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Effects of Cyclophosphamide on Allergic Contact Dermatitis

(cyclophosphamide effects/allergic contact dermatitis)

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A 10 mg dose of Cyclophosphamide was administered intraperitoneally to guinea pigs for 5 days at various stages of sensitization, and the effects of the drug for allergic contact dermatitis were studied by vascular permeability. This permeability was markedly increased in the group that was sensitized after administration of Cyclophosphamide, but the condition was inhibited in the group given the drug after sensitization but prior to provocation. Histopathological observations were made using indices of epidermic thickening (hypertrophy) and number of epidermic infiltrating cells. A more severe dermatitis was noted in the group given Cyclophosphamide prior to sensitization, as epidermic thickening in the animals in which the lesser concentration of DNCB had been applied, while there was an infiltration of cells in animals to which a higher concentration of DNCB had been given.

There are individual differences in the development of allergic contact dermatitis. In other words, there are those who have potent reactions while others show no response. To cite an example, many of those with malignant tumors have decreased cellular immuno-competence, and thus DNCB has been used as a method of testing. Various studies have been made in efforts to ascertain the mechanism for such reaction, but detailed findings on the resulting dermatitis have not been reported. We induced changes in organisms by the administration of Cyclophosphamide and observed the effects on allergic contact dermatitis.

MATERIALS AND METHODS

White male guinea pigs weighing 250–350 g were used. One vial containing 100 mg Cyclophosphamide was dissolved in physiological saline, and 10 mg was given i. p. for 5 consecutive days.

The animals were sensitized by shaving an area on the tail and applying 0.1 ml of 0.5% DNCB acetone solution, and the condition was later provoked by shaving an area on the back and applying 0.01 ml of the same solution

in varying concentrations of 0.2%, 0.1%, 0.05% and 0.025% 10 days after sensitization.

Vascular permeability was observed at various time stages of Cyclophosphamide administration, sensitization and provocation. That is, a control group (9 animals) of untreated, sensitized and provoked guinea pigs, a group (12 animals) administered Cyclophosphamide at the same time they were sensitized, a group (8 animals) were given Cyclophosphamide 5 days before sensitization and a 4th group (5 animals) received Cyclophosphamide from the 6th day after sensitization. Sixteen hours after provocation, 1% Evans blue 60 mg/kg was given i. v. and the site was observed 30 min later (Fig. 1).

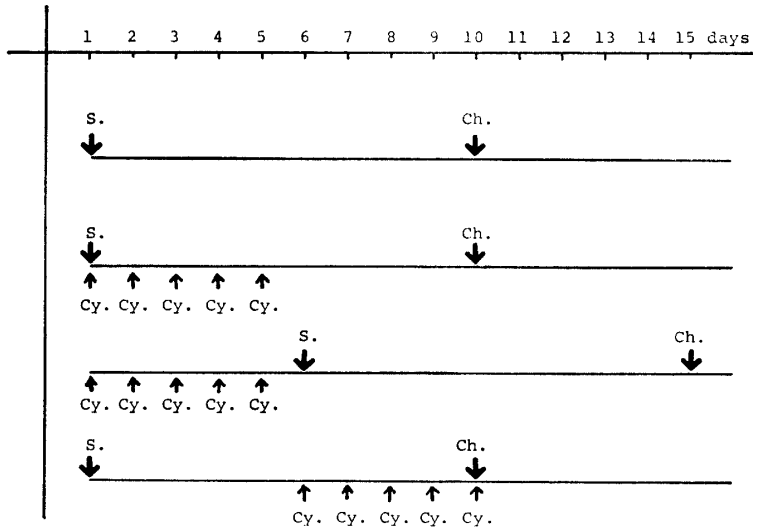

Fig. 1. Various combinations of sensitization and Cy. administration.
S.=Sensitization, Ch.=Challenge, Cy.=Cyclophosphamide

Observation under optical microscope was performed using the group administered Cyclophosphamide prior to sensitization (20 animals) and untreated controls (20 animals). Skin samples were taken from 5 animals each which had been given provocation doses of 0.2%, 0.1% and 0.05% after 12, 24, 48 and 72 hours, and the specimens were stained with H-E. The number of epidermic cells in the cell layers was counted at two randomly selected sites for each histological specimen. Also, the number of cells infiltrating the epidermis within a fixed area was counted by adopting a 10 mm ocular micrometer to the microscope and magnifying the specimen 400 times. The mean values were taken and the state of the inflammatory lesions as determined after 72 hours were processed statistically.

