Increased lymphocyte transport by lipid absorption in rat mesenteric lymphatics

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MIURA, SOICHIRO, EIICHI SEKIZUKA, HIROSHI NAGATA, CHIKARA OSHIO, HARUYUKI MINAMITANI, MAKOTO SUEMATSU, MASAYUKI SUZUKI, YOSHIKI HAMADA, KENSUKE KOBAYASHI, HITOSHI ASAKURA, AND MASAHARU TSUCHIYA. *Increased lymphocyte transport by lipid absorption in rat mesenteric lymphatics.* Am. J. Physiol. 253 (Gastrointest. Liver Physiol. 16): G596–G600, 1987.—The effect of olive oil administration on lymphocyte transport through mesenteric lymphatics was examined to see the possible involvement of nutritional absorption in lymphocyte traffic from the intestinal mucosa. After the olive oil administration to rats, remarkable increase in lymphocyte flux was observed within 2 h in lymph samples collected from rats with lymphatic fistula. The use of a high-speed microscopic video system made it possible to analyze accurately the lymphocyte transport in rapid movement that could not be detected by any of the ordinary video systems. The direct observation of mesenteric collecting lymphatics by this system showed an increment of lymphocyte transport from the intestinal mucosa by lipid absorption in 2 h. The contraction frequency of intestinal collecting lymphatics was also enhanced by olive oil administration. The densitometric analysis on video image was applied to estimate the extent of lipid absorption. The combination of a high-speed video system and the densitometric analysis revealed that the increase in lymphocyte flux occurred before lipid absorption reached its maximum and also demonstrated that the lymphocyte transport returned to control levels under the maximal absorptive condition. The results suggest that the fat absorption could be an important factor influencing the lymphocyte transport in the lymphatic system of intestine.

high-speed microscopic video system; lymph fistula rats; densitometric analysis

THE GASTROINTESTINAL MUCOSA is continually bathed in fluid containing food, microbial antigens, and infective microorganisms. There is now evidence that the population of immunoglobulin A-secreting cells in the gut is maintained by a traffic of large lymphocytes that enter the blood by way of the thoracic duct and then migrate from the blood into the intestinal lamina propria where they complete their differentiation into plasma cells (6). This constant traffic of lymphocytes may play a role in the cell-to-cell interaction necessary for functioning of the immune defense system in the intestinal wall. The distribution of lymphocyte set and subsets in the small intestinal mucosa and in Peyer's patches is well documented. Recently, homing specificity of lymphocytes has been considered to be determined by the specific interactions between recirculation lymphocytes and postcapillary high endothelial venules in Peyer's patches (11). However the factors that may influence the leaving of lymphocytes from the intestinal mucosa to intestinal lymphatics are not fully understood. Because intestinal lymphatics are the major route of absorption of nutrients, including long-chain fatty acids, the absorptive activity of dietary lipid might affect the movement of lymphocytes, which are deemed important in terms of the immunological activity in the intestinal mucosa. In the present study, we try to elucidate the effect of fat absorption on lymphocyte traffic through mesenteric collecting lymphatics and demonstrate the presence of close correlation between lipid absorption and lymphocyte transport by using a high-speed microscopic video system and a densitometric analysis.

MATERIALS AND METHODS

Materials. Male Wistar rats weighing ~200 g were used throughout the study. They were maintained on standard laboratory chow diet (Oriental Yeast, Japan). Olive oil was obtained from Kanto Chemical, Japan.

Measurement of lymphocyte flux of intestinal lymph system in rats with lymphatic fistula. After an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt), the main mesenteric lymphatic duct near the cisterna chyli was cannulated as described by Bollman et al. (3). After surgery, animals were maintained in Bollman's cages, and saline was infused intravenously from the jugular vein at a flow rate of 2.4 ml/h to maintain lymph flow. All animals were subjected to experiments 24 h after surgery. To examine the effect of lipid administration on lymph flow and lymphocyte transportation in the intestinal lymphatics, 1 ml of olive oil was administered into the stomach of the rats by a stomach tube. Lymph samples were collected in ice-cold vials containing heparin to prevent coagulation. The volume, number of lymphocytes, protein, and triglyceride concentration of lymph samples were monitored at 1-h intervals for 4 h after the olive oil administration. Lymphocyte count

