Contact Sensitivity in Rhesus Monkeys to Poison Ivy Extracts and Fluorodinitrobenzene. (29691)

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Reports on the sensitization of rhesus monkeys to poison ivy extracts have been conflicting. Straus(1) cited induction of a specific sensitivity to poison ivy after percutaneous application of poison ivy extract (PIE) in rhesus monkeys, while Zeligman (2) using similar procedures failed to sensitize rhesus monkeys to either poison ivy extracts or one of its components, pentadecylcatechol (PDC). Von Adelung(3) failed in his attempt to sensitize a monkey to poison oak extract.

In light of these conflicting data the principal aim of this investigation was to determine if a reproducible procedure for sensitization of rhesus monkeys to PDC or PIE could be effected. Upon production of successful sensitization, steps would be taken to determine if desensitization of rhesus monkeys could be achieved by various administrations of PDC or PIE. Finally, any toxicity resulting from administration of these allergens would be evaluated.

Despite the difficulties in sensitization of the rhesus monkeys in this study, efforts were made to assess the toxic effects resulting from percutaneous, parenteral and oral administration of PDC and PIE. To test the ability of these monkeys to respond in a delayed fashion, sensitization by a chemically unrelated contact allergen, 1-fluoro-2, 4-dinitrobenzene (FDNB) also was attempted.

Materials and methods. Male rhesus monkeys, (*Macaca mulatta*) weighing about 2.2 kg were obtained from commercial sources. The substances used in attempts at sensitization were PDC, (a synthetic compound),* PIE,† and FDNB.‡

The PIE was assayed in sensitized guinea pigs and the concentration of its activity expressed as micrograms of PDC(4,5).

PDC or PIE were applied percutaneously in an acetone or alcohol vehicle or by subcutaneous injections in complete Freund-type adjuvant. Adequate contact of PDC or PIE with the monkey skin was assured by covering the topically applied material with Saran Wrap§ and securing it by use of jackets as described by Straus(1). FDNB was applied percutaneously in acetone solutions. The abdomen served as the site for the sensitizing percutaneous applications while the dorsal surfaces served as the skin test sites. When sensitization was attempted by subcutaneous injections of PDC in complete adjuvant, the cervical axillary, and inguinal regions served as sites. One ml of emulsion was prepared from equal parts of buffered saline (pH 7.2) containing 1.5 mg of dried *Mycobacterium butyricum* (Difco) and Arlacel-Drakeol (35:65) containing 6 mg PDC.

Feedings of 0.5 ml doses of PDC or PIE incorporated in vegetable oil were introduced into the throat by means of a 5 inch bulb-tipped 15 gauge hypodermic needle attached to a syringe in order to avoid contaminating the skin. The intramuscular injections of 0.5-1.0 ml of PDC or PIE incorporated in vegetable oil, were given in the leg.

A schedule of the procedures is listed chronologically in Table I. It should be noted that 2 monkeys of Group II were started on the PDC regimen, but were changed later to a regimen of PIE.

Skin tests for contact sensitivity were conducted according to the procedures of Dunn, Mason and Smith(6) and of other studies from this laboratory(4,5) by placing 5 μl of an acetone solution containing varying concentrations of reagent on the skin within the confines of a brass ring having an inside diameter of 7 mm. The reactions were recorded at 48 hours (time of maximum intensity).

Daily observations were made on all ani-

* Kindly supplied by Dr. C. R. Dawson, Columbia University.

† Purchased from Hollister-Stier Laboratories.

‡ Purchased from Eastman Organic Chemicals.

§ Dow Chemical Co.

TABLE I. Chronology of Treatment of Monkeys.

Method of administration	Day	Group I 507-512 Total PDC, mg	Group II 513-515, 517, 518 Total PIE,§ mg	Group III† 508, 512, 514, 5161‡ Total PDC or FDNB, mg	Group IV 673, 810, 811, 812 Total PIE,§ mg
Percutaneous	1, 7, 13, 30	20.5	3.5		2.5
Intradermal	34				.6
Percutaneous	40,‖ 69,‖ 79	.6	.5		
Subcutaneous	97			(PDC) 6.0	
Percutaneous	130			(FDNB) 5.3	
		507, 509	510,* 511,* 513, 515, 517, 518		
Oral (biweekly)	97-125	20.0	20.0		
Intramusc. (weekly)	97-125	20.0	20.0		
Percutaneous	142, 170	.7	.5		
Oral (biweekly)	178-201	20.0	20.0		
Intramusc. (weekly)	178-201	20.0	20.0		

