Reutilization of DNA Breakdown Products from Lymphocytes in Lumen of Intestine

By Masahiko Kotani, Akira Yamashita, Fumio Rai, Kanji Seiki and Isoo Horii

Migration of lymphocytes into the lumen of the intestine has been reported. In the present study, this migration has been quantitated. Large numbers of lymphocytes were demonstrated in the lumen of the intestine of the rat. To ascertain the fate of these cells, lymphocytes labeled with tritiated thymidine were obtained from donor rats and injected into the intestinal canal of recipients; the radioactivity of serum of portal vein blood and thoracic duct lymph plasma was then measured. The results indicate that labeled DNA breakdown products derived from the lymphocytes were absorbed from the lumen of the bowel. The question of reutilization of DNA breakdown products from lymphocytes migrating into the intestine is really a fundamental problem.

Material and Methods

Young male rats of the Wistar strain, weighing 85 to 115 Gm., were used in these experiments.

Experiment 1. Quantitation of Lymphocytes in Lumen of Small Intestine

Five unfasted rats were used. A midline incision was made through the abdominal wall of animals, under nembutal anesthesia. Two portions of the jejunum and ileum were selected, in each of which segments 2.3 to 3.6 cm. in length were isolated by ligatures. The lumen of each of the two isolated segments of the intestine was gently washed to and fro with 0.10 to 0.15 ml. of physiologic saline (pH 7.4) through a syringe. The washing was repeated four times at intervals of 30 minutes. The abdominal cavity was closed during these intervals. Particular care was paid through the whole procedure not to damage the blood vessels and the intestinal mucosa. The number of lymphocytes in each part of the small intestine in a 30 minute period per 5 cm. length was estimated from the mean value of lymphocyte counts in the last four washings, these cells being carefully differentiated from loose epithelial cells. The type of lymphocytes in the saline washes of the intestine was studied in smears stained with May-Giemsa. Lymphocytes of 7μ or less in diameter were considered as small, those more than 7μ and less than 10μ as medium, and those 10μ or more as large.

Experiment 2. Measurement of the Radioactivity of Blood and Lymph after Injection of Labeled Lymphocytes into Lumen of Small Intestine

Five donor rats were each injected intraperitoneally with 1 μc. H3-thymidine (specific activity 5.0 c./mM) per Gm. body weight on 4 occasions at 4-hour intervals on the
first and second days, on two occasions at the same intervals on the third day, and were sacrificed 24 hours after the last injection. A mixed cell suspension from thymus and mesenteric lymph nodes was prepared in physiologic saline (pH 7.4) using standard methods. The cells from each animal were gently washed 3 times in 20 ml of physiologic saline to remove any extracellular radioactive material and were finally resuspended in 2 ml of physiologic saline. At least 95 per cent of these cells were lymphocytes, of which 46 to 53 per cent were labeled as determined autoradiographically (3 months exposure. Emulsion NR-M1, Konishiroku Photo. Co., Tokyo). The number of labeled lymphocytes given to each recipient is indicated under "Results." Eighty-seven to 93 per cent of the cells showed viability by the nigrosin test before administration into the intestine.

Recipient rats were fasted 24 hours before administration of the cell suspension, water being given ad libitum. Two ml of the cell suspension were slowly injected into the duodenum of each animal under Nembutal anesthesia, careful attention being paid not to damage the intestinal mucosa. The injection site was ligated to prevent leakage. The abdominal wall was closed after injection. At various times after injection, the abdominal cavity was reopened, and blood was withdrawn from the portal vein and the abdominal aorta. Lymph from the thoracic duct was collected by the method of Reinhardt.9

Tritium activity of blood serum and lymph plasma was determined using the Packard Tri-Carb liquid scintillation spectrometer (Series 314A). Bray's scintillator mixture10 was used. The counting efficiency for H3 was about 5 per cent. H3 activity was determined from the difference of counts per min. (c.p.m.) per ml of the experimental samples and those of control background samples obtained from normal rats.

