was used as the acceptor. This inhibition was also reversed by coenzyme Q.

Tappel (*7*) suggested that the inhibition by naphthoquinone derivatives appears to be related to their lipophilic nature. The results presented here may suggest that the reversal effect of coenzyme Q on the inhibition of the reductase by lipophilic compounds may be due to the lipophilicity of coenzyme Q. Recently, Green and co-workers (*4*, *8*) have considered coenzyme Q as a "floating" component of the respiratory chain. Indeed, Redfearn (*2*) and Chance (*9*) have reported that only a fraction of the coenzyme Q in mitochondrial preparations undergoes oxidation-reduction commensurate to the overall rate of electron transfer. The reversal effect of coenzyme Q on these inhibitions, in a certain sense, is in accord with the floating concept.

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Immunological Tolerance Induced in Animals Previously Sensitized to Simple Chemical Compounds

Abstract. *Adult guinea pigs develop a delayed hypersensitivity to intradermally injected neoarsphenamine. If the animals are treated intravenously with high doses of this compound before sensitization, a specific and permanent immunological tolerance develops. Permanent tolerance is also obtained in previously sensitized animals when an intravenous injection of neoarsphenamine is followed 6 hours later by an intradermal one. When, however, the interval between the two injections is extended to 7 or 14 days, no tolerance is observed.*

In the "Sulzberger-Chase" type of tolerance as defined by Medawar (*1*) a specific and long-lasting state of unresponsiveness to simple chemicals is induced in adult guinea pigs by administering the compounds before sensitization. The experiments initiated by Frei (*2*) and Sulzberger (*3*) with neoarsphenamine have not been reproduced on a broad scale (*4*) because of the irregular responses obtained when different strains of guinea pigs or different batches of the sensitizing compound were used (*5*).

Tolerance studies with simple chemical compounds that provoke allergic contact dermatitis have therefore been pursued by "feeding" adult animals with compounds like dinitrochlorobenzene and picryl chloride before sensitization (*6*), by feeding or injecting these substances intraperitoneally to pregnant animals in order to produce tolerance in their offspring (*7*), or by intravenous injection to adult animals (*8*, *9*). But up to now the state of permanent tolerance has never been achieved if previously sensitized animals were used instead of unsensitized animals (*10*).

We have reproduced and confirmed Sulzberger's experiments and have obtained quite regularly a state of cutaneous hypersensitivity manifested by a tuberculin-type response to neoarsphenamine injected intradermally. Furthermore, we have been able to induce tolerance in untreated animals as well as in previously sensitized animals. Our experiments were performed as follows.

Sensitization of untreated animals was studied in an experiment in which 22 spotted Himalayan guinea pigs of both sexes weighing 450 to 500 g and derived from a closed colony were sensitized by intradermally injecting on the shaved flank 0.35 mg of neoarsphenamine (*11*) per kilogram of body weight. The neoarsphenamine was dissolved in 0.1 ml normal saline. This injection provoked a pale reddish papule, 5 to 7 mm in diameter, which persisted for 24 to 36 hours (primary toxic reaction). Seven to 14 days later, 14 animals spontaneously developed a "flare-up" at the site of injection characterized by a larger, strongly red, infiltrated papule, 9 to 13 mm in diameter, which persisted 4 to 8 days. Twenty-one days after the first injection, the animals received an identical dose of neoarsphenamine (test) in the other flank, and 12 to 24 hours later 18 of the 22 developed a large, strongly red, infiltrated papule, 12 to 16 mm in diameter, which persisted for 3 to 5 days leaving some desquamation (tuberculin-type reaction). Histologically, these lesions are indistinguishable from the tuberculin reaction observed in animals we infected with BCG (Bacille Calmette-Guérin) and tested with purified protein derivative, and they correspond morphologically to those described by Waksman (*12*) and Arnason and Waksman (*13*).

Subsequent intradermal injections (tests) always provoked the same tuberculin-type reactions which contrasted with the small primary toxic reactions seen in these animals after the first injection or in unsensitized control animals. This state of cutaneous delayed hypersensitivity persisted for 21 weeks, as demonstrated by subsequent tests performed first at intervals of 1 week, and then at intervals of 1 and 2 months. The hypersensitivity was further evidenced by the fact that subsequent intradermal tests provoked a generalized dermatitis in 5 out of 18 animals. Four additional groups of eight animals each were sensitized in the same manner but were tested weekly for only 2 to 3 months; these showed a similar proportion of flare-ups and sensitization.

In animals so sensitized, no contact dermatitis could be provoked by epicutaneous application of neoarsphenamine, nor could an anaphylactic shock be elicited by intravenous injection of this haptene.

