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CELL PROLIFERATION IN GERMINAL CENTERS OF THE RAT SPLEEN.*

By

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INTRODUCTION:

The purpose of this paper is to present data which gives some insight into the rate of cell proliferation within the lymphatic nodules of the rat's spleen. In this study the technique of labeling DNA with the specific precursor tritiated thymidine was used. The observations were made on the Malpighian corpuscles of the spleen utilizing autoradiographic methods.

The lymphatic nature of the Malphighian corpuscles was first recognized by Kölliker in 1855 (1). Lymphatic nodules are observed in various forms but the most characteristic histological appearance consists of an aggregation of cells in which a central zone of pale staining cells can be distinguished from a zone of dark staining, closely packed smaller cells. This central zone was named "Keimzentrum" or the "germinal center" by Flemming in 1885 (2). Flemming observed the high mitotic activity in the germinal centers and felt that this area was actively forming lymphocytes. Other terms that have been used for the "germinal center" are "secondary nodule" or "Innenraum". The "germinal center" is surrounded by darkly staining, small cells and has been termed "mantle zone" or "marginal zone". The "mantle zone" is surrounded in turn by a well defined circular area of larger cells (mixed large lymphocytes, histiocytes and perhaps other cells). This zone has also been called "marginal layer","Knotchenrandzone" and "Follikel-Aussenzone". The cells in this zone are arranged more loosely than in the "mantle zone". Since the original description of lymphatic nodules by Flemming various ideas have been advanced in respect to their possible function. The question of their function remains a center of continuing controversy. Some investigators have supported Flemming's suggestion that the pale centers of the nodules are "germinal centers" for the formation of new lymphocytes, although it

is well known that these are not the only sites of new cell production (3-6). This concept was challenged by Marchand in 1913 (7). He pointed out that the "germinal centers" are usually sharply demarcated. This makes it difficult to visualize potential genetic relations between "germinal centers" and cells and the surrounding small lymphocytes. Since then, numerous experimental and pathological studies have been reported in support of Marchand's doubts (9-10). Hellmann (8) suggested that a major function of the "germinal centers" was to act as "reaction centers" against toxins. However there is also ample evidence that "germinal centers" are produced as a reaction to bacterial infections and repetitive antigenic stimulation (11-13). White, however, roduced evidence that "germinal centers" are produced only after induction of the anamnestic (secondary response) and not after the primary antigenic stimulus (14). Most recently this has been conclusively demonstrated by Cottier et al. (15). The high mitotic index of "germinal centers" signifies that there is a significant production of new cells. Whether these newly formed cells in the "germinal centers" should be called lymphocytes, "germinoblasts", or some other name is a problem of nomenclature to be resolved later. Certainly now that it is known that the "germinal centers" are formed after induction of the secondary immune response, it appears reasonable to assume that the cells have some function connected with antibody production. The precise nature of the function and the fate of the cells remains obscure. Conway (16) had earlier tried to combine the opposing views of Flemming and his supporters and those of Marchand, Hellman and Latta and their supporters by suggesting that the differences which had been observed are due to observation of the "germinal centers" in different phases of cyclic changes that are believed

to occur in the lymphatic nodules.

The present study was designed to obtain data on various time parameters of cell renewal in the lymphatic nodules of the rat spleen. Tritiated thymidine (H^3TDR) was administered as a "flash-injection" in order to label all cells which were in DNA synthesis at the time of the injection. Changes of the labeling indices and of the intensity of labeling were studied by serial killing of animals after labeling and then studying the sequence of events in the spleens by autoradiography. Preliminary results of this study have been reported previously (17).

MATERIALS AND METHODS

a) Experimental Approach

Seventy-five adult albino rats, weighing about 200 gm were used. Each animal was given 0.25 μc/gm of body weight, intravenously, through the tail vein, of tritiated thymidine (1.9 C/mM)*. All animals were housed under standard conditions. At least two rats were killed at hourly intervals for the first six hours, then every two hours until 24 hours had elapsed and then every six hours until 60 hours had elapsed and thereafter at 72, 96, 120 and 162 hours after the administration of H^3TDR. Upon killing, the spleens were removed and fixed in neutral formalin. Paraffin sections were prepared seven microns in thickness. After deparaffinization, stripping film (Kodak AR10) was applied (18). The film was exposed for 70 days. The autoradiographs were then developed in D19 developer, fixed, washed and stained with hematoxylin-eosin at a carefully controlled pH

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* Produced by Schwarz Bioresearch, Mt. Vernon, New York.)

through the stripping film.

b) Evaluation of Autoradiograms

The splenic autoradiograms were surveyed and at least three lymphatic nodules in each splenic section were identified that were similar in morphological appearance with the characteristic pattern of a center of lightly stained larger cells surrounded by a sharply demarketed cuff of small, darkly staining cells which in turn was surrounded by a typical zone of more loosely arranged larger cells. These lymphatic nodules were then photographed.* After photographing, special 12 x 17 inch black and white prints were prepared on special paper which showed a minimal variability in dimensions due to photographic processing. The photographic prints were used as guides for further microscopic study of the autoradiograms and for permanent records. By combining microscopic observation of the nodule under study, with observation of the photograph, the "germinal center" was outlined carefully on the photograph as well as the surrounding "mantle zone" as illustrated in Fig. 1. Then at least 200 cells in the "germinal center", and at least 500 cells in the "mantle zone" were examined by direct microscopy and the number of grains found overlying the nucleus was recorded on the photograph. If no grains were found, the cells were recorded as zero. Care was taken, that every cell in the top focal plane of the section was examined and its grain count recorded. Thereafter, the percentage of labeled cells for the "germinal center" and for the "mantle zone" was determined in addition to the mean grain count.

The presence of pyknotic nuclei (tingible bodies) within macrophages or

* The photography and valuable help and cooperation of Mr. Robert A. Smith is gratefully acknowledged.

outside of cells in the "germinal centers" are well known. The number of "tingible bodies" per hundred "germinal centers" cells was counted. In addition the index of labeling of tingible bodies as a function of time was determined.

Since much information can be obtained about the time spent in the different portions of the generative cycle by observing the progression of label through mitosis, labeling of mitotic figures was studied. Since there were not enough mitoses in the "germinal centers" studied microscopically and photographically, a total of 50 mitoses were counted in similar "germinal centers" of other lymphatic nodules of the same section in order to determine the index of labeling as a function of time in these mitoses. Mitoses were further divided into large and small cells.

RESULTS

"Germinal Centers"

a) Labeling indices of "germinal center" cells.

In Fig. 2 the labeling index of "germinal center" cells of the rat spleen are shown as a function of time. Each small point on the graph represents the labeling index of one "germinal center" in which 200 successive adjacent cells, regardless of size or without attempting a cytological classification, were counted. The large points are the means of the labeling indices for all "germinal centers" for each time interval after H^3TDR injection. The heavy black line is a free hand curve suggesting the changes of the labeling pattern with time.

