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STUDIES ON THE EFFECT OF SYSTEMIC ADMINISTRATION OF SENSITIZERS IN GUINEA-PIGS WITH CONTACT SENSITIVITY TO INORGANIC METAL COMPOUNDS

I. THE INDUCTION OF IMMUNOLOGICAL UNRESPONSIVENESS IN ALREADY SENSITIZED ANIMALS

L. POLÁK AND J. L. TURK

Department of Immunology, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Homerton Grove, London

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SUMMARY

A permanent state of specific immunological unresponsiveness can be produced in guinea-pigs already sensitive to $K_2Cr_2O_7$ by a combination of the intravenous injection of 20 mg/kg $K_2Cr_2O_7$ and an epicutaneous test with this compound given 24 hr after the intravenous injection. The later the first epicutaneous skin test after the intravenous injection is delayed, the shorter the period of unresponsiveness. An intravenous injection of less than 20 mg/kg also produces only a temporary state of desensitization. The time that animals became resensitized was proportional to the dose of $K_2Cr_2O_7$ given intravenously.

Intravenous injection of Be lactate into guinea-pigs sensitive to BeF_2 produces only a temporary desensitization lasting no longer than 48 hr.

It is suggested that the state of permanent unresponsiveness induced to $K_2Cr_2O_7$ after the animals have already been made sensitive is an example of 'high zone' immunological tolerance.

INTRODUCTION

The induction of immunological unresponsiveness to simple chemical contact sensitizing agents by the systemic administration of a large dose of the chemical as late as 24 hr after attempted sensitization was first described by Sulzberger (1929) with neoarsphenamine. These findings were enlarged and studied further by Frey, de Weck & Geleick (1966a). In a further communication (Frey, de Weck & Geleick, 1966b) these workers found that they could induce a prolonged state of specific immunological unresponsiveness in animals

Correspondence: Dr J. L. Turk, Department of Immunology, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Homerton Grove, London, E.9.

already sensitized by giving a large dose of the sensitizer intravenously associated with an intradermal injection of a small dose of the substance given between 6 hr prior to and 12 hr after the intravenous injection. A smaller dose intravenously or a longer period between the intravenous injection and the intradermal injection only produced a temporary state of desensitization. This state of temporary desensitization in these cases was similar to that produced to dinitrochlorobenzene (DNCB) (Frey & Geleick, 1962) and by Uhr (1958) to ovalbumin and diphtheria toxoid.

In initial studies on the effect of the intravenous injection of large doses of potassium dichromate ($K_2Cr_2O_7$) intravenously to guinea-pigs which were already sensitive, we found that it was possible to produce a prolonged state of specific unresponsiveness similar to that described by Frey *et al.* (1966b) for neoarsphenamine. It was, therefore, decided to study further the effect of the intravenous injection of different doses of the compound and also to find out to what extent the unresponsiveness could be modified by prolonging the time between the intravenous injection of the compound and the subsequent application of potassium dichromate to the skin to determine whether the animal was still sensitive or had become unresponsive. These results were compared with results obtained in guinea-pigs sensitized with beryllium fluoride (BeF_2).

MATERIALS AND METHODS

Animals

Albino guinea-pigs of the Hartley strain, bred in the Institute of Dermatology, were used, weighing between 400 and 600 g. They were fed on pelleted diet RGP (E. Dixon & Son, Ware, Herts) liberally supplemented with fresh greens and hay.

Sensitization

Guinea-pigs were sensitized by the intramuscular injection of 1 mg $K_2Cr_2O_7$ (Analar, Hopkin & Williams) in 1 ml Freund's complete adjuvant containing *M. tuberculosis* H37Ra (Difco). The emulsion was injected intramuscularly in five divided doses given at the same time into the muscles of the neck and thighs. Two weeks later they were injected intradermally with 25 μg $K_2Cr_2O_7$ in 0·1 ml of 0·15 M-NaCl. This was repeated again once or twice at weekly intervals. At the same time they were painted on the clipped skin of the flank with 0·5% $K_2Cr_2O_7$ in 1% Triton X 100. This was repeated weekly until they had reached a steady state of sensitization. This took approximately 4–8 weeks.