**Experiment 3. Determination of Nature of Labeled Material in Serum after Injection of Labeled Lymphocytes into Lumen of Small Intestine**

Cells were prepared from thymus and mesenteric lymph nodes of four donor rats in the same manner as in Experiment 2, suspended in physiologic saline (pH 7.4) and introduced 2 ml into the duodenum of each of four recipient rats. The number of cells administered to each animal was 534.3 \( \times 10^6 \) of which 48 per cent were labeled lymphocytes. Ninety-two per cent of the cells showed viability by the nigrosin test before administration into the intestine. Six hours after administration of the labeled lymphocytes, blood was withdrawn from the portal vein and abdominal aorta.

Tritiated water (THO) activity, nonvolatile H3 activity and total H3 activity were determined on the pooled serum from the four recipients. Total H3 activity and THO activity of serum were determined by liquid scintillation spectrometry according to the method of Rubini et al.11 In this experiment, 0.6 N-perchloric acid (PCA) was added to the sample for the purpose of removal of protein, and Bray's scintillator mixture was used for aqueous H3 counting instead of the ethanol and PBD-xylene mixture used by Rubini et al. Nonvolatile H3 activity was estimated from the difference between total H3 activity and THO activity of the same sample.

After determination of total H3 activity and THO activity, the pooled serum from the portal vein of the four recipients was used for paper chromatographic separation of acid-soluble components. The neutralized extracts from serum were prepared by the method of Potter et al.12 and dried at 25 to 30 C. under reduced pressure with a Rincro rotary evaporator. Aliquots of the concentrated extract, to which thymine (T), thymidine (Tdr) and thymidylic acid (dTMP) had been added as reference compounds, were analyzed by paper chromatography. followed by radioactivity measurements. Filter paper strips of 1.8 cm width (No. 51A: Toyo Roshi Kaisha, Ltd., Tokyo) were used for descending chromatography, using an isobutyric acid-ammonia-EDTA solvent system.13 The same paper strips impregnated with polyethyleneimine (PEI) were used for ascending ion-exchange chromatography.14 The reference compounds were located by scanning the paper with an ultraviolet lamp. After development of the chromatograms, the strips were cut in pieces (1.8 cm. \( \times \) 1.0 cm.). The radioactivity on each piece was determined.
using the Tri-Carb liquid scintillation spectrometer. Bray's scintillator mixture was used. The number of counts per minute for each cm. length of the chromatograms was plotted against distance from the starting line.

Experiment 4. Autoradiography of Gut Epithelium, Bone Marrow and Lymphoid Tissues

The recipient rats in Experiment 2 were used for autoradiographic study after withdrawal of blood from the portal vein and abdominal aorta. Smears made from cell suspensions of the bone marrow, spleen, mesenteric lymph node and thymus were fixed in absolute methyl alcohol for 4 minutes. Epithelial cells of the jejunum, ileum, appendix and colon were fixed in 33 per cent methanol for 60 minutes and then smeared. Autoradiographs of these smears were stained with May-Giemsa solution after 6 months' exposure (Emulsion NR-M1).

Experiment 5. Autoradiography of Portal Blood and Thoracic Duct Lymph

Cells were prepared from thymus and mesenteric lymph nodes of two donor rats in the same manner as in Experiment 2, suspended in physiologic saline (pH 7.4) and introduced 2 ml. into the duodenum of each of four recipient rats. Ninety-two to 90 per cent of the cells showed viability by the nigrosin test before administration into the intestine. The number of H\(^3\)-thymidine labeled lymphocytes administered to each animal was 132.4 \(\times 10^6\) in two animals of a group and 157.2 \(\times 10^6\) in two animals of the other group. Blood and lymph were separately collected from one animal of each group. Blood was withdrawn from the portal vein in a small amount at 0.5, 1, 2, 3 and 4 hours after injection. The abdominal cavity was closed during intervals. Lymph was collected at hourly intervals for 4 hours. Autoradiographs of smears of blood and concentrated lymph by centrifuging at 1500 r.p.m. for 5 minutes were stained with May-Giemsa solution after 2 months of exposure (Emulsion NR-M2).

