

ALLERGENS IN VEGETABLE OILS

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THE parenteral administration of vegetable oils in man may provoke a sensitization easily demonstrated by intracutaneous testing.⁶ These oils contain allergens which certainly are not specific for each kind of oil, since intracutaneous tests with different oils have been shown to yield cross reactions.⁶ The skin tests are followed by the same kind of local reactions when the oils are entirely refined, that is, completely freed of proteins, carbohydrates, and free fatty acids. Therefore, such impurities can be discarded as possible causes of sensitization by natural vegetable oils. It must be admitted, therefore, that the allergen is either a constituent of the fatty oil by itself, or a lipid with very similar physical—chemical properties which was not separated from the glycerides during the refining procedure. The purpose of this investigation is to test the hypothesis that triglycerides may be the allergens contained in vegetable oils. This can be done by examining the allergenic properties of chemically pure, synthetic glycerol-fatty acid esters.

MATERIAL AND METHODS

Synthesis of Chemically Pure Triglycerides.—Synthesis of glyceryl trioleate (triolein) and glyceryl tripalmitate (tripalmitin) were carried out by the procedure of Belluci,² while glyceryl tributyrinate (tributyrin) was obtained by the usual esterification technique.

The absolute glycerol used for all esterifications was purified by twofold vacuum distillation.

Chemically pure oleic acid was obtained by twofold vacuum distillation and further refining the oleic acid by lead and lithium precipitation, according to the method of Täufel and Seuss.¹³ The lithium salt of the oleic acid was recrystallized as many times as necessary to obtain a perfectly white salt and an absolutely colorless concentrated solution. The oleic acid liberated from its lithium salt was found to have the expected acid and iodine values.

Chemically pure palmitic acid was obtained by several recrystallizations of pure commercial palmitic acid, until the substance yielded a stable and well-defined melting point. Analysis of the synthetically obtained glyceryl trioleate and tripalmitate yielded values in agreement with the theoretical saponification and iodine values. The melting point of the tripalmitin was 64° C.

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For the synthesis of glyceryl tributyrates, pure butyric acid anhydride was used. The tributyrin then obtained was further purified by vacuum distillation.

Sensitization Technique.—Sensitization experiments were performed with chemically pure butyric acid, tributyrin, tripalmitin, and triolein, obtained as described in previous paragraphs of this article. The liquid preparations were given intracutaneously to healthy volunteers in single doses of 0.1 c.c. every twenty-four hours except on Sundays. Solid tripalmitin was rubbed into scarified skin areas. This technique was adopted to avoid dissolution of tripalmitin in fat solvents, which might in themselves be biologically active. The sites of injection or scarification were controlled daily with regard to redness and swelling. Total leukocyte and eosinophil counts with the phloxine method were done every second day.

RESULTS

1. *Butyric Acid.*—The intracutaneous injection of butyric acid provoked a strong local irritation. During injection a burning pain was felt, which lasted for several minutes. Then an erythematous papule about 10 mm. in diameter appeared and lasted for about twenty-four hours. During a series of twelve injections in fourteen days, this immediate reaction occurred after each injection, without any change in timing or local aspect. The total leukocyte and eosinophil counts remained unchanged. The experiment was stopped after two weeks because of painful local reaction.

2. *Tributyrin.*—In this experiment a painful local reaction was also elicited, along with an erythematous papule, 3 to 4 mm. in diameter, which disappeared within twenty-four hours. During an experiment of three weeks' duration, the same cutaneous reaction occurred each time. The total leukocyte and eosinophil counts remained unchanged. It is probable that the local reaction was due to the small quantities of free tributyrin contained in the injected material, as tributyrin very rapidly undergoes a slight hydrolysis during storage.

3. *Tripalmitin.*—This ester, applied by the scarification method, did not produce any local reaction or any change of the total leukocyte and eosinophil counts during an experiment lasting five weeks.