RESULTS

Vascular permeability was markedly inhibited in the group in which Cyclophosphamide was administered at the same time as sensitization as well as in those that were administered the drug 6 days after sensitization, whereas

it was enhanced in those which were sensitized after administration (Table I), (Fig. 2).

TABLE I. *State of Vascular Permeability under Various Combinations of Cyclophosphamide (Cy.) Administration*

	Challenge DNCB concentration %	+++	++	+	±	−	Total
Control group	0.2	—	4	4	1	—	9
	0.1	—	—	3	3	3	9
	0.05	—	—	—	—	9	9
	0.025	—	—	—	—	9	9
Cy. administered concomitant with sensitization	0.2	—	—	1	1	10	12
	0.1	—	—	—	—	12	12
	0.05	—	—	—	—	12	12
	0.025	—	—	—	—	12	12
Sensitized after Cy. administration	0.2	7	1	—	—	—	8
	0.1	1	5	2	—	—	8
	0.05	—	—	4	3	1	8
	0.025	—	—	—	—	8	8
Cy. administered 6 days after sensitization	0.2	—	—	—	—	5	5
	0.1	—	—	—	—	5	5
	0.05	—	—	—	—	5	5
	0.025	—	—	—	—	5	5

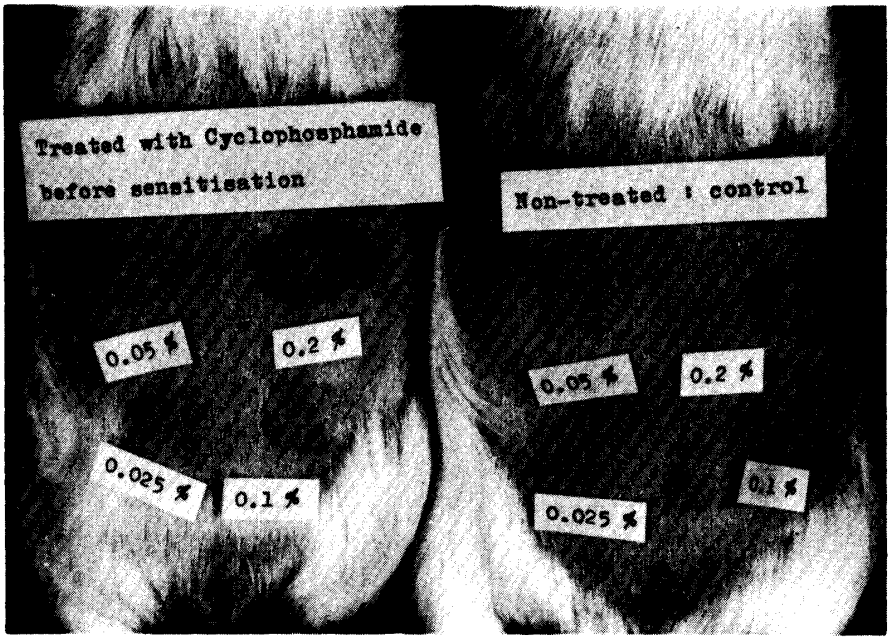

Fig. 2. Dye permeability in Cyclophosphamide administered prior to sensitization group and controls.

The course of epidermis hypertrophy thickening showed that in the controls, thickening increased up to 24 hours in those given doses of 0.2%, 0.1% and 0.05%, but showed a decrease at 48 hours, and increased again at 72 hours. In the group in which sensitization preceded drug administration, the pattern for those that received a dose of 0.2% was slightly different and there was no significant difference at the 5% level even after 72 hours.

However, those given doses of 0.1% and 0.05% showed definite significant differences at the 1% level (Figs. 3, 5–8).