"Germinal center" cells were significantly labeled at the first observation period of 30 minutes. For 1-1/2 days the mean labeling index varied roughly between 20 and 50 per cent. During this interval, there was considerable variation in the labeling between single germinal centers. From the free hand curve describing the pattern in the mean labeling indices, there is a suggestion that

the labeling index increases from roughly 20 per cent to almost 50 per cent between 30 minutes and 6 hours after injection. Thereafter, there is an apparent decrease to roughly 30 per cent at 12 hours after the injection and an apparent secondary increase to in excess of 40 per cent 24 hours after injection. Thereafter there appears to be a steady decrease in the labeling index until the end of the experiment as expected with dilution in the grain count and loss of cells from the labeled category. Admittedly, the great variability in individual labeling indices of lymphatic nodules makes a precise interpretation of the data impossible. However, as will be discussed later, the apparent increase in labeling index described may have a rational basis. During the first 1-1/2 days while the grain count was diminishing, the index of labeling remained above 30 per cent. This is of importance in later interpretation.

The labeling indices are highly dependent upon the arbitrary threshold grain count for positivity. Accordingly, in Fig. 3 the labeling index for different grain counts is plotted. During the first 24 hours, frequent sacrifices were made. Accordingly, in this interval data is pooled for the 0-6, 7-12, 13-18, and 19-24 hours after the injection of thymidine. Thereafter, the data were collected at 6, 12, or 24 hour intervals and accordingly not pooled. Grain thresholds were set at 2,4,8,16 and 32 grains. From observation of Fig. 3, it is apparent that all cells with more than 32 grains have disappeared within 42 hours and that all cells with more than 16 grains have disappeared after 60 hours. Between 2-1/2 and 5 days, the labeling index for the 2, 4, and 8 grain classes remains at a relatively constant level.

In Fig. 4, the progression of label through the "tingible bodies" is presented. In the course of evaluating "germinal centers" 1 to 5 "tingible bodies" were found per hundred "germinal center" cells. The data were pooled for 0-2,

3-6, 7-12, 13-18, and 20-24 hours. Thereafter they are plotted for the time intervals at which animals were killed. The labeling of "tingible bodies" was found as early as 30 minutes after H^3TDR injection. Thereafter the per cent labeled increased to a maximum of about 4? per cent, 24 hours after the injection. There was a decrease to about 17 per cent roughly 44 hours after injection and a secondary increase to 36 per cent 60 hours after injection, followed by a disappearance of labeling 72 hours after the injection. Labeled tingible bodies reappeared to an apparent tertiary maximum on the fifth day and were not seen in the last observation periods seven days after the injection.

The progression of labeling through mitosis in the "germinal centers" is illustrated in Fig. 5. Mitoses were classified according to their size into large, medium and small mitoses. Since the large and medium mitoses had identical patterns the results of these are pooled. The small mitoses are those that measure less than 14 μ. To date labeling has been counted in mitotic figures for only the first 12 hours. At 30 minutes after H^3TDR injection, 7 per cent of all mitoses were labeled. Thereafter the per cent labeling increased rapidly to 100 per cent after 2 hours in the medium and large mitoses and 70 per cent in the small mitoses. The per cent labeling in mitotic figures remained roughly constant until 5 hours after the injection of H^3TDR. Then a sharp decline in per cent labeling was observed in all types of mitoses. From 6-12 hours the labeling in the large and medium size mitoses remained at 70-80 per cent and 40-60 per cent in the small mitoses.

b) Grain Counts over "Germinal Center" Cells.

The mean grain counts over "germinal center" cells are plotted in Fig. 6 as a function of time. Each small point represents the mean grain count

over the cells of one "germinal center". The large points are the calculated means for all observations at each time interval. A mathematical analysis using the weighted means indicate that the data can be fitted by a curve consisting of a constant and an exponential component. The half-time of the grain count diminution of the exponential component is 13.4 hours.

"Mantle Zone" Cells.

a) Labeling of Cells in the Lymphocytic Cuff Surrounding "Germinal Centers" ("Mantle Zone").

In the "mantle zone" around the "germinal centers" at least 500 cells were counted. In many instances when the entire "mantle zone" was outlined clearly on the microphotograph all cells in the zone were counted. In Fig. 7 changes in the per cent labeling of cells in this zone are shown. The small points represent the labeling per cent for each "mantle zone". The large points are the means of all "mantle zones" recorded for each time interval. First, the marked difference in the labeling indices of "germinal center" cells as compared to "mantle zone" cells is evident. In the "mantle zone", the per cent labeling one hour after injection is about 1-3 per cent. At 12 and 36 hours after injection, there is a suggestion of an increase in the per cent labeling of questionable significance. The per cent labeling remains essentially constant actually for the 5-7 days. The cells labeled initially after H^3TDR injection are predominantly large cells with a nucleus similar in size to the large cells in the "germinal center". After a few days label appears over small dense cells indistinguishable from the other small cells in the "mantle zone". There is at no time any evidence of an appearance of a wave of labeled cells moving centrifugally through the "mantle zone" from the center. The labeled cells in the "mantle zone" are distributed at random. Mitoses are extremely rare in the "mantle zone".

Accordingly, there was no attempt to observe the movement of the label through mitosis in the "mantle zone."

b) Grain Counts Over Labeled Cells in "Mantle Zones".

The data on the grain count as a function of time over lymphocytic cells in the cuff surrounding "germinal centers" is shown in Fig. 8. The small points represent the mean grain count of the labeled cells (> 2 grains) in the "mantle zone" of lymphocytic nodules in the rat spleen. The large points are the calculated weighted means for all observations at the particular times involved. The initial grain counts as well as the grain counts throughout the observation period (162 hours) are always higher than those over "germinal center" cells. Initially, the grain counts over labeled "mantle zone" cells is at least twice as high as over "germinal center" cells. The grain counts over "mantle zone" cells then diminishes to about one half of the initial value within 12 hours. Thereafter, the grain count diminution is very slow, However, the grain counts remain 3-4 times as high in the "mantle zone" as over the "germinal center" cells throughout the entire observation period. A mathematical analysis of the slope of the diminution indicates that it can be expressed as a 2 exponential component curve with a final slope representing a half-time of 260 hours or more. The first component has a half-time of 4.2 hours.

DISCUSSION

The interpretation of all DNA labeling is fraught with many difficulties and assumptions discussed in earlier publications (18-24). Since the study of "germinal centers" is based on serial killing of animals there are obviously biological, unavoidable differences in the amount of H^3TDR utilized for DNA synthesis from animal to animal although the amount administered was constant. In addition to the normal biologic variation to be expected there may be differ-

ences in circulation at the microscopic level that will induce a further variation into the amount [illegible] available at a particular "germinal center". Furthermore, accumulative evidence (23, 24) suggests that labeling data may be influenced by reutilization of H^3TDR labeled building blocks of disintegrating cells. As pointed out later in the discussion, this problem deserves particular attention in "germinal centers" known to contain large numbers of pyknotic cells. Perhaps the more important and fundamental difficulties are inborn within the system that is under study. If it is correct that the lymphatic nodules undergo cyclic changes and transform from a phase of active, rapid growth to that of a "functional" or "disintegrating" nodule, then selection and comparison of nodules for study becomes important. An attempt was made to study only nodules of similar histologic appearance, however, there is no way in which one can be certain that the histologically similar follicles selected are in identical phases of their assumed cyclic transformation. This problem is emphasized more recently by the studies of Cottier et al (15) in which it has been shown apparently conclusively that "germinal centers" develop in lymph nodes only upon secondary immunological stimulus. In the spleen, which is the subject of our studies, it may well be that there is continuous antigenic stimulation. Accordingly, the "germinal centers" of the spleen may well be in a "steady state" proliferation rather than progressing through cyclic changes from intermittent antigenic stimulation. However, there is no evidence that this is the case. The preceding difficulties are frankly admitted and all interpretations may be in part erroneous because of one or more of the factors discussed or previously published.