**Results**

**Experiment 1**

Table 1 shows the number of lymphocytes in the small intestinal canal for 30-minute periods. The lymphocytes were more numerous in the ileum than in the jejunum. The total number of lymphocytes in the small intestinal canal in a 30-minute period was calculated by multiplying the mean number of lymphocytes in the jejunum and ileum per 1 cm. by the whole length of the intestine. The number of lymphocytes in the whole length of the small intestinal tract was estimated as 211.8 \(\times 10^6\) per day. Of 410 lymphocytes in the saline washes of the intestinal tract of three rats, 80.2 per cent were small, 15.9 per cent medium and 3.9 per cent large.

**Experiment 2**

Figure 1 shows H\(^3\) activity of serum of the portal vein and abdominal aorta at various times after administration of labeled lymphocytes with H\(^3\)-thymidine into the small intestine in three groups of animals. The number of labeled lymphocytes introduced into each recipient was 92.7 \(\times 10^6\) in the first group, 88.0 \(\times 10^6\) in the second group and 93.9 \(\times 10^6\) in the third group. H\(^3\) activity in serum after injection of labeled lymphocytes into the intestine was of sufficient magnitude to indicate that DNA breakdown products from the lymphocytes were absorbed from the gut. The absorption was thought to be most
REUTILIZATION OF DNA BREAKDOWN PRODUCTS

C.P.M./ml.

<table>
<thead>
<tr>
<th></th>
<th>V. portae</th>
<th>A. abdominalis</th>
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<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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<tr>
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</table>

Fig. 1. — H₃ activity (c.p.m./ml.) of serum of the portal vein and abdominal aorta at various times after injection of lymphocytes labeled with H₃-thymidine into the intestine in three groups of animals. The number of labeled lymphocytes administered to each animal was 92.7 × 10⁶ in the first group (○), 88.0 × 10⁶ in the second group (△) and 93.9 × 10⁶ in the third group (□).

Intensity 4 to 8 hours after administration of the labeled cells. Serum H₃ activity was always higher in the portal vein than in the abdominal aorta in samples taken 2 to 16 hours after injection of the labeled cells.

Figure 2 shows H₃ activity of serum of the portal vein and abdominal aorta of animals with thoracic duct fistula at various times after administration of labeled lymphocytes with H₃-thymidine into the intestine. The number of labeled lymphocytes administered to each recipient was 108.5 × 10⁶. H₃ activity in serum suggests that DNA breakdown products from the lymphocytes in the gut were absorbed directly into the portal vein, because lymph from the gut was drained through the fistula to the outside. Serum H₃ activity was again higher in the portal vein than in the abdominal aorta. H₃ activity of the thoracic duct lymph plasma from the animal employed for the collection of blood at 8 hours after injection of the labeled cells is shown in Figure 2. H₃ activity of thoracic duct lymph plasma indicates that DNA breakdown products from the lymphocytes in the gut were also absorbed into the lymphatic vessels.

To confirm the absorption of DNA breakdown products from the lymphocytes into the lymphatic vessels, about 3 times larger numbers of H₃-thymidine labeled lymphocytes were injected into the intestine; thoracic duct lymph was then collected at hourly intervals for 7 hours. The number of labeled lymphocytes introduced into the recipient was 337.5 × 10⁶. The results are shown in Figure 3. H₃ activity of thoracic duct lymph plasma was elevated as compared with that at each corresponding time in Figure 2. H₃ activity of serum of the portal vein at the end of this experiment is also shown in Figure 3 and indi-
Table 1.—The Number of Lymphocytes in the Small Intestinal Canal