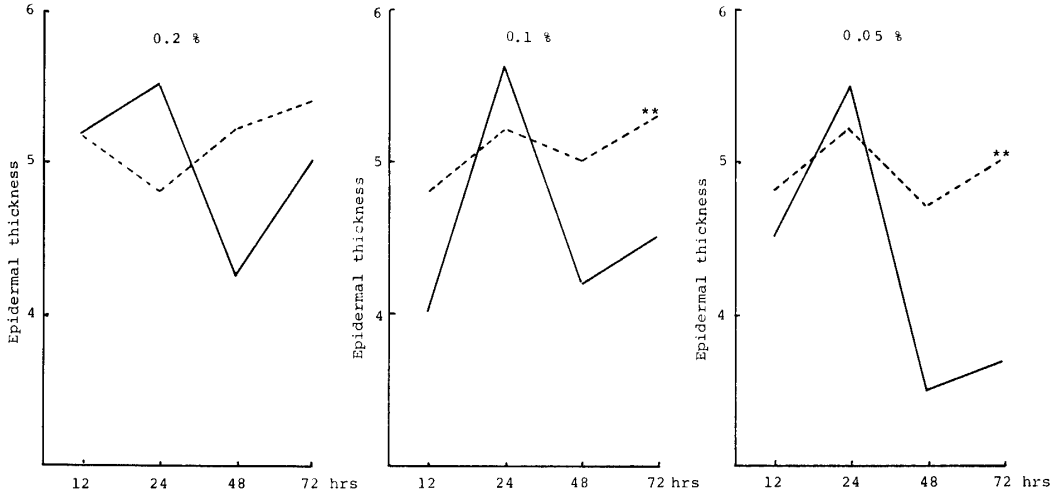

Fig. 3. Epidermal thickness in Cy. administrered group prior to sensitization.
—— Control
---- Cyclophosphamide administered
** Significant at 1% level

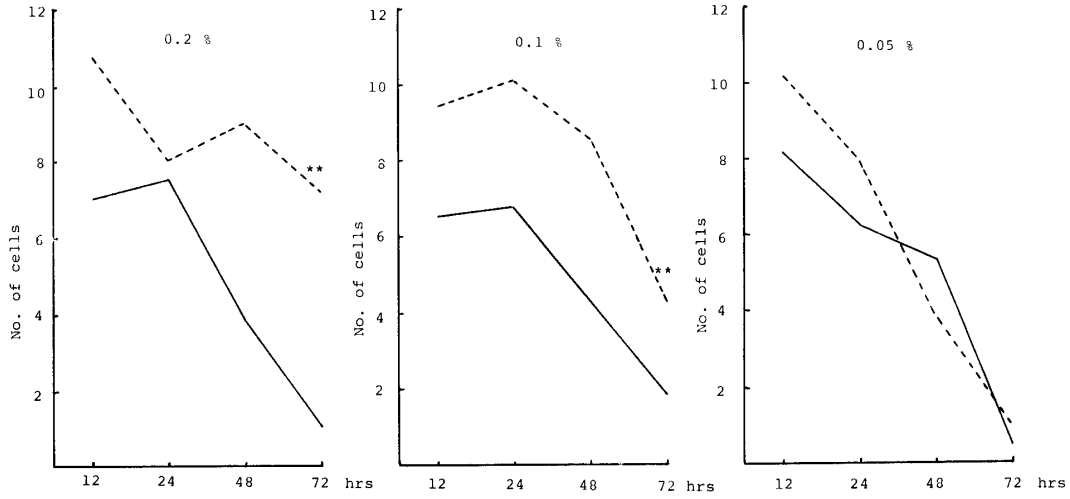

Fig. 4. Intraepidermal infiltrative cells in Cy. administered prior to sensitization.
—— Control
---- Cyclophosphamide administered
** Significant at 1% level

The epidermic infiltrating cells showed a temporary increase after 24 hours in the controls given doses of 0.2% and 0.1%, and decreased thereafter, but the pattern in those given a 0.05% dose was slightly different. In the group that was given the drug prior to sensitization, those that received a 0.2% dose showed a temporary decrease, which increased at 48 hours and then decreased again. The difference after 72 hours was significant at the 1% level for those that received doses of 0.2% and 0.1%(Figs. 4, 5–8).

