Protein Synthesis in Rat Lymphocytes: Radioautographic Studies of Availability and Utilization of Labeled Amino Acids in Vivo

By William O. Rieke and M. Roy Schwarz

One of the earliest applications of radioautographic and radiochemical technics to the problem of protein synthesis in various animal tissues in vivo was made by Leblond, Everett and Simmons. This study clearly showed that these technics were well suited for the localization and quantification of protein synthesis as indicated by the incorporation of a radioactive amino acid. The validity of using radioactive amino acid incorporation as revealed by radioautography for an indicator of new protein synthesis has been affirmed recently by Warshawsky and Droz and Droz and Warshawsky. These investigators studied tissues of mice which had received C14-leucine and showed that although up to 50 per cent of the total radioactivity could be lost from labeled tissues during radioautographic processing, 90-97 per cent of the retained radioactivity was firmly bound to protein.

Current interest in the production of immunologically active protein by cells of the lymphoid series provides a strong incentive for the application of radiologic technics to lymphocytes. While there have been numerous radiochemical and immunoradiochemical studies measuring antibody or protein synthesis by mixed lymphoid cells from spleen or lymph node (see review by Harris and Harris, also references 5–10), there has been no high-resolution radioautographic investigation of the normal protein metabolism of individual types of lymphocytes in vivo. Tischendorf and Linnartz-Niklas have published a radioautographic study of the localization of S35-methionine in the lymphoreticular organs of mice, rats and rabbits in vivo. However, resolution with this isotope permitted only the general conclusion that labeling was greater over areas such as germinal centers where large lymphocytes and monocytes were preponderant than over areas containing mainly small lymphocytes. Additional histologic detail as well as support for this conclusion have thus far been provided only by studies of limited duration accomplished in vitro. Thus, Meneghelli found that while slices of rabbit mesenteric lymph node exposed to 1-C14-glycine showed maximal labeling in perifollicular cortical cells, there was also labeling in cells of the medullary cords. In the cords, large lymphocytes labeled much more intensely than medium lymphocytes, which in turn

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* A summary and comparison of labeling characteristics of the various cell sizes in the different lymphoid tissues is presented in table 2.

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labeled better than small lymphocytes or reticular cells. Cooper similarly has found that human lymph node cells treated with H\textsuperscript{3}-leucine in vitro showed heavier labeling on the large and medium lymphocytes than on the small.

In order to supplement existing information on the "normal" protein metabolism of lymphocytes in vivo as well as to provide a baseline for subsequent studies, the present investigation was accomplished in healthy animals which had not been stimulated with exogenous antigen. Because the validity of the interpretations drawn depended upon a knowledge of the duration of availability of the tracer employed, the study included radiochemical and radioautographic investigations of the availability time of the principal radioisotope utilized (H\textsuperscript{3}-methionine).

**MATERIALS AND METHODS**

Three types of experiments were undertaken employing inbred Lewis rats and radioautographic technics previously described.  

1. **Incorporation and Metabolism of H\textsuperscript{3}-methionine**

Seven male rats weighing 135-170 Gm. were injected intravenously with 4 $\mu$Ci./Gm. body weight of DL-methionine-T (H\textsuperscript{3}-methionine, Volk Radiochemical Co., Skokie, Ill.) having a specific activity of 148 mc./mM. The animals were sacrificed at intervals of 1/4, 4, 8, 18, 24, 36 and 96 hours after injection and smears were made from thoracic duct lymph, teased thymus and mesenteric lymph node. The smears were processed for radioautography, and film exposures of 2, 5 and 8 weeks were allowed. The 8-week exposures were consistently employed to determine per cent of labeled cells and grain counts over cells. In making grain counts, 20 to 100 cells were scored at random and appropriate corrections for background reduction were applied after study of cell-free areas on each slide. Because serum proteins were labeled by the isotope, the corrections for background—i.e., non-cellular—reduction was higher at short intervals after isotope. The data showing grain counts and per cent label were recorded and, in addition, an attempt was made to show total tissue labeling by multiplying the average grain count by the per cent label at the various intervals and plotting the product against time (figs. 1-3).