* Animals previously a part of Group I. † Animals taken at random from Groups I & II. ‡ Animal not previously treated. § PIE expressed as mg PDC activity. ‖ Quantity for skin tests included.

mals for illness, and complete blood counts were done weekly. In addition, serum levels of glucose, urea nitrogen, creatinine and glutamic-oxalacetic transaminase were determined once a month for the first 2 months, and then 24 hours prior to sacrifice. Skin and tissue biopsies, performed as indicated, and post mortem tissues taken from the viscera of all monkeys and from the brains of 3 were subjected to gross and histological studies.

Results. Day 40 after percutaneous exposure to a total of 0.5 mg PDC or PIE,‖ 2 monkeys reacted to the homologous antigen while 9 monkeys failed to react. Using skin test doses ranging from 6.25 to 100 μg in 2-fold increments, monkey 511 reacted down to 25 μg PDC while monkey 515 was positive at 12.5 μg PIE. Day 69 after an additional percutaneous administration of 0.3 mg allergen, monkey 511 became nonreactive to PDC while the reaction of monkey 515 to PIE dropped 8-fold. Monkey 514, not reacting at 6 weeks, reacted weakly to 50 μg PIE at 10 weeks.

The regimen outlined in Table I was continued in order to determine whether any toxic effects or alteration in sensitivity occurred as a result of the parenteral or oral administration of PDC or PIE. Blood counts and chemical analyses of sera remained within the normal range during treatment(7).

‖ Expressed as μg PDC.

Ten weeks after initial exposure to poison ivy extract, one monkey (515) upon skin testing developed a severe generalized exudative rash which subsequently healed. On testing 10 days later the reaction sites remained negative and no generalized rash occurred.

At autopsy, 280 days after the beginning of treatment gross and histologic study of the viscera of all monkeys and the brains of 3 showed no abnormalities related to the feeding or injection of PDC or PIE. Even the monkey which had the episode of generalized rash showed no visceral abnormalities.

Three monkeys taken at random from Groups 1, (508, 512) and II (514) plus an untreated monkey (5161) comprised Group III (Table I). Group III monkeys were negative to percutaneous tests with 100 μg of PDC, even though each monkey had received 6 mg PDC in complete adjuvant 3 weeks prior to testing. However, the same monkeys reacted to 62.5 μg of FDNB seven weeks following percutaneous application of this substance, whereas untreated monkeys were non-reactive to 4000 μg of FDNB.

Attempts at sensitization to poison ivy extract were repeated (Group IV, Table I), and monkeys of this group (673, 810, 811, 812) also failed to react to 100 μg PIE.

Discussion. Complete blood counts, serum chemistries, gross and histologic observations indicated the lack of pathology due to repeated percutaneous, oral and intramuscular

administration of solutions containing PDC or PIE in vegetable oil. The one acute episode of generalized eczema was transitory; its etiology remains unknown and may not have been related to the treatment.

Our results have indicated that the methods employed failed to induce a well developed delayed contact sensitivity to PDC or PIE in monkeys.

Reactivity was induced in 3 of 16 animals. The sensitization induced in these monkeys to PDC and PIE both in terms of percentage sensitized and degree of sensitization attained can be considered minimal when compared to humans(8,9) or guinea pigs(4). Kligman (8), and Straus(9) reported successful sensitization of 73 to 85% of all non-sensitive humans to PDC or PIE while almost 100% sensitization of normal guinea pigs to PDC has been reported(4).

Dermal reactivities of the order of 0.1-0.25 μg have been demonstrated in PDC sensitized guinea pigs(4) and naturally sensitized humans(10). The most sensitive monkey in this investigation reacting to 12.5 μg of PIE showed 50- to 100-fold less dermal reactivity than guinea pigs or humans. Subsequent skin testing neither demonstrated a sensitizing effect of the skin test nor detected any sensitization which might have been slow in developing. Moreover, administration of PDC in complete adjuvant was ineffective in producing contact sensitivity, a procedure known to sensitize nearly all normal guinea pigs(4). The rhesus monkey does not therefore appear to be a suitable model for studies involving contact sensitivity to poison ivy.