Induction of tolerance in untreated animals was studied in a second series of experiments in which 60 mg of neoarsphenamine per kilogram of body weight in a 3-percent aqueous solution was injected into a vein of the hind leg of 12 animals. As in Sulzberger's experiment, these animals had received an intradermal injection of 0.35 mg of this substance per kilogram of body weight 24 hours earlier. Twenty-one

days later, the animals were sensitized intradermally with neoarsphenamine (0.35 mg/kg) as described earlier. From the 28th day onward, these animals were tested intradermally, first at weekly intervals and later at intervals of 1 and 2 months over a total period of 21 weeks. In none of these animals could either a flare-up, a tuberculin-type reaction, or a generalized dermatitis be provoked; they showed only a primary toxic reaction.

A further group of eight animals was treated with neoarsphenamine intravenously before any intradermal injection. Twenty-one days later they were sensitized intradermally and from the 28th day onward they were tested weekly for 9 weeks. In these animals as well, only small papules (primary toxic reactions) were provoked, as seen in unsensitized controls, which contrasted with the large papules (tuberculin-type reactions) seen in sensitized animals already described.

Induction of tolerance in previously sensitized animals was studied in a third experiment in which a group of 28 animals was sensitized intradermally with neoarsphenamine as described. Fourteen showed a spontaneous flare-up 7 to 14 days later, and 23 became sensitized, as demonstrated by an intradermal test on the 21st day. On the 28th day, the 23 sensitized animals were given neoarsphenamine (60 mg/kg) intravenously, and 6 hours later they were given an intradermal dose (0.35 mg/kg). This test, as well as all subsequent tests performed, first at intervals of 1 week and later after 1 and 2 months over a period of 28 weeks, was completely negative. This state of immunological unresponsiveness is specific, since the intravenous injection of neoarsphenamine did not interfere with the immunological response to dinitrochlorobenzene in five animals sensitized concomitantly with both compounds.

In order to determine whether this state of tolerance is due either to an overloading with, or a long persistence of, the haptene in the body, or both, further experiments were performed with a varying interval between the intravenous and the intradermal injections. When 6 or 12 hours elapsed, all 31 animals used became permanently tolerant. When the interval was extended to 1 or 3 days, 7 of 12 animals became unresponsive. No tolerance was achieved in 17 animals when 7 or 14 days elapsed between both injections (confirmation of results obtained by Sulzberger).

It seems, therefore, that for this particular compound tolerance in previously sensitized animals is obtained only when an intravenous injection of the haptene is followed some hours later by an intradermal one. That tolerance may not be due to the persistence of haptene in the body is indicated by the fact that animals receiving injections at intervals of 6 or 12 hours are still tolerant when tested 14 days later, whereas those injected at intervals of 7 and 14 days showed sensitivity, the time available for elimination of the intravenously given haptene being the same in all groups. To determine if tolerance persists because of renewed haptene supply by repeated testing, the first control test was performed in further groups of similarly tolerant animals as late as 3 months after induction of tolerance. Again, all 23 animals used remained unresponsive.

In comparable experiments (*9*) which we performed in animals sensitized to dinitrochlorobenzene (contact dermatitis), an intravenous injection of the sodium salt of dinitrobenzenesulfonic acid induced complete but only temporary inhibition of an epicutaneous test performed 6 hours later, the original degree of sensitivity being restored after 3 to 5 days. In the present experiments, previously sensitized animals injected with neoarsphenamine intravenously and 6 hours later with neoarsphenamine intradermally remained unresponsive for 28 weeks. It is generally recognized that tolerance will be maintained only so long as enough antigen remains available. Differences in the duration of unresponsiveness obtained with dinitrochlorobenzene compared with that obtained with neoarsphenamine might reflect merely variations in the pharmacokinetics of the haptenes used, but could also be due to fundamental differences in the manner of impairment of the immunological response.

In conclusion, the induction of tolerance of the "Sulzberger-Chase" type is possible in previously sensitized animals, as is the case in the "Felton" and the "protein-overload" type (*1*). It should be noted, however, that the immunologic reaction to neoarsphenamine that we succeeded in repressing is of the tuberculin type, whereas in Chase's "feeding" experiments as well as in the experiments of Coe and Salvin (*10*) and our own performed with dinitrochlorobenzene, we were dealing with a contact dermatitis.

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Reevaluation of Assays Used To Show RNA Induction of Glucose-6-Phosphatase in Ascites Cells

Abstract. *The standard procedures —Glucostat method and Fiske-Subbarow phosphorus determination—are inadequate for the assay of glucose-6-phosphatase activity in Ehrlich ascites cells. Therefore, RNA induction of this enzyme in ascites cells, even if it occurs, cannot be demonstrated by these procedures.*

According to recent reports (*1*, *2*), Ehrlich ascites cells, known to lack glucose-6-phosphatase and tryptophan pyrrolase activities, can be induced to exhibit these activities by treatment with RNA from liver, an organ possessing high levels of these enzymes. Furthermore, the induced tryptophan