2. **Availability Time of H\textsuperscript{3}-Methionine**

Five female rats weighing 130-140 Gm. were injected intravenously with 8 $\mu$Ci./Gm. body weight of H\textsuperscript{3}-methionine. The animals were sacrificed at intervals of 5, 15, 30, 60 and 140 minutes after injection. One ml. of blood was withdrawn from each animal by cardiac puncture at the exact times noted. The blood was allowed to clot so that serum could be separated and used for paper chromatographic analysis. With the exception of the 5-minute animal, smears of thoracic duct lymph and mesenteric lymph node were prepared for radioautography from each of the animals as above. The smears were exposed for 2 and 6 weeks, and the 2-week exposures were employed for grain counts.

To determine availability time of the isotope, 0.01 ml. of serum from each of the animals was spotted on Whatman No. 1 chromatography paper between spots of non-radioactive methionine (5 $\mu$g.) and subjected to ascending chromatography for 18 hours in a solvent of n-butanol, acetic acid and water (2.4:1:0.6, v:v:v). The chromatograms were dried, sprayed with ninhydrin reagent and the Rf values of the various spots were determined. The average Rf of 12 non-radioactive methionine spots was 0.59. The entire length of each chromatographic path was divided transversely, as indicated by the spots, into strips of paper approximately 30-40 mm. long. Care was taken that the sixth strip in each case centered on the Rf of methionine as shown by the adjacent non-radioactive methionine spots. The paper strips were placed in counting vials containing p-dioxane-
naphthalene solvent and were assayed for radioactivity in a Tri-Carb liquid scintillation counting system. Blanks containing paper strips cut from locations next to the chromatographic path were assayed to determine the background count. Internal tritium standards were then added and the vials assayed again to ascertain the efficiency and allow conversion of the previously measured radioactivity to total disintegrations present in the samples. Calculations of total radioactivity were made for the paper strips taken at the origin of the chromatographs (proteins), at the methionine locations, and at the sum of all other spots. Since 0.01 ml. of serum was used in the chromatographs and the circulating serum volume of the animals was taken to be approximately 4 ml., the total radioactivity in the various locations was multiplied by 400. This allowed calculation of the per cent of the injected dose which remained in the serum. The activity in the strip cut from the origin was taken to represent protein, although it was recognized that other high molecular weight compounds may also have been present.

3. Labeling by a High Dose of Radioactive Amino Acid

One male rat weighing 95 Gm. was given a single subcutaneous injection of 30 μc./Gm. body weight of DL-leucine-4, 5-T obtained from Nuclear of Chicago, Des Plaines, Ill., and having a specific activity of 5.4 c./mM. Five hours after H3-leucine, the animal was sacrificed and smears for radioautography were prepared from the lymph, thymus and mesenteric lymph node. Radioautographic exposures of 1, 2 and 4 weeks were made, and nearly all cells were found to be labeled at the earliest exposure.
Fig. 2.—The curves for disappearance of radioactivity in medium lymphocytes are seen to resemble those for large cells except that the pattern for lymph node cells more closely follows that of the thymus rather than of the lymph.

Fig. 3.—The curves showing disappearance of radioactivity from small lymphocytes emphasize that considerable label is lost from circulating cells of the lymph. In contrast, label in the thymus remains relatively high. X1900.
RESULTS AND DISCUSSION

Availability Time of \( H^3 \)-Methionine

The disappearance of intravenously administered \( H^3 \)-methionine from the blood was found to be exceedingly rapid. As shown in table 1, only 1.5 per cent of the injected dose remained as circulating methionine 5 minutes after administration, and this amount decreased progressively during the 21/4-hour period measured thereafter. The disappearance of \( H^3 \)-methionine was accompanied by progressively increasing amounts of radioactivity in circulating proteins and decreasing amounts of activity in non-protein, non-methionine substances. These findings are in close agreement with those reported by Humphrey and Suliteanu who studied the disappearance of hydrolysates of C\(^14\)-labeled \textit{Chlorella} proteins injected into rabbits.\(^{15} \)