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Body Weight (Gm.)</th>
<th>Segment* (cm.)</th>
<th>Jejunum</th>
<th>Number of lymphocytes /5 cm. length ($\times 10^6$)</th>
<th>Heum</th>
<th>Number of lymphocytes /5 cm. length ($\times 10^6$)</th>
<th>Mean Number of Lymphocytes /5 cm. length ($\times 10^6$)</th>
<th>Length of Small Intestine (cm.)</th>
<th>Calculated Lymphocytes in Small Intestine ($\times 10^6$)</th>
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<tr>
<td>1</td>
<td>105</td>
<td>20.0–23.6</td>
<td>31.95</td>
<td>46.6–49.1</td>
<td>327.15</td>
<td>179.55</td>
<td>73.1</td>
<td>2.625</td>
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<tr>
<td>2</td>
<td>105</td>
<td>35.5–38.0</td>
<td>146.33</td>
<td>54.5–56.8</td>
<td>687.50</td>
<td>416.92</td>
<td>76.2</td>
<td>6.354</td>
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<tr>
<td>3</td>
<td>86</td>
<td>8.5–11.1</td>
<td>82.94</td>
<td>45.1–47.6</td>
<td>312.50</td>
<td>197.72</td>
<td>53.6</td>
<td>2.120</td>
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<tr>
<td>4</td>
<td>83</td>
<td>14.0–16.5</td>
<td>141.88</td>
<td>45.5–48.5</td>
<td>815.11</td>
<td>478.50</td>
<td>56.5</td>
<td>5.407</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>88</td>
<td>15.0–17.8</td>
<td>197.10</td>
<td>52.8–55.8</td>
<td>670.00</td>
<td>433.55</td>
<td>63.3</td>
<td>5.489</td>
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<tr>
<td>6</td>
<td>85</td>
<td>15.3–18.3</td>
<td>67.29</td>
<td>43.3–46.0</td>
<td>639.20</td>
<td>353.25</td>
<td>63.5</td>
<td>4.486</td>
<td></td>
</tr>
</tbody>
</table>

Mean   ± S.E.  ± 3.8  111.25  ±22.71  ±77.06  ±47.23  ±3.2  ±0.632

*Distance from pylorus.

Table 2.—Serum H⁻ Activity at 6 Hours after Injection into the Small Intestine of Lymphocytes (256.5 $\times 10^6$) Labeled with H⁻-thymidine

<table>
<thead>
<tr>
<th>Serum</th>
<th>Total H⁻ Activity (c.p.m./ml.)</th>
<th>THO Activity (%)</th>
<th>Nonvolatile Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portal vein</td>
<td>638.0</td>
<td>69.0</td>
<td>31.0</td>
</tr>
<tr>
<td>Abdominal aorta</td>
<td>571.0</td>
<td>71.0</td>
<td>29.0</td>
</tr>
</tbody>
</table>
RECTILIZATION OF DNA BREAKDOWN PRODUCTS

Fig. 2—$H^3$ activity (c.p.m./ml.) of serum of the portal vein and abdominal aorta of animals with thoracic duct fistula at 1.5, 4 and 8 hours after injection of $H^3$-thymidine labeled lymphocytes into the intestine. The number of labeled lymphocytes administered to each animal was $108.5 \times 10^6$. $H^3$ activity of thoracic duct lymph plasma (TDL) from the animal sacrificed at 8 hours after injection is also shown at every 1-hour interval.

...cates that the absorption of DNA breakdown products into the portal vein has occurred simultaneously.

Experiment 3

Total $H^3$ activity, nonvolatile $H^3$ activity and THIO activity of serum of the portal vein and abdominal aorta at 6 hours after injection of labeled lymphocytes with $H^3$-thymidine into the intestine are shown in Table 2. About 30 per cent of total $H^3$ activity in serum was in nonvolatile material.

Figure 4 shows the radioactivity pattern obtained after ascending paper chromatography of serum of the portal vein. About 15, 18 and 42 per cent of the total radioactivity on the strip were present in T, Tdr and dTMP, respectively. These components were identically found in portal vein serum following descending paper chromatography. In the present work, large fragments of DNA in the acid-insoluble fraction were not detected.

