-four days. Erythroblastic cells in close association with a small blood vessel in the region of Peyer's patch. Photo $\times 750$.

9 Rabbit, age forty-four days. Mononuclear eosinophiles in the tunica propria of the caecum near its junction with the ileum. Early developmental stages in their formation are seen. Photo $\times 750$.

10 Rabbit, age twenty-one days. Mononuclear and binuclear eosinophiles in the tunica propria, directly underneath the epithelium, in the region of the appendix. In some cells the eosinophilic granules are so densely massed as to make the cytoplasm appear in the photograph as almost homogeneous. Photo $\times 750$.

11 Rabbit, age twenty-one days. Eosinophiles in the tunica propria and in among the epithelial cells in the region of the appendix. Photo $\times 750$.

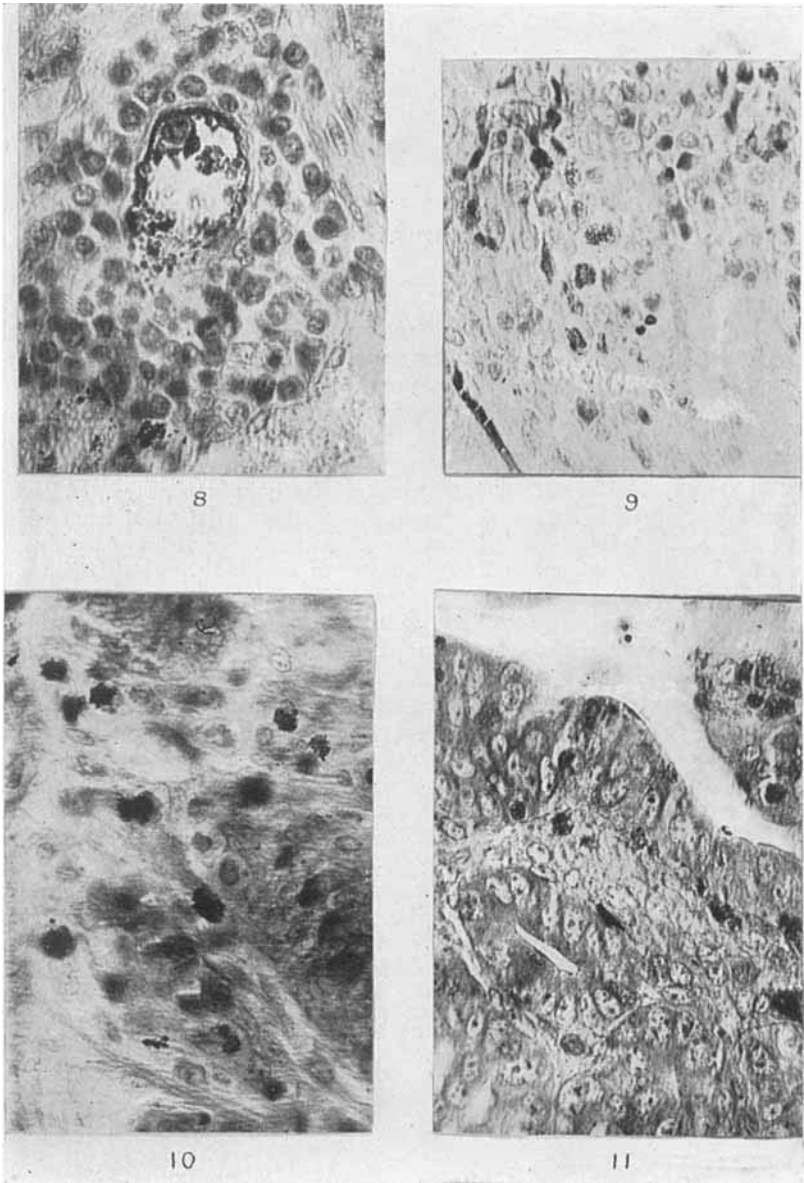


PLATE 4

EXPLANATION OF FIGURES

12 Rabbit, adult. Showing the heavy infiltration of small lymphocytes in a portion of the epithelium covering a lymphatic nodule of Peyer's patch (*B*). These invading cells occupy spaces in the epithelium, with a varying number of cells in each space. The nuclei of four epithelial cells are indicated. The upper part of the drawing shows a portion of the epithelium of a neighboring villus (*C*); with numerous goblet cells pouring their secretion out upon the free surface (*A*). Note the absence of goblet cells in the epithelium covering the nodule (*B*). Wright's stain. $\times 1400$.

13 Rabbit, adult. Cells from the center of a lymphatic nodule of Peyer's patch following an injection of a 5 per cent aqueous solution of trypan blue. Two acidophilic macrophages which have phagocytized particles of the dye are shown. Two normal lymphoid hemoblasts are also indicated. Stained with safranin. $\times 1500$.

14 Rabbit, seventeen days. Erythroblastic and granuloblastic cells in the subnodular connective tissue in the region of Peyer's patch. The nuclei of the erythroblasts are pyknotic. Several erythrocytes which have lost their nuclei are seen. Two lymph vessels and one arteriole are present. The erythroblastic cells are in close association with them, while the granuloblastic cells are more distant from them. Stained with eosin-methylene blue. $\times 1400$.

15 Rabbit, seventeen days. A portion of a large erythropoietic focus in the subnodular connective tissue in the region of Peyer's patch. Several lymphoid hemoblasts from the adjacent nodule are indicated in the drawing. Stained with eosin-methylene blue. $\times 1400$.

16 Rabbit, forty-four days. Mononuclear (granuloblasts) and binuclear (granulocytes) eosinophiles in the tunica propria of the intestine in the region of Peyer's patch. Note the rod-like appearance of the granules. Several lymphoid hemoblasts are also present. Hastings-Nochts stain. $\times 1400$.