As mentioned in the introduction, there has been much controversy over the origin and function of "germinoblasts" and the origin of the cells in the

"mantle zone". Klemperer (25) in 1938 stated "the present conception holds, that the secondary follicles are sites of lymphocyte formation as well as centers of reaction to toxic substances". In this statement, Klemperer attempted to combine the views about the significance of lymphatic nodules held by Flemming and his supporters (2-6) and by Hellmann and his supporters (7-10). This concept remained attractive since Groll and Krampf (26), Wätjen (27), Hueck (28), Jager (29) and Conway (16) suggested repeatedly that the seemingly opposing views may all be representing merely different phases of a developmental cycle during which a "germinal center" is born, grows, functions and disintegrates.

In direct consideration of our own observations we will discuss the following questions: 1) what are the genetic relations between "germinal center" cells and the surrounding "small lymphocytes" of the "mantle zone"? 2) What is the origin and fate of "tingible bodies"? 3) How rapidly are lymphocytic and other cells proliferating in the "germinal centers" and the surrounding "mantle zone"?

Before proceeding to the specific questions, it can be stated generally that H^3TDR labeling of proliferating lymphopoietic is as satisfactory as for other hemopoietic tissues studied earlier (19-21). However, the use of H and E stained sections makes the specific cytologic identification of cells more difficult and in some instances impossible whereas panchromatic staining of blood and marrow smears facilitates identification of individual cells. However in smears and imprints one loses the microscopic anatomical relationships that are retained in cut sections.

It appears unlikely for many reasons that the "germinal center" cells are the precursors of the small lymphocytes in the "mantle zone". In the first place if this were so, one would expect a centrifugal wave of labeling to pro-

ceed outwards from the germinal center into the mantle zone. This was not seen. Secondly, since there is "flash" labeling, albeit very little, in the "mantle zone" there must be some endogeneous cell proliferation within this area. Thirdly, if there were a systematic migration of newly formed cells into the "mantle zone" from the "germinal centers" one would expect logically an increase in the per cent of labeling in the "mantle zone" to that of the more highly labeled germinal center until the input of labeled cells is equalled by the output of labeled cells. This clearly was not seen. Fourthly, the grain count data is simply incompatible with the concept of migration of labeled cells into the "mantle zone" because the grain count starts higher in this area by a factor of 2 and climbs to a factor of 4 by the end of the experiment while the grain count progressively diminishes within the "germinal center". For the preceding reasons it is believed that Flemming's concept is refuted.

In the discussion that followed the presentation of this paper the question was asked by Speirs if it were possible for the "mantle zone" cells to migrate into the "germinal center". This question can not be satisfactorily answered by our data. However, the data do no favor such an idea. After a flash H^3TDR label no further labeling occurs (except that contributed by possible reutilization of DNA or degradation products of DNA). If labeled "mantle zone" cells migrate into the center to form germinal center cells, the labeling index within the mantle should decrease or if migration follows mitosis, the labeling index would remain constant with a progressive decrease in the intensity of label. Since this was not observed, it is rather unlikely that migration of labeled "mantle zone" cells into the "germinal center" occurs. The other possibility namely that "mantle zone" cells may act as a focus of new germinal center development remains a possibility that can not be answered by the current experiments.

The present data illuminates the problems concerned with the origin of the "tingible bodies." The latter were considered to be of nuclear origin apparently by Ebner in 1902 on the basis of tinctorial characteristics (30). Since "germinal centers" are supplied with arterial blood by a fine network of capillary loops (29) one might believe that "tingible bodies" represent dying lymphocytes transported via the blood stream. Heiberg in 1923 (32) considered "germinal centers" to be the graveyards of lymphocytes. Cottier in his earlier studies (31) suggested that tingible bodies may originate from "germinal center" cells. The question of whether disintegrating phagocytized cells may serve as building blocks or induction material for the new formation and transformation of cells was brought up by Trowell (33, 34) and Ehrich (35, 36) and Hamilton (37, 38). Our studies do not permit any conclusions in respect to induction of cell transformation or initiation of cell division.

However, the labeling pattern of "tingible bodies" is pertinent in respect to their origin. Some tingible bodies were labeled as early as 30 minutes after H^3TDR injection. Thereafter there was a rapid increase in the fraction of labeled tingible bodies. The labeling per cent ultimately reached values similar to that of the "germinal center" cells. From other studies (39, 40) it is well known that small lymphocytes are labeled only to a small proportion after H^3TDR flash injection and that it takes about three weeks of 12 hourly injections to label small lymphocytes up to 40 per cent (41). Little et al (42) utilizing continuous intravenous H^3TDR in rats were unable to label 100 per cent of small lymphocytes in the peripheral blood after 90 days of continuous intravenous injection. Thus, it is highly unlikely that the source of "tingible bodies" are small blood lymphocytes. Similar reasoning is applicable for all blood lymphocytes and other leukocytes in the blood since none of the peripheral blood cell types are labeled

close to 40 per cent within 12 hours after a flash injection of H^3TDR under normal conditions. Thus it is scarcely possible that the peripheral blood appears as a likely source of "tingible bodies" in the rat spleen "germinal centers". Actually, the close similarity of the labeling pattern between "germinal center" cells and "tingible bodies" suggests that they are the result of local cell death of "germinal center" cells. Even though no serious attempt was made to count the incidence of tingible bodies in different types of "germinal centers" it was our impression that they were less frequent in "small" young appearing centers than in the larger "germinal centers". Since labeled "tingible bodies" were found as early as 30 minutes after H^3TDR injection it is believed that cells in DNA synthesis or in G_2 (premitotic phase) are dying and may never undergo cell division. If many of the cells which synthesize DNA do not divide and remain as a static population, the grain count halving time for "germinal center" cells would be seriously overestimated. However, if these cells which have synthesized DNA do not divide but are rapidly dying as suggested by labeling in tingible bodies, the estimate of generation time based on grain count halving time may not be significantly in error. From the preceding we conclude that "tingible bodies" are formed primarily from dying cells which had probably originated within the splenic lymphalic follicles. Perhaps this is the ultimate fate of most "germinoblasts".

From the data collected one can make rather good estimates of various time parameters of the generative cycle just as has been done with other tissues (41, 63). However, in the present study no attempt was made to distinguish different cell types in the "germinal centers". It is clear that "germinal centers" are comprised of a variety of cell types. Besides the predominant rapidly proliferating "germinoblasts", reticular cells and macrophages are seen. It is believed that

the presence of these cells however do not alter the over all conclusions of the "germinoblasts".

