Rectal Mucosa Damage in Rabbits After Subchronical Application of Suppository Bases

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The effect of suppository bases on rabbit rectal mucosa was investigated using six triglyceride bases, polyethylene glycol, and a triglyceride base combined with monoglycerides or fatty acids and methyl esters of those acids. Rectal irritation was evaluated and scored according to defined pathological features. "Pure" triglycerides and a triglyceride to which a nonionic surfactant was added caused severe mucosal damage with ulceration and inflammation. Hyperemia was characteristic for irritation by polyethylene glycol suppositories. Mucosal damage by a pure triglyceride combined with monoglycerides or fatty acids and methyl esters of those acids was similar but statistically less pronounced than with all other bases.

KEY WORDS: suppositories; rectum; polyethylene glycol; triglyceride; monoglyceride; fatty acids; ulcer; hyperemia.

INTRODUCTION

Suppositories remain the most widely used dosage form in rectal therapy, although different other delivery systems such as microenemas, hydrogels, rectal capsules, and osmotic pumps are being used for therapeutic or research purposes.

Suppository bases can be divided into fatty bases (mixtures of mono-, di-, and triglycerides) and the water-soluble or water-dispersible bases (e.g., polyethylene glycol). Except for polyethylene glycol, suppository bases are considered to cause no major damage to the rectal mucosa. We emphasize that reports on the irritative effect of suppository bases without medication always concerned short-term experiments (1–3).

Therefore we investigated the effects of different suppository bases on the rectal mucosa of rabbits in subchronical experiments.

MATERIALS AND METHODS

Suppository Bases

Nine suppository bases were tested: a hydrophilic composition, five "pure" triglyceride bases, and three triglyceride formulations with adjuvants. The hydrophilic base was polyethylene glycol (Flandria, Zwijnaarde, Belgium), 1500:4000 (w/w, 3:7). The five "pure" triglyceride bases were three lauric glycerides [Novata B (Henkel & Cie GmbH, Düsseldorf, Germany), Suppocire AM (Gattefossé, St.-Priest, France), and Witepsol H15 (Hüls AG, Witten, Germany)], a nonlauric glyceride [Mesuro PS (Vandemoortele N.V., Izegem, Belgium)], and cocoa butter (Flandria, Zwijnaarde, Belgium). The lauric glycerides contained mainly C12–C14 saturated fatty acids, while the nonlauric glyceride and cocoa butter contained mainly C16–C18 saturated and unsaturated fatty acids. All triglyceride bases were mono-, di-, and triglycerides mixtures containing more than 80% triglycerides and less than 2.5% monoglycerides.

We also tested Suppocire AP (Gattefossé, St.-Priest, France), a lauric triglyceride supplemented with a nonionic surfactant (polyethylene glycol fatty acid esters), and two formulations of Mesuro PS to which 5% monoglycerides (Dimodan LS, Grindsted, Antwerpen, Belgium) or a mixture of 9% fatty acids of Mesuro PS origin and 1% methyl esters of those fatty acids (Vandemoortele, Izegem, Belgium) was added. The mono-, di-, and triglyceride content of the pure triglyceride bases and Suppocire AP as well as the composition of the monoglyceride, the fatty acids, and the fatty acid methyl esters is shown in Table I. From these bases 1-g suppositories were prepared.

Suppository Administration

To male New Zealand albino rabbits weighing ±2.5 kg, provided with water and food ad libitum, three suppositories were administered daily (8, 16, and 24 hr) during 14 days. To guarantee a 30-min contact time between the base and the rectal mucosa, the anus was kept shut over this period with a clothes-peg. Each suppository base was tested on 20 rabbits. For the Novata B base 33 rabbits were used.

Controls and Placebo

Two control groups were used: rabbits receiving neither a suppository nor a clothes-peg and rabbits where the clothes-pegs were applied on a time schedule similar to the suppository administration in order to evaluate mucosal changes due to their application.

A nonmelting paraffin suppository (Flandria, Zwijnaarde, Belgium) was used as a placebo to study the influence of mechanical trauma on the rectal mucosa.

Each test was performed on 20 rabbits.

