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Effect of Physiological Stress on the Delayed Hypersensitivity Reaction. (30533)

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Delayed hypersensitive reactions can be produced in mice that have been sensitized by injection of water in oil emulsions of various proteins or by percutaneous application of minute amounts of certain chemicals (1-4,6). The severity of the reactions can be quantitatively assessed by measurement of the reacting zone and is decreased by pretreatment with the anti-inflammatory steroid cortisone(1,4,7). Passive transfer of serum from a sensitized donor to a normal recipient does not confer sensitivity to the recipient; however, transfer of immune lymphocytes can confer sensitivity. The factor responsible for sensitivity is considered a cell-bound antibody (1,5,6). The circulating level of cortisone or the reaction threshold to cortisone is altered in a stressed animal(7). These facts led to the hypothesis that stressed, sensitized animals would have a less severe delayed hypersensitivity reaction than would non-stressed sensitized animals. The results obtained in experiments designed to test this hypothesis are reported here.

Materials and methods. Male Swiss mice weighing 20-25 g at start of the experiment were used. They were fed mouse pellets and water *ad lib.*

The mice were sensitized by two 50 μg percutaneous applications of 1-chloro-2,4-dinitrobenzene* in freshly prepared acetone solution. The applications were made 14 days apart. The challenge dose, 20-25 μg, was applied 14 days after the second sensitizing dose.

Two types of stress were used: heating at 37°C for 60 minutes in a bacteriologic incubator or heating followed by intravenous injection of 0.2 ml of pyrogen free sterile saline.†

The reaction zones were measured with a millimeter rule to the nearest millimeter in 2 directions 90° apart; the resulting area was

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TABLE I. Effect of Stress on Expression of the Delayed Hypersensitivity Reaction in Mice Sensitized to 1-Chloro-2,4-dinitrobenzene.

Group	Animals reacting /Animals tested	% +	Mean reaction zone, mm²	% Inhibition of reaction
Control	33/35	93.4	78.6 ± 37.8	.0
Heat + iv saline—48 hr	16/17	94.0	70.8 ± 33.3	9.8
Idem 24 "	14/16	83.3	66.4 ± 30.6	15.5
" 1 "	17/17	100.0	57.4 ± 24.6	27.0
Heat—24 hr	17/17	100.0	61.4 ± 34.7	22.0
" 1 "	16/17	94.0	59.8 ± 28.2	23.0
Not sensitized	0/10	.0	.0	100.0

computed as if it were a square.

Results. These experiments were designed to determine a) if stress would alter the severity of the expression of the delayed hypersensitivity reaction, b) effectiveness of 2 kinds of stress and c) effect of timing. The sensitized mice were randomly assigned to groups. Each group was stressed at a different time interval prior to challenge or by a different kind of stress. The results indicated that stress of heating or from heating followed by intravenous injection was capable of altering the severity of the delayed hypersensitivity reaction as measured by reaction zone areas. In all cases the mean area of reaction zones of stressed animals was less than that of the control group. The timing of the stress in relationship to the challenge was important in the quantitative results obtained. Stress given 48 hours before challenge was less effective in decreasing the reacting area than was stress given at 24 or one hour prior to challenge. A summary of pooled data from 2 experiments is given in Table I.

Discussion. The stress procedures used to alter the severity of the delayed hypersensitivity reaction included a widely used aid in preparing small laboratory animals for intravenous injection and a placebo injection. A statistical analysis of the data indicates a limited significance in the differences between groups; however, a consistent trend towards a less severe reaction is observed in all stressed groups. Further research is therefore required to effect a complete evaluation. However, the advantage of including stress controls is clearly indicated.

Summary. Mice sensitized to 1-chloro-2,4-dinitrobenzene and stressed by heating or heating followed by intravenous injection of pyrogen free sterile saline gave quantitatively less severe delayed hypersensitivity reactions when exposed to the sensitizing agent than did non-stressed mice.

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