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THE ALLERGENICITY OF PARAPHENYLENDIAMINE. II

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Abstract. On the basis of intracutaneous sensitization experiments on guinea pigs with 0.001% paraphenylendiamine (PPD), benzoquinone together with quinhydrone and hydroquinone, benzoquinone proved to be the strongest sensitizer. Whereas PPD-sensitized animals frequently reacted to benzoquinone, the reverse occurred only to a lesser extent. The sensitization capacity of quinhydrone is regarded as due mainly to its benzoquinone component. Benzoquinone, whose intracutaneous sensitization capacity was shown to be approximately equivalent to that of DNCB, thus seems to have an important, possibly a decisive, role in PPD-sensitization.

On the basis of patch testing with paper chromatographic strips, which we have undertaken, benzoquinone is considered as an allergen among the intermediates of paraphenylendiamine (PPD) (1). We have endeavoured to explore this question more fully by guinea pig sensitization experiments with chemically well-defined PPD-intermediates since human experiments could have quite serious consequences.

MATERIALS AND METHODS

Intracutaneous rather than epicutaneous sensitization was chosen, partly on account of its greater sensitization capacity (see, e.g. 2) and partly because of the difficulties in reading as a result of the dark colour obtained from epicutaneous sensitization in addition to inflammation (see, e.g. 8). Mayer also usually applied the intracutaneous method of PPD-sensitization of guinea pigs (6).

Female guinea pigs with a mean initial weight of 270 grams were intracutaneously injected during a period of 10 days. The injections were made on the left flank, in a skin area of about 2×2 cm, with the following substances which had been freshly prepared as water solutions: PPD (*p*-phenylendiamine BDH, Laboratory reagent, Poole, England)—20 guinea pigs; Benzoquinone (E. Merck no. 2410, Darmstadt, Germany)—20 guinea pigs; Hydroquinone (Kebo no. A 3180, p.a. Stockholm, Sweden)—18 guinea pigs; Quinhydrone (E. Merck no. 2284, Darmstadt, Germany)—18 guinea pigs.

The concentration was determined in accordance with experiments with the following solutions: 0.1%, 0.01%, 0.001%. The amount injected was 0.1 ml.

Readings were made after provocation with equipotent (see below) solutions, 17 days after the end of the sensitization period. The definitive reading was made 48 hours after provocation according to the following criteria: + = redness, slight infiltration; + + = redness, more pronounced infiltration; + + + = necrosis. Biopsies were performed simultaneously at all the sites. As controls, 18 non-sensitized guinea pigs were used.

In an attempt to ascertain the most suitable sensitization concentration a second reading was made after a further 17 days (see 6).

RESULTS

Table I shows the primary irritant effect of the substances used. Benzoquinone and quinhydrone gave necrotic reactions with 0.1% concentration and weaker reactivity with 0.01%. Hence 0.001% was chosen as the most suitable sensitizing concentration for all four substances. The sensitization capacity of the concentration was tested in comparison with that of 0.1% PPD, after the first and second readings. Table II shows that the sensitization capacity of a 0.001% PPD solution was quite high at the first reading, but was obviously weaker than when a 0.1% solution is employed. Consequently, a second reading

Table I. *Primary irritant effect of intracutaneously applied PPD and other substances tested in guinea pigs*

Concentrations %	PPD	Hydroquinone	Quinhydrone	Benzoquinone
0.1	–	–	+ + +	+ + +
0.01	–	–	+	+
0.001	–	–	–	–

Table II. *Comparison of sensitizing effect of intracutaneously applied PPD 0.1 % and 0.001 % in guinea pigs*

	First test			Second test		
	++	+	−	++	+	−
7 animals sensitized by PPD 0.1 %	1	6	−	3	4	−
7 animals sensitized by PPD 0.001 %	−	5	2	−	6	1

Table III. *Positive sensitization and cross reactions of intracutaneously applied 0.001 % PPD and other substances in guinea pigs*

	Eliciting of sensitivity after 17 days (i.c.)			
Sensitizing agents	PPD	Hydro-quinone	Quin-hydrone	Benzo-quinone
PPD	16/20	4/20	15/20	16/20
Hydroquinone	6/18	4/18	4/18	9/18
Quinhydrone	6/18	2/18	15/18	14/18
Benzoquinone	5/20	1/20	18/20	19/20
Control animals	0/18	0/18	0/18	0/18