Although these authors made no attempt to identify specific compounds and assumed that all dialyzable radioactive substances were amino acids, they also found that only 1 per cent of the administered amino acids remained free in the serum 5 minutes after injection. A figure of 0.25 per cent has been quoted by McFarlane for the activity remaining in free amino acids 2 hours after injection of labeled \textit{Chlorella} hydrolysates in the rat.\(^{16} \)

This correlates well with the present finding of 0.3 per cent at 21/2 hours in the rat.

Grain counts over large lymphocytes from the same animals used for measuring serum availability made it obvious that disappearance curves are not an adequate indication of tissue availability. Although large lymphocytes had acquired over 60 per cent of their maximal labeling intensity by 15 minutes, it was not until 1 hour after isotope administration that grain density became maximal (table 1). It was not possible to discern whether the increasing grain density was a consequence of continued incorporation of labeled methionine which had re-entered the serum from tissues, or whether it reflected the conversion of radioactive compounds which had been extractable during fixation to non-extractable compounds. Most probably both mechanisms were important. The fact that 98.5 per cent of the \( H^3 \)-methionine was removed from the serum in the first 5 minutes and only another 1.2 per cent during the succeeding 21/4 hours argues for exchange and re-entry of the tracer from
tissues to serum. The probability of such an exchange is supported by work from other investigators showing that re-entry occurs readily from intracellular free amino acid pools in the rabbit which are on the order of 100 times the size of the free amino acid pool in plasma. From table 1 it may be seen that even 1 hour after isotope injection the concentration of circulating H3-methionine was well within the range of that commonly used to produce labeling with in vitro systems. Thus, continued incorporation of label was most likely. In addition, however, the demonstration by Borsook et al. that very high quantities of intracellular radioactivity are in a non-protein form at short intervals after administration of labeled amino acids makes it probable that at least some of this activity was removed during fixation for radioautography.

Whatever the mechanisms which increased the grain density, it is clear that when radioautography is used with in vivo systems to measure protein synthesis the effective availability time of an injected precursor must be considered to be at least 1 hour. It is to be emphasized, in addition, that utilization of maximum grain density as a measure of availability time assumes that there is no turnover of radioactivity within cells during the time they are being observed. Although there probably was turnover during the 1st hour after isotope when exchange of labeled amino acid between tissues and serum was high, this could be neglected, for the net grain density continued to increase. The interval after the 1st hour could not be as readily dismissed. Even though there is evidence that most of the intracellular activity was protein-bound after the first hour, it is seen from table 1 that during the succeeding 1½ hours the average grain density over large lymphocytes from lymph and lymph node together decreased. The decrease per hour was approximately 2.6 per cent. From the results in the second group of animals shown in figure 1, it may be calculated that the decrease in radioactivity from 4 to 18 hours after isotope was fairly uniform and averaged 5.4 per cent per hour. Because the decrease in per cent during the time from 1 to 2 hours after isotope in the first group of animals was less than that expected from a projection of the relatively constant rate of decrease seen in the second group of animals, it is probable that large lymphocytes continued to label during the 2nd hour. The amount of labeling was, however, small and it may be calculated that availability as measured by labeling was 97–98 per cent completed at the end of the 1st hour.

Labeling of Large Lymphocytes

Large lymphocytes in smear preparations of rat tissues have previously been defined by this laboratory as rapidly proliferating cells originating from some "stem" cells and having a nuclear index (the product of two perpendicular nuclear diameters) of at least 110. By mitosis, large lymphocytes are believed to give origin to more of their kind as well as to smaller members of the lymphocyte series.

