

EXPERIMENTAL CONTACT SENSITIZATION OF GUINEA PIGS TO VEGETABLE OILS

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OLIVE OIL, peanut oil, and corn oil are commonly used as diluents in experimental contact sensitization because they are considered to be essentially nonantigenic.

During the course of recent experimental work with contact sensitization in guinea pigs, it became evident that these oils are not antigenically inert and the following study was undertaken to provide a clearer picture of their potential as contact antigens in the guinea pig.

MATERIALS AND METHODS

Animals.—Three groups of guinea pigs, obtained from two commercial suppliers,* were used. Two of the groups were albino guinea pigs of the Hartley strain and the third strain was Himalayan. All of the guinea pigs weighed approximately 500 grams at the start of each experiment. They were kept in groups of 2 to 4 in standard rabbit cages and were fed on Rockland Rabbit Pellets with supplements of lime juice and fresh greens. Since the responses in all three groups of guinea pigs were essentially identical and seemed unrelated to sex, no further distinction will be made with respect to either the source of the animal or its sex in the rest of this report.

Antigens.—Mazola corn oil, Planter's peanut oil, Re Umberto virgin olive oil, and Progresso olive oil were obtained from local grocers. A third batch of olive oil (Conti, USP) had been stored in its original metal container for about 10 years at room temperature. It showed no evidence of rancidity, discoloration, bacterial contamination, or loss of edibility.

For tests of the unsaponifiable fraction, the Conti olive oil was saponified with ethanolic potassium hydroxide and the unsaponifiable fraction was extracted with ether.¹ After evaporation of the ether, the unsaponifiable fraction was diluted to approximately 2 per cent with reagent grade acetone (Fisher) for use as a test material.

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Received for publication March 27, 1962.

*Hemlock Hollow Farms (Wayne, New Jersey), and Laboratory Animal Corporation (Plainfield, New Jersey).

Test Procedure.—A medicine dropper, delivering approximately 20 drops of solution per milliliter, was used to apply single drops of the test solutions to the backs of the guinea pigs. The hair was removed by gentle clipping immediately before the test.

The reactions to the substances used in these experiments consisted of varying degrees of erythema without whealing or vesiculation. They were noted as early as 6 hours after testing in some of the animals and usually persisted for 2 or 3 days. The tests were read at 24 hours and were recorded as positive only if they showed an even, well-defined erythema. No distinction was made among degrees of positivity for the purposes of this report.

TABLE I. SKIN REACTIVITY OF GUINEA PIGS REPEATEDLY EXPOSED TO VEGETABLE OILS

ANIMAL NO.	INITIAL APPLICATION	TIME OF APPLICATION OF OIL AFTER INITIAL APPLICATION					
		2 WK.	4 WK.	6 WK.	12 WK.	16 WK.	20 WK.
<i>Conti Olive Oil</i>							
1	0	+	+	0	0	0	0
2	0	0	+	+	+	Died	
3	0	0	+	+	+	+	+
4	0	0	0	+	+	0	0
5	0	0	+	0	0	Died	
6	0	+	+	0	0	0	0
7	0	0	0	0	0	0	0
8	0	0	+	0	+	+	+
9	0	+	+	+	+	+	+
10	0	0	0	+	+	0	+
11	0	0	0	+	0	0	0
12	0	0	0	0	+	+	+
<i>Umberto Olive Oil</i>							
1	0	+	0	0	0	0	0
2	0	0	+	+	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
<i>Corn Oil</i>							
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	+	+	0	0	0
5	0	0	0	0	+	0	0
6	0	0	+	+	0	0	0
<i>Peanut Oil</i>							
1	0	0	0	+	+	0	0
2	0	0	0	+	+	0	0
3	0	0	0	0	+	0	0
4	0	0	0	0	0	0	0

EXPERIMENT 1

Single drops of the Conti olive oil were applied to the skin of the backs of 12 guinea pigs at 2 to 6 week intervals over a period of 5 months. Four guinea pigs were similarly treated with Umberto olive oil, 6 with corn oil and 4 with peanut oil.

Results.—It was noted, as detailed in Table I, that none of the animals showed positive reactions to the initial application of any of the oils. Subsequently, however, 50 per cent or more of the animals showed positive reactions at some time during the period of testing.