The rhesus monkeys used in this study came from the jungles of India, where members of Anacardiaceae (other than poison ivy) such as the marking nut, the mango, and the cashew nut trees are native(11,12). Dermatitis producing antigens found in members of this family share immunologic cross reactivity(8,13,14). One might speculate that the rhesus monkeys did not become sensitized to PDC or the catechols in PIE due to species resistance, or frequent prior exposure causing either primary unresponsiveness or sensitization followed by desensitization. That these monkeys are resistant to PDC or poison ivy sensitization by virtue of prior exposure seems quite possible in light of the capacity of the monkey to exhibit contact sensitivity to FDNB. It has been demonstrated that guinea pigs made unresponsive to PDC still have the ability to be sensitized by FDNB (5).

A repetition of this study using rhesus monkeys bred and reared in captivity might resolve the question of whether prior exposure could account for the refractoriness of the wild animal to sensitization.

Summary. Percutaneous applications of pentadecylcatechol or poison ivy extract to 16 monkeys resulted in sensitization of only one pentadecylcatechol treated and 2 poison ivy treated monkeys. The most sensitive monkey showed 50- to 100-fold less dermal reactivity than guinea pigs or humans. Three monkeys picked at random from the group of non-reactors gave well developed contact reactions to FDNB after primary exposure to it. The specific unresponsiveness of these monkeys to pentadecylcatechol and poison ivy extract might be attributed to their prior contact with antigenically related antigens in their natural habitat. A regimen of oral and parenteral administration of pentadecylcatechol or poison ivy extract produced no toxic effects as judged by blood count and serum chemistry. Gross and histologic studies on the viscera of 12 monkeys and on the brains of 3 failed to show any abnormalities related to the feedings or injection of the antigens.

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Effects of Plasma from Normal and Endotoxin-Tolerant Humans on Endotoxin Fever in Rabbits. (29692)

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Endotoxin tolerance, the relative refractory state that follows a series of injections of endotoxin in both man and experimental animals, has been passively transferred in rabbits(1). Greisman *et al* recently reported that in humans the same dose of endotoxin administered on 2 consecutive days evoked a greater febrile response on the second day(2). Passive transfer of pooled plasma from tolerant subjects reduced the expected enhanced response of the second day to a level comparable to the first(2). This finding was interpreted as the passive transfer of endotoxin tolerance between humans.

Tolerance to the febrile effect of endotoxin has been observed following certain infectious diseases(3,4) while, in contrast, episodes of familial Mediterranean fever will diminish tolerance(5). Furthermore, administration of the pyrogenic steroid, etiocholanolone, enhances febrile reactivity to endotoxin(6). The availability of an experimental model in which the risks of human to human transfer do not exist would be of aid in investigating the factors that may play a role in these states of altered reactivity to endotoxin. The effect of the passive transfer of normal and tolerant human plasma on the febrile reactivity of rabbits to endotoxin is the subject of this report.

Materials and methods. Four normal male volunteers ranging in age from 19 to 24 years comprise the donor group. Lipexal,®* the endotoxin of *Salmonella abortus equi*, employed throughout these studies, was diluted to desired concentration with pyrogen-free physiological saline immediately prior to injection. Pyrogen-free needles, syringes and glassware were used. Two subjects (E.S. and D.D.) received 17 daily injections of endotoxin beginning with a dose of 1 mμg/kg which was increased to a daily dose of 0.25 μg given for 13 days. The other 2 volunteers (J.M. and J.D.) received 12 daily injections of 2 mμg/kg. Febrile tolerance developed in all 4 subjects.

Volunteers remained in bed for 1 hour prior to and 8 hours following each injection during which time rectal temperatures (°C) were obtained at half-hour intervals. Blood was obtained using precautions to insure sterility from each individual prior to and following the induction of tolerance. The blood was anticoagulated with heparin and the plasma separated by centrifugation and stored in sterile flasks at —20°C until used.

Recipient animals were 2 kg New Zealand albino rabbits of either sex. The day prior to testing rabbits were trained in wooden stocks with loosely fitting collars. Temperatures were monitored with thermistors inserted 4 to 6 inches into the rectum and connected to a recording telethermometer (Yellow Springs Instrument Co.). On the day of passive transfer temperatures were re-

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