In table 2 it is seen that 100 per cent of large lymphocytes were labeled 4 hours after a single injection of H3-methionine. Nearly 100 per cent were
The per cent label and average grain count of rat lymphocytes following intravenous administration of 4 μc./Gm. body weight of H3-methionine are shown. 

Another experiment showed that 100 per cent of small lymphocytes can be labeled with a single injection if the dose of isotope is sufficiently high. See text.

labeled at 15 minutes after isotope, a time when significant quantities of methionine were still available for incorporation. The labeling intensities of these cells in any one tissue varied only moderately, and a factor of 2.5 was found to encompass the range from minimal to maximal labeling. The large lymphocytes in the thymus showed consistently less uptake than large cells elsewhere. This characteristic has also been observed with H3-thymidine labeling of thymic cells and presumably is a consequence of a vascular barrier impeding diffusion and/or of a relatively large pool of metabolites in the gland. In agreement with the in vitro study of human lymphocytes reported by Cooper, large lymphocytes were found to label approximately twice as heavily per square micron of area as small lymphocytes (figs. 4, 6 and 7). The range in labeling intensities observed on large cells was, however, slightly less than half that noted by Cooper. As shown in figures 6 and 7, it was common to find labeling over both the nucleus and cytoplasm at early and late intervals after isotope. Baserga and Kiesieski have reported that in radioautographs of Ehrlich ascites cells treated with H3-leucine, many of the silver grains found over nuclei actually did not represent nuclear label but arose

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<tr>
<th>Time after Isotope</th>
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<th>Medium Lymphocytes</th>
<th>Small Lymphocytes</th>
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<td>% Label</td>
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<td>Thoracic Duct Lymph</td>
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from radioactivity in a thin layer of cytoplasm which was interposed between the nuclei and the photographic emulsion. While the present results gave no reason to question this interpretation in the usual case, there were occasional large lymphocytes, especially at early intervals, which showed intense nuclear labeling with virtually no silver grains over a broad band of surrounding cytoplasm. At least in such cases there can be little doubt that nuclei also incorporate amino acids and bind them in an insoluble compound which most probably is protein.

The disappearance of radioactivity from large lymphocytes with time was divisible into two phases (fig. 1). During the first phase from 4 to 24 hours after isotope, radioactivity decreased rapidly with a half-time of approximately 7 to 8 hours. Large lymphocytes from the thymus as well as from thoracic duct lymph and lymph node showed very similar rates of loss of label during much of this period. Analysis of the extent to which dilution through mitosis was responsible for decrease in activity was dependent upon determination of the generation time of large lymphocytes and the manner of partition of newly synthesized protein between daughter cells. Grain counts over telophase figures in thymus and lymph node at intervals up to 8 hours after H3-thymidine indicated that within the limits of the technic there is equal distribution of new protein between daughter cells. Estimates of generation time were made by employing material from previously published studies on H3-thymidine labeling of lymphocytes, and indicated that large lymphocytes divide approximately every 12 hours in lymph node and every 14–16 hours in lymph. Although weaker labeling and apparent extensive reutilization of metabolites in the thymus made determination of the generation time of the large thymocyte more difficult, it appeared to be of the same order as that of the large lymphocyte in the lymph or slightly longer. Comparison of these data with the disappearance rate of radioactivity showed that about 25 per cent more activity was lost from large lymphocytes in lymph node and thymus and 50 per cent more from the large cells in lymph than could be explained by dilution through mitosis. Although part of this loss may have been only metabolic turnover within cells, lymphatic tissues have been shown to produce beta and gamma globulins. Because large lymphocytes in particular have been implicated in this role, it may be that the excess loss of radioactivity reflected secretion of these proteins. If this be so, it is of interest that circulating as well as tissue-fixed cells show this activity. Moreover, it is noteworthy that all large lymphocytes are continuously synthesizing protein of some type, even though previous studies with fluorescein-conjugated antibodies have shown that not all large cells contain the same species of protein at the same time.

