monitis, lung congestion or generalized hyperemia in most cases. However, these changes were commonly observed in control mice also. Amyloid deposition was noted occasionally in liver and spleen. In 2 animals bloody cysts were encountered in association with enlarged lymph nodes; and in 3 others inflammatory lesions were found in the region of the aorta. Whether these represent sites of localization of glucan particles is not known. There is no evidence, therefore, that the deaths at various times following tumor-necrotizing doses of glucan were related to response of the hosts to the carbohydrate, and no light is thrown on the immediate response to lethal doses through microscopic evaluation of the tissues.

*Summary.* Hydroglucan, a carbohydrate substance derived by Dr. W. J. Nickerson of Rutgers University, from glucan (a component of the yeast cell wall) had previously proven to be an effective oncolytic agent against certain experimental mouse tumors. In the present studies, glucan is shown to be equally effective against the same tumors, and to have no observable toxic effects at tumor-destructive levels.

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## Effect of Macrophages on Primary Immunization of Lymph Node Cells Against Homografts in Diffusion Chambers.* (29509)

STEPHEN B. HULLEY, HAROLD F. DVORAK, AND BYRON H. WAKSMAN†
*Department of Pathology and Neurology Research Laboratory, Massachusetts General Hospital, Boston, Mass.*

Primary immunization in a partially isolated system has been obtained by incubating normal rabbit lymph node fragments with homologous skin in standardized Millipore diffusion chambers maintained in an indifferent host(1). The immunized cells, on injection intradermally into the original skin donor, were found to produce a localized graft-*vs*-host lesion, the *transfer reaction,* comparable to that produced by cells immunized *in vivo*(2,3,4). This lesion is specific and provides a measure of the degree of homograft immunity achieved. The cells, whether immunized in chambers or *in vivo,* may act by transferring information to cells of the animal in which the lesion is actually elicited. This is suggested by several findings: that their reactive capacity is destroyed by RNase(5), that crude RNA prepared from them can endow normal rabbit lymph node cells with specific reactivity(6), and that the principal cell type participating in lesion formation is derived from the host and not from the transferred cells(7).

It has been frequently suggested, on purely morphologic grounds, that primary im-

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† Present address: Dept. of Microbiology, Yale University.

munization may require the interaction of macrophages, which ingest antigen, with cells of another type, possibly lymphocytes. A transfer of information may be involved, since the cells which produce antibody appear to be distinct from those which take up antigen(8,9,10). Structural units, consisting of lymphocytes or plasma cells in close association with macrophages, have been demonstrated in cultures of lymph node or spleen derived from immunized animals(11,12,13). Recently primary formation of antibody in partially or entirely isolated systems has been achieved by incubating normal lymph node cells with RNA derived from macrophages which had ingested antigen ($T_2$ bacteriophage)(14,15). The present paper shows that addition of macrophages to chambers containing partially purified suspensions of lymph node lymphocytes and homologous skin enhances the resulting homograft immunization.

*Materials and methods.* Cells from Dutch rabbits were incubated with ear-skin slices from New Zealand rabbits in Millipore chambers implanted in the peritoneal cavities of indifferent New Zealand hosts(1). At 5-7 days, the cell suspensions were harvested, washed, and injected (in 0.15 ml) intradermally in the depilated flank of the antigen donor. Reactions were read blind and graded on the basis of diameter and degree of induration(1). In Table II and Fig. 5, diameter is expressed in mm and degree of induration by a subjective score, flat, pale pink reactions being scored as ± or + and raised, indurated reactions, dark red in color as ++ or +++. Only tests which could be compared with suitable control lesions in the same rabbit were regarded as acceptable.