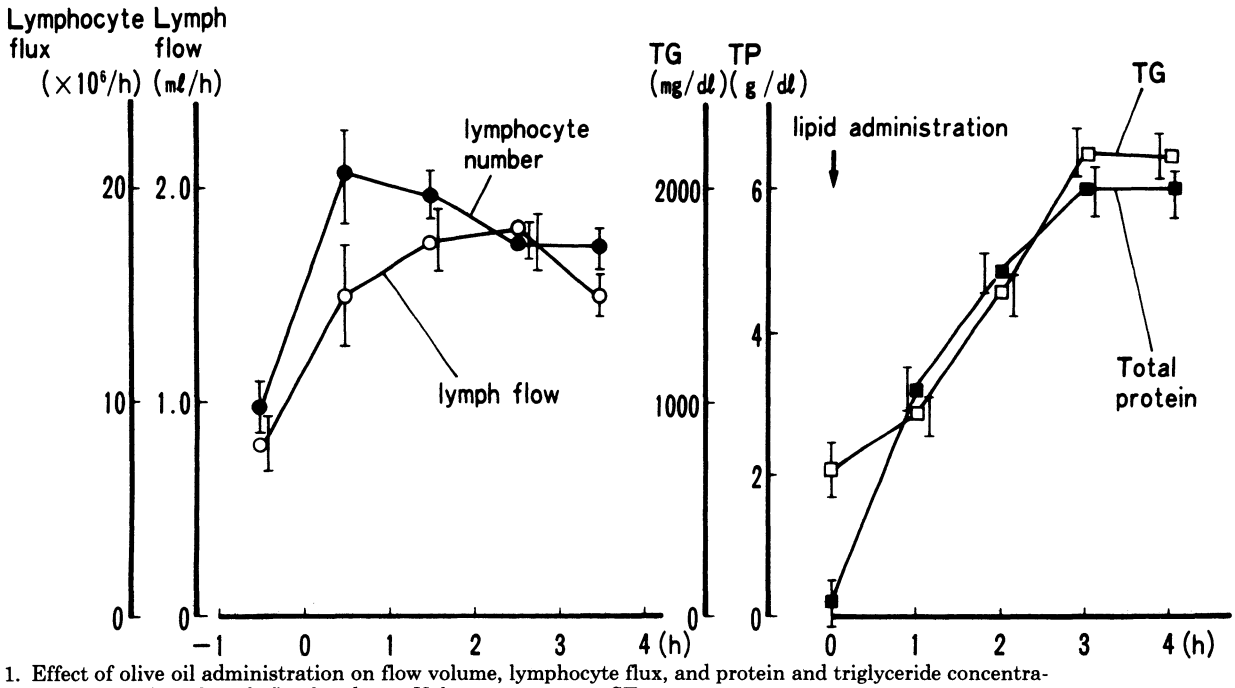

FIG. 1. Effect of olive oil administration on flow volume, lymphocyte flux, and protein and triglyceride concentration of lymph samples from lymph-fistulated rats. Values are means ± SE.