The data presented (Fig. 5) clearly shows that labeled metaphases are present 30 minutes after the injection of H^3TDR. This then represents an upper limit to the minimum time for a cell to complete DNA synthesis and to go through the pre-mitotic rest phase (R_2) and prophase. Since all of the medium and large sized mitoses were labeled by 2 hours, an upper limit for R plus prophase can be set. Since the labeling of large and medium sized mitoses remains at 100 per cent for five hours and thereafter diminishes rapidly, one can estimate a minimum time for DNA synthesis in these cells as being of the order of 4-1/2 hours. The distance between the 50 per cent values on the ascending and decending curve in Fig. 5 for the large and medium cells, gives an estimate of the average DNA synthesis time of these "germinal center" cells of about 5 hours. A maximum DNA synthesis time can not be deduced from the present data since a second cycle was not observed and the labeling index did not decrease to zero. In addition, the duration of the post mitotic rest phase (R_1) and an accurate estimate of the generation time from progression of label through mitosis can not be derived from the data in Fig. 5. However, since the labeling of mitosis of the large and medium cells does not decrease to zero at any time suggests that there may be very marked "straggling" of cells in R_1 or that they have their origin in populations of markedly different R_1 or DNA synthesis times. In respect to labeling of "small" mitosis, the fact that 100 per cent labeling was not obtained indicates that these cells come from

a population in which the maximum $R_{\frac{1}{2}}$ may be longer than the minimum DNA synthesis time or that these mitoses belong to a cell population with greatly different time parameters for the various phases of the generative cycle.

The half-time for the grain count diminution over "germinal center"-cells of 13.4 hours provides an estimate of the average generation time of these cells. Preliminary data on the grain count diminution over mitotic figures in "germinal centers" (44) suggests a half-time of 6-7 hours, considerably shorter than that derived from interphase cells. This indicates that the generation time for the cell population successfully proliferating may be considerably shorter than suggested from the grain count diminution rate over interphase cells. If cells synthesize DNA but do not divide and die some time after completion of DNA synthesis, this could lead to a slower grain count diminution rate over interphase cells than one would expect if all cells divide after completion of DNA synthesis.

The second component of the grain count curve is not significantly different from zero with a grain that remains at about twice the background. Comparable observations were made in granulocytopoiesis (41), erythropoiesis (45) and in blood lymphocytes (38). There are alternate explanations discussed earlier (22, 41, 45) but now reutilization of H^3TDR from degradation of dying cells seems the more likely (23, 24, 45, 47).

The cells of the "mantle zone" have time parameters of the generative cycle entirely different than that of the germinal center cells. However, there might be a functional relationship (exchange of material for instance). A few cells labeled in the "mantle zone" divide during the first hour after H^3TDR injection, the grain count diminution indicates that the 4.2 hours mean grain count is reduced by 50 per cent. Since there is no evidence of an early "shoulder" (which would be produced by an R_2 significantly longer than 30 minutes) the time of 4.2 hours would reflect the DNA synthesis time of the labeled "mantle zone" cells. After that, the slope having a 260 hour half-time is in reality not significantly different from being flat. Therefore, it is consistent with the suggestion of a very long R_1. Apparently, most of the labeled "mantle zone" cells do not undergo a further division during the 162 hour observation period and may well have a generation time in excess of this. It is of interest, that the labeling index in the "mantle zone" is only 1.5 per cent at any one time. If one assumes that the cells in "mantle zone" are a homogenous cell population in a steady state, the generation time would be calculated by

$$\frac{t_s}{I_L}$$

and would be $\frac{4.2}{0.015}$ = 280 hours.

There is no evidence that the "mantle zone" consists of a homogeneous cell population. In fact there are two obvious cell types--the predominant small lymphocyte and the infrequent large labeled lymphocyte. If the different size cells represent phases of a homogeneous population then the small lymphocyte would become transformed into a large cell as it entered DNA synthesis. This idea is actually consistent with what has been observed in the cultures of human peripheral blood where small lymphocytes enlarge, synthesize DNA and then divide (48).

The splenic lymphatic follicles as alluded to earlier may represent a unique situation not comparable directly to lymph node follicles. In respect to the latter White (14) and Cottier et al (15) have demonstrated clearly that "germinal centers" develop only after a secondary antigenic stimulus. It is conceivable, but not proved, that the position of the spleen in the circulation affords a continuous antigenic stimulation that may maintain "germinal centers" in a more or less of a "steady state" equilibrium of cell growth.

From studies on extracorporeal irradiation of the circulating blood (49, 50, 51) it has been shown that a marked peripheral lymphopenia can be induced from which there is a prolonged recovery time of weeks. Histologically there is severe depletion of the "mantle zone" of the splenic and lymph node follicles, and depletion of the cortical areas of the lymph nodes with intact "germinal centers". These observations appear consistent with those of this paper.

SUMMARY

1. The purpose of this paper was to present data relevant to the genetic relations of cells in lymphatic nodules of rat spleen and to time parameters of their proliferative kinetics. These data are based on autoradiographic studies using tritiated thymidine as a specific DNA label. They suggest that there is no genetic relation between the rapidly proliferating cells in the "germinal centers" and the cells in the surrounding "mantle zone" of densely packed small cells. They suggest further, that there is a high degree of cell death in "germinal centers" as evidenced by "tingible bodies" which probably originate from "germinal center" cells in or immediately after DNA synthesis. The data are comparable with but do not prove the older hypothesis that "germinal centers" are transient "micro-organs" which go through a cycle of birth-growth- and disintegration.

2. The autoradiographic data of "germinal center" cells in interphase and in mitosis are compatible with an average DNA synthesis time of about 5 hours and a minimum of 4.5 hours. The minimum time for the pre-mitotic rest period (R_2) is 30 minutes or somewhat less, the maximum for cells giving rise to medium or large sized mitoses ≤2 hours, but >1 hour. The grain count diminution rate over interphase cells suggests a generation time of 13.4 hours as an average for all "germinal center" cells, regardless of their type. This may overestimate the generation time of cells actually undergoing mitoses. The grain count diminution rate over mitoses is faster and has a half time of 6-7 hours. The grain count data are compatible with reutilization of H^3 labeled DNA breakdown products.

3. About 1-2 per cent of "mantle zone" cells surrounding the "germinal center" become labeled after H^3TDR flash injection, indication their capacity to synthesize DNA. The grain count data over these labeled cells are compatible with the suggestion, that these cells undergo one division within the first 12 hours after H^3TDR injection. Thereafter, further divisions may not occur for days as evidenced by a grain count diminution rate with a half-time of 260 hours or more. The data thus support the suggestion, that "mantle zone" cells represent part of a "storage pool" of lymphocytes with a rather slow rate of proliferation under steady state conditions.

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Figure 1. Example of a "microphotographic map" (original size: 11 x 14 ins.) of a lymphatic nodule of rat spleen used in the evaluation of autoradiographs in this study. Note the outline of the "germinal center" and the cells evaluated in it and of the surrounding " mantle zone."