Tissue Processing

Eight hours after the last suppository administration the rabbits were exsanguinated. Rectum and anus were removed, opened lengthwise, and cleaned with a 0.9% NaCl solution before fixation in a 4% buffered formaldehyde solution. They were examined macroscopically before being cut longitudinally in three or four sections, covering the entire width of the rectum. The tissue specimens were then routinely embedded in paraffin, sectioned at 5 μm, stained

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Table I. Composition of "Pure" Triglyceride Suppository Bases and of the Additives Used

Composition of "pure" triglyceride bases and of the Suppocire AP base Percentage of mono-, di-, and triglycerides and of PEG fatty acid esters						
	Mesuro PS	Cocoa butter	Suppocire AM	Suppocire AP	Witepsol H15	Novata B
Monoglycerides	—	±1	0.5	2.5	0.3	1.9
Diglycerides	2.9	±2	3.0	28.0	10.7	19.5
Triglycerides	97.1	±97	96.5	55–60	89.0	78.6
PEG-fatty acid esters	—	—	—	10–15	—	—
Lauric/nonlauric base	Nonlauric	Nonlauric	Lauric	Lauric	Lauric	Lauric

Composition of the additives Percentage of free fatty acids, fatty acid methyl esters, and mono-, di-, and triglycerides			
	Monoglyceride	Fatty acid	Fatty acid methyl ester
Free fatty acids	3.3	100	—
Fatty acid methyl ester	—	—	100
Monoglycerides	91.0	—	—
Diglycerides	5.7	—	—
Triglyceride	—	—	—
Percentage of (glyceride/methyl ester) fatty acid according to its chain length			
C 8:0–C 14:0	<1.0	<1.0	<1.0
C 16:0	10.2	7.5	17.5
C 18:0	11.5	6.5	6.5
C 18:1	15.0	67.5	67.5
C 18:2	59.2	6.2	6.2
C 20:0–C 22:0	<1.0	<1.0	<1.0

with hematoxylin–eosin, and examined with a light microscope.

Macroscopic evaluation was used only as a confirmation for the light-microscopic scoring of hyperemia or surface injuries (ulceration or erosion, regeneration, and polyp formation).

Evaluation of Rectal Irritation

The mucosa, the surface of the intestine, exists of crypts formed by epithelial cells extending vertically into the lamina propria. The lamina propria occupies only a small volume of the mucosa. Under the mucosa, the muscularis mucosa and the submucosa can be found.

Mucosal capillary congestion with red blood cells in the lamina propria was defined as hyperemia. A red-stained rectum, being macroscopically visible, was an indication of the diffuse presence of erythrocytes (Fig. 4d). Edema was seen when the mucosa appeared thicker than normal, with the crypts failing to reach the muscularis mucosa and the space between the crypts being increased. Inflammation in the scored preparations was always acute. This was confirmed by the increase in inflammatory cells (polymorphonuclear cells or neutrophilic cells) in the lamina propria (Fig. 1a). This in contrast to chronical inflammation, where plasma-cell infiltrates are predominant in the lamina propria. Erosion was defined as the detachment of surface epithelium and the presence of fibrin and inflammatory cells in the lamina propria. When necrotic material and fibrin exudates were observed, the erosion evoluated to an ulceration. Regeneration occurred when a new epithelial layer was present on the ulceration (arrow in Fig. 1b). Irregular healing of ulcerations can induce polyp formation (pseudopolyps or "inflammatory polyps"). These are localized lesions projecting from the mucosa (Figs. 1c and d and Fig. 4c) (4).

To allow a statistical interpretation of the results, a scoring table was used (Table II) based on the severity of the inflammation. Each rectum was scored microscopically only according to the type and degree of mucosal damage. The