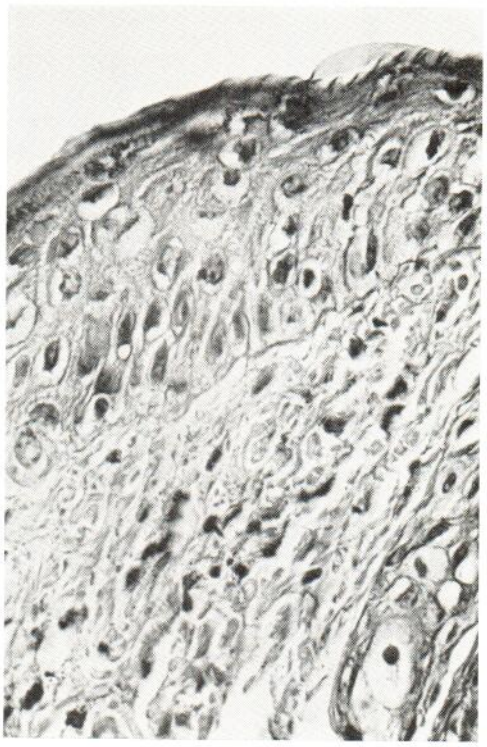

Fig. 1. Microscopic reaction in guinea pig sensitized to PPD.

was not made in subsequent experiments. The difference between the + and ++ reactions was only slight, and no differentiation was subsequently made between them.

In Table III the macroscopic reactions are summarized, when using 0.001 % solutions. It is evident that: (*a*) both benzoquinone and quinhydrone have a somewhat stronger sensitization capacity than PPD; (*b*) frequent reactions (the same frequency as with the original sensitizer PPD) to benzoquinone and quinhydrone were observed in the PPD-treated animals; the reverse

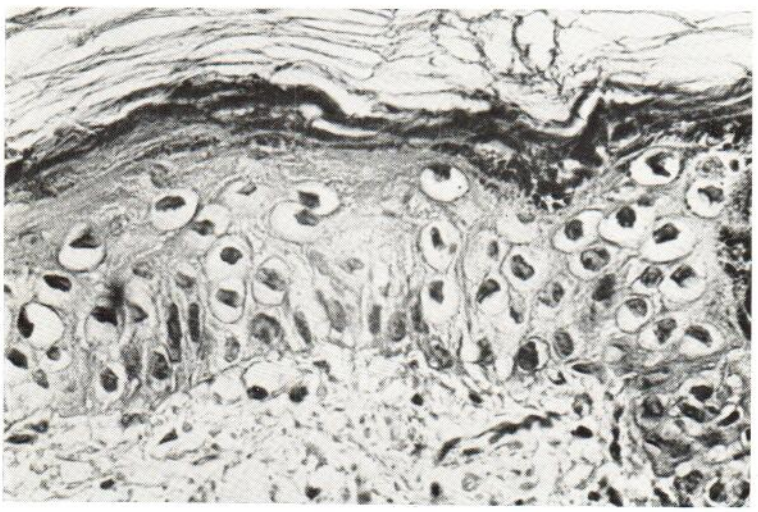

Fig. 2. Microscopic reaction in guinea pig sensitized to benzoquinone.

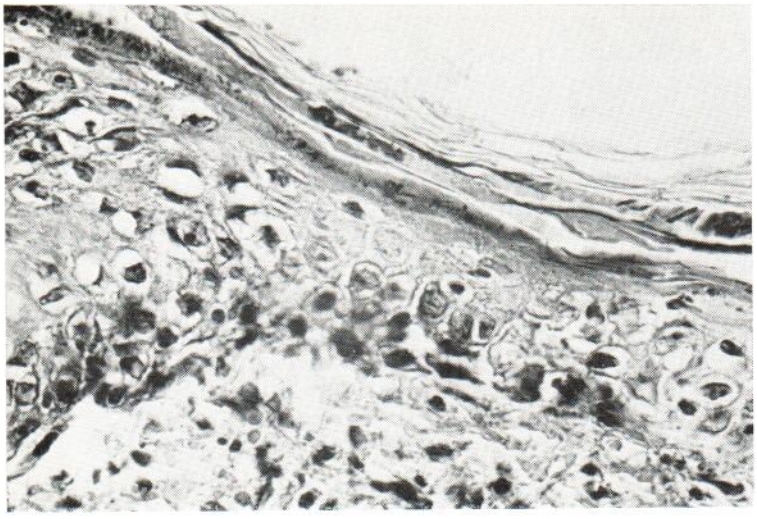

Fig. 3. Microscopic reaction in guinea pig sensitized to quinhydrone.

was, however, not the case (PPD provoked comparatively few reactions in animals sensitized with benzoquinone and quinhydrone respectively); (*c*) the reactions to benzoquinone and quinhydrone were, on the whole, similar, but the former provoked somewhat more frequent reactions; (*d*) in this comparison the sensitization capacity of hydroquinone was relatively low.