Normal *mesenteric lymph node cells* were used throughout, 5-15 $\times$ $10^8$ cells being obtained from a single rabbit. In many experiments, for removal of phagocytic cells, these were incubated with glass wool(16,17) at 37°C on a rotating drum (2 revolutions per minute) for 50-60 minutes. In others, they were incubated 40 minutes with iron granules (12 ml of cell suspension + 1 ml saline containing 20 mg iron granules of 3 $\mu$ diameter and 20 mg gum acacia) and centrifuged briefly, only the upper portion of the supernatant being used(18). Yields were 15-16%; and viabilities ranged from 60 to 90%. Neither method resulted in a significant decrease in the proportion of reticulum cells, monocytes, and large lymphocytes found in smears. *Peritoneal exudate cells* (90% macrophages, 90-99% viable) were harvested 5 days after intraperitoneal injection of sodium caseinate and washed once(19). Yields were 3-12 $\times$ $10^7$ cells per rabbit. *Alveolar macrophages* were obtained by washing out the bronchial tree of normal rabbits (or rabbits injected intravenously 8-10 days earlier with an adjuvant mixture containing 0.07 ml oil, 0.03 ml Arlacel A, and 0.2 mg heat-killed tubercle bacilli) with Hanks' solution(20,21). Yields of 2.2-8.4 (or 8-42) $\times$ $10^7$ cells were obtained, of which 93% were macrophages, 92-99% viable. Aliquots of these cells were killed by heating 20 minutes at 100°C. As *non-phagocytic controls* we used cells expressed from minced normal testis (70% mature sperm). All manipulations were carried out in chilled Hanks' solution containing penicillin (100 units/ml), and streptomycin (0.125 mg/ml). Cell viability was estimated on the basis of exclusion of 0.1% trypan blue.

*Chambers* were made of 47 mm nylon-reinforced Millipore filters (85 $\mu$ thick, pore size 0.45 $\mu$) glued to Lucite rings (38.1 mm inside diameter). Initially these contained approximately $10^8$ cells and 8-10 2 mm pieces of skin. Experiments are not reported in which there was evidence of leakage of chambers or infection of their contents (presence of large numbers of polymorphonuclears in the cell population obtained, cell viability below 30%, visible bacteria, purulent transfer reactions).

*Results.* The numbers of cells inoculated into chambers containing lymph node cells alone and chambers containing lymph node cells plus macrophages were closely comparable, as were the cell yields obtained after incubation and cell viability (always greater than 75%) (Table I). Differential counts revealed little change with time in the rela-

tive proportions of different cell types in the chamber fluid (Table I). No attempt was made to characterize cells adhering to the chamber walls. Macrophages in the fluid generally increased in size from 8-14 $\mu$ to 12-20 $\mu$ during culture and occasionally formed multinucleate giant cells, 70 $\mu$ in diameter (Fig. 3). Other cell types remained more or

TABLE I. Number of Cells in Chambers Before and After Incubation with Ear Skin.

| Chamber contents | Before incubation: Viable cells* × $10^6$ | Before incubation: Avg % Small lymphocytes | Before incubation: Avg % Macrophages | After incubation: Viable cells* × $10^6$ | After incubation: Avg % Small lymphocytes | After incubation: Avg % Macrophages |
|---|---|---|---|---|---|---|
| Lymph node cells | 84 (50-120) | 90 | 3 | 4.7 (1.5-18 ) | 91 | 4 |
| Lymph node cells + macrophages | 85 (50-120) 19 (10- 40) | 75 | 18 | 3.4 (1.4- 9 ) | 79 | 15 |
| Macrophages | 19 (10- 35) | 5 | 92 | .76 ( .1- 1.2) | 14 | 86 |

* Avg values with range in parentheses.

TABLE II. Size of Transfer Reactions Produced by Lymphoid Cells Incubated with Homograft Antigen With and Without Added Macrophages.