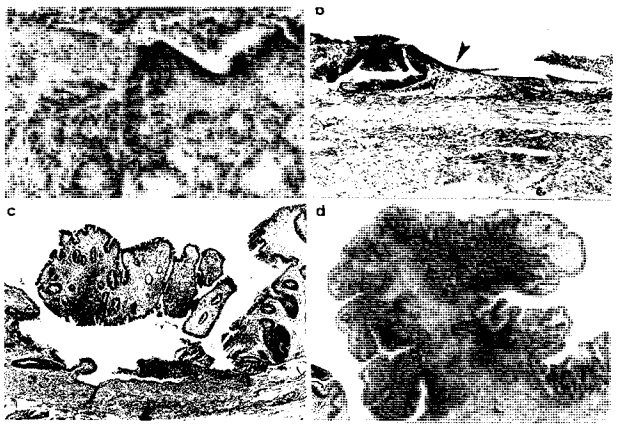

Fig. 1. Rectal mucosa featuring (a) hyperemia and inflammation after polyethylene glycol application, (b) ulceration with epithelial regeneration at the edge (arrow) after Suppocire AM treatment, (c) inflammatory polyp and underlying ulcer after using Novata B suppositories, and (d) inflammatory polyp at the anorectal junctional zone in Suppocire AP-treated rabbits. (a, b) ×75, (c) ×32, and (d) ×28; reduced 50% for reproduction.

Table II. Scoring Table for the Evaluation of Rectal Irritation

Pathological feature		Degree of irritation	Score
Hyperemia	0	Negative	0
	+	Focal	1
	+ +	Diffuse	2
Edema	0	Negative	0
	+	Focal	1
	+ +	Diffuse	2
Inflammatory cells	0	Normal numbers	0
	+	Focal	3
	+ +	Diffuse	4
	+ + +	Heavy diffuse	5
Erosion, ulceration, regeneration, and/or polyps	Neg.	Absent	0
	Pos.	Present	15

scorings were performed in a blind fashion by three observers simultaneously.

Histological sections showing severe hyperemia, diffuse inflammation, ulceration, and inflammatory polyps are shown in Figs. 1a–d. When erosion, ulceration, regeneration, and/or inflammatory polyps were present, a score of 15 was attributed. As these types of lesions are the consequence of epithelial damage, we assumed that they had a major impact compared to hyperemia, edema, or inflammation. The maximal score per rectum was 24. For the different suppository bases, controls, and placebo, a mean total score was calculated by grouping the individual data. For each pathological characteristic the number of positive scoring rabbits was then expressed as percentages. This was also done for the degree (focal, diffuse, or heavy diffuse) to which hyperemia, edema, and inflammation occurred.

Statistical Evaluation

Statistical analysis was performed on the total scores and on the values obtained for hyperemia, edema, and granulocytes. The presence of erosion, ulceration, polyps, and regeneration was evaluated separately.

Two nonparametric tests were used: the Kruskal–Wallis one-way analysis of variance by ranks when more than two groups were compared and the median test when two groups were compared. A probability value of $P < 0.05$ was considered to be statistically significant (5).

RESULTS

The mean total scores for each suppository base, control group, or placebo are shown in Fig. 2.

For some representative groups Figs. 3a–d show the number of positive scoring rabbits for each pathological characteristic and for the degree in which hyperemia, edema and inflammation occurred.

Because a high variability (important interanimal variation) was observed for the response of the rabbits to the suppository bases, it was necessary to use groups of 20 rabbits.

Hyperemia or surface injuries such as erosions, ulcers, regeneration, and polyps were seen macroscopically (Figs. 4a–d). Light microscopy showed that the mucosal damage was always limited to the epithelium and the lamina propria. In the submucosa we did not find any abnormalities (such as edema, inflammation, or misplaced glands).

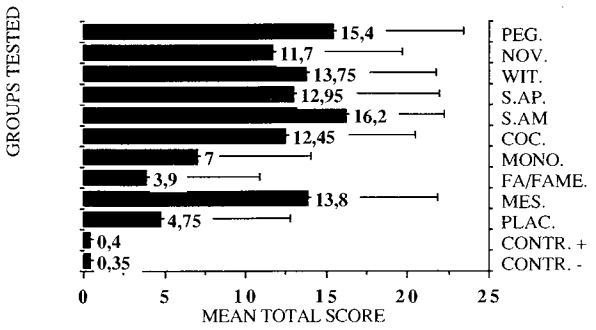

Fig. 2. The mean total scores (±SD) for each group tested. CONTR.−, control without clothes-peg; CONTR.+, control with clothes-peg; PLAC., placebo; MES., Mesuro PS; FA/FAME, Mesuro PS + 9% fatty acids + 1% fatty acid methyl esters; MONO., 5% monoglycerides; COC., cocoa butter; S.AM, Suppocire AM; S.AP, Suppocire AP; WIT., Witepsol H15; NOV., Novata B; PEG., polyethylene glycol. $n = 20$ except for NOV., where $n = 33$.

