Comparative Response of Normal Human Thymus and Lymph Node Cells to Phytohemagglutinin in Culture

By ALAN WINKELSTEIN AND CHARLES G. CRADDOCK

Although thymus lymphoid cells (thymocytes) are morphologically similar to peripheral lymphocytes, the exact relationship between these two cell lines remains to be clarified. Based on various animal experiments, several investigators claim that thymocytes are destined to leave the thymus and function as immunologically competent lymphocytes in the periphery.1,4 Others point out, on the basis of kinetic studies, that thymocytes may constitute a distinct population of lymphoid cells which subserve a function that is still undefined.5-7

In small mammals, the intact thymus has been shown to contain lymphoid cells with a short lifespan and a rapid turnover rate.6,7 These cells are believed to have autonomous proliferative activity not influenced by factors which promote lymphocyte proliferation.8 In addition, these cells are relatively weak effector cells in various tests of immunologic competency.9,10 In contradistinction, lymphocytes have been categorized as recirculating,11 long-lived cells, with a slow turnover rate,12,13 and they are strong effector cells in immunologic reactions.14 The major stimulus for lymphopoiesis is believed to be exposure to environmental antigens, whereas the proliferative activity of thymocytes is unaffected by parenteral antigenic challenges.9

The recent finding that an extract of kidney beans, phytohemagglutinin (PHA), will promote lymphopoiesis in vitro provides an additional means of studying lymphoid physiology.15-20 It has been repeatedly demonstrated that, in short-term tissue culture, an excess of 85 per cent of peripheral blood lymphocytes from normal human donors will be induced to transform and proliferate in response to this nonspecific mitogen.20 Control cultures that do not contain an added stimulatory agent show a very low level of transformation. As an accompaniment of the transformation process, stimulated cells will actively synthesize both DNA and RNA15,16,21-23 and are believed capable of elaborating specific immunoglobulins.24

Since PHA appears to be a potent stimulating agent for normal human

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Lymphocytes, its capacity to stimulate thymus lymphoid cells would be useful in further elucidating the relationship of these two cell lines. Previous reports, however, have presented conflicting data on the responsiveness of thymocytes. Furthermore, most studies have not related the high basal rate of thymus cell proliferation in the intact animal5-7 to the in vitro results. In an effort to define the capacity of thymocytes to proliferate in culture, we have investigated the comparative response of normal human thymocytes and lymphocytes to PHA stimulation. These results have been related to the basal proliferative activity in culture. Our results, which are consistent with the findings of Claman,25 suggest that the normal human thymus contains a dual population of cells: a major component with autonomous proliferative activity which is not responsive to PHA, and a minor cell component which proliferates in response to PHA.

MATERIALS AND METHODS

A block of thymus tissue and an inguinal lymph node were obtained aseptically from 17 hematologically normal patients undergoing open heart surgery. No patient had any known disorder that might interfere with the stimulatory effects of PHA, and none exhibited any disease other than the cardiac disorder at the time of surgery. The patients ranged in age from 4 to 46 years and were equally distributed between acquired and congenital heart disorders.

The procedures employed were essentially the same as that of other workers.15-18,20 The surgically excised tissue was aseptically minced and a measured aliquot of cells added to media consisting of 8 ml. TC199 and 2 ml. fetal calf serum. All cultures were prepared in sealed, rubber capped, glass vials. Streptomycin (100 μg./ml.) and penicillin (100 U./ml.) were routinely included in each culture. The final cell concentrations were: lymph node cells, 0.5-1.0 × 10⁶ cells/ml.; and thymocytes, 1.0-1.5 × 10⁶ cells/ml.

For morphologic assessment and tritiated thymidine (H3T) incorporation studies, 6 to 10 cultures were routinely prepared for each specimen. Duplicate cultures were evaluated for a basal proliferative activity by incubating the culture with 1.0 μc. H3T per 5 × 10⁶ cells (New England Nuclear Corp., specific activity, 6.7 C./mM) for 1 hour. These cultures were terminated at this point and processed as described below. Two to 4 cultures, each containing 0.25 ml. PHA,* and 2 to 4 control cultures, without any added stimulatory agent, were incubated for 3 days at 37 C. One hour prior to harvesting, H3T, 1.0 μc. per 5 × 10⁶ cells, was added to each culture. Mitotic arresting agents were not employed as the accumulation of metaphase plates was not germane to this study.

