# The Passive Transfer of Chemical Hypersensitivities in Rabbits (35497)

DENIS R. BURGER, WILLIAM S. COZINE, JR., and DAVID J. HINRICHS
(Introduced by W. S. Jeter)

*Department of Microbiology, University of Oregon Medical School, and Surgical Research Laboratory, Veterans Administration Hospital, Portland, Oregon 97207; Department of Biology, University of Windsor, Windsor, Ontario; and Department of Bacteriology and Public Health, Washington State University, Pullman, Washington 99163*

The cellular transfer of chemically induced, delayed hypersensitivities in guinea pigs was demonstrated by Landsteiner and Chase (1) in 1942. The passive transfer of other delayed hypersensitivities in a variety of species with leukocytes has confirmed their initial observations (2–4). Passive transfer of dinitrochlorobenzene sensitivity with extracts of guinea pig leukocytes was reported by Jeter *et al.* (5) in 1954. Shortly thereafter, Lawrence (6) demonstrated the cell-free transfer of delayed hypersensitivities in man with extracts of peripheral leukocytes. Lawrence (7) has since isolated and characterized this "transfer factor." Although the cell-free transfer of delayed hypersensitivities in man seems to be accepted, passive sensitization with subcellular material in laboratory animals has been questioned (8, 9).

Recently, Burger and Jeter (10) reported experiments which clearly demonstrated the passive transfer of contact sensitivity in guinea pigs with a cell-free factor. The conditions for isolation of this factor from peritoneal exudative cells, lymph node cells and alveolar cells were described.

Tsuji *et al.* (11) described passive transfer of tuberculin sensitivity in rabbits with extracts of alveolar macrophages and serum. Bloom and Chase (12) questioned the interpretation of these experiments and confirmation from other laboratories has not appeared. In a recent paper, Cozine *et al.* described immunologic responses in rabbits following topical treatment with DNFB. In addition to an antibody response, the rabbits developed a contact delayed hypersensitivity to the simple chemical compound. The passive transfer of this dermal reactivity should establish the nature of this reaction and define the passive transfer characteristics of delayed hypersensitivity in rabbits.

The purpose of this study was to investigate the cellular and cell-free passive transfer of contact sensitivity in rabbits.

*Materials and Methods.* New Zealand white male rabbits, weighing 2–3 kg, were sensitized to 2,4-dinitrofluorobenzene (DNFB) or to 2,4-dinitrochlorobenzene (DNCB) by topical application of 2% chemical in alcohol each day for 7 days. Details have been described (5). Five days after the last application, the animals were skin tested with 0.5% (v/v) DNFB in olive oil or 1% (w/v) DNCB in olive oil. The reactions were read at 48 hr according to the following grading scheme: 0, no detectable reaction; 1, patchy erythema; 2, homogeneous erythema; 3, homogeneous erythema and induration; and 4, homogeneous erythema, induration, and raised reaction site. Each animal with dermal reactivity of 3 or 4 received 50 ml of sterile light mineral oil intra-abdominally. Ninety-six hr later, peritoneal exudative cells were collected in three washes of the abdominal cavity with Hanks' balanced salt solution (HBSS). The washings were pooled and immediately centrifuged at 1500 rpm for 15 min. Suprascapular lymph nodes were dissected from the animals, trimmed, minced, and the cells were expressed through a stainless steel screen. Lungs were removed and the alveoli were lavaged with HBSS. In two experiments, spleens were removed, minced, and the cells were expressed through a stainless steel wire screen. In all cases, the cells were collected as

TABLE I. Skin Test Results from Rabbits Passively Sensitized with Transfer Products from 2,4-Dinitrofluorobenzene-Sensitive Donors.

| Expt. no. | No. of cell donors | Cell source[a] | Whole cells | Transfer product; cell-free incubation fluid | | |
|---|---|---|---|---|---|---|
| | | | | Undialyzed | Dialysate | Retentate |
| 31 | 8 | PEC | 3[b] | 2 | | |
| | | LNC | 3 | 3 | | |
| 32 | 8 | PEC | 2 | 2 | | |
| | | LNC | 2 | 3 | | |
| 33 | 6 | PEC | 4 | 3 | | |
| | | LNC | 2 | 3 | | |
| 34 | 7 | PEC | 3 | 3 | | |
| | | LNC | 2 | 2 | | |
| | | ALC | 2 | 2 | | |
| 35 | 8 | PEC | 3 | 3 | | |
| | | LNC | 4 | 2 | | |
| | | ALC | 2 | 2 | | |
| 36 | 8 | PEC | 3 | | 2 | 0 |
| | | LNC | 2 | | 4 | 0 |
| 37 | 6 | PEC | 3 | | 2 | 0 |
| | | LNC | 3 | | 3 | 0 |
| 38 | 6 | PEC | 2 | | 2 | 0 |
| | | LNC | 3 | | 3 | 0 |
| 39 | 3 | ALC | 3 | | 3 | 0 |
| | | SC | 3 | | 3 | 0 |
| 40 | 3 | ALC | 3 | | 3 | 0 |
| | | SC | 3 | | 2 | 0 |

[a] PEC, 2–3 × $10^9$ peritoneal exudative cells in HBSS; LNC, 4–8 × $10^8$ lymph node cells in HBSS; ALC, 3–8 × $10^8$ alveolar cells in HBSS; and SC, spleen cells in HBSS. Each suspension was divided into equal parts; one part was used for the whole cell transfer, and the second was incubated in HBSS for 4 hr at 37°, and the incubation fluid was tested for transfer activity.

[b] Skin tests of recipients were performed with 0.5% DNFB in olive oil 48 hr following iv injection of cells or cell-free transfer factor. The results were graded 48 hr after skin testing as follows: 0 = no detectable reaction; 1 = patchy erythema; 2 = homogeneous erythema; 3 = homogeneous erythema and induration; 4 = homogeneous erythema, induration, and raised reaction site.

rapidly as possible, usually within 30 min.

Half of the cells from each source was injected iv into rabbits immediately after collection. The remaining cells were held in HBSS at a concentration of $10^9$ cells/7.5 ml in siliconized tubes for 4 hr at 37° with frequent gentle agitation. After incubation, the cells were sedimented at 1500 rpm for 20 min. In some experiments the fluid phase was injected iv into the recipient rabbits. In other experiments the incubation fluids were dialyzed for 24 hr at 4° against 10 volumes of 0.15 *M* NaCl and the dialysate concentrated by ultrafiltration. In these instances the retentate and dialysate were injected into separate rabbits. The recipients were skin tested 48 hr later with 0.5% DNFB or 1% DNCB in olive oil applied to a clipped area of the flank. No depilatories were employed. The specificity of the response was tested with 1% citraconic anhydride and 15% *o*-chlorobenzoyl chloride in olive oil. Untreated rabbits were

TABLE II. Skin Test Results from Rabbits Passively Sensitized with Transfer Products from 2,4-Dinitrochlorobenzene-Sensitive Donors.

| Expt. no. | No. of cell donors | Cell source[a] | Whole cells | Transfer product; cell-free incubation fluid | | |
|---|---|---|---|---|---|---|
| | | | | Undialyzed | Dialysate | Retentate |
| 41 | 6 | PEC | 3[b] | 3 | | |
| | | LNC | 3 | 2 | | |
| | | ALC | 3 | 3 | | |
| 42 | 5 | PEC | 2 | 2 | | |
| | | LNC | 3 | 3 | | |
| | | ALC | 2 | 3 | | |
| 43 | 7 | PEC | 0 | 0 | | |
| | | LNC | 0 | 0 | | |
| | | ALC | 0 | 0 | | |
| 44 | 6 | PEC | 2 | 2 | | |
| | | LNC | 2 | 2 | | |
| | | ALC | 2 | 3 | | |
| 45 | 6 | PEC | 3 | | 2 | 0 |
| | | LNC | 2 | | 2 | 0 |
| 46 | 6 | PEC | 2 | | 3 | 0 |
| | | LNC | 2 | | 2 | 0 |

[a] PEC, 2–3 $\times$ $10^9$ peritoneal exudative cells in HBSS; LNC, 4–8 $\times$ $10^8$ lymph node cells in HBSS; and ALC, 3–8 $\times$ $10^8$ alveolar cells in HBSS. Each suspension was divided into equal parts; one part was used for the whole cell transfer, and the second was incubated in HBSS for 4 hours at 37°, and the incubation fluid was tested for transfer activity.

[b] Skin tests of recipients were performed with 1% DNCB in olive oil 48 hr following iv injection of cells or cell-free transfer factor. The results were graded 48 hr after skin testing as follows: 0 = no detectable reaction; 1 = patchy erythema; 2 = homogeneous erythema; 3 = homogeneous erythema and induration; 4 = homogeneous erythema, induration, and raised reaction site.

tested with all of the reagents for control purposes.

*Results.* When DNFB was employed as the sensitizing agent, whole peritoneal exudative cells (PEC) passively sensitized the recipient rabbits in all of eight experiments. When DNCB was employed, five of six attempts were successful. In parallel experiments, in which the same number of cells were incubated in HBSS for 4 hr at 37°, the cell-free incubation fluids or dialysates of them transferred contact sensitivity to DNFB in all eight attempts and to DNCB in five of six attempts (Tables I and II).

When lymph node cells were employed, eight of eight transfers from DNFB-sensitive animals and five of six transfers from DNCB-sensitive animals were positive. Cell-free incubation fluids or dialysates passively transferred DNFB sensitivity eight of eight times and DNCB reactivity five of six times (Tables I and II).

Alveolar cells from animals sensitive to DNFB were active four of four times and three of four times in animals sensitive to DNCB. Cell-free incubation fluids or dialysates were just as effective as whole cells in these experiments (Tables I and II).

Spleen cells from animals sensitized to DNFB were harvested in two experiments. In both cases, whole cells and cell-free dialysates successfully transferred the sensitivity to normal recipients (Table I).

Recipients of either cells or cell-free materials showed no dermal reactivity to olive oil alone, 15% *o*-chlorobenzoyl chloride, or 1% citraconic anhydride. Normal rabbits tested along with the recipient animals, did

not react to any of the reagents at the concentrations employed. Cells and incubation fluids isolated from untreated animals contained no passive transfer activity.

*Discussion.* These results indicate that a chemically induced contact sensitivity in rabbits can be effectively transferred to normal animals with leukocytes from several sources. In addition, cell-free materials isolated from competent leukocytes were also found to be effective in transferring this sensitivity in rabbits.

Conflicting reports on subcellular passive transfer have appeared since Jeter *et al.* (5) reported successful transfer of DNCB sensitivity with cellular extracts in guinea pigs. However, the work of Guthrie *et al.* (14) suggested that the speed of the collection processes was critical to passive transfer success. Burger and Jeter (10) explored this possibility by holding viable leukocytes for 4 hr in HBSS prior to passive transfer. The majority of passive transfer activity was found in the cell-free incubation fluids. These cell-free fluids were collected without cellular disruption and transferred the sensitivity with the same competence and specificity as did the viable cells.

Cell-free fluids were employed here to investigate the subcellular passive transfer of chemical sensitivities in rabbits. Passive sensitization was accomplished with transfer factor from peritoneal exudative cells, lymph node cells, alveolar cells, and spleen cells from animals sensitive to DNFB and DNCB. Seven experiments demonstrated that the transfer factor was dialyzable. This agrees with the results of Lawrence (7) and Baram *et al.* (15) in man and of Burger and Jeter (10) in guinea pigs.

Our results suggest that transfer factor is not a peculiarity of man or monkey, but represents a fundamental biological principle underlying delayed-type sensitivities in other species. The purification and characterization of transfer factors from different delayed-type sensitivities in different species should establish this principle. A biochemical analysis of transfer factors from man has proven particularly difficult because of the problems associated with research in that species. The characterization of transfer factors from guinea pigs and rabbits is, therefore, necessary to establish the molecular basis for delayed-type hypersensitive responses.

*Summary.* Passive transfer of contact sensitivity in rabbits was demonstrated with peritoneal exudative cells, lymph node cells, alveolar cells, and spleen cells from sensitive animals. Cell-free incubation fluids from these four leukocytic populations were as effective in transferring delayed reactivity as were whole cells. The active material was found to be dialyzable. The results suggest a common mechanism for the delayed-type hypersensitive response in several species.

1. Landsteiner, K., and Chase, M. W., Proc. Soc. Exp. Biol. Med. **49**, 688 (1942).

2. Haxthausen, H., Acta Dermato-Venereol. **27**, 275 (1947).

3. Stavitsky, A. B., Proc. Soc. Exp. Biol. Med. **67**, 225 (1948).

4. Kirchheimer, W. F., Weiser, R. S., and Van Liew, R., Proc. Soc. Exp. Biol. Med. **70**, 99 (1949).

5. Jeter, W. S., Tremaine, M. M., and Seebohm, P. M., Proc. Soc. Exp. Biol. Med. **86**, 251 (1954).

6. Lawrence, H. S., J. Clin. Invest. **33**, 951 (1954).

7. Lawrence, H. S., Advan. Immunol. **11**, 195 (1969).

8. Bloom, B. R., dissertation, The Rockefeller Institute, 1963.

9. Bloom, B. R., Chase, M. W., and Goldstein, G., Bacteriol. Proc. **61**, 108 (1961).

10. Burger, D. R., and Jeter, W. S., Bacteriol. Proc. **69**, 70 (1969).

11. Tsuji, S., Oshima, S., Oshiro, M., and Izumi, T., J. Immunol. **93**, 838 (1964).

12. Bloom, B. R., and Chase, M. W., Progr. Allergy **10**, 151 (1967).

13. Cozine, W. S., Jeter, W. S., and Beavers, C. D., Proc. Soc. Exp. Biol. Med. **134** (1), 74 (1970).

14. Guthrie, R. K., Ellis, W. L., and Brock, C. H., Proc. Soc. Exp. Biol. Med. **126**, 1 (1967).

15. Baram, P., Yuan, L., and Mosko, M. M., J. Immunol. **97**, 407 (1966).

Received Sept. 24, 1970. P.S.E.B.M., 1971, Vol. 136.