Experiment 4

Definite nuclear labeling was readily apparent in cells of the small intestinal mucosa, bone marrow, mesenteric lymph node, spleen and thymus 2 hours after injection of $H^3$-thymidine labeled lymphocytes into the intestine. The number of labeled cells increased gradually after the introduction of labeled lymphocytes. Labeling was noted most frequently in cells of the intestine and then in those of the bone marrow (Fig. 5). The cells in the appendix were
Fig. 3.—$^3$H activity (c.p.m./ml.) of thoracic duct lymph plasma (TDL) at hourly intervals for 7 hours after injection of $^3$H-thymidine labeled lymphocytes into the intestine. The number of labeled lymphocytes introduced into the animal was $337.5 \times 10^6$. $^3$H activity of serum of the portal vein at the end of the experiment was also shown in this figure.

Fig. 4.—Radioactivity pattern obtained after ascending paper chromatography of the concentrated acid-soluble extract from serum of the portal vein at 6 hours after injection of lymphocytes ($256.5 \times 10^6$) labeled with $^3$H-thymidine into the intestine. The relevant $R_f$ values of thymidyllic acid (dTMP), thymidine (Tdr) and thymine (T) were 0.22, 0.82 and 0.71, respectively. The radioactivity within each peak is expressed in per cent of the total radioactivity on the strip.
Fig. 5.— Autoradiographs of transfused cells (1), jejunal cells (2), appendix cells (3), bone marrow cells (4), mesenteric lymph node cells (5), spleen cells (6), and thymus cells (7). (1) = 3 months exposure, × 1200. (2)–(7) = 6 months exposure, × 1700. (2)–(3) are at 2 hours after injection of H^3-thymidine labeled lymphocytes into the intestine, and (4)–(7) at 24 hours. May-Giemsa stain.

thought to be more frequently labeled than in the other parts of the intestine. In the lymphoid tissues, the labeling was markedly less in frequency and intensity; labeled cells were particularly scarce in the thymus. The majority of labeled cells in the lymphoid tissues were of the larger type of lymphocyte (cytoplasm staining deeply with May-Giemsa). The number of silver grains was commonly 2 to 3 + background in each cell in the lymphoid tissues (Fig. 5).

Experiment 5

In each of two animals, 1000 lymphocytes were counted in smears of portal blood at different times. The total number of lymphocytes counted in smears of thoracic duct lymph for 4 hours was 8000 in each of two animals. However, no definitely labeled cells were found either in blood or in lymph, although the donor cells were heavily labeled after the same periods of exposure using the same emulsion.
Discussion

In Experiment 1, blood cells other than lymphocytes were not found in the saline washes of the intestinal tract. The number of lymphocytes in the saline washes was not associated with an increase in the number of detached epithelial cells after the ligation of the intestine. The movement of lymphocytes through the epithelium into the intestinal lumen is apparently a physiologic process. The number of lymphocytes in the small intestinal canal can be calculated to be sufficient to replace the blood lymphocytes of a 100 Gm. rat more than 5 times daily. The number is also equal or two-thirds that of the daily output of lymphocytes from the thoracic duct (220-260 × 10⁶). The greater number of lymphocytes in the ileum than in the jejunum may depend on the relatively greater amount of lymphatic tissue in the former.

In Experiment 2, the suspension of H³-thymidine labeled lymphocytes, obtained from thymus and mesenteric lymph nodes, was injected into the intestine, and then H³ activity of blood serum and thoracic duct lymph plasma was measured. The results show that DNA breakdown products from the lymphocytes in the gut were absorbed and transferred by way of both portal vein and thoracic duct. The counting efficiency of H was about 5 per cent in the present work, so that much more radioactive material must have been present in blood and lymph.

In Experiment 3, considerable amounts of nonvolatile H³ activity was found in serum at 6 hours after injection of labeled lymphocytes into the intestine. Moreover, significant amounts of thymine, thymidine and thymidylate were evidenced in serum by paper chromatography. These results indicate that part of the DNA breakdown products from the lymphocytes in the gut are absorbed at the available level for reutilization.

