EFFECT OF CYCLOPHOSPHORAMIDE, 6-MERCAPTOPURINE, ACTINOMYCIN D AND VINCALEUKOBLASTINE ON THE ACQUISITION OF DELAYED HYPERSENSITIVITY (DNCB CONTACT DERMATITIS) IN THE GUINEA-PIG*

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Immediate type allergy deals with antibodies that are relatively self-contained molecules and which are frequently found in the blood serum. These antibodies account for the classical reactions of agglutination, complement fixation and so forth. Further, several investigators have shown that an appropriately timed course of whole body irradiation or of one of several cancer chemotherapeutic drugs may sometimes be arranged so as to inhibit antibody formation to first exposure to antigen (1, 2). For instance, a rabbit given high doses of 6-mercaptopurine and then injected with bovine serum albumin (BSA) fails to make anti-BSA antibody, whereas BSA injected control rabbits produce anti-BSA antibody of high titer (3).

In contrast to immediate hypersensitivity, a convincing array of unrelated observations indicates that the specific antibody-like stuff of delayed hypersensitivity is intimately associated with the circulating lymphocyte and is *not* found freely circulating in the blood serum (4). Included in delayed allergy are tuberculin hypersensitivity, the homograft reaction, contact dermatitis and, at least in part, organ allergies such as allergic encephalitis. Pretreatment with high doses of whole-body x-ray will inhibit the acquisition of delayed hypersensitivity (5, 6). However, the literature raises considerable question as to whether a similar suppression of delayed hypersensitivity can be achieved with the cancer chemotherapeutic drugs (7–11). This study is directed at just that issue, namely—can a delayed type sensitization following first exposure to antigen be importantly frustrated by a cancer chemotherapeutic drug?

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Cyclophosphoramide (Cytoxan®) was supplied by Paul A. Walter, M.D., Mead Johnson Laboratories, Evansville 21, Indiana; 6-mercaptopurine by Donald S. Searle, M.D., Burroughs Wellcome & Co., Tuckahoe, N. Y.; vincaleukoblastine by J. A. Armstrong, M.D., Eli Lilly and Co., Indianapolis 6, Indiana; and Actinomycin D by Elmer Alpert, M.D., Merck Sharp and Dohme, West Point, Pa.

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METHOD

Cyclophosphoramide (Cytoxan®) was the test cancer chemotherapeutic drug. This compound has found wide clinical use especially in lymphomatous malignancy; in large dosages it will produce leukopenia in man and guinea-pig.

Our model for delayed hypersensitivity is DNCB (1-Chloro-2,4-dinitrobenzene) contact dermatitis in the guinea-pig. Our sensitizing and eliciting (challenging) procedures are an adaptation of classical procedures.† Stock albino guinea pigs are purchased from a local dealer; in a given experiment control and experimental animals are drawn from the same lot. Animals are sensitized by a single intradermal injection into the shaved pre-sacral skin of 0.1 cc. of a warmed solution of 1% DNCB in propylene glycol. A few days prior to challenge a large area of the back is prepared by Zip® wax epilation. The challenge is made with 0.05 cc. of 0.1% DNCB in absolute ethyl alcohol which material is pipetted onto a pre-marked circular area 2.1 cm. in diameter. A warm air current from a commercial hair dryer facilitates evaporation of solvent; care is taken to uniformly distribute the DNCB alcohol solution (Fig. 1 and 2). Reactions are read at 24 and 48 hours and recorded according to classical criteria. Numerous control studies have shown this DNCB challenge dose to be non-irritating to the *non*-sensitized guinea-pig.

The experimental animals received daily IP injections of cyclophosphoramide (10 mgm/d. × 14 d). On day 8 the experimental group as well as a control group were injected with a sensitizing dose of DNCB as described above. Both groups were challenged at various later times; to avoid the possibility of sensitizing the animals with our challenge dose, we selected non-recurring aliquots

† As standardized by Bertil Magnusson, M.D.