Sensitization to BeF_2 was produced by painting one ear with 0·05 ml of a 20% solution of BeF_2 in detergent each day for 3 days. The animals were tested by skin painting with 1% BeF_2 in 1% Triton X 100 on the flank. This concentration did not produce any reaction in non-sensitized animals.

Skin testing

Guinea-pigs were painted on the clipped skin of the flank with 0·5% $K_2Cr_2O_7$ or 1% BeF_2 in 1% Triton X 100. Skin reactions were read 24 hr later and graded:

++	Bright red confluent raised reaction.
+	Definite red reactions but not raised.
0	Negative.

Intravenous injection of $K_2Cr_2O_7$ and BeF_2

$K_2Cr_2O_7$ was injected intravenously through the marginal vein of the ear as a 1, 0·5, 0·2, 0·01 or 0·001% solution depending on the amount of material to be injected. Only strongly sensitive animals were used and the intravenous injection was followed by a first skin test given either after 6 hr, 24 hr, 48 hr, 4 days, 2 weeks and 3 months.

Guinea-pigs sensitive to BeF_2 were injected intravenously with 5 mg/kg beryllium lactate 1 week after a previous positive contact reaction. They received their first skin test after 24 hr and this was repeated 48 hr, 96 hr and 1 week after the intravenous injection.

EXPERIMENTAL RESULTS

Unresponsiveness of animals following intravenous injection of 20 mg/kg $K_2Cr_2O_7$

Guinea-pigs sensitized to $K_2Cr_2O_7$ were injected intravenously with 20 mg/kg $K_2Cr_2O_7$. This dose was the LD_{50} for this compound in the guinea-pig. If animals were skin tested before 24 hr after the injection they were found to be immunologically unresponsive for a

TABLE 1. Unresponsiveness of animals previously sensitized to potassium dichromate, following intravenous injection of 20 mg/kg potassium dichromate

Interval between intravenous injections and first epicutaneous skin test	Proportion of animals responsive when tested after											
	Hours			Days			Months					
	6	24	48	4	7	14	1	2	3	4	5	6
6 hr	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/5	0/5	0/4	0/4
24 hr		0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
48 hr			0/10		5/9	5/9	6/9	5/9	6/9	5/9	6/8	6/8
4 days				0/9	2/8	4/8	4/8	5/8	7/8	7/8	7/7	7/7
14 days						3/10	7/10	6/9	9/9	7/7	7/7	7/7
3 months									7/9	7/9	6/8	3/3
Control (no intravenous injection)							8/8	7/7	7/7			

period of at least 6 months. However, if the first skin test was left as long as 48 hr after the intravenous injection over half the animals became responsive within 7 days although a number remained unresponsive for as long as 6 months. If the first skin test was delayed for 4 days a similar pattern was found in that all the animals were unresponsive when first tested, one remained unresponsive for as long as 4 months but many began to react again between 1 and 2 weeks after the injection. With the first skin test left to 2 weeks after intravenous injection three out of ten animals already reacted when first tested and most became reactive between 1 and 2 months. If the first skin test was left to 3 months only, two out of nine animals were still unresponsive. These results are shown in tabular form in Table 1 and graphically in Fig. 1. The unresponsiveness was always immunologically specific in that guinea-pigs when unresponsive to $K_2Cr_2O_7$ would react to another contact agent, e.g. oxazolone, and to tuberculin.