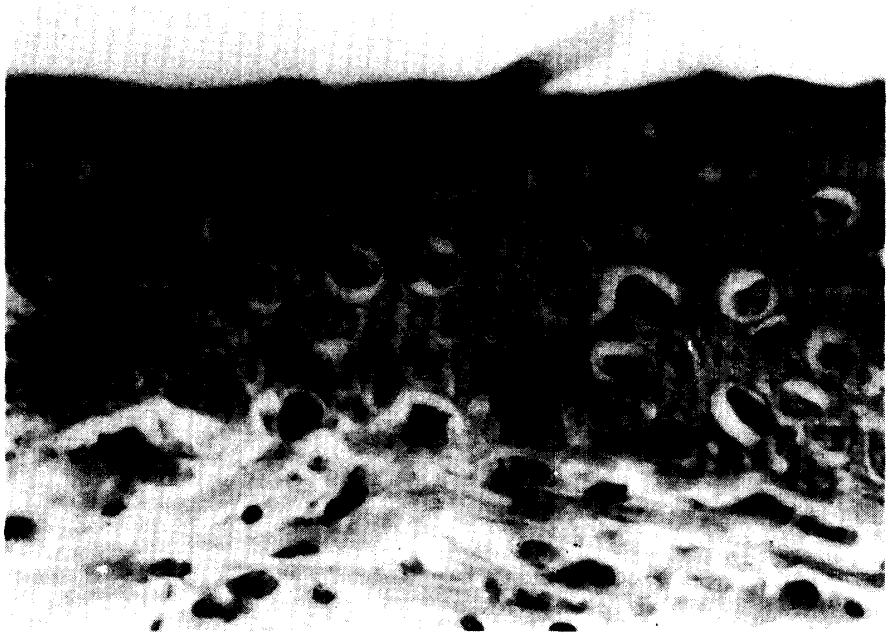

Fig. 5. Allergic contact dermatitis in guinea pigs. (induced with 0.2% DNCB, after 72 hrs) Controls : Thickening of epidermis is marked, but there is little intra-epidermal infiltration of cells.

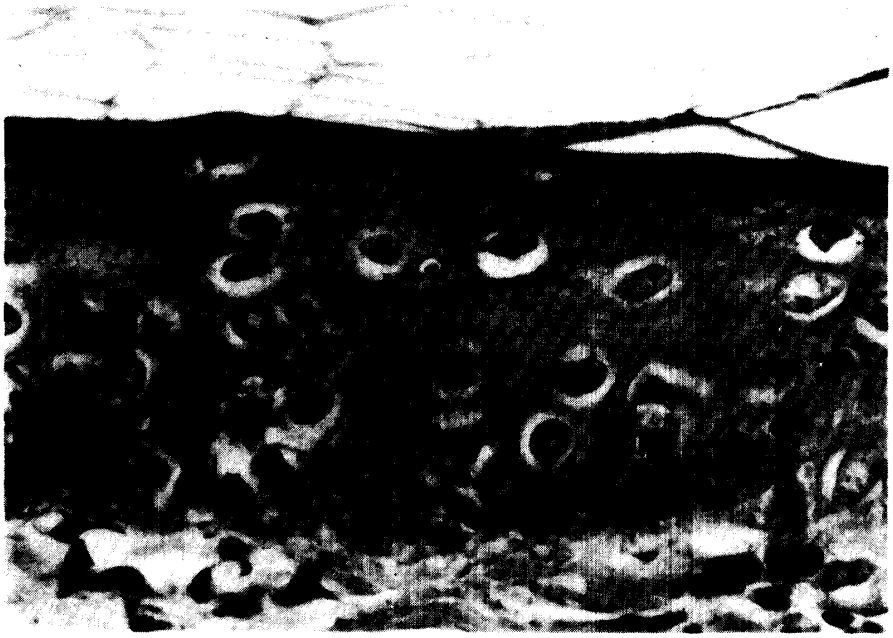

Fig. 6. Allergic contact dermatitis in guinea pigs. (induced with 0.2% DNCB, after 72 hrs) Cyclophosphamide administered prior to sensitization group : Note the thickening of epidermis and intraepidermal infiltration of cells.

Fig. 7. Allergic contact dermatitis in guinea pigs. (induced with 0.05% DNCB, after 72 hrs) Controls : Little thickening of epidermis and intraepidermal infiltration of cells are observed.

Fig. 8. Allergic contact dermatitis in guinea pigs. (induced with 0.05% DNCB, after 72 hrs) Cyclophosphamide administered prior to sensitization : Marked thickening is observed and few intraepidermal infiltrating cells are present.