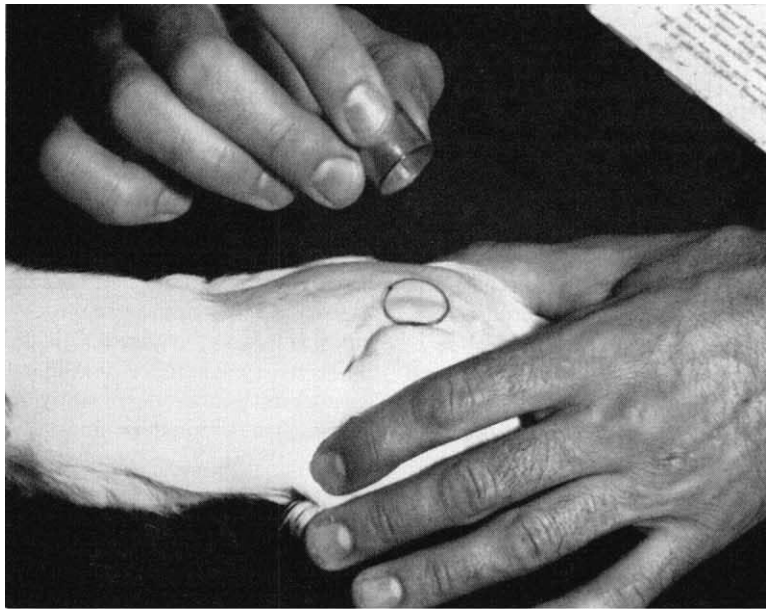

FIG. 1. A non-irritated area on the ZIP®-epilated back of the guinea pig is marked prior to challenge.

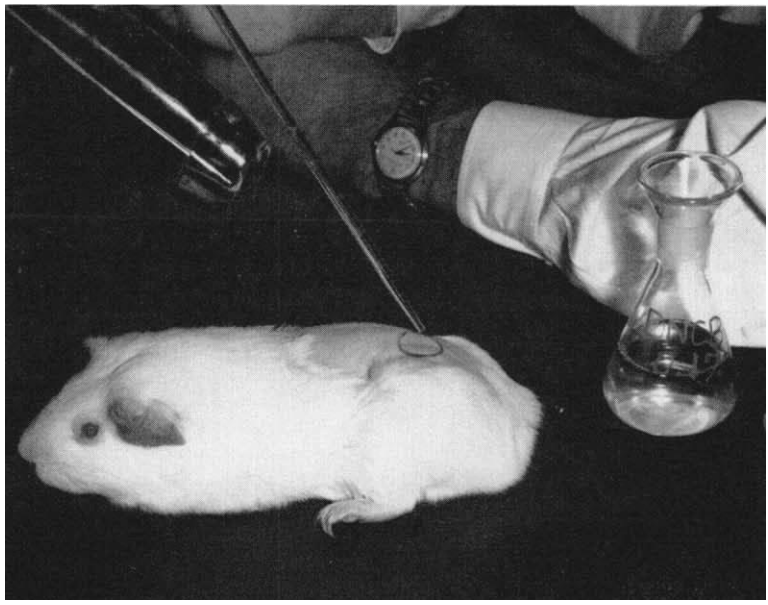

FIG. 2. The challenge dose is pipetted onto the test site.

of animals from each group for successive eliciting tests as often as possible.

In a correlative experiment we set about to determine the differential effect on delayed hypersensitivity of a variety of anti-cancer drugs. We used Actinomycin D, vincaleukoblastine, 6-mercaptopurine and cyclophosphoramide. These compounds will be recognized for the heterogeneity of their chemistry, pharmacology, toxicology and therapeutic spectrum; of the four, only cyclophosphoramide induced marked leukopenia in the guinea-pigs. However, all these drugs are potent cellular poisons and were administered in dosages close to or exceeding their LD_{50}s (Fig. 3). Surviving animals were all sick during drug administration and for a short time afterwards. All

4 experimental drug groups as well as an untreated control group were injected with a sensitizing dose of DNCB on the third day after start of drug treatment.

RESULTS

Cyclophosphoramide clearly delays the onset of DNCB contact dermatitis. (Fig. 4). This could mean either (a) inhibition of sensitization or (b) inhibition of challenge reaction (masking of sensitization). To decide between these alternatives we undertook the following control experiment. Twenty guinea-pigs were sensitized to DNCB; *then* they were treated with cyclophosphoramide (10 mgm./d × 14 d). After start of cyclophosphoramide these already sensitized animals were challenged with DNCB every 2–3 days. Their challenge responses appeared entirely like that of a *non*-cyclophosphoramidized DNCB-sensitized control group. Cyclophosphoramide did not significantly alter the picture of the DNCB eliciting reaction.

Projecting back to the main experiment, it is clear that the cyclophosphoramide induced delay in onset of DNCB contact dermatitis is due to inhibition of the sensitization. We are not dealing with sensitized animals who experimental circumstance has made temporarily refractory to challenge.

The attempts to delay onset of hypersensitivity with Actinomycin D, vincaleukoblastine and 6-mercaptopurine were entirely unsuccessful in the correlative experiment; these animals became sensitive at the same time and to the same degree as the control animals. The failure with these drugs is particularly noteworthy since the guinea-pigs of all three groups, and especially of the Actinomycin group, showed a marked morbidity throughout the entire incubation period of sensitivity (Fig. 3). Our positive controls, the cyclophosphoramide animals, became sensitive some fourteen days after the other guinea-pigs.