Cultures were all harvested in an identical fashion. After an even dispersion of cells had been obtained, an 8 ml. aliquot was removed for radiochemical determinations of quantitative H3T uptake (see below). The remaining 2 ml. suspension was mixed with 8 ml. distilled water and incubated for 15 minutes at 37 C, then centrifuged at 1500–2000 r.p.m. for 10 minutes. The cells were fixed by the dropwise addition of 2 ml. of a freshly prepared 3:1 mixture of absolute alcohol and glacial acetic acid. This suspension was centrifuged, the supernate discarded, and the process repeated twice. After the final fixation, the cell suspension was evenly distributed on gelatin-coated slides. Two slides were routinely stained with a modified Giemsa stain, consisting of 0.5 ml. Giemsa stain, 2 ml. phosphate buffer (pH 6.4-6.8) and 47.5 ml. distilled water. Autoradiographs were prepared as previously described.26 Films were exposed at 4 C. for 7 days. The labeling index was computed on the basis of a 300 cell count and expressed as the percentage of labeled cells. In general, there was no difficulty in distinguishing labeled from unlabeled cells, as

*In these experiments PHA-M (Difco) was used. The desiccated powder was reconstituted with 5 ml. media TC199. Each batch was tested for mitogenic activity.
most of the former showed intense labeling with the high specific activity material employed. No cell was considered labeled unless the grain count was at least 5 above background.

Radiochemical determinations of quantitative H3T uptake were obtained by washing the cells 3 times with a 1 per cent citric acid solution. This wash removed most of the cytoplasm and any tritium not incorporated into DNA. The remaining cell nuclei were demonstrated free of unincorporated H3T by showing that 3 washes with a solution of saline and cold thymidine contained no residual radioactivity. The final cell concentration was determined and the cell nuclei were transferred to a cellophane combustion bag, dried at 60°C, and combusted in a 95 per cent oxygen atmosphere. The tritiated water formed was condensed by rapidly cooling the combustion flask with dry ice and counting, as previously described, in a Packard liquid scintillation counter. With the use of H3T as the labeled precursor and with the treatment described, all tritiated water obtained after combustion was derived from tritiated DNA. The final count consisted of an average of 3 separate determinations and was expressed as the average count/min./\(10^6\) cells.

Tritiated cytidine (H3C) studies were performed on 5 patients by a modification of the method of Epstein and Stohlman. The technique of culturing was identical to that described above. One hour prior to harvesting, H3C, 3.0 μc. per 5 × 10⁶ cells (New England Nuclear Corp., specific activity 2.34 C./mM), was added to each culture and incubated at 37°C. For each specimen, two cultures were analyzed for a basal isotope uptake by incubating for 1 hour and then terminating the culture. Two to 4 cultures with PHA and a like number without PHA were incubated for 3 days. Cultures were harvested by mixing a 4 ml aliquot of an evenly dispersed cell suspension with 8 ml distilled water and incubating for 15 minutes. The cells were harvested by centrifuging at 1500–2000 r.p.m. They were then evenly spread on gelatin-coated slides and fixed in methanol. Autoradiographs were prepared using the same method as employed in the H3T studies. The films were exposed for 1 week. The criteria for labeling with H3C were identical to those used with the H3T studies.

Peripheral blood cultures, from heparinized venous blood, were prepared in a fashion similar to that employed for the cell suspensions. Leukocyte-rich suspensions, obtained by dextran sedimentation and centrifugation, were adjusted to the desired cell concentration by addition of fetal calf serum. Each peripheral blood culture contained 1.0–1.5 × 10⁶ cells/ml. No attempt was made to separate lymphocytes from other peripheral blood leukocytes.

In addition, preliminary studies aimed at determining production of specific immunoglobulins were performed on cultured thymocytes. Slides were prepared for immunofluorescence by washing an aliquot of each culture in 5 per cent bovine serum albumin twice and fixing the cells in 95 per cent ethanol at 37°C. Slides were stained by a 2-layer indirect immunofluorescent technic as previously described. Each slide was independently evaluated by two observers. Details of these methods and results will be the subject of another communication.