4. *Triolein.*—When injections were performed with triolein, stored for some time and containing, therefore, small quantities of free oleic acid, a burning pain was felt and a papule, 5 to 6 mm. in diameter, appeared within one hour and disappeared after twenty-four hours. When freshly prepared triolein was injected, however, no pain was evoked and no trace of local reaction could be observed (Fig. 1) ten minutes, thirty minutes, one hour, two, twelve, twenty-four, forty-eight and seventy-two hours after its first application. Twenty-five further injections could be given without any reaction. However, on the twenty-eighth day of the uninterrupted series of skin tests, a local redness and swelling, 8 mm. in diameter was observed within twenty-

four hours after injection (Fig. 2). After that, each skin test was followed by an increasingly strong local reaction so that during the fifth week of the injection series, papules 12 to 15 mm. in diameter were provoked. At that time, analogous reactions were observed at the sites of the early injections, which previously had not been followed by any local signs (Fig. 3). Redness and swelling remained unchanged for several days and disappeared only

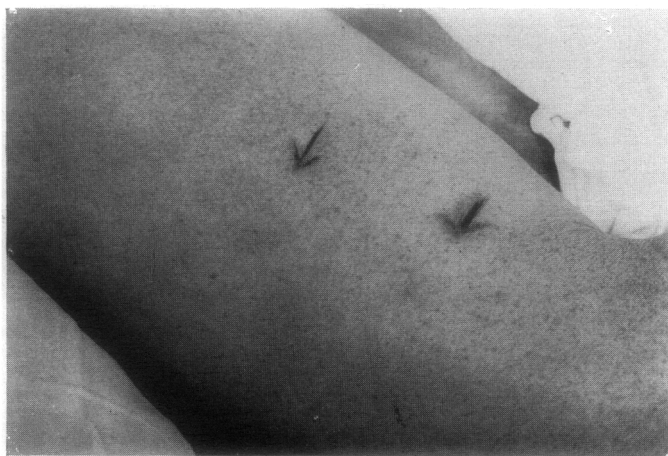


Fig. 1.—Sites of initial intracutaneous applications of 0.1 c.c. triolein, twenty-four and forty-eight hours after injection. No reaction is seen.

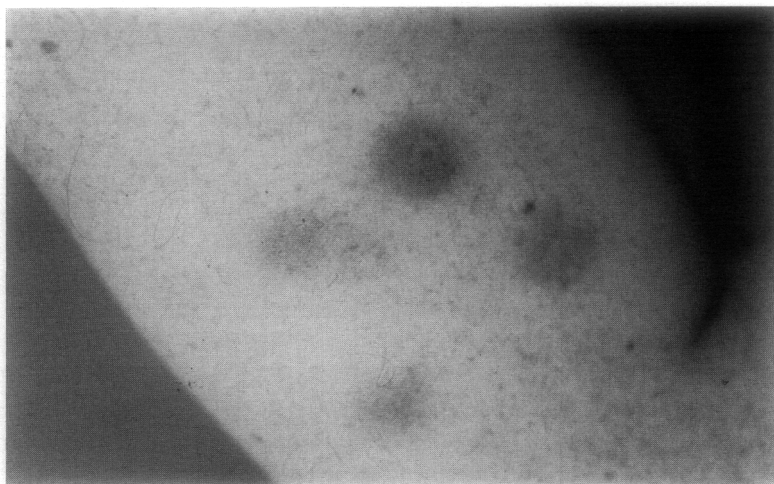


Fig. 2.—Sites of four serial intracutaneous triolein injections (twenty-fourth to twenty-eighth injections) in the course of the fifth week of the series of injections. Redness and swelling at the sites of injection (left upper arm, same person as in Fig. 1).

in the course of three weeks. A total of thirty-one injections of 0.1 c.c. was administered in the course of this experiment. The total leukocyte and eosinophil counts did not change significantly. The highest eosinophil count was 185 cells per cubic millimeter (4 per cent).

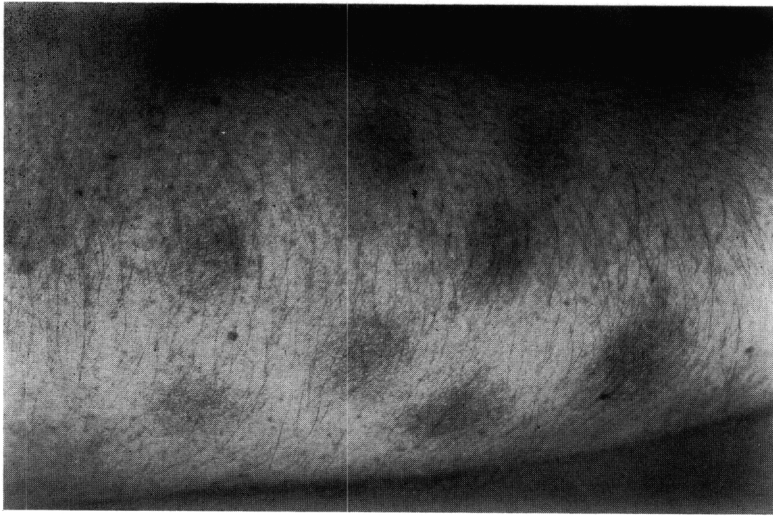


Fig. 3.—Late flaring up at the sites of previous injections. The wheals occur at the sites of intracutaneous injections given three weeks earlier (right forearm, same person as in Figs. 1 and 2).