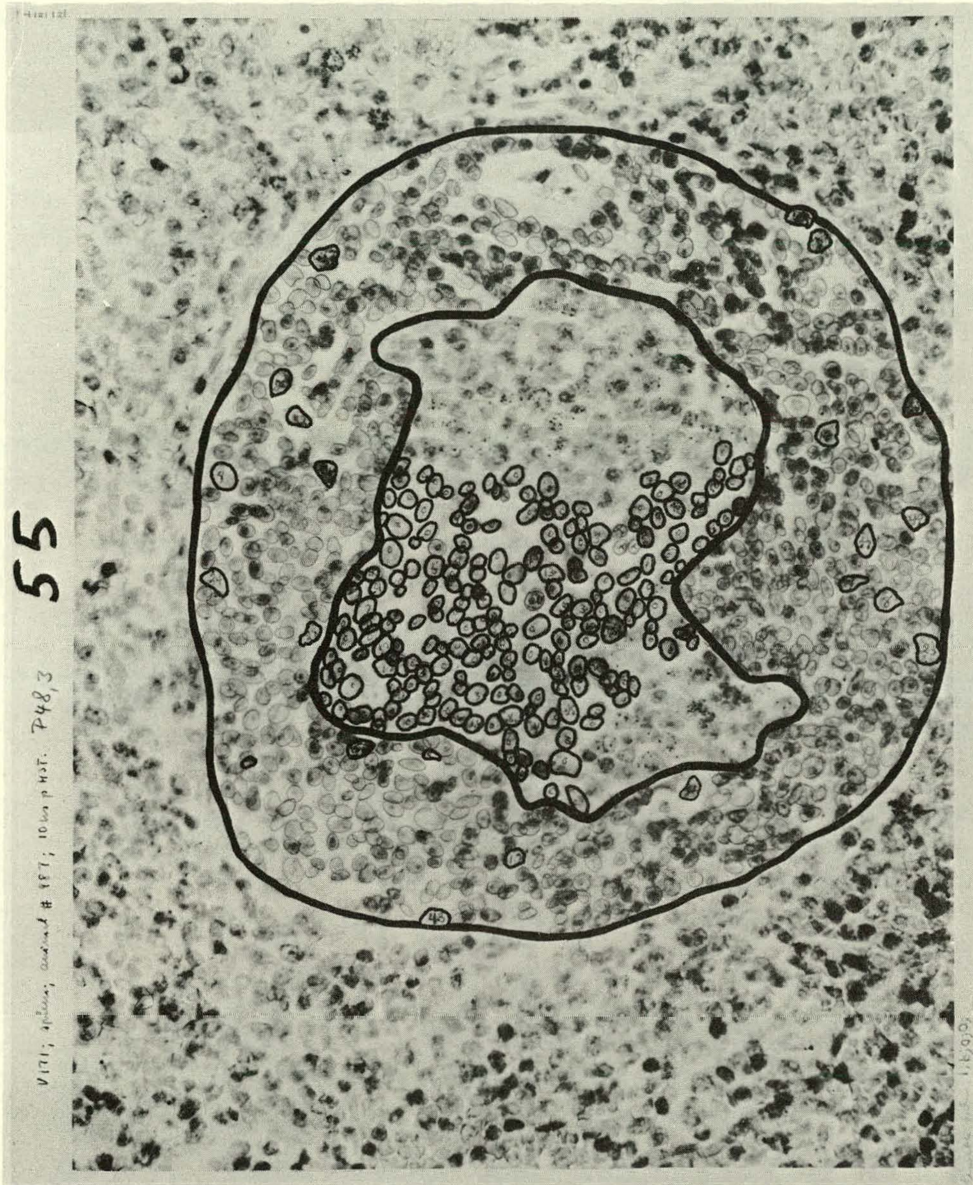

FIGURE 1

Figure 2. Changes of labeling indices of "germinal center"-cells as a function of time after i. v. "flash" injection with H^3TDR. - Small points: mean of each "germinal center" evaluated. Large points: mean for each time interval.

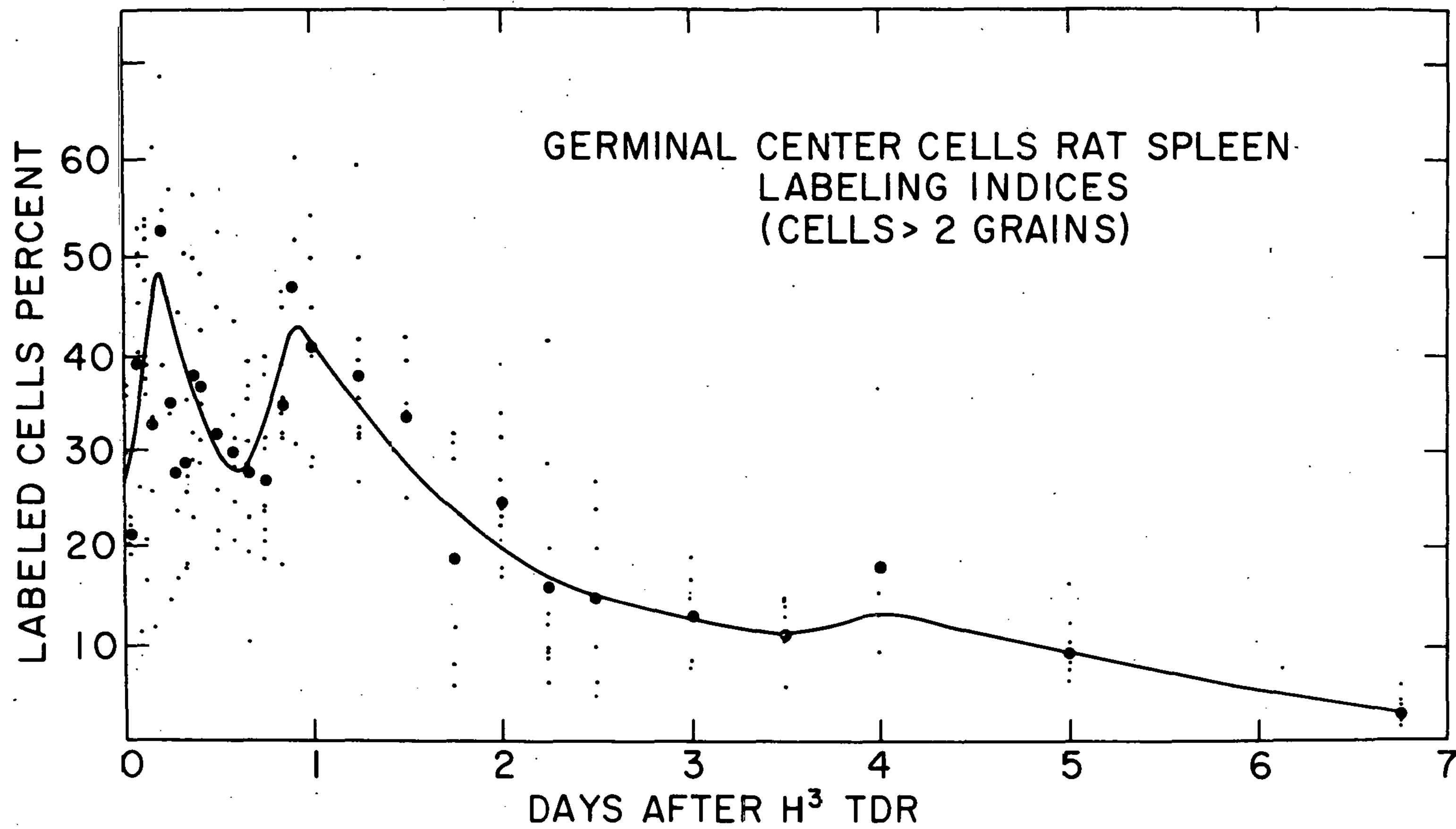

FIGURE 2

Figure 3. Pooled labeling indices of "germinal center"-cells as a function of time for different thresholds of autoradiographic positivity scoring.