From the above, it was learned that Cyclophosphamide administered prior to sensitization induced a more severe and prolonged inflammation.

DISCUSSION

To evaluate the severity of dermatitis in animals, gross findings such as erythema, induration and edema have been considered (1—11). However, these are expressions of dermic reactions, and in order to observe actual alterations in cases of contact dermatitis, it is also essential to study, at the same time, the changes in the epidermis at the primary site of the reaction.

Therefore, in this experiment, the dermic reaction was determined by the degree of vascular permeability and the epidermic changes were observed in a review of the histopathological material.

Further, as subjectivity tends to interfere in the performance of gross observations, evaluation of the degree of pigment permeability was made by comparison against a chart prepared and used previously (12).

Histopathological findings showed that definite inflammatory reaction could not be observed in the vascular permeability test in those given a dose of 0.025%, therefore only conditions provoked by applications in access of 0.05% were studied.

The main findings in allergic contact dermatitis included thickening of the epidermis, epidermic cell edema, intercellular edema, lymphocytic and histiocytic infiltrations into epidermic and dermic vessel surroundings and dermic edema (13). Of the above histological findings, epidermal thickening and epidermic infiltration of lymphocytes and histiocytes in which differences can be detected fairly easy were selected as indices to observe the degree of inflammation.

Epidermal thickening differs depending on the concentration and number of sensitizations and can be induced by tuberculin and arthus reactions, thus it is not a finding peculiar to contact dermatitis, but since this is one of the expressions, it cannot be ignored (14). In our experiment, as sensitization was induced by a single application of acetone solution, marked epidermal thickening was not expected. However, the results indicate that the treated and untreated showed little difference after 72 hours in lesions provoked by 0.2% concentration, but there was a definite increase in thickening after 24 hours between the two groups in lesions provoked by 0.1% and 0.05% concentrations. Study of the pattern showed that lesions produced by concentrations of 0.2% and 0.1% were of lesser severity than in the controls at 24 hours, while in cases of a 0.05% application, the findings were similar to the observations in the controls.

When the degree of inflammatory reaction is to be evaluted on the basis of epidermic thickening, a comparatively long period of observation is required, and it was felt that the degree of provocative reaction can affect thickening as much as that at the time of sensitization. The significance of the 72 hour findings of epidermic thickening is considered to reflect the

phase of inflammatory repair in guinea pigs and is assumed to be proportional to the severity of the inflammatory lesion. Therefore, in strong inflammatory lesions provoked by 0.2% solution, it is felt the difference between the two has been covered.

Infiltration of lymphocytes and histiocytes into the epidermis is one of the most important indices in allergic contact dermatitis, and its specificity has been well described in the series of work reported by Groth (15–18). According to the results of our experiment, the lesions provoked by 0.2% solution were more severe and prolonged than those of 0.1% and 0.05% solutions. Thus the epidermal infiltration of cells is both the most characteristic finding of this dermatitis and also the most appropriate means of evaluating the degree.

It is assumed from the vascular permeability and histopathological findings that pretreatment with Cyclophosphamide results in a stronger and more persistent inflammation. Gross evaluation of the enhancing and inhibiting effects of Cyclophosphamide on contact dermatitis has been reported in the past, and when this drug is administered during sensitization, the sensitization is inhibited, but administered prior to sensitization, it is enhanced (4, 9).

With regard to the mechanism involved, it is reported that when pyroninophilic cells, the origin of T-cells, are pretreated with Cyclophosphamide, they show a greater increase in number than the untreated cells on the 7th and 10th days after sensitization (19), and in support of this finding there is one report describing an increase in the number of T-cells in the lymphocytes after administration of Cyclophosphamide (20). These findings are considered to indicate the effects sustained by such immunologically competent cells as T- and B- cells. Turk *et al.* reported that on the basis of various experiments, the reaction to B-cell derived humoral antigen regulates normal cellular immunity (9) and since such action is completely obstructed by the administration of Cyclophosphamide, it is considered to be the effects of B-cells, particularly, suppressor B-cells (21).

More and more knowledge is being gained on T-cells and B-cells such as the presence of the various subsets of Herper-T, Effector-T and Suppressor-T cells are being discovered, and such are probably involved in allergic contact dermatitis. The results of one experiments may serve in the elucidation of the related mechanisms.

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