RESULTS

Human thymocytes, in short-term culture, show a proliferative activity significantly different from either peripheral blood or lymph node lymphocytes. Kinetic studies suggest that the normal human thymus contains two cell populations, as proposed by Claman: one which transforms and proliferates in
response to phytohemagglutinin (PHA), and a second component which shows autonomous but unsustained proliferative activity and does not respond to PHA stimulation. Results are summarized in Table 1 and depicted in Figure 1.

Because of the inherent variability in duplicate cultures, several measurements were independently evaluated in these studies. The chief indicators used included: (1) morphological assessment of transformation; (2) autoradiographic labeling with the specific DNA precursor, tritiated thymidine (H3T); (3) quantitative incorporation of H3T into newly formed DNA. On a limited number of specimens, these studies were supplemented by autoradiographic labeling with the combined DNA and RNA precursor, tritiated cytidine (H3C). The results of the H3C studies are summarized in Table 2. The combination of multiple indicators for both DNA synthesis and cellular stimulation were employed in order to minimize any potential error that might result if only a single measure was employed. Furthermore, they permitted a fuller evaluation of the behavior of thymus lymphoid cells in culture.

In lymphocyte cultures in which cellular transformation occurred, all measurements show concordantly positive results. However, based on our initial results with peripheral blood cultures, the most reliable indicator of cell stimulation, in our hands, appeared to be H3T autoradiography. This technic provides a sensitive and highly reproducible measure of stimulation. Duplicate
Table 1.—Proliferative Activity of Thymocytes and Lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>1-Hour Incubation</th>
<th>% Transformed Cells</th>
<th>% H3T Labeled Cells</th>
<th>Counts/ min./10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral blood lymphocytes†</td>
<td>1.3 ± 0.3*</td>
<td>0.1 ± 0.1</td>
<td>25 ± 6</td>
<td></td>
</tr>
<tr>
<td>Lymph node lymphocytes‡</td>
<td>2.4 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>100 ± 40</td>
<td></td>
</tr>
<tr>
<td>Thymocytes§</td>
<td>3.2 ± 0.4</td>
<td>10.1 ± 1.4</td>
<td>611 ± 176</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>3 Day Incubation with PHA</th>
<th>% Transformed Cells</th>
<th>% H3T Labeled Cells</th>
<th>Counts/ min./10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>49.6 ± 2.5</td>
<td>20.6 ± 3.1</td>
<td>1008 ± 278</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>3 Day Incubation without PHA</th>
<th>% Transformed Cells</th>
<th>% H3T Labeled Cells</th>
<th>Counts/ min./10^6 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.9 ± 0.3</td>
<td>0.1 ± 0.1</td>
<td>37 ± 7</td>
<td></td>
</tr>
</tbody>
</table>

*Mean value ± standard error.
†Average values for 8 specimens.
‡Average values for 12 specimens.
§Average values for 17 specimens evaluating after 1-hour incubation and 13 specimens analyzed after 3-day incubation with and without PHA.
Table 2.—H³ Cytidine Autoradiographic Labeling

<table>
<thead>
<tr>
<th></th>
<th>Number of Specimens</th>
<th>1-Hour Incubation</th>
<th>3 Days with PHA</th>
<th>3 Days without PHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lymphocytes</td>
<td>7†</td>
<td>1.1 ± 0.7</td>
<td>69.9 ± 8.5</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>Lymph node lymphocytes</td>
<td>4</td>
<td>0.4 ± 0.3</td>
<td>72.3 ± 4.0</td>
<td>5.5 ± 2.7</td>
</tr>
<tr>
<td>Thymocytes</td>
<td>5</td>
<td>22.9 ± 3.3</td>
<td>51.6 ± 7.1</td>
<td>10.2 ± 1.6</td>
</tr>
</tbody>
</table>

*Mean value ± standard error.
†Six specimens analyzed for 1-hour incubation.

cultures from the same individual show less than a 10 per cent variation in labeling index. Furthermore, autoradiography is less subjective than the morphologic assessment of transformation and the results are more reproducible than the measurement of quantitative H3T uptake. The radiochemical measurement of H3T incorporation is subject to a considerable error in the final enumeration of cells after washing. Although H3C autoradiography is less specific, results with this measurement appear to correlate well with H3T autoradiography.