The second phase in the disappearance of radioactivity began at approximately 24 hours after isotope and was characterized by a gradual but steady decline in labeling throughout the remainder of the experiment. The change in the disappearance rate at 24 hours (fig. 1) at first suggested that large lymphocytes contain two classes of proteins with different turnover times much like the “exportable” and “sedentary” proteins found in retinal rods by
However, large lymphocytes are not a stable population like retinal rods but are constantly renewing. Moreover, while the disappearance rates during the first phase were similar for large cells in all three locations studied, they became dissimilar during the second phase. By 96 hours after isotope administration, isotope radioactivity had disappeared completely from large lymphocytes in the lymph, whereas small quantities remained in lymph node lymphocytes and larger amounts persisted in the thymus. It seems unlikely
that cells which initially behaved similarly with respect to protein metabolism would later show proteins of three separate renewal rates. Rather it seems that the present observations may be coupled with previous studies showing phagocytosis of labeled lymphocytes by reticular cells\textsuperscript{19,24,25} and utilization of labeled metabolites by various tissues\textsuperscript{28,29} to suggest that the gradual disappearance of radioactivity during the second phase was actually the consequence of reutilization of labeled metabolites. The concept of reutilization is strengthened both by the fact that label disappeared completely from thoracic duct lymphocytes which were circulating away from tissue sites of reutilization and by the observation that reutilization was the most prolonged in the thymus where the pool of at least certain metabolites is known to be large.\textsuperscript{20}

**Labeling of Medium Lymphocytes**

Medium lymphocytes are recognized in smear preparations as cells with nuclear indexes greater than 56 $\mu^2$ but less than 110 $\mu^2$ (fig. 4). They have previously been described as arising both by division of large lymphocytes and by enlargement of small cells.\textsuperscript{18} Most of the population is active in mitosis. In table 2 it is seen that like large cells, 100 per cent of medium lymphocytes were labeled at short intervals after H\textsuperscript{3}-methionine. Although the absolute labeling intensity of medium lymphocytes was less than that of large lymphocytes, the silver grain density per square micron was comparable. The range of labeling intensities was greater on medium than on the large cells with a few (usually intensely basophilic) lymphocytes being labeled 4–5 times as heavily as the usual cell. The over-all labeling patterns and disappearance of radioactivity were remarkably similar to those exhibited by large lymphocytes with the exception that thoracic duct lymphocytes did not parallel the cells in the lymph node (fig. 2). The medium cells in lymph showed a later and higher peak radioactivity than lymph node cells. Presumably this reflected the gradual entry into the lymph of medium cells which had just been formed in the node by division of heavily labeled large lymphocytes.

Although the generation time of medium lymphocytes is not known, and cannot be calculated readily because of the dual origin of the population,\textsuperscript{19}

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Fig. 4.—Radioautograph showing labeled medium and small cells from thoracic duct lymph 15 minutes after injection of 4 $\mu$C. Gm. body weight of H\textsuperscript{3}-methionine. X1500.

Fig. 5.—Radioautograph of cells from thoracic duct lymph 5 hours after the injection of 30 $\mu$C. Gm. body weight of H\textsuperscript{3}-leucine. Label is seen over all the cells even though this radioautograph was exposed for only 1 week. X1500.

Fig. 6.—Radioautograph of thoracic duct lymph to show the labeling of a large lymphocyte 15 minutes after H\textsuperscript{3}-methionine. X1500.

Fig. 7.—Radioautograph of a large lymphocyte from thoracic duct lymph 8 hours after injection of H\textsuperscript{3}-methionine. Labeling remains heavy. Note the accumulation of grains over the cytoplasm in the area of the nuclear hof. This area is commonly the site of the Golgi apparatus.

Fig. 8.—Radioautograph of a teased preparation of mesenteric lymph node to show labeling of cells 15 minutes after H\textsuperscript{3}-methionine. X1500.