In Experiment 4, autoradiography of proliferating tissues revealed that absorbed DNA breakdown products were actually reutilized. Heavily labeled lymphocytes were not found in any tissue examined, although large numbers of the injected lymphocytes were intensely labeled (Fig. 5). This fact indicates that lymphocytes introduced into the intestine were probably not absorbed intact.

In Experiment 5, autoradiography of portal blood and thoracic duct lymph gave a direct evidence that any recycling of instilling cells into the small intestine did not occur.

A reutilization of lymphocyte DNA for cell proliferation has been shown experimentally by many investigators. The present study has shown that, under physiologic conditions, lymphocytes could serve as DNA trephocytes after emigration into the gut and breaking down within it. The existence of DNase in the intestinal or pancreatic juice supports this work. Some problems remain. It is, in general, believed that the thymic and lymph node lymphocytes differ in certain respects. Which cells migrate normally into the gut? The results might be altered quantitatively or qualitatively if they were separately introduced into the gut. In the present work, radioactivity measurements of the degradation products of labeled DNA of lymphocytes placed in the gut
were made on serum freed of macromolecules by acid precipitation. It would be of great interest if large fragments were present in portal blood or thoracic duct lymph, since one could postulate, as others have done,30,31 that informational transfer might occur. At the present time, studies on these problems are under way in our laboratory.

**Summary**

Migration of a very large number of lymphocytes \((211.8 \times 10^6\text{ per day})\) into the intestinal canal of rats, which weighed about 100 Gm., was found. Lymphocytes in the lumen of the intestine were 80.2 per cent small, 15.9 per cent medium and 3.9 per cent large. Any recycling of instilling cells into the intestine could not be observed. Lymphocytes labeled with H\(^3\)-thymidine, obtained from both thymus and mesenteric lymph nodes of donor rats, were washed and injected into the intestine of recipient rats. H\(^3\) activity of the blood and thoracic duct lymph plasma after administration of labeled lymphocytes showed that DNA breakdown products from the lymphocytes in the gut were absorbed and transferred by way of both the portal vein and the thoracic duct. Evidence that the activity was actually incorporated into the DNA of proliferating cells of the recipient was demonstrated by autoradiographic means.

**Summario in Interlingua**

Esseva constatate le migration de un grandissime numero de lymphocytes \((211.8 \times 10^6\text{ per die})\) ad in le canal intestinal de rattos que habeva un peso de circa 100 g. Le lymphocytes in le lumine del intestino consisteva ad 80.2 pro cento de micres, ad 15.9 pro cento de medies, e ad 3.9 pro cento de grandes. Nulle recvclage per le instillation de cellulas ad in le intestino poteva esser observate. Lymphocytes obtenite tanto ab le thymo como etiam ab nodos lymphatic del mesenterio de rattos donatori e marcate con thymidina a tritium esseva eluite e injicite ad in le intestino de rattos recipiente. Le activitate de tritium in le sanguine e le plasma lymphatic del ducto thoracic post le administration de marcate lymphocytes demonstrava que productos de degradation de acido deoxyribonucleic ab le lymphocytes in le intestino esseva absorbite e transferite via le vena portal e via le ducto thoracic. Evidentia in supporto del conception que le activitate esseva de facto incorporate ad in le acido deoxyribonucleic de cellulas proliferante del rattos recipiente esseva trovate per medio de autoradiographias.

**ACKNOWLEDGMENTS**

We wish to thank Drs. S. Sugino and H. Shinomo, Virus Institute, University of Kyoto, for invaluable advice with this work. We are also grateful to Dr. F. C. Courtice, Department of Experimental Pathology, the Australian National University, and to Dr. W. O. Reinhardt, Department of Anatomy, University of California San Francisco Medical Center, for helpful advice in the preparation of this paper.

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