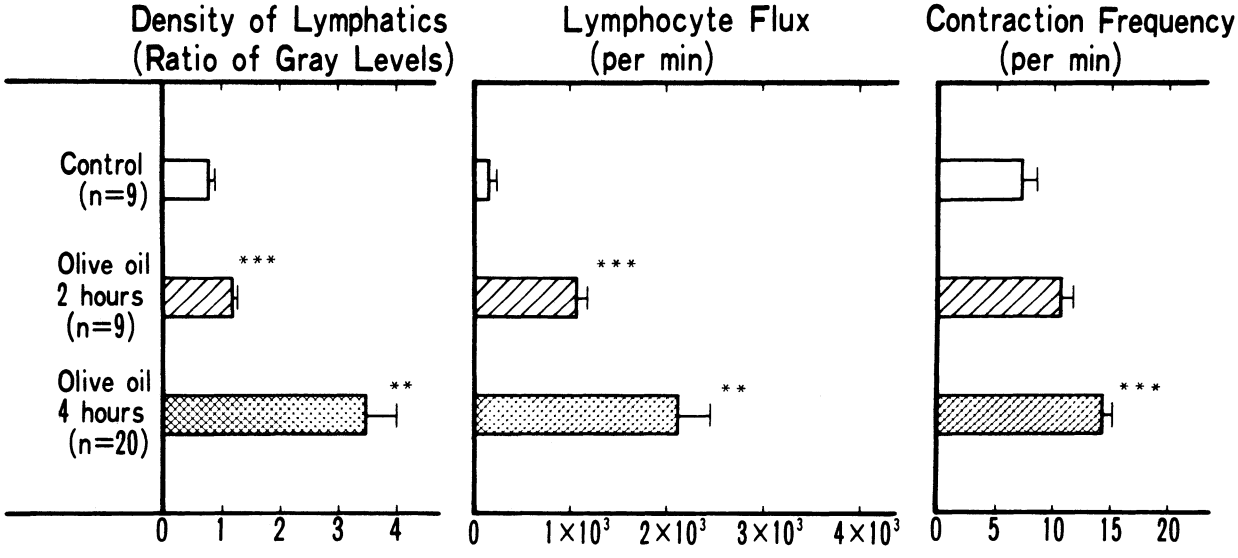

FIG. 2. Measurement of lymph density (gray level ratio), flux of lymphocytes, and contraction frequency of rat collecting lymphatics by a video image analysis system. Values are means ± SE for controls (*open bar*) at 2 h (*dashed bar*) and 4 h (*meshed bar*) after olive oil administration. ** $P < 0.01$, *** $P < 0.001$ compared with control values.

of lymph samples was determined by Celltrak (Bio-Dynamics, Indianapolis, IN). By Giemsa staining of intestinal lymph, >99% of white blood cells were revealed to be lymphocytes. Protein was assayed according to the method of Bensadoun and Weinstein (2) using bovine serum albumin as the standard. Triglyceride concentration was determined by the method of Van Handel and Zilversmit (14).

Observation of intestinal collecting lymphatics under intravital microscope. Collecting lymphatics of mesentery near the intestinal loop were chosen to make direct observation of peripheral lymph, which does not reach lymph nodes to be modified. After laparotomy, the intestinal loop corresponding to two-thirds of the small intestine was gently pulled out, and the mesentery was spread over a glass plate equipped with a small water bath that was maintained at 38°C. Collecting lymphatics 80–150 μm in diameter were observed under an intravital high-speed microscope system consisting of an inverted microscope (Nikon Diaphot-TMD, Tokyo), a high-speed video camera, and a recorder (NAC MHS-200, Tokyo). The video image was recorded on a VHS video tape at a speed of 200 frames/s.

Analyses of video image. Measurements of lymphocyte flux, diameter, and density changes of lymph vessels were carried out by using a video image analysis system that comprised a video recorder (Toshiba, A-61X, Tokyo), a digital image processer (Nippon Avionics, TVIP-2000, Tokyo), and a microcomputer (NEC, PC-9800, Tokyo). The contractile activity of collecting lymphatics