The mucosal irritation of recta treated with "pure" triglycerides was similar for all bases. Hyperemia occurred only focally. There was active inflammation with increased polymorphonuclear cells and crypt abscesses (polymorphonuclear cells present in the lumen of the crypt). A significant difference for the occurrence of edema between the five "pure" triglycerides was found (Kruskal–Wallis test, $P < 0.05$). Erosion evolving in ulceration with replacement of surface epithelium by fibrin, mucus, and inflammatory cells was a frequent finding, as well as the regeneration of the mucosal damage and inflammatory polyps.

The effects of Suppocire AP on the rabbit rectal mucosa were similar to these induced by "pure" triglycerides (Kruskal–Wallis test, $P = 0.26$).

The sections of recta treated with polyethylene glycol revealed the same abnormalities as in the pure triglyceride and the Suppocire AP groups, but hyperemia was more pronounced than with polyethylene glycol (Kruskal–Wallis test, $P < 0.05$). For the presence of erosion, ulceration, regeneration, and polyps and for the total scores, no significant difference was found among the pure triglycerides, the Suppocire AP, and the polyethylene glycol.

The addition of fatty acids and fatty acid methyl esters or the addition of monoglycerides to Mesuro PS decreased the mucosal damage dramatically. Scores for each type of abnormality and total scores for these bases differed significantly from those for pure triglycerides, Suppocire AP, or polyethylene glycol (Kruskal–Wallis or median test, $P < 0.05$).

Both control groups showed no differences. They had very low total scores and were significantly different from the triglycerides, the Suppocire AP, polyethylene glycol, and all formulations with Mesuro PS.

The scores of the placebo suppositories were not significantly different from those of both control groups (median test on total scores, $P = 0.27$ for control group without clothes-peg and $P = 0.12$ for control group with clothes-