DISCUSSION

It has been shown that repeated injections of triolein may produce sensitization. This sensitization can be followed up by observing the behavior of cutaneous oil papules. The intracutaneous oil deposits do not elicit any local reaction until, after several weeks of continuous skin testing with the oil, the injections produce a cutaneous reaction of the tuberculin type, that is, redness and swelling occurring within twenty-four to twenty-eight hours after injection. Simultaneously with the first positive skin tests, similar reactions occur at the sites of earlier injections, which originally had not been followed by any reaction. This phenomenon is due to the slow absorption of the triolein, which remains for several weeks in the skin. This slow absorption also explains why the cutaneous signs do not disappear after a few days as with tuberculin, but last for several weeks. Allergic skin reactions following testing with refined vegetable oils behave identically.⁶ This sensitization by refined vegetable oils and by chemically pure synthetic triolein shows that allergy to plant oils is due to the fatty acid glycerides and not to chemical impurities.

Triolein probably behaves as a hapten, as it is not likely that such a simple substance has the properties of a complete antigen. It might be significant that it is the triglyceride of an unsaturated acid, the oleic acid, that is the sensitizing agent and not the glyceride of the saturated palmitic and butyric acids. This agrees with the observations made by Hass⁹ that unsaturated oil fractions are biologically more active than saturated fractions.

The behavior of the blood eosinophils after application of vegetable oils and chemically pure synthetic fatty acid glyceride is of interest. Protracted intramuscular injections of sesame and almond oils in man and guinea pigs provoke a significant rise of the number of blood eosinophils.^{3, 5-8, 10} During

daily administration of 1.0 to 2.0 c.c. oil to man and 0.25 c.c. to guinea pigs, the eosinophil count begins to rise after about ten days, reaches values of 20 to 80 per cent, and drops again to normal within three to four weeks after cessation of the intramuscular oil injections. This blood eosinophilia is accompanied by a high-grade bone marrow eosinophilia. After the return of the eosinophil count to normal, a single oil injection does not produce any rise of the eosinophils. However, a new protracted series of oil injections produces again an eosinophilia analogous to the first one. This blood eosinophilia has the characteristic features of the resorption eosinophilia, as defined by Essellier and co-workers,^{3, 4, 6, 8} that is, constant period of latency, progressive rise and drop, and proportionality of the maximum number of blood eosinophils to the amount of oil injected, that is, the intensity of the eosinopoietic stimulus. These differential features leave no doubt that the eosinophilia occurring after intramuscular oil injections is not of an allergic nature.

In contrast with the protracted intramuscular application of relatively large amounts of vegetable oils, the intracutaneous sensitization with almond and sesame oil^{5, 6} and with triolein is not accompanied by any significant rise of the blood eosinophil count. This behavior of the blood eosinophils is well accounted for by the difference in the quantities of oil injected. In the course of the series of intramuscular injections leading to a resorption eosinophilia, total amount of 12 to 173 c.c. of oil were injected. In the experiments with intracutaneous triolein, however, the total amount of oil given was only 3.1 c.c. It is obvious that the resorptive and inflammatory reactions elicited by such small quantities of oil are not sufficient to produce a significant rise of the blood eosinophils. The different behavior of the blood eosinophils during intracutaneous and intramuscular administration of oil is therefore easy to understand. The fact that the positive skin tests are not accompanied by a blood eosinophilia does not speak against the allergic nature of these reactions. Although the eosinophil granulocytes contain serologically specific substances,¹² it is well established that eosinophilia is not an obligatory symptom of the allergic reaction.¹

SUMMARY

Serial intracutaneous injections of chemically pure, synthetic glyceryl trioleate (triolein) lead to the same allergic reactions as vegetable oils. The sensitization by natural vegetable oils can be attributed to fatty acid triglycerides and not to impurities. This sensitization is not accompanied by a rise of the blood eosinophils. The absence of eosinophilia fits in with the known fact that eosinophilia is far from constant in allergy and with our finding that large amounts of oil are needed to produce a resorption eosinophilia.

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