It should be noted that the technic of 1-hour pulse labeling with H3T provides a relative rather than absolute measure of cell proliferation. The brief period of exposure to H3T will not label all stimulated cells. However, it can be reasonably assumed that stimulated cells randomly enter the mitotic cycle. During any fixed time interval, a similar proportion of stimulated cells in culture from different specimens will be synthesizing DNA and thus incorporate the specific isotopic precursor. It has been shown previously, both in vivo and in vitro, that the increment of H3T incorporation per cell synthesizing DNA is maximal during the first hour of exposure. Since the time of exposure and the quantity of radioactive material are constant, the percentage of labeled cells provides a relative measure of cell proliferation for each individual culture and therefore may be justifiably compared.

As expected, lymph node lymphocytes show a proliferative activity similar to that observed by peripheral blood lymphocytes. Lymph node and blood lymphocytes show little evidence of autonomous DNA synthesis, as indicated by the incorporation of H3T of 1 per cent or less by these cells in the first hour of incubation. After 3 days, in the presence of PHA, a large proportion of cells are morphologically transformed and frequent mitotic figures can be identified, even in the absence of a metaphase arresting agent. Closely paralleling the morphologic evidence of transformation are the determinations of H3T incorporation. A representative autoradiograph of a PHA stimulated lymph node culture is shown in Figure 2. Within experimental limitations, the proliferative activity of lymph node lymphocytes after PHA stimulation is identical to blood lymphocytes. The slightly higher H3T autoradiographic labeling index of lymph node lymphocytes is not significantly different. Our findings with respect to the proliferative activity of blood lymphocytes is in accord with similar observations by other investigators.15,16,21,22

There was significant in vitro proliferation in 5 out of 12 lymph node prep-
Fig. 2.—A representative H3T autoradiograph of a lymph node cell culture stimulated with PHA. After a 1-hour incubation with H3T, a significant proportion of stimulated cells are labeled with the specific DNA precursor. Autoradiography, using the technics employed in these studies, provides a relative measure of cell proliferation which can reliably assess the stimulatory effects of PHA.

arations after 3 days in culture without PHA. This activity may be related to the cellular heterogeneity of lymph node cells, or it may represent a proliferative response on the part of lymphocytes to some unrecognized antigen. These nodes were obtained from the relatively reactive inguinal area where lymph node histology is quite variable.

Tritiated cytidine labels a high per cent of cells after PHA stimulation (72.3 per cent of lymph node cells and 69.9 per cent of blood lymphocytes). In the basal state and in 3-day control cultures, there is a low level of isotope incorporation.

In contrast to the kinetics of blood and lymph node lymphocytes, thymus lymphoid cells show a distinctive pattern of proliferation. The maximum DNA synthesis, as measured by H3T autoradiography, occurs in the first hour of incubation. We have considered this behavior of thymus cells during the first hour in culture to represent the high basal proliferative activity of thymocytes. Whereas less than 1 per cent of either blood or lymph node lymphocytes show evidence of H3T labeling during the first hour, 10.1 per cent of the cells in a thymus culture have incorporated H3T. The difference between lymph node lymphocytes and thymocytes is highly significant (p < 0.001). The other measurements of nucleic acid synthesis confirm the high prolifera-
RESPONSE OF THYMUS AND LYMPH NODE CELLS TO PHA

Table 3.—Serial H’T Autoradiographic Labeling

<table>
<thead>
<tr>
<th></th>
<th>1 Hour</th>
<th>1 Day</th>
<th>2 Days</th>
<th>3 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without PHA</td>
<td>10.1 ± 1.4*</td>
<td>5.0 ± 1.9</td>
<td>0.1 ± 0.1</td>
<td>0.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(4)</td>
<td>(3)</td>
<td>(13)</td>
</tr>
<tr>
<td>With PHA</td>
<td>9.4 ± 1.2</td>
<td>4.9 ± 1.1</td>
<td>2.0 ± 1.1</td>
<td>6.5 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(13)</td>
</tr>
</tbody>
</table>

*Mean value ± standard error (number in parenthesis refers to the number of specimens evaluated).