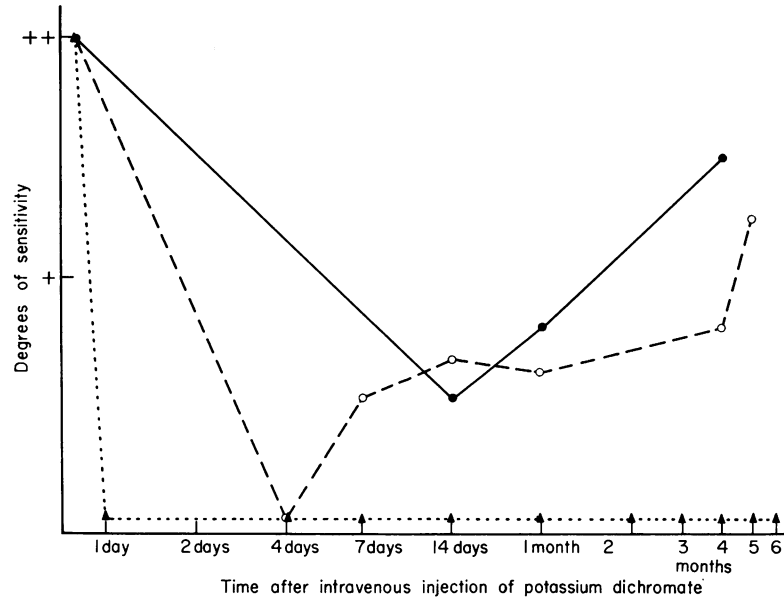

FIG. 1. Unresponsiveness of potassium dichromate sensitized guinea-pigs by intravenous injection followed by epicutaneous painting with 0·5% potassium dichromate. Intravenous injection constant at 20 mg/kg. Interval between intravenous injection and first epicutaneous painting: ▲, Up to 1 day; ○, 2 days; ●, 4–14 days.

Dose dependency of unresponsiveness

If the first skin test was kept constant at between 6 and 24 hr after the intravenous injection of $K_2Cr_2O_7$ it can be shown that (Table 2) the unresponsiveness was markedly dependent on the dose of $K_2Cr_2O_7$ injected intravenously. Whereas with a dose of 20 mg/kg (the LD_{50}) unresponsiveness would persist for at least 6 months, with half the dose (10 mg/kg), although animals were initially unresponsive, most regained reactivity within 1 week.

TABLE 2. Unresponsiveness of animals previously sensitized to potassium dichromate, following intravenous injection of different doses (interval between intravenous injection and first epicutaneous test up to 24 hr)

Dose of potassium dichromate injected (mg/kg)	Proportion of animals responsive when tested after				
	24 hr	1 week	2 weeks	4 weeks	6 months
20	0/10	0/10	0/10	0/10	0/8
10	0/6	5/6	—	6/6	—
2	4/7	6/7	7/7	—	—
0·1	4/7	7/7	—	—	—
0·01	7/7	—	—	—	—

With a dose of 2 mg/kg only about half the animals became temporarily unresponsive and this persisted for between 1 and 2 weeks. These results are shown graphically in Fig. 2. A dose of 0·1 mg/kg produced a temporary unresponsiveness lasting only 24 hr in three out of seven animals. A dose of 0·01 mg/kg failed to produce any unresponsiveness in seven animals tested.

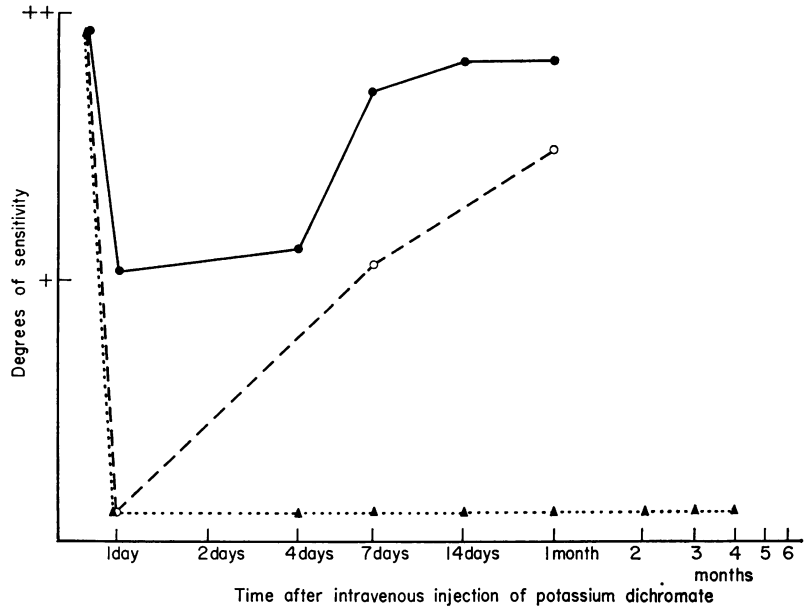

FIG. 2. Unresponsiveness of potassium dichromate sensitized guinea-pigs by intravenous injection followed by epicutaneous painting with 0·5% potassium dichromate. Interval between intravenous injection and epicutaneous test constant up to 24 hr. Dose of $K_2Cr_2O_7$: ▲, 20 mg/kg; ○, 10 mg/kg; ●, 2 mg/kg.