Fig. 9.—Radioautograph of a teased preparation of thymus to show labeling of cells 15 minutes after H\textsuperscript{3}-methionine. X1500.
the rate of disappearance of radioactivity makes it highly probable that, at least in the medium cells in the lymph, more label is lost than can be accounted for by mitosis alone. Thus, like large lymphocytes, they may be producing and secreting proteins. This possibility is strengthened by previous immunochromical demonstrations that medium as well as large lymphocytes contain beta and gamma globulins. The latter part of the disappearance curves in figure 2 are presumed to indicate the same sort of reutilization of labeled metabolites as discussed above for large lymphocytes. It may be noted that the degree of reutilization followed the same order among the tissues as described for the large lymphocytes in these tissues.

**Labeling of Small Lymphocytes**

Small lymphocytes have been defined as a non-mitotic population composed of cells which in smears of rat tissues have nuclear indexes of 56\(^2\) or less. Two subpopulations have been described in the rat on the basis of their circulating life span and distribution in hemopoietic tissues. Members of the one population are short-lived and circulate for only 5-6 days, whereas the other class has long-lived cells at least some of which circulate between blood and lymph for as long as 6 months. It has further been shown that over 90 per cent of the small lymphocytes in the thymus are of the short-lived variety whereas 90 per cent of the small cells in thoracic duct lymph are long-lived and recirculate between blood and lymph.

Like large and medium lymphocytes, small cells label immediately after an injection of \(H^3\)-methionine. Although table 2 shows that not all of the small lymphocytes were labeled by 4 \(\mu\)c/Gm. body weight of \(H^3\)-methionine, it was found that larger amounts of isotope would label 100 per cent of them. Thus, either by doubling the dose of \(H^3\)-methionine and exposing radioautographs for 6 weeks or by giving 30 \(\mu\)c./Gm. body weight of \(H^3\)-leucine and exposing the slides for 2 weeks, all of the cells could be labeled (fig. 5). Labeling intensities of small cells were fairly uniform with grain counts at early intervals after isotope ranging between 3 and 10. There was no indication of the small population of heavily labeled cells noted by Cooper from in vitro studies with human lymphocytes. This, however, probably is not as much a difference between the two studies as it is a matter of where the boundary between medium and small cells is established.

In figure 3 the labeling patterns of small lymphocytes are shown. It is to be noted that the values on the ordinate are one-tenth as much as the values on the ordinates in figures 1 and 2. This, of course, is a consequence of the weaker labeling of small cells as compared to large and medium cells. In general, the disappearance of radioactivity from small cells in the lymph and lymph node occurred in two phases, just as was observed for large and medium lymphocytes. Because it is known that 90 per cent of the small cells in lymph recirculate and, hence, were not being renewed while radioactivity was disappearing most rapidly from them, there is little doubt that there was turnover of radioactive protein. Mitchell and Nossal have reported that in
contrast to plasma cells there is little turnover of protein in small lymphocytes incubated in vitro with H\textsuperscript{3}-leucine.\textsuperscript{33} While their contrast is valid, the present data indicate that there is significant turnover of protein by small lymphocytes in vivo and that the time course of this is considerably longer than the 5-hour periods employed in the in vitro study. In the absence of definitive immunochemical evidence of immune protein production by small lymphocytes, it cannot be determined whether the observed turnover is a consequence of intracellular catabolism and renewal, or of secretion. In a preliminary report, however, Zucker-Franklin has suggested that human small lymphocytes produce gamma globulin.\textsuperscript{34}

The disappearance of radioactivity from small cells in the thymus was much more gradual, and at 96 hours after H\textsuperscript{3}-methionine approximately 5 times as many small thymocytes were labeled as small cells in the lymph node (table 2). Because the production of small cells in the thymus is known to be very rapid with the half-time renewal rate for the population being 36 hours,\textsuperscript{32} it was not possible to demonstrate significant protein turnover by these cells. Moreover, although half of the cells were replaced every 36 hours, radioactivity had decreased only 21 per cent in the 36-hour period after the time of maximal labeling. Thus it seemed that the extensive reutilization of metabolites noted in large and medium cells in the thymus was even better shown by the labeling patterns of the small thymocytes.