The proliferative activity of thymocytes in the basal state. By both the criteria of quantitative radiochemical H3T incorporation and H3C labeling index, thymocytes show significantly greater activity than lymphocytes. In contrast to the isotopic studies, few cells in the thymus suspension at 1 hour appear to be transformed, suggesting a limitation in the use of morphologic criteria of blast transformation as the sole indicator for cell proliferation in this tissue.

Thymocytes, after 3 days in culture without PHA, show virtually no isotopic incorporation or morphologic evidence of transformation. The addition of this nonspecific mitogen results in both morphologic blast transformation and autoradiographic evidence of nucleic acid synthesis by some thymocytes. Whereas control cultures, on the average, show only 2.1 per cent transformation and 0.8 per cent H3T autoradiographic labeling, PHA stimulated cultures result in morphologic transformation of 15.1 per cent and H3T labeling of 6.5 per cent. These differences are both significant (p < 0.001). Autoradiographic measurement of cytidine uptake shows comparable results. It should be emphasized that although there is some stimulation of thymocytes by PHA, the degree of stimulation is small. Indeed, quantitative radiochemical estimates of H3T uptake by thymocytes showed no significant difference between 3-day cultures with or without PHA. This disparity between autoradiographic and radiochemical estimates of DNA labeling may reflect the difficulty of detecting small percentage differences with radiochemical technics. It also may result from the existence of non-DNA thymidylate pools in the thymus.6,29 The differences in transformation and H3T labeling between lymph node cells and thymocytes are highly significant (p < 0.001) by all 3 criteria.

In serial cultures, PHA does not influence the autoradiographic incorporation of H3T of thymocyte cultures until the second day. The data on the serial uptake of this isotope in both PHA-stimulated and control cultures are summarized in Table 3 and graphically illustrated in Figure 3. The basal labeling index is not influenced by the presence of PHA in cultures of thymocytes. After 1 day in culture, both the stimulated and control cultures show an equal decline in the labeling index. The per cent of labeled cells is reduced by approximately 50 per cent of the basal level after 24 hours. In the control cultures, the progressive decline continues so that after 2 days there are virtually no labeled cells present. However, in cultures containing PHA on day 2, the labeling index is slightly higher than in the control cultures. This difference becomes highly significant after 3 days. In a few cultures incubated for 4 days, the labeling index was approximately equal to the proliferative activity...
observed on the third day. In evaluating the response of thymocytes to PHA stimulation, it is significant that other investigators have reported that PHA does not influence DNA synthesis by blood lymphocytes until after the first 24 hours in culture.15,16,21,22

Preliminary results on gamma globulin production by stimulated cells, using immunofluorescent labeling with type-specific rabbit antigamma G, gamma A and gamma M-human immunoglobulin sera, show that despite the high basal proliferative activity, thymocytes in the first hour in culture do not fluoresce with any of the antisera. Lymphocytes that are stimulated with PHA show a significant percentage of cells positive for each type of immunoglobulin, and thymocytes, stimulated with PHA, show a low percentage of cells that fluoresce with each antiserum. Although these studies are preliminary, it is quite clear that despite the high rate of cell production in the first hour of thymus culture, there is virtually no detectable gamma G, gamma A or gamma M immunoglobulin production. These results will be reported in more detail in another communication.

**DISCUSSION**

Previous reports relating the proliferative activity of thymocytes in short-term tissue culture have presented conflicting data. For example, Reike and Schwarz reported that suspensions of rat thymus cells in concentrations 5 to 50 times that of lymph node cells responded to PHA.38 Chapman, however, found that in rabbits, neither specific antigens or PHA produced significant DNA stimulation.31 McIntyre and Segel, using P^{32} incorporation into DNA,
were unable to observe a significant degree of cell stimulation in suspensions of human thymus lymphoid cells. \textsuperscript{32} In another report, Strosselli et al. found that PHA did not cause transformation of thymocytes. \textsuperscript{33} Stastny and Ziff reported that in cultures of rabbit thymus cells, tested with either PHA or Streptolysin S, there was only a small increase or no increase in H3T uptake. However, the addition of living autologous polymorphonuclear leukocytes resulted in an increase in DNA synthesis. \textsuperscript{34} None of these investigators determined the initial proliferative activity and related this to the response with PHA stimulation.