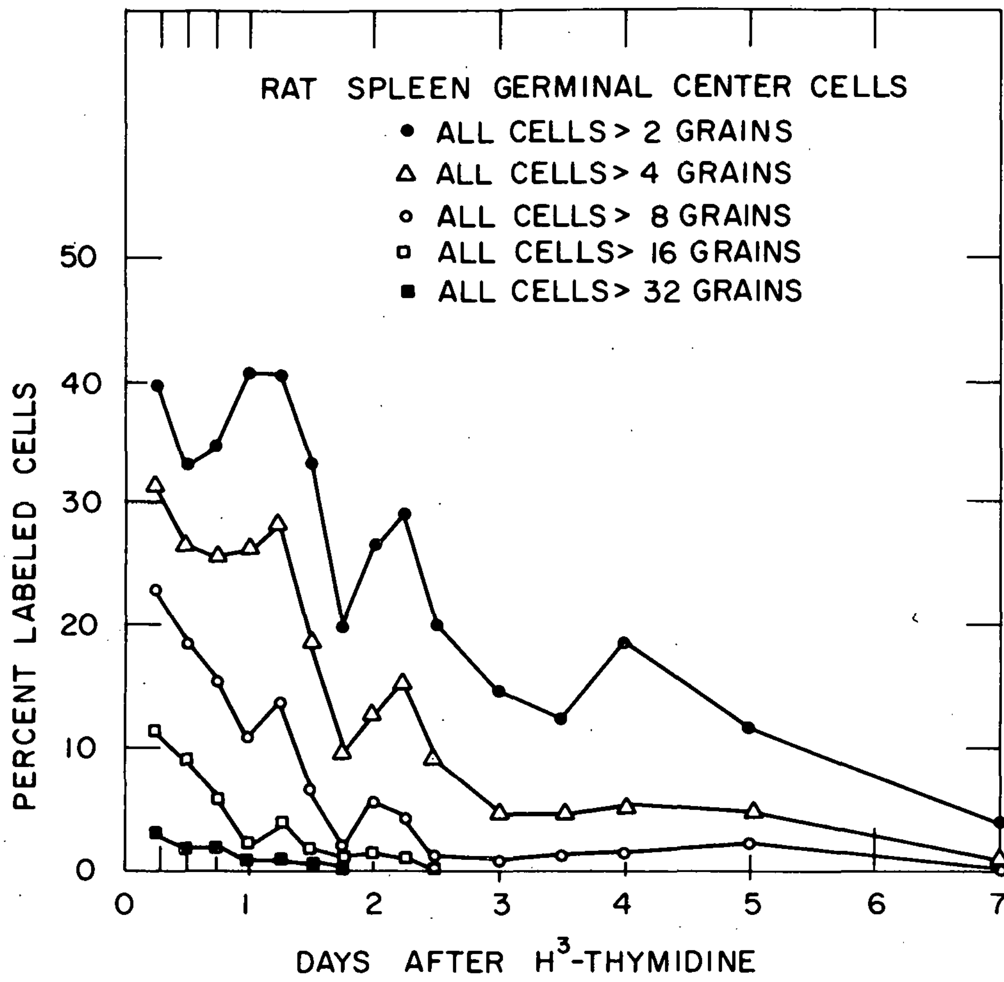

FIGURE 3

Figure 4. Pooled labeling indices for tingible bodies with more than 2 grains as a function of time after H^3TDR "flash injection."

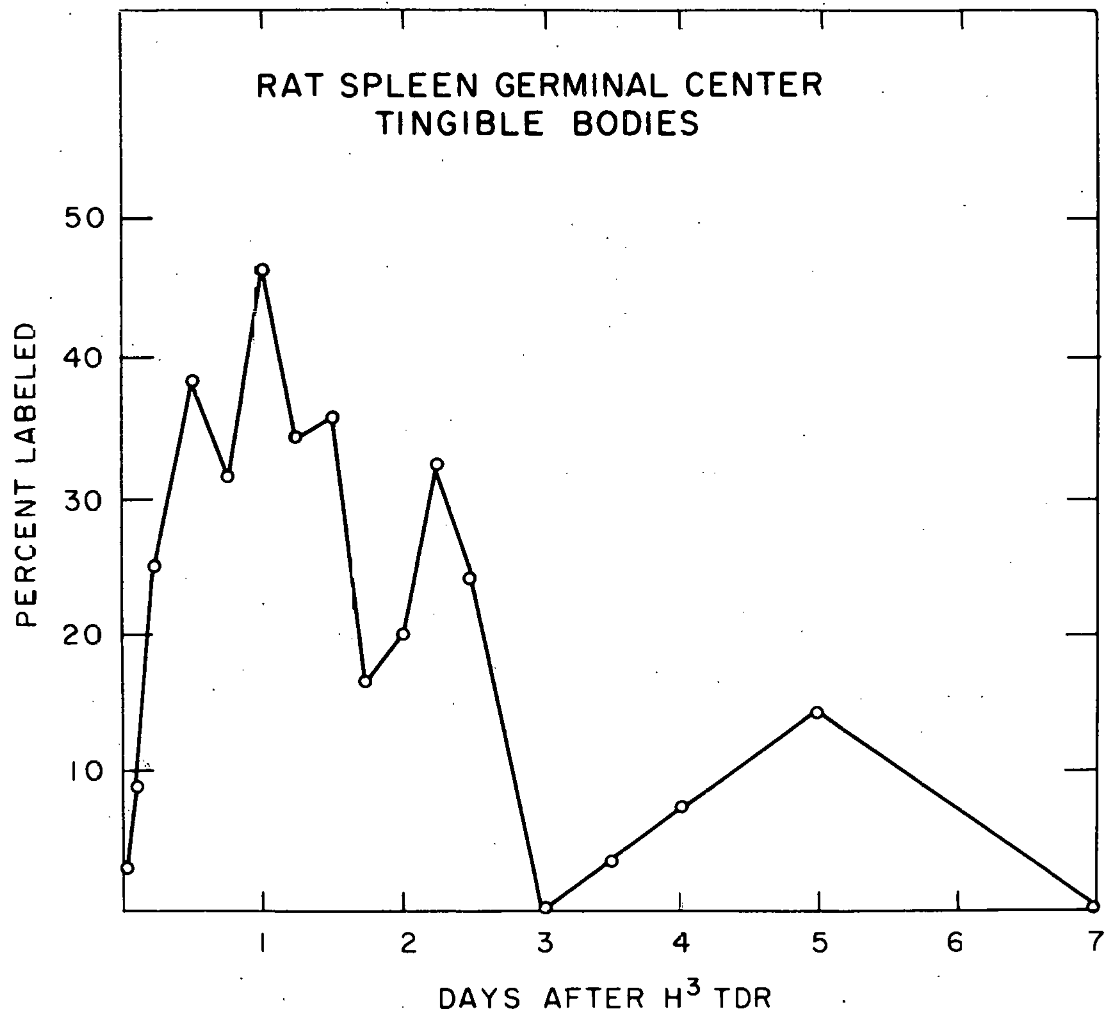

FIGURE 4

Figure 5. Labeling indices of large and medium sized and small sized mitoses after H^3TDR injection.