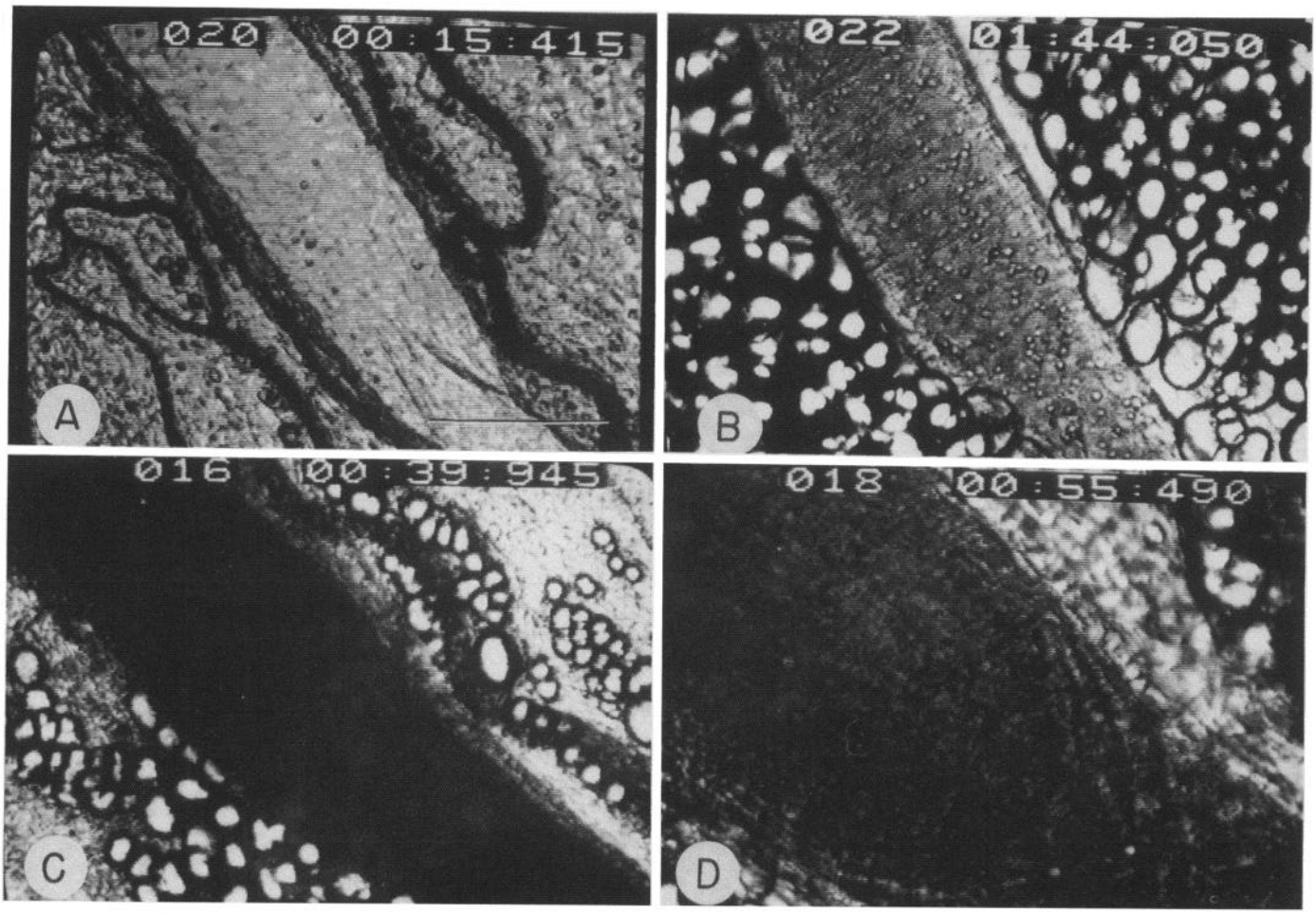

FIG. 3. Video images of intestinal collecting lymphatics under an intravital high-speed microscope system. *A*: control rats. A small number of lymphocytes is seen in lymphatics. Gray level ratio (density of inside lymphatics/surrounding tissue) is 0.7. *Bar* at right corner indicates 100 μm. *B*: 4 h after administration of olive oil. Marked increase in lymphocyte flux is shown in lymphatics where ingested lipid has just reached. Gray level ratio shows 2.7. *C*: picture of other lymphatics at 4 h after olive oil administration. Lymphatics are seen as a black stream with gray level ratio of 10.5, which is supposed to correspond to state of maximal lipid absorption. *D*: higher magnification of *C*. Lymphocyte transport through lymphatics is rather suppressed to almost same levels as those of controls.

and the total flux of lymphocytes passing through intestinal lymph vessels were determined by frame-to-frame analysis. The interval between two frames was 5 ms. The flux of lymphocytes was expressed as number per minute per 100 μm of lymphatic diameter. Diameter changes of lymphatics were measured at the equidistant positions along the lymphatic vessels by postulating vessels as being cylindrical. For the assessment of the increment of lipid absorption, densitometric analysis of intestinal lymphatics on video image was applied. When lipid transportation was increased in the intestinal lymphatics, many chylomicra and very low-density lipoprotein (VLDL) particles appeared on the video image as black particles. Therefore the concentration of lipid content in lymph could be estimated from the measurement of gray levels of the image at the central point of lymph vessels. We express the extent of lipid absorption on video image by gray level ratio that is defined as follows: $D = \mathrm{Ily}/\mathrm{Iti}$, where Ily is the gray level at the central point of a lymph vessel and Iti is the gray level at the surrounding tissue, which does not change.

Statistical analysis. All results are expressed as means ± SE. Differences in mean values between groups were tested with variance analysis for significance and the level of significance by unpaired Student's t test. P values <0.05 were considered significant.