There was good parallelism between the *microscopic* and macroscopic findings. In the epithelium intracellular edema and, in most cases, spongiosis were also observed. In the corium there were edema, perivascular infiltration and diffuse infiltrates consisting mainly of lymphocytes, some leukocytes, several fragments of leukocyte nuclei and individual eosinophils (Figs. 1–4). The controls did not show any macroscopic reactions. Microscopically both epithelium and corium were normal.

DISCUSSION

The intracutaneous sensitization method gave an unexpectedly high frequency, considering that sensitization with PPD and the other substances was performed with an amount of 10 μg. Similar results were obtained with 2,4-dinitrochlorobenzene (DNCB), well-known for its marked sensitization capacity. With DNCB, Klaschka, for example, sensitized 13 out of 15 guinea pigs with 10 μg (4). Skog obtained a high sensitization fre-

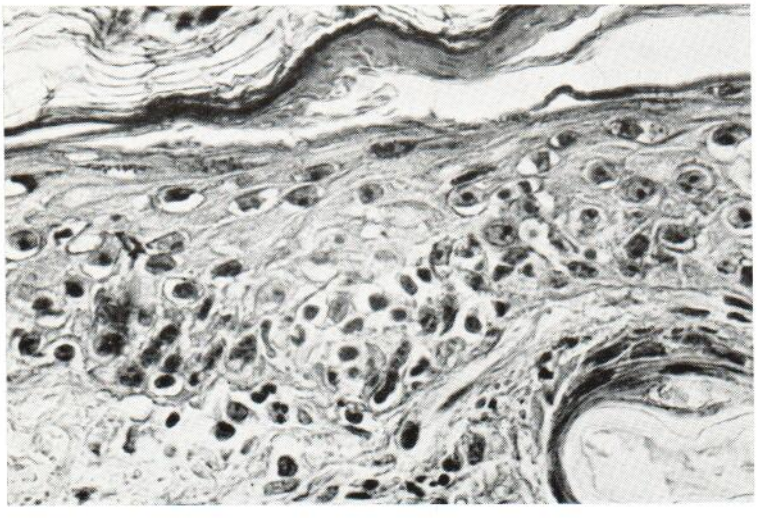

Fig. 4. Microscopic reaction in guinea pig sensitized to PPD, the sensitivity elicited by benzoquinone.

quency with 15 μg of DNCB (7), and Frei & Geleick obtained positivity in 3 of 8 guinea pigs with even smaller sensitization quantities, i.e. 20 μg/kg (2). This implies that PPD has a very marked sensitization capacity when administered intracutaneously. In contrast to DNCB, its primary irritant effect is minimal, even at higher concentrations (8). Magnusson (5) also considers that water is the most suitable solvent for intracutaneous PPD sensitization experiments. In this series there was a high correlation between macroscopic and microscopic reactions, which is not otherwise an indispensable phenomenon in experimental sensitization. The microscopic findings are in very good agreement with similar observations of DNCB sensitization (4). From the macroscopic/microscopic experiments it is evident that benzoquinone and, to a certain extent, quinhydrone also, are more potent sensitizing substances than PPD. According to the results mentioned under (*a*) and (*b*) in this respect the situation may be characterized as follows:

PPD $\rightleftharpoons$ Benzoquinone.

Quinhydrone, a dark-green substance, is formed from equimolecular quinone and hydroquinone. These two components saturate each other (3) —which is the reason for having included hydroquinone in the present sensitization experiments. Hydroquinone was shown to have a relatively less pronounced sensitization capacity. Benzoquinone not only had the strongest capacity, but was also a somewhat more potent sensitizer than quinhydrone. There is thus good reason to believe that the sensitization capacity of quinhydrone is due mainly to its benzoquinone component.

Benzoquinone proved to be the strongest sensitizer in the group; stronger than the actual initial product, PPD. This is in good agreement with the previous experiment (1), of the present investigators in which it was stated that benzoquinone and its derivatives are the actual allergens in the case of some tropical trees and of primin. Hence, on the basis of our experiments, it is probable that benzoquinone and its derivatives have an important, possibly a decisive, role in PPD-sensitization.

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