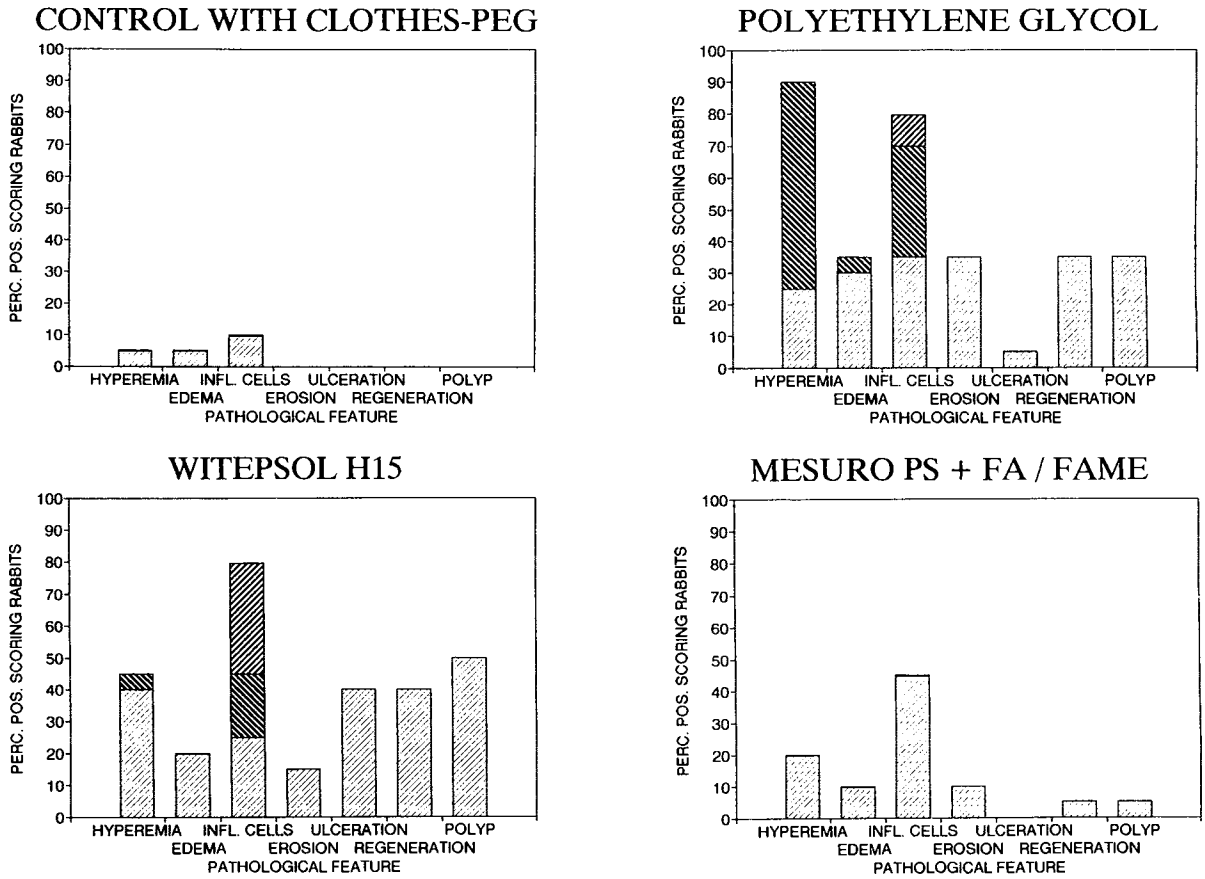

Fig. 3. Number of positive-scoring rabbits for each pathological characteristic and degree (focal, diffuse, or heavy diffuse) of hyperemia, edema, and inflammation (all data are expressed as percentages). (+) ▨; (+ +) ▩; (+ + +) ▧. (+) Focal; (+ +) diffuse; (+ + +) heavy diffuse. Infl cells, inflammatory cells; FA/FAME, Mesuro PS + 9% fatty acids + 1% fatty acid methyl esters.

peg). The Mesuro PS formulations with additives did not differ significantly from the placebo group [median test on total scores, $P = 0.20$ for Mesuro PS suppositories with monoglycerides (5% Dimodan LS) and $P = 0.19$ for Mesuro PS suppositories with 9% fatty acids and 1% methyl esters].

DISCUSSION

Rectal ulceration with therapeutic use of suppositories has been reported but this effect was always attributed to the drug, and never to the suppository base. Case reports mentioned either long-term application (e.g., indomethacin) (4,7) or drug abuse (analgesics or drugs used for the treatment of migraine) (8–10).

The rabbit was chosen as a model because its rectum is histologically comparable to the human rectum. The size of a rabbit rectum is similar to the size of the rectum of a 6-month-old baby.

Differences between the total scores of both control groups were not observed, indicating that rectal irritation was not due to increased strain of the rectoanal muscles induced by the clothes-peg technique.

Administration of the placebo suppositories caused focal inflammation. Because of the nonmelting nature of these suppositories at the animal's body temperature, the injuries may be due to suppository administration or to their presence in the rectal cavity. However, those data exclude the possibility that mucosal damage induced by polyethylene glycol and triglyceride bases was due to their insertion only.

Polyethylene glycol caused severe mucosal hyperemia in 65% of the rabbits. This was the main characteristic of polyethylene glycol-induced rectal irritation, although diffuse infiltration of granulocytes, erosion, regeneration, and inflammatory polyps were also observed. Recta treated with Suppocire AP base (containing a polyethylene glycol derivative) also showed serious mucosal damage not significantly different from triglyceride bases. The irritative effect of polyethylene glycol and its derivatives after a single exposure to the gastrointestinal tissue has been reported (11). Due to this effect the absorption of poorly absorbed polar molecules (antibiotics, peptides) was increased (12,13). The use of polyethylene glycol lauryl ether was described as a method to produce rectal ulcers after single application (14).

An unexpected result was the severe mucosal damage observed after frequent application of the triglycerides. Most of the literature on this subject describes fatty bases as being nonirritant. A report where rectal irritation induced by semisynthetic triglycerides was compared to cocoa butter after chronical application to dogs concluded that these bases did not induce inflammation (15). In other studies, several semisynthetic triglycerides were considered to be nonirritant be-