RESULTS

The effect of olive oil administration on the lymph flow volume, lymphocyte flux, and protein and triglyceride concentration of lymph samples from rats with lymphatic fistula is shown in Fig. 1. After the olive oil administration, the number of lymphocytes showed a rapid increase and reached twice the initial value within 1–2 h. Then it slightly decreased at 3 and 4 h. An increase in lymph flow was also observed after the olive oil administration, although it occurred rather later than the increase in lymphocyte flux. On the other hand, triglyceride and protein concentrations of intestinal lymph were gradually and constantly increased after the administration of olive oil and reached their peak values at 3–4 h. The results from lymph fistula rats revealed that the increase in lymphocyte flux occurred rapidly and considerably earlier than the maximal lipid transportation into

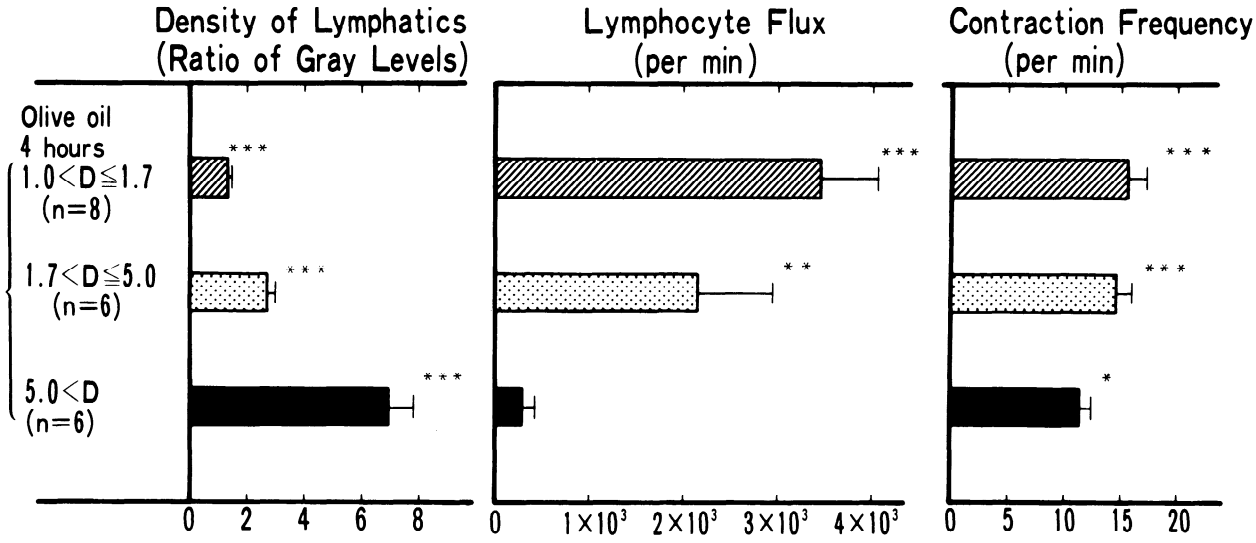

FIG. 4. Video image analysis of intestinal lymphatics at 4 h after olive oil administration. Lymphatics were divided into 3 groups according to value of gray level ratio. Values are means ± SE for lymphatics of gray level ratio of $1.0 < D \leq 1.7$ (*fine-dashed bar*), $1.7 < D \leq 5.0$ (*dotted bar*), and $5.0 < D$ (*black bar*). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with control values which are shown in Fig. 2. D = Ily/Iti, where Ily is gray level at central point of a lymph vessel and Iti is gray level at surrounding tissue.

intestinal lymph.

In Fig. 2 are summarized the data of lymphocyte flux and contraction frequency of lymphatics obtained from in vivo observation of mesentery collecting lymphatics through a high-speed video system and an image-processing system. A high-speed video system made it possible to determine the accurate number of lymphocyte passing through lymphatics, even under the condition of rapid lymph flow. Previously, by use of an ordinal video system, we could not detect rapid lymphocyte transport because lymph flow often reached the speed of more than 2–3 mm/s (12). In lymphatics of control rats, a small number of lymphocytes were seen and the density of central lymphatics was lower than that of the surrounding tissue as shown in Fig. 3*A*. After the administration of olive oil, the color of lymphatics became darker because of the absorbed lipid particles. The gray level ratio reached the mean value of 1.19 at 2 h and 3.45 at 4 h. The lymphocyte flux was significantly increased after olive oil administration and reached about a 3-fold increase compared with the control value at 2 h and showed about a 10-fold increase at 4 h. The contraction frequency of collecting lymphatics also seemed to be enhanced 2 h after olive oil administration, even though not statistically significant. The enhanced contraction frequency of intestinal lymphatics reached significant levels at 4 h later. The results from the intestinal lymphatics 4 h after the olive oil administration were divided into three groups, according to the results of densitometric analysis (Fig. 4). The increase in lymphocyte flux was significant only in the lymphatics whose gray level was below 5.0. These lymphatics may correspond to the intestinal loops where ingested lipid has just reached, as shown in Figure 3*B*. However, when the gray level ratio of lymphatics exceeded 5.0, which is supposed to correspond to the state of maximal lipid absorption, the lymphatics were seen as a black stream as shown in Fig. 3*C*. The lymphocyte flux was paradoxically small, and it is rather suppressed to almost the same levels as controls, as shown in Fig. 3*D*. It should be noticed that increased contraction frequency was still maintained, even though the lymphocyte flux was suppressed in the lymphatics with gray level ratio over 5.0.

DISCUSSION

Among macromolecular substances both long-chain fatty acids and lymphocytes utilize intestinal lymphatics as a major transport pathway from intestinal mucosa to the systemic circulation (1). In the present communication, we demonstrate a close correlation between lipid absorption and lymphocyte traffic in the intestinal lymphatics. In the study of antigenic stimulation on lymphoid architecture, Trnka and Cahill (13) have shown that antigenic stimulation produced 10–25 times increased blood flow through the stimulated tissue within hours, corresponding to the increase of lymphocyte traffic through the area. On the other hand, studies on the effect of food intake have demonstrated that lipids strongly increase the formation of lymph (5). The increase in lymph flow after the lipid infusion may partially result from the increment of luminal absorption, but also it may be due to increased plasma proteins, which are permeable from the intestinal microcirculation (15). The exact mechanism of this increase in lymphocyte flux after lipid infusion is not known. It is conceivable that the increase in blood flow in the intestinal mucosa may contribute to the increase in lymphocyte transport in intestinal lymphatics.

In vivo observation of rat mesenteric lymphatics is crucial to estimate directly the lymphatic transport from the intestinal mucosa because the data obtained from rats with lymphatic fistula may reflect some modifications by mesenteric lymph nodes. The results obtained from the high-speed video and the video image-process-

ing system show that in situ lymphocyte transport from the intestinal mucosa was in fact stimulated to more than 10 times by lipid administration. The contraction frequency of intestinal lymphatics was also enhanced by olive oil, although the extent of the increase was not greater than that of lymphocyte number. This suggests that an increased lymphocyte transport by lipid absorption is not simply due to the increased flow of intestinal lymph. Recently, Moore and Lachman (8) reported that in sheep the release of lymphocytes into popliteal nodal afferent lymph was modified by an infusion of vasoactive neurotransmitter substances into afferent nodal lymphatics (7, 8). O'Dorisio et al. (9) mentioned the possibility that peptidergic neurons in the intestine may regulate migration of lymphocytes in Peyer's patches or in intestinal lamina propria. Further investigations are needed to elucidate the role of humoral and neural factors in the mechanism of increased lymphocyte traffic after lipid absorption.

The present study demonstrates that lymphocyte traffic from intestine is enhanced before lipid absorption reaches its maximum. It is interesting that at 4 h after olive oil administration the darker the lymphatics were, the less lymphocytes were found in them. The exact reason for this transport suppression of lymphocyte at the maximal absorption of lipid remains unknown. Palay and Karlin (10) observed deficient basement membrane and occasional intercellular gaps of lymphatic endothelium in rat intestinal villi. Casley-Smith (4) identified in this same species that chylomicra and lipoprotein passed through open junctions of lymphatic endothelium in jejunal villi. These particles are considered to enter lymphatics of the rat jejunum mainly through lymphatic endothelial cells, while a route for transcellular transport of chylomicra is also present. We assume that lipoprotein particles and mucosal lymphocytes might pass through the same common channel when they enter central lacteals. When lipid absorption begins, gates of the channel are open wide to allow the increased lymphocytes transport. However, at the maximal lipid absorption the channel is supposed to be occupied by abundant lipoprotein particles, leaving few spaces for lymphocytes traffic.

The lamina propria and Peyer's patches in intestinal mucosa are considered to be the major source of lymphocytes in intestinal lymph. The clinical significance of increased lymphocyte transport during lipid absorption is not known, but we can suppose the possibility that these lymphocytes deliver certain information to the peripheral systemic tissues before the absorptive nutrients reach these places and that nutritional absorption through the small intestine may contribute to the maintenance of the immune defense mechanism.

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