**SUMMARY**

Injections of H\textsuperscript{3}-methionine and H\textsuperscript{3}-leucine were combined with radiochemical and radioautographic technics to study the availability time of H\textsuperscript{3}-methionine and the protein synthetic ability of rat lymphocytes in vivo. Although 98.5 per cent of H\textsuperscript{3}-methionine was removed from the serum 5 minutes after injection, sufficient quantities persisted and/or re-entered the serum from tissues to cause increasing grain counts in radioautographs of large lymphocytes for 1 hour after isotope administration. A small amount of additional labeling occurred during the 2nd hour, but it is calculated that labeling is 97–98 per cent complete by 1 hour.

All of the large and medium lymphocytes were labeled in the thymus, lymph node, and thoracic duct lymph at short intervals after injection of 4 \(\mu\)c./Gm. body weight of H\textsuperscript{3}-methionine. Evidence is presented that protein synthesis occurs in the nucleus as well as in the cytoplasm and that newly formed protein is equally distributed between daughter cells following mitosis. Previous immunochemical studies are combined with information on generation time and disappearance rates of radioactivity to suggest that large and medium lymphocytes are constantly producing and releasing proteins. Large and medium cells in lymph and lymph node are more active in this than are similar cells in the thymus. Evidence of reutilization of labeled metabolites in the lymph node and especially in the thymus is discussed.

Although not all small lymphocytes were labeled by 4 \(\mu\)c./Gm. body weight of H\textsuperscript{3}-methionine, it was shown that larger doses of isotope would label 100
per cent of them. Small lymphocytes in thoracic duct lymph evidenced significant turnover of labeled protein during the 1st day after isotope administration.

**Summario in Interlingua**

Injectiones de methionina a H³ e leucina a H³ esseva combinate con tecnicas radiochimic e radioautographic pro studiar le periodos de disponibilitate de methionina a H³ e le potentia de synthetisar proteina de lymphocytos de ratto in vivo.

Ben que 98,5 pro cento del methionina a H³ esseva eliminate ab le sero intra 5 minutas post le injection, un sufficiente quantitate persisteva e/o retornava ab le tissus pro causar un augmento del numeration de granos in le radioautographias de grande lymphocytos durante 1 hora post le administration del isotopo. Un micre mesura de marcase additional occurreva durante le secunde hora, sed il esseva calculate que inter 97 e 98 pro cento del marcase es complete al fin de 1 hora.

Omne le grande e intermedie lymphocytos esseva marcate in le thymo, le nodos lymphatic, e le lympha del ducto thoracic brevemente post le injection de methionina a H³ in un dosage de 4 μc per g de peso corporee. Es presentate datos a evidentar que le synthese de proteina occurre si ben in le nucleo como in le cytoplasma e que le novemente formate proteina es distribuite equalmente inter le cellulas descendente post mitoses. Preve studios immunochimic es combine in con information in re le tempore de generation e le rapiditate del disparition del radioactivitate in supporto del conclusion que grande e intermedie lymphocytos es constantemente active in le production e liberation de proteinas. Le grande e intermedie cellulas in le lympha e le nodos lymphatic es plus active in isto que simile cellulas in le thymo. Es discutite le datos que reflecte un reutilisation de marcate metabolitos in le nodos lymphatic e specialmente in le thymo.

Ben que non omne le micre lymphocytos esseva marcate per le administration de methionina a H³ in un dosage de 4 μc per g de peso corporee, il esseva demonstrate que plus forte doses del isotopo pote marcar 100 pro cento de illos. Le micre lymphocytos in le lympha del ducto thoracic manifestava un significative metabolismo de marcate proteina durante le prime die post le administration del isotopo.

**REFERENCES**


5. Keston, A. S., and Katchen, B.: Incorporation of glycine-2-C⁴ into homo-
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