Recently, Claman reported results on the proliferative response of human thymocytes. \textsuperscript{25} His data suggest that the thymus contains a dual population of lymphoid cells: one group that is unresponsive to PHA and shows a high basal proliferative activity, and a second population which is responsive to PHA. In our initial report, \textsuperscript{35} we demonstrated the high basal proliferative activity of human thymocytes, which contrasted to the negligible basal proliferation of either blood or lymph node lymphocytes. After 3 days, thymus cultures stimulated with PHA showed considerably less labeling compared to the basal level, suggesting that these cells did not proliferate in response to this nonspecific mitogen. However, additional data demonstrated the presence of a minor component which responds to PHA stimulation. These observations thus confirm and extend Claman’s conclusions. Our studies suggest that the major lymphoid component constitutes a population of cells with autonomous proliferative activity and a rapid replacement rate. The second and probably minor cellular component constitutes cells that are responsive to PHA stimulation. These stimulated cells appear to respond identically to peripheral lymphocytes.

The evidence is indirect for the conclusion that a small fraction of the total thymus lymphocyte mass is PHA responsive. An accurate assessment of the ratio of PHA responsive to unresponsive cells in the thymus cannot be measured because of such factors as cell death and multiple division of stimulated cells. The rate of cell death assumes significance in evaluating the response of thymocytes because of the apparent high rate of cell death in unstimulated cultures. Probably a significant number of cells which do not respond to PHA are lost in the stimulated cultures. Furthermore, it has been determined by others that a significant proportion of stimulated cells will have completed at least one division and some will have divided twice after 3 days. \textsuperscript{36} Both of these variables would tend to produce an apparent increase in the percentage of PHA responsive cells. Even if these variables are not considered, it would be reasonable to assume that if a majority of cells were responsive to PHA, the labeling index and per cent transformation should approach that observed in lymph node cultures. By both criteria, lymph node cells show at least 5 times the proliferative activity of thymocytes.

The failure of most thymocytes to proliferate in response to PHA stimulation cannot be attributed to any known selective activity of this agent. Although the exact mode of action of PHA remains to be clarified, it has been shown that it induces transformation in at least 85 per cent of peripheral blood
lymphocytes from normal donors. It is classified as a nonspecific agent because of its capacity to stimulate normal lymphocytes irrespective of any prior sensitization. This is in contrast to the so-called specific agents, believed to act as antigens, of which tuberculin protein is the prototype. This latter group is stated to react only with lymphocytes from a previous sensitized donor and induces transformation in a small fraction of the total lymphocyte population. The difference in response of thymocytes and lymphocytes to PHA stimulation is best attributed to differences in reactivity of the two populations in culture. It is not possible to exclude other factors which might alter the proliferative response of thymocytes. These cells might require different in vitro conditions for optimal growth. Alternatively, thymus cell suspensions may contain an inhibitory factor that prevents a maximum response by thymocytes to PHA stimulation. Neither of these alternatives, however, accounts for the high initial proliferative activity observed by thymocytes in culture.

It is of interest to correlate the in vitro proliferative activity of human thymocytes and lymphocytes with the kinetics of these lymphoid cells in the intact animal. Many studies have demonstrated that the rate of cell division in the thymus is considerably higher than that observed in peripheral lymphoid tissues. For example, thymus lymphoid cells in rats, given H3T one hour prior to sacrifice, show a higher labeling index and a more rapid rate of DNA renewal than lymphocytes in either the mantle zone of lymphoid follicles or around blood vessels. This high basal proliferative rate in vivo is probably reflected by the differences in the per cent labeling with H3T of thymocytes and lymphocytes in the initial 1-hour incubation. Furthermore, the rapid rate of disappearance of H3T labeled DNA from the intact animal thymus suggests a short lifespan for these cells. This compares with the progressive fall in labeling index seen in thymus cultures without PHA. This latter observation suggests that the majority of thymus cells in culture are incapable of repetitive divisions and rapidly die.