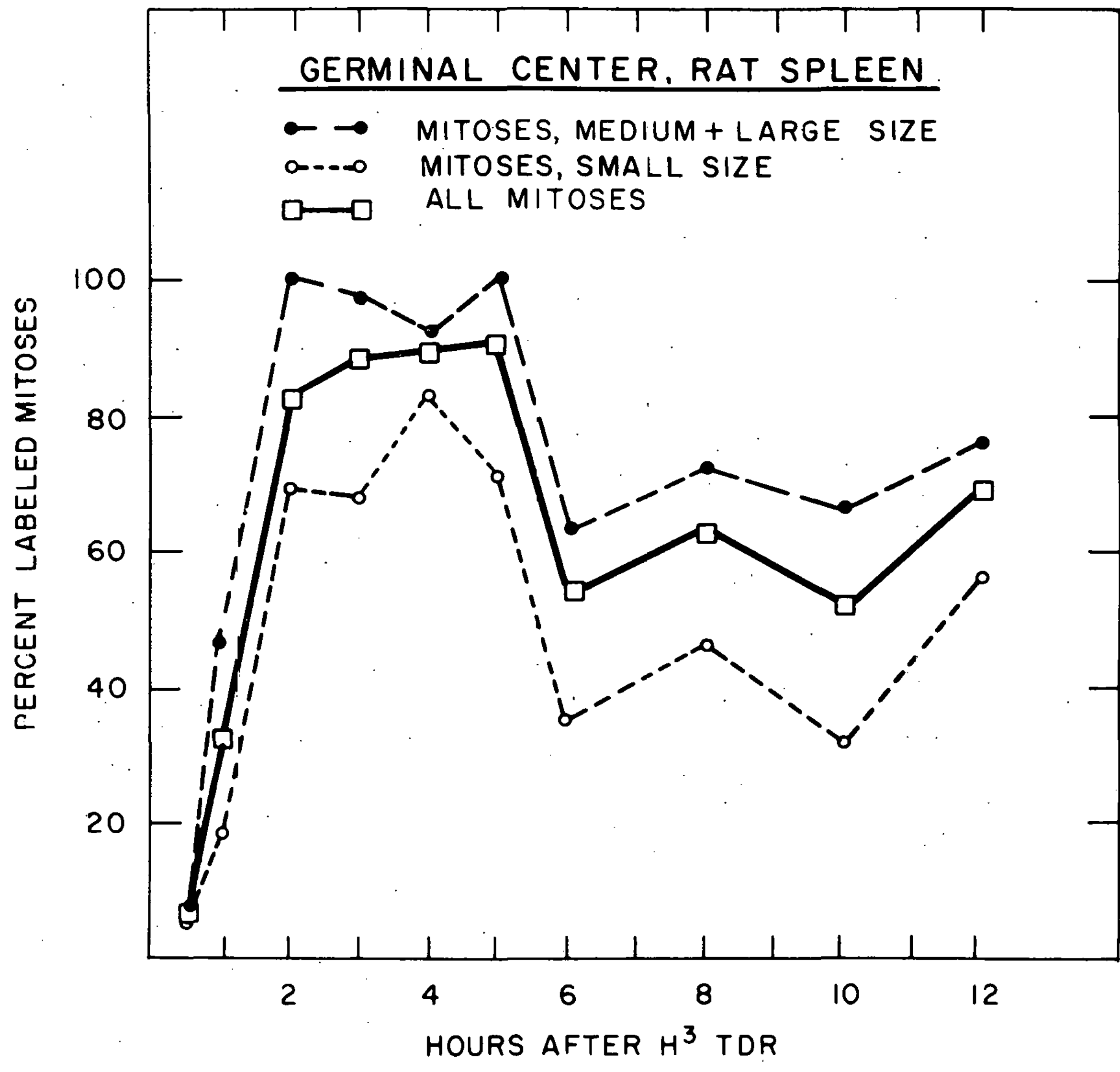

FIGURE 5

Figure 6. Grain count dilution over "germinal center"-cells as a function of time after H^3TDR injection.

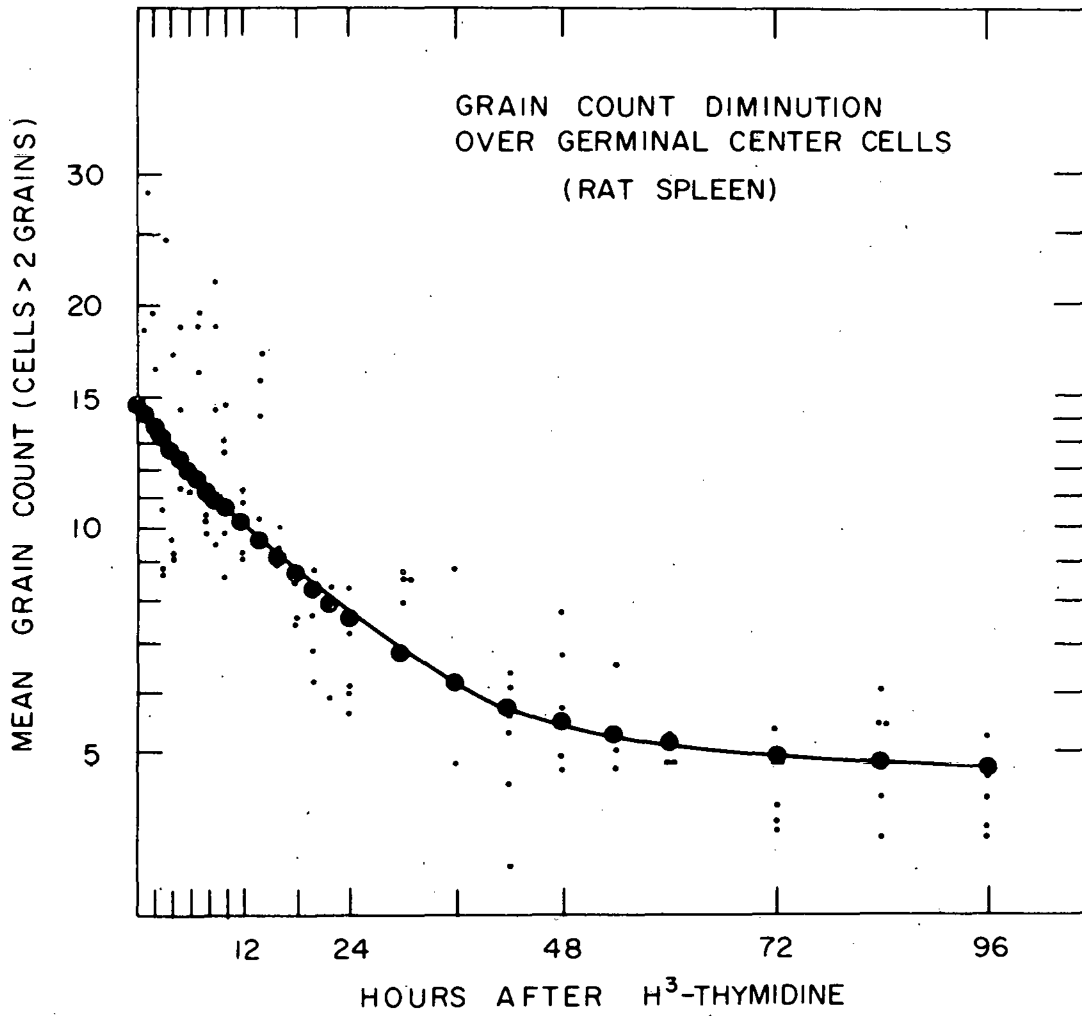

FIGURE 6

Figure 7. Labeling indices of "mantle zone"-cells as a function of time after H^3TDR flash injection.