How does cyclophosphoramide inhibit the onset of delayed hypersensitivity? Cyclophosphoramide is a potent and unique cellular poison with particularly high activity against nuclear protein. The leukocyte is especially susceptible to its toxic properties. Stated in a general way it seems likely that the drug sabotages the antibody-making machinery just at that time when it has received rush production orders for large numbers of a new commodity. The exact and necessary site(s) of damage is not known; but that it is temporary under the conditions of our experiments is attested to by the later appearance of fully-formed high-quality DNCB sensitivity.

	Intraperitoneal Dosage	Three Week Survivors*
Cyclophosphoramide	10 mgm/d × 10 d.	16/20
6-Mercaptopurine	50 mgm/d × 10 d.	15/20
Vincaleukoblastine	10 gamma/d × 10 d.	12/20
Actinomycin D	10 gamma/d × 10 d.	4/20

* Average weight of guinea-pigs at start of experiment was 400 grams.

FIG. 3

SUMMARY

Cyclophosphoramide (Cytoxan®) pretreatment greatly delays contact dermatitis type sensitization to DNCB in the guinea-pig. Actinomycin D, vincaleukoblastine and 6-mercaptopurine in toxicologically comparable amounts were completely unable to prolong the incubation of the sensitivity.

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	Days* after DNCB Sensitizing Injection				
	Day 6	Day 10	Day 13	Day 17	Day 20
Control animals	8/8—pos.	4/4—pos.	4/4—pos.	4/4—pos.	8/8—pos.
Cyclophosphoramide animals	0/4—pos.	0/4—pos.	1/8—?pos. 7/8—neg.	0/4—pos.	8/8—pos.

* The day of the 24 h. reading.

FIG. 4

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DISCUSSION

Dr. Rudolf L. Baer (New York, N. Y.): I enjoyed Dr. Maguire's paper very much. In addition to the non-specific methods mentioned by him, namely x-rays and anticancer drugs, there are also specific measures for interfering with the primary sensitization of the delayed type to "simple" chemical compounds.

Three methods are known which apparently achieve this. The first one was described by Sulzberger and is based on the intravenous administration of the specific allergenic agent prior to cutaneous exposure. The second one was described by Dr. Chase and was mentioned by Dr. Dubos today, namely feeding of the specific sensitizing agent prior to cutaneous exposure. The third method is the one used by Rosenthal, Harber and me, namely exposure during fetal life either via feeding of pregnant guinea pigs or via intraperitoneal administration in pregnant guinea pigs.

The anticancer drug which Dr. Maguire used, apparently produces a very short-lived suppression of the sensitization. With these specific other methods, it appears possible that the interference with the sensitization lasts much longer. However, I know of no studies which tell us exactly how long this interference ["specifically acquired tolerance"] does last.

Dr. Walter F. Lever (Boston, Massachusetts): I would like to ask Dr. Maguire how long the reaction lasted that appeared on the sixth day in the non-treated animals, and also how long the reaction lasted in those animals who had been given the cytoxan and in whom the reaction first appeared on the twentieth day.

Dr. Cyril H. March (New York, New York): I would like to ask whether white cell counts were performed in the animals which had received the cytotoxic agents and whether lymph node cytology was examined, whether there was any difference between the counts after cytoxan or after the other cytotoxic agents.

Dr. Charles G. Mendelson (Detroit, Michigan): Along the same lines as Dr. March, I believe that Dobson and probably Obaley and others in treating patients with mycosis fungoides with cytoxan have found a marked rise in eosinophils almost routinely above 40 per cent in their cases.

In this light, would Dr. Maguire have any idea as to what response there might be as far as the adrenal and steroid changes which might be effective in the aberrance of the controlled and treated animals.

Dr. Henry C. Maguire, Jr. (in closing): I wish to thank the discussors for their comments.

To answer the questions—Dr. Lever, we did not follow the controls and the Cytoxan® treated animals' hypersensitivities in terms of months. Certainly, for the next several weeks, these animals remain unequivocally positive.

Dr. March, we did white blood counts and this is discussed in the written presentation of this paper.

Briefly, Actinomycin D, VLB and 6MP, the first three drugs, very weakly depress the circulating white count in the guinea pig. Cytoxan has a more profound effect on the circulating white count. It is our feeling that this leukopenia is a crucial matter in the inhibition of the primary sensitization reaction.

We did not do lymph node histology.

Dr. Mendelson brings up the possibility (if I am interpreting his question correctly) that we may have been stimulating the adrenals and, actually, getting a secondary blockade from adrenal discharge.

We did not do measurements of steroid levels. However, in terms of LD-50's, we gave the actinomycin-D, VLB and 6-MP, the three drugs that did not work, in more toxic quantities than the Cytoxan®. And therefore, if we might be permitted to speculate, we probably had more steroid, a greater adrenal discharge, with these three compounds than with the Cytoxan®.