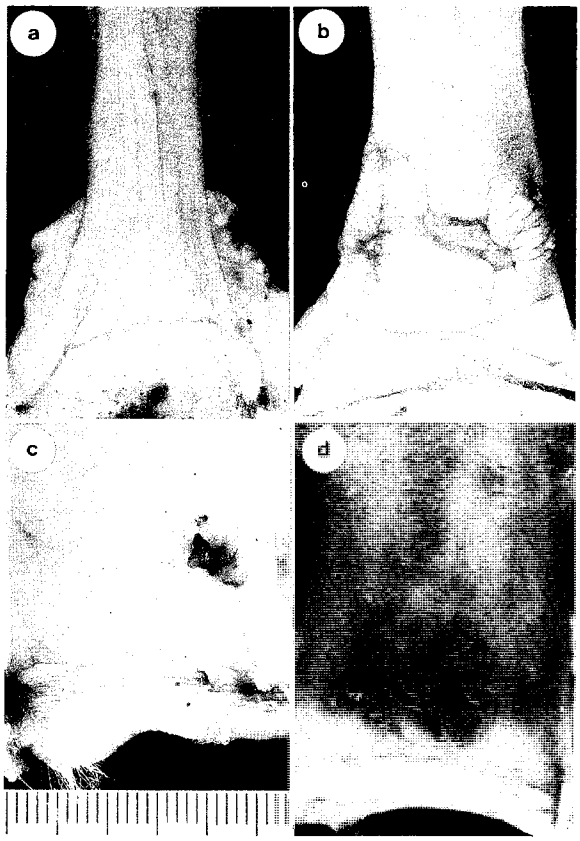

Fig. 4. Rabbit rectoanal preparations: (a) normal rectum after placebo treatment; (b) ulcerated rectoanal area after Witepsol H 15 suppositories; (c) inflammatory polyp developed after application of Suppocire AP suppositories; (d) hyperemic rectal mucosa typical for PEG administration.

cause the mucosal damage after single daily application did not exceed the natural capacity of the epithelium to prevent its disruption (1,2,16). In a recent study by Van Hoogdalem *et al.* (17) where, after single application, hyperemia, edema, and localized epithelial cell detachment were observed, these findings were considered to be reversible damaging effects, not necessarily excluding clinical applicability. In our study all triglycerides caused severe lesions similar to the solitary ulcer syndrome in man (8–10). The composition of the triglycerides regarding diglyceride content, glyceride fatty acid chain length (C12–C14 or C16–C18), and their degree of saturation did not influence the results. Considering the fact that polyethylene glycol and triglyceride bases induced rectal ulcers in rabbits, one might assume that rectal ulcerations in humans observed after chronic use of suppositories (6,7,8–10) were induced not only by the drug but also by the suppository base.

From these results it should be clear that in the early stages of suppository formulation, irritation studies in rabbits should be performed to determine if local irritation occurs after subchronic application of suppository bases with and without the active substance.

Adding 5% monoglycerides or a combination of 9% fatty acid and 1% methyl ester of those fatty acids decreased the mucosal damage. These results are in agreement with the literature data on the use of MGK (glyceride mixture with ±60% monocaprylate) (3), fatty acids, or fatty acid derivatives in single-dose experiments (18). However, these results are in contrast with the results obtained by Van Hoogdalem *et al.* (17) using glycerylmonooctanoate and glycerolmonodecanoate and fatty acids. In this study cefoxitin solutions were administered with and without the different adjuvants. The cefoxitin solution in saline already caused fairly high scores. This finding and the fact that (i) these monoglycerides had other fatty acid chain lengths, (ii) the amounts used were different, and (iii) PEG 400 was added to the monoglyceride formulations may contribute to the discrepancy in the observed results (PEG suppositories can induce complete desquamation of epithelial cells in the rat rectum after single application) (1).

From our experiments and because monoglycerides, fatty acids, and fatty acid derivatives have been tested successfully as absorption enhancing products for poorly absorbed drugs such as cephalosporins (18,19), the use of monoglycerides and fatty acids and derivatives could be very promising as absorption promoting agents. However, the influence of the fatty acid chain length, the amount of additives used, and the type of ester on the reduction of the mucosal damage remains unclear, as only one monoglyceride and one mixture of fatty acids and fatty acid methyl esters were added to Mesuro PS.

Summarizing, this study demonstrated that frequent application of polyethylene glycol or pure triglycerides induced damage resulting in ulceration and inflammation of the rectal mucosa. This effect was similar for triglycerides supplemented with polyethylene glycol fatty acid esters. However, rectal mucosa damage was significantly reduced with triglyceride suppositories where monoglycerides or fatty acid and fatty acid methyl esters were added.

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