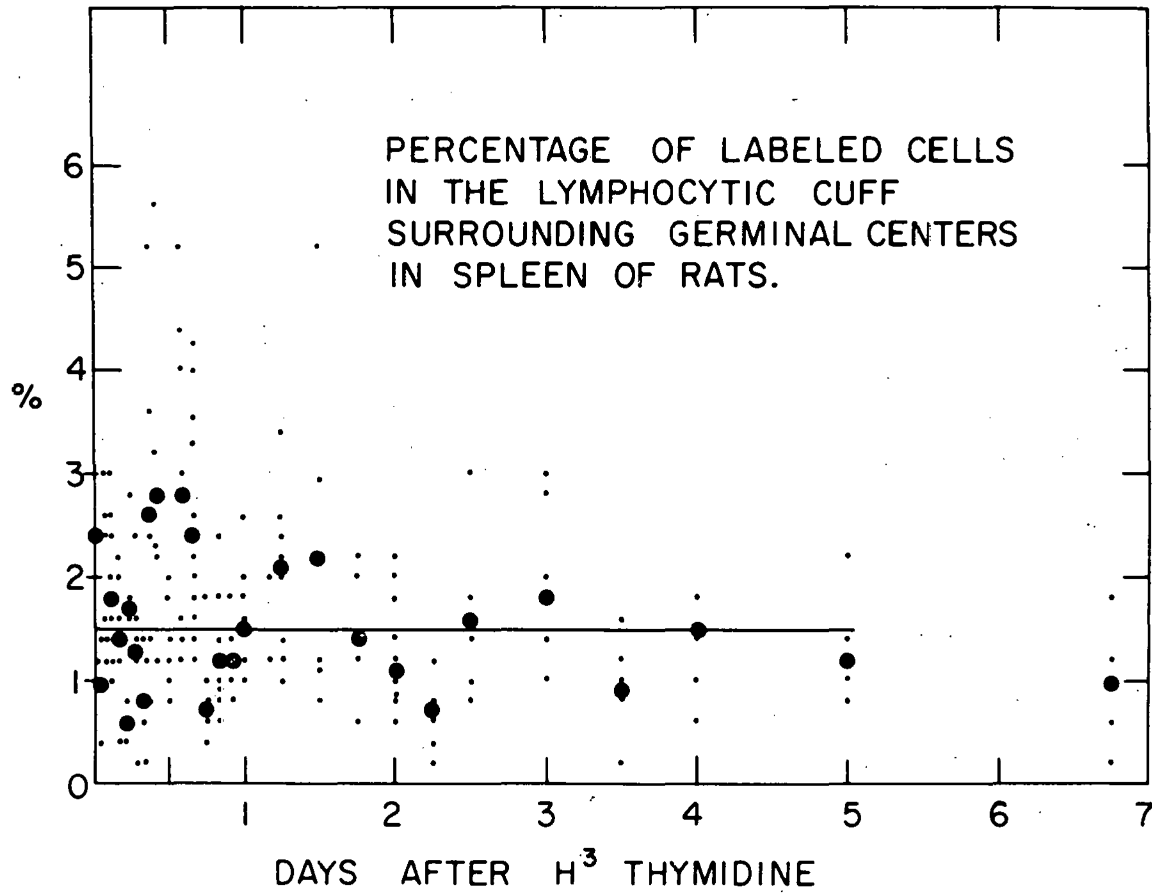

FIGURE 7

Figure 8. Grain count distribution over labeled cells in the "mantle zone" surrounding "germinal centers" in rat spleen.

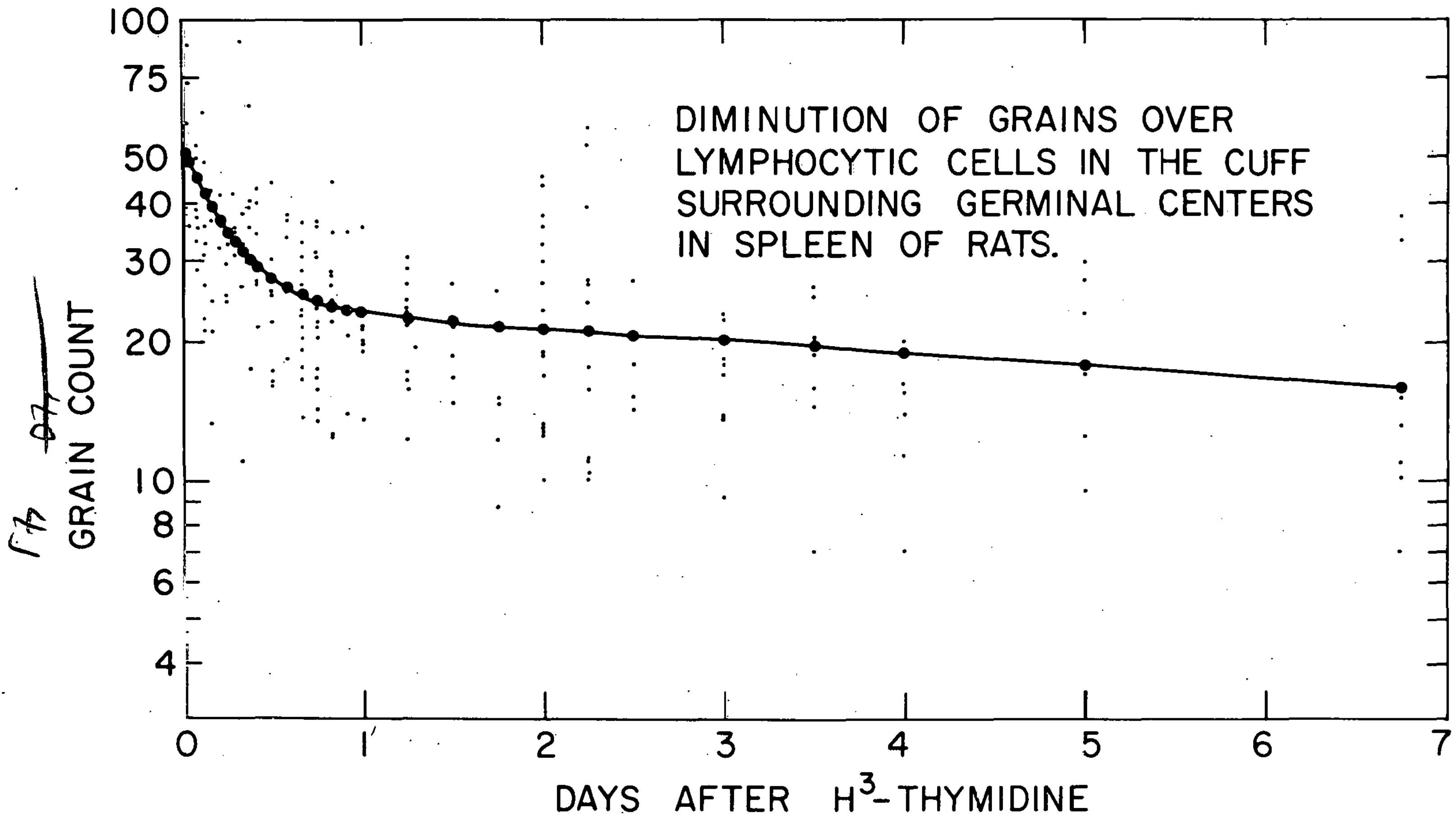

FIGURE 8