1. *Conti olive oil*: Eleven of the 12 guinea pigs treated with Conti olive oil developed positive reactions. Three showed positive reactions as early as the second application of the oil (2 weeks after the initial application). Four animals had their first positive reactions at the third application and 3 more at the fourth application. A single animal first reacted positively to the fifth application of the oil, 12 weeks after its initial application.

In 2 of the animals, the reactivity was observed in only one test, but the other reactive animals showed two or more consecutively positive tests, and usually maintained the ability to react for a month or more. Five of the animals were still reactive at the termination of the experiment.

2. *Umberto olive oil*: Two of the 4 animals showed positive reactions. One animal reacted only to the second application of the oil, but the second reacted to both the third and fourth applications.

3. *Corn oil*: Three of the 6 guinea pigs were found to react to the corn oil. Two reacted one month after their initial exposure to the oil and one reacted at 3 months.

4. *Peanut oil*: Three of the 4 guinea pigs reacted positively to peanut oil. The reactivity was noted in 2 of the animals at the time of the fourth application of the oil and at the fifth application in the other.

TABLE II. CROSS-REACTIVITY TO VEGETABLE OILS IN GUINEA PIGS SENSITIVE TO CONTI OLIVE OIL.

	CONTI OLIVE OIL	RE UMBERTO OLIVE OIL	PROGRESSO OLIVE OIL	CORN OIL	PEANUT OIL
1	+	+	+	0	0
2	+	+	+	0	0
3	+	+	+	0	0
4	+	+	+	0	0
5	+	0	+	0	0
6	+	+	+	0	0
7	+	0	+	0	0
8	+	+	+	0	0
9	+	+	+	0	0
10	+	+	0	0	0
11	+	+	0	0	0
12	+	+	+	0	0
13	+	0	+	0	0
14	+	+	+	0	0
15	+	+	+	0	0
16	+	0	0	0	0
17	+	+	+	0	0
18	+	+	+	0	0
19	+	+	0	0	0
20	+	+	+	0	0
21	+	+	+	0	0
22	+	+	+	0	0

EXPERIMENT 2

Guinea pigs sensitive to Conti olive oil were tested for cross-reactivity to the Re Umberto and Progresso brands of olive oil, and to the corn and peanut oils. The five oils were applied simultaneously to different areas on the backs of each of 22 animals.

Results.—As outlined in Table II, 18 of the 22 animals sensitive to Conti olive oil showed cross-reactions with Re Umberto oil. A similar number showed cross-reactions with the Progresso olive oil, but no cross-reactivity to peanut oil or corn oil was demonstrated.

EXPERIMENT 3

Eight animals sensitive to Conti olive oil and 4 nonsensitive control animals were tested with single drops of the unsaponifiable fraction of Conti olive oil.

Results.—Each of the 8 animals sensitive to Conti oil showed positive reactions to its unsaponifiable fraction. The four nonsensitive animals failed to react.

DISCUSSION

The high incidence of reactivity to vegetable oils noted in these experiments is unusual. An initial suspicion that the strain of animals being used in preliminary work was peculiarly hypersensitive seems eliminated by the appearance of similar reactivity in two independent strains of guinea pig.

The reactions appear to be true allergic contact-type sensitizations. In no case did the initial application of an oil produce a reaction and the subsequent positive reactions occurred within the period of incubation customary with experimental contactants. The cross-reactivity of two other brands of olive oil in Conti-sensitive animals, and the failure of the corn and peanut oils to cross react in the same animals, suggest that the hypersensitivity is specific.

The apparent demonstration of a common antigenic factor among the olive oils, possibly confined to the nonsaponifiable fraction of the oils, suggests that the age of the batch of Conti oil was not a critical factor in its antigenicity. Aging may well have increased its antigenic potency, probably through an oxidative change; but there it seems unlikely that an abnormal antigen was formed.

It is of interest to note that the oils used in these experiments behaved as "weak" antigens in much the same sense that the term has been used by Epstein and Kligman.² In contrast to a "strong" antigen, such as 1-chloro-2,4-dinitrobenzene, a weak antigen tends to sensitize a smaller proportion of animals. It might be added, further, that sensitization produced by a weak antigen tends to appear more slowly and to be relatively transient. When the animals of the present group were sensitized with 1-chloro-2,4-dinitrobenzene, a typical strong antigen, the rate of sensitization was close to 100 per cent and strong sensitization persisted for over 6 months in the majority of animals.

REFERENCES

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