| Exp | Cells placed in chamber* | Cells injected × $10^6$ | Transfer reactions at 48 hr: Control | Transfer reactions at 48 hr: Experimental |
|---|---|---|---|---|
| 1 | $5 \times 10^7$ GLN + $2 \times 10^7$ AM | 1.6 | | 6+++ |
| | $5 \times 10^7$ GLN | 1.6 | 3±, 3± | |
| | $2 \times 10^7$ AM | 1.0 | 4± | |
| 2 | $10^8$ LN + $2 \times 10^7$ AM | 1.8 | | 8+++, 8++ |
| | $10^8$ LN + $3 \times 10^7$ TC | 2.1 | 3±, 3± | |
| | $3.5 \times 10^7$ AM | .6 | 2± | |
| 3 | $8 \times 10^7$ LN + $10^7$ PE | 2.4 | | 7+++ |
| | $8 \times 10^7$ LN | 3.2 | 4± | |
| 4 | $10^8$ LN + $1.6 \times 10^7$ AM | 1.8 | | 7++, 6++ |
| | $10^8$ LN | 1.8 | 5+ | |
| 5 | $10^8$ FeLN + $2.6 \times 10^7$ AM | 2.5 | | 5+++ |
| | $10^8$ FeLN | 3.0 | 3± | |
| 6 | $10^8$ LN + $4 \times 10^7$ PE | 1.8 | | 5+ |
| | $10^8$ LN | 2.0 | 4± | |
| 7 | $1.2 \times 10^8$ GLN + $10^7$ AM | 2.5 | | 6+, 5+ |
| | $1.2 \times 10^8$ GLN | 2.5 | 3±, 3± | |
| | $10^7$ AM | .9 | 1± | |
| | $1.2 \times 10^8$ GLN + $10^7$ AM | 4.0 | | 3± |
| | $1.2 \times 10^8$ GLN | 4.0 | 4± | |
| 8 | $10^8$ GLN + $2 \times 10^7$ AM | 1.9 | | 3± |
| | $10^8$ GLN + $2 \times 10^7$ dead AM | 1.9 | 6+ | |
| | $10^8$ GLN | 1.5 | 5± | |
| 9 | $9 \times 10^7$ GLN + $2 \times 10^7$ AM | 1.4 | | 6++, 5+++++, 4+ |
| | $9 \times 10^7$ GLN + $2 \times 10^7$ dead AM | 1.3 | 6+++ | |
| | $9 \times 10^7$ GLN | 1.5 | 4+++++ | |
| 10 | $5.4 \times 10^7$ LN + $10^7$ AM | 4.0 | | 7++ |
| | $5.4 \times 10^7$ LN | 4.0 | 6++, 6+ | |
| | $5.4 \times 10^7$ LN + $10^7$ AM | 1.2 | | 5±, 4± |
| | $5.4 \times 10^7$ LN | 1.2 | 7++ | |
| | $10^7$ AM | 1.2 | 3± | |
| 11 | $5 \times 10^7$ GLN + $2 \times 10^7$ AM | 1.8 | | 6+ |
| | $5 \times 10^7$ GLN | 2.0 | 7++ | |
| | $2 \times 10^7$ AM | .1 | 0, 0 | |

* LN, GLN, FeLN: Lymph node cells, untreated or treated with glass wool or iron granules. PE: Peritoneal exudate cells. AM, dead AM: Alveolar macrophages, untreated or heat-killed. TC: Testicular cells.