The differences in the initial labeling index of thymocytes and lymphocytes in culture may also reflect differences in proliferative stimuli for these two cell populations in the intact animal. Whereas the major stimulus for lymphopoiesis appears to be exposure to environmental antigens, the turnover rate of thymocytes is not significantly altered by this exposure. Based on the comparative response of thymocytes and lymphocytes in mice, Metcalf concluded that thymocytes show autonomous proliferative activity and that the stimulus for thymocyte proliferation probably resides within the thymus itself. In vitro, the high labeling index of thymocytes in the basal state, when compared with the negligible activity of lymphocytes, suggests that a significant proportion of thymus cells in culture show a similar autonomous proliferative activity which is probably not influenced by exogenous stimuli that promote lymphopoiesis.

There has been conflicting data on the immunologic competency of thymus cells, but most investigators conclude that, on a per cell basis, thymocytes are distinctly weaker in effecting an immune response than peripheral lymphocytes. In this context, these in vitro data suggest that the thymus contains a relatively small number of immunologically competent cells which are
diluted by a large mass of cells which are unreactive in this respect. This might account for the need for 10–20 times as many thymus cells as lymphocytes to equally effect an immune response. These observations do not, however, pertain to the possibility that thymus cells may develop into immunologically active cells after reaching peripheral tissues in the intact animal.

**Summary**

Human thymocytes, in short-term tissue culture, show a proliferative activity distinct from that observed by either lymph node or blood lymphocytes. As expected, the behavior of lymph node lymphocytes in culture is very similar to that of peripheral blood lymphocytes. The only difference between these 2 groups of cells was the finding of spontaneous proliferation by normal lymph node cells after 3 days in culture without phytohemagglutinin (PHA).

Whereas blood and lymph node lymphocytes show a negligible uptake of H3T in the basal state, approximately 10 per cent of thymus cells incorporate H3T, indicating significant autonomous proliferation. This is unaffected by PHA and is unassociated with globulin synthesis as judged by immunofluorescent technics. After 3 days in culture, there are significantly more transformed cells and more cells which incorporate H3T into DNA in thymus cell cultures containing PHA than in the control cultures. However, the labeling index of stimulated thymus cultures is less than either the basal rate of proliferation of thymocytes or 3-day cultures of PHA stimulated blood and lymph node lymphocytes. These observations suggest that the normal human thymus contains at least two populations of lymphoid cells: a major component which shows autonomous and unsustained proliferative activity and does not respond to PHA, and a second and probably minor cellular component which transforms and proliferates in response to PHA.

**Sommaire in Interlingua**

Thymocytos human monstra in histoculturas brevidurative un activitate proliferatori distincte ab illo observate in lymphocytos ab nodos lymphatic o ab le sanguine. Non inexpectatemente, le comportamento de culturate lymphocytos ab nodos lymphatic es similissime a illo de lymphocytos ab sanguine perpheric. Le sol differentia inter iste 2 gruppos de cellulas esseva le constatation de un proliferation spontanee de normal cellulas de nodo lymphatic post 3 dies de culturation sin phytohemagglutinina.

Durante que lymphocytos ab le sanguine e ab nodos lymphatic monstra un negligibile accetaption de thymidina a tritium in le stato basal, approximativemente 10 pro cento del thymocytos incorpora thymidina a tritium lo que indica un significative grado de proliferation autonome. Isto non es afficite per phytohemagglutinina e es sin association con le synthese de globulina a judicar per technicas immunofluorescentic. Post un culturation de 3 dies, significativemente plus alte augmentos es constatate in le numeros de cellulas transformate e de cellulas incorporante thymidina a tritium ad in acido deoxyribonucleic in culturas de thymocytos continente phytohe
glutinina que in culturas de controlo. Tamen, le indice de marçage in stimulate culturas de thymocytes es inferior al rapiditate basal de proliferation de thymocytes o de culturas de etates de 3 dies de stimulate lymphocytes ab sanguine e ab nodos lymphatic. Iste observationes suggestiona que le normal thymo human contine al minus duo populationes de cellulas lymphoide: (1) un componente major que monstra autonome e non-continue activitate proliferative e que non responde a phytohemagglutinina e (2) un secunde e probablemente minor componente que se transforma e monstra proliferation in responsa a phytohemagglutinina.

**ACKNOWLEDGMENTS**

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