Unresponsiveness to BeF_2

Following the intravenous injection into fourteen beryllium sensitive guinea-pigs of 5 mg/kg beryllium lactate, a dose which killed 30% of animals, all guinea-pigs were found unresponsive on first skin testing 24 hr later, but eight out of fourteen had become responsive again when skin tested 48 hr later, when skin tested 96 hr after intravenous injection all the survivors (nine out of nine) had regained their responsiveness to contact sensitivity to BeF_2.

DISCUSSION

The development of immunological unresponsiveness of guinea-pigs already sensitized to simple inorganic metal sensitizers following intravenous injection of the sensitizer, can be compared with that produced to other sensitizers DNCB (de Weck, Frey & Geleick, 1964) and neoarsphenamine (Frey *et al.*, 1966b). Intravenous injection of dinitrobenzene sulphonic

acid ($DNBSO_3$) in a marginally sublethal dose produces a state of unresponsiveness lasting no more than 48 hr. However, with neoarsphenamine these workers were able to produce a prolonged state of unresponsiveness regularly if they followed a large intravenous dose of the sensitizer with an intradermal injection of a small dose within 6–12 hr. It would appear, therefore, that a similar phenomenon to that produced with neoarsphenamine can be produced with $K_2Cr_2O_7$ whereas the intravenous injection of BeF_2 has an effect more like that of $DNBSO_3$. Prolonged unresponsiveness to $K_2Cr_2O_7$ can only be produced by the injection of the highest dose tolerated by the animals (20 mg/kg). A lower dose appears to have an effect little different from that produced by $DNBSO_3$. It would, therefore, appear that we are dealing with two different phenomena. One is a state of peripheral densensitization, which is produced by $DNBSO_3$ (de Weck *et al.*, 1964; Turk, 1965), BeF_2 and low doses of neoarsphenamine or $K_2Cr_2O_7$, and high doses followed by a first skin test left for some time after the intravenous injection. In this case there is no evidence of any effect on the central immunological mechanisms and once the concentration of sensitizer drops below a critical level the animal becomes resensitized. However, with a high dose of sensitizer intravenously, which would be above the lethal level for $DNBSO_3$ or BeF_2, followed within 24 hr by a skin test, the effect is one of a specific central inhibition of the immunological mechanisms, so that the animal becomes permanently and specifically unresponsive. Between these two extremes a state of prolonged unresponsiveness can exist lasting for a month or more, after which the animal becomes gradually resensitized.

Following the work of Mitchison (1964) it has become accepted that immunization can precede a state of specific immunological paralysis or unresponsiveness. This had, however, been shown previously with simple protein antigens (Glenny & Hopkins, 1924) and transplantation antigens (Brent & Gowland, 1962). This type of paralysis would appear to need a large dose of protein antigen to be effected and has been referred to as the 'high zone' type to distinguish it from the 'low zone' type in which microgram amounts of protein antigen will cause a specific state of unresponsiveness, in a previously unsensitized animal, to further stimulation with a protein antigen.

The need for a skin test within 24 hr of intravenous injection of sensitizer, to produce prolonged unresponsiveness regularly, has to be explained. It is likely that the intravenous injection causes the inactivation of sensitized cells in the periphery. It may be that the subsequent skin test makes certain cells in the central lymphoid tissues which would normally be susceptible to stimulation by low doses of sensitizer, susceptible to inactivation by the high dose of sensitizer injected intravenously. The effective dose to cause inactivation of these central cells has to persist for a certain period of time. The difference between $K_2Cr_2O_7$ and neoarsphenamine, and BeF_2 and $DNBSO_3$ might be that in the first group the effective dose of sensitizer persists in the tissue long enough to cause central inactivation, whereas in the second group the compound is eliminated in such a way that the level falls below that which can cause inactivation of the central mechanisms, before it can occur.

It may, however, be that a large proportion of central cells, which have the potential of being specifically activated, are also inactivated by the intravenous injection. However, a small number of cells remain which are only susceptible to conjugates formed between the sensitizer and the skin. These cells will then get inactivated by the subsequent application of the sensitizer to the skin. Because the amount of conjugate formed between the sensitizer and skin protein is so low, skin painting has to be within a limited period after the intravenous injection to be effective.

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