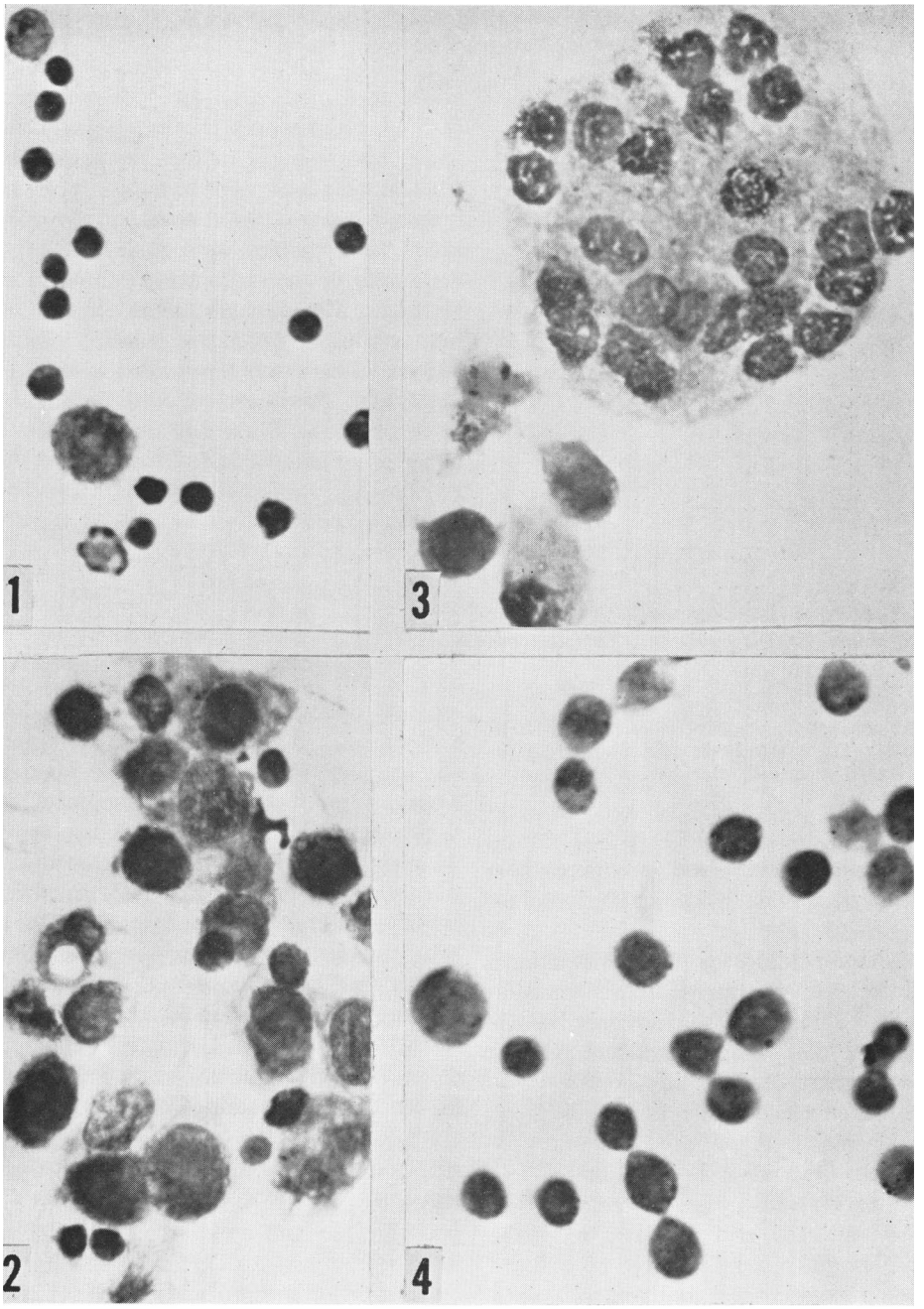


FIG. 1-4. Smears from chamber fluid. All Wright's stain, × 830.

FIG. 1. Lymph node cells following incubation. Note small lymphocytes and central pale macrophage.

FIG. 2. Mixture of lymph node cells plus macrophages following incubation. Heterogeneous population of large, vacuolated macrophages with poorly defined nuclei and borders. Several small and medium lymphocytes are also present.

FIG. 3. Macrophages following incubation. Note giant, multinucleate cell.

FIG. 4. Alveolar macrophages before incubation.

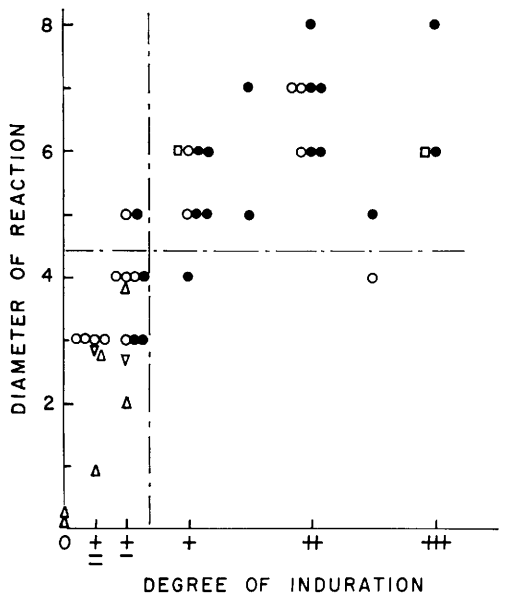


FIG. 5. Reactions graded by degree of induration (0-++) and diameter in millimeters. Each point denotes a single transfer reaction. Experimental points: ●, lymph node cells plus macrophages; ○, lymph node cells, glass wool treated lymph node cells or iron granule treated lymph node cells. Control points: △, alveolar macrophages or peritoneal exudate cells; △, lymph node cells plus testicular cells; □, lymph node cells plus heat killed alveolar macrophages.

less constant in appearance (Fig. 1-4). Macrophages were often observed in clumps, frequently in association with small lymphocytes.

The 48 hour readings of transfer reactions produced by cells recovered from chambers are given in Table II and Fig. 5. Only lesions which showed the typical histologic picture (sheets or islands of large histiocytes, perivascular mononuclear infiltration, and focal areas of necrosis) at 48 or 72 hours(1,4) are included. Occasional lesions showed extensive primary necrosis; