distension, the peristalsis slowly fatigues; this effect can be reversed by naloxone[10].

Previous experiments have shown that the inhibition of propulsive intestinal motility that occurs following this type of abdominal surgery in rats mimics the postoperative paralytic ileus seen in humans[13]. This inhibition is not clearly related to release of known neural transmitters in the intestine. Therefore, it seemed possible that endorphins could be involved and that administration of naloxone would block - and thus reveal - this influence. In our experiments, however, no such response to naloxone occurred, which implies that the decreased intestinal motility following cecectomy is not related to release of endogenous endorphins in the intestine (or from the brain, as a central response to pain). The paralytic ileus seen in humans after intestinal surgery may thus be similarly unrelated to release of endorphins. It is unclear whether the slight decrease in propulsive intestinal motility following naloxone administration to operated animals has anything to do with endorphins.

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A rapid method for measuring the phospholipid synthetic activity of incubated lymphocytes

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Summary. A rapid and simple method for estimating the phospholipid synthetic activity of incubated lymphocytes by a Florisil column technique following the uptake of ^{14}C-oleic acid was established. Stimulation of phospholipid synthesis by PHA and inhibitions caused by Tween 20 or Tween 80 and heating were evaluated easily with this method.

It is known that the phospholipids are synthetized rapidly in cultured lymphocytes following the administration of various precursors such as fatty acids, phosphate etc. Incorporation of ^{14}C-oleic acid, ^{14}C-acetate[1], ^{32}P-phosphate[2], ^{14}C-glycerol and ^{14}C-choline[3] into phospholipids extracted from lymphocytes is enhanced after the stimulation of mitogens such as PHA, ConA etc. On the other hand, these incorporations are supposed to be inhibited following impairment of lymphocytes.

We developed a rapid and simple method for estimating the phospholipid synthetic activity of lymphocytes by measuring labelled phospholipids, which were extracted from the incubated cells and purified by Florisil column.

Lymphocytes were separated from lymph nodes of male Wistar rats weighing 150-220 g following the method reported previously[4]. Separated lymphocytes were suspended in RPMI-1640 solution (GIBCO, Grand Island, USA) to be 2×10^7 cells/ml. Usually, ^{14}C-oleic acid was applied as a

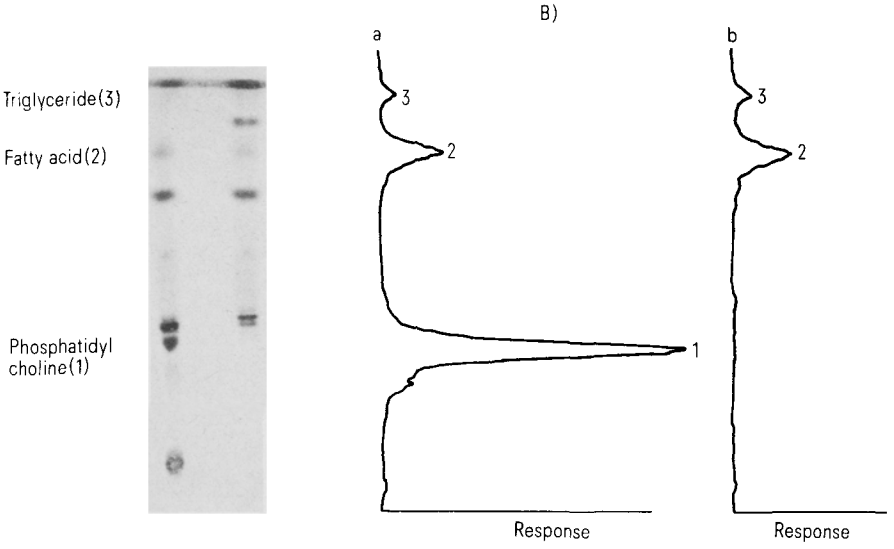

Fig. 1. Thin layer chromatograms (A) and radiochromatograms (B) of the lipids extracted from the incubated lymphocytes before and after the Florisil column chromatography. *a* Before the column treatment; *b* after the treatment (effluent). In both chromatograms A and B, phosphatidyl choline indicated as 1 in *a* disappeared completely.

Effects of PHA, Tween and heating on incorporation of ^{14}C-oleic acid into phospholipid of lymphocytes

Incubation	µg/ml	Radioactivities (cpm)
Control*		13,971± 649
PHA*		18,826± 564
Control		54,925±2571
Heated		2,772± 190
Tween 20	10	51,784±3592
	50	37,172± 933
	100	19,241± 858
Tween 80	10	45,644± 898
	50	32,765±2076
	100	23,594±1195

*Added 2.5 mg albumin to 1 ml medium.

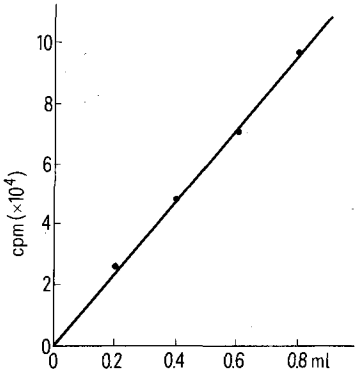

Fig. 2. Calibration curve of radioactivity of ^{14}C-phospholipids treated with Florisil column.

radioactive precursor incorporated into newly synthesized phospholipids. Suspension of oleic acid in RPMI-1640 solution was prepared by sonication of a mixture of 1 µg/ml ^{14}C-oleic acid (Radiochemical Centre Amersham, Great Britain) and 3 µg/ml oleic acid (Sigma Chemical Co., USA) resulting in concentration of total oleic acid of 2 µg/ml (0.2 µCi/ml).

0.5 ml of lymphocyte suspension described above was mixed with an equal volume of labelled oleic acid suspension and allowed to stand for 1 h in a CO_2-incubator kept at 37 °C to obtain a standard value.

To measure the stimulating effect of mitogens upon the phospholipid synthesis of lymphocytes, 2.5 mg/ml of bovine albumin (fraction v, Grand Island Biological Co., USA) was added to the cultivating media (RPMI-1640) in both the controls and the samples, because the addition of albumin caused promotion of uptake of ^{14}C-oleic acid into cellular outer membrane, and magnified the differences in radioactive counts between the samples and the controls[1]. Then the experimental specimens were incubated in a CO_2-incubator kept at 37 °C for 3 h instead of 1 h, because, according to the report of Resh et al.[1] effects of mitogen were not so appreciable within 1 h. The samples were added 20 µg/ml PHA as a mitogen. To observe the degree of impairment of lymphocytes, phospholipid synthetic activity of lymphocytes heated at 56 °C for 30 min was examined. Furthermore, samples containing Tween 20 or Tween 80 (10-100 µg/ml) were tested after 1 h incubation at 37 °C.

Incubated suspension was transferred into a homogenizer and 2 ml added of washed solution of incubation bottle with RPMI-1640, then centrifuged at 1500 rpm for 5 min. Supernatant was withdrawn by a pipette carefully and N_2 gas was blown through the homogenizer to remove as much water as possible. 0.8 ml of a mixture of chloroform-methanol (2:1 v/v)[5] was added to the sediment and homogenized for 2 min. Homogenized sample mixed with 0.3 ml washed mixture of the pestle, was poured into a Florisil column[6].

Modified Florisil chromatography was carried out as follows: A glass tube of 5.5 mm inner diameter and 200 mm length was sealed with cotton at the bottom, then packed with 0.5 g of Florisil powder (100–200 mesh, Floridin Co., USA) activated at 100 °C for 40 min. Lymphocytes homogenate described above was poured into the column and fatty acids, cholesterol esters and triglycerides etc. were allowed to effuse through the column by pouring 6 ml of washed solution of homogenizer with a 4% acetic acid-ethyl ether mixture[6].

Following this treatment, it was proved that phospholipids remained in the column by means of a thin-layer chromatogram on silica gel[7,8] and a scanning radioactive chromatogram (Aloka Co., Japan) as shown in figure 1, A and B. Dried powder of Florisil containing labelled phospholipids was transferred into counting vial with a scintilation mixture of 5 g 2,5-diphenyloxazole, 0.1 g 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene in 1000 ml toluene, then radioactivity was measured directly by liquid scintillation counter (Packard Instruments, Downers Grove, USA). Calibration curve of ^{14}C-phospholipids shown in figure 2 was found to be linear.

Table 1 summarizes the incorporation of ^{14}C-oleic acid into phospholipids of lymphocytes incubated with PHA, Tween 20 or Tween 80, and heated ones. Significant increase of incorporation of ^{14}C-oleate into phospholipids after 3 h incubation with PHA was observed.

Resh et al.[1] reported that incorporation of ^{14}C-oleic acid after 3 h incubation with PHA was about 2fold over the control, while our result revealed about 135%. The reason for this discordance is supposed to be the difference between experimental animals, because Resh et al. observed rabbit lymphocytes while we tested rat lymphocytes. The lymphocytes heated at 56 °C for 30 min revealed loss of fatty acid turnover from oleate to phospholipids because of inactivation of phospholipase and acyl-CoA (lysolecithin acyltransferase) after heating. Our rapid and simple method for measuring the labelled phospholipids extracted from lymphocytes is available to evaluate the effects of stimulants and detect the cellular receptor for stimulants with a short period of lymphocyte culture. Trace amounts of surface active substances, such as Tween 20 and 80, inhibited the incorporation of ^{14}C-oleic acid into phospholipids remarkably as shown in the table. Effects of emulsifiers in experimental additions upon the lymphocytes should be kept in mind and can be detected by our method. The effects of stimulants and/or inhibitors on phospholipid metabolism of lymphocytes detectable easily by our method might provide a clue to clarify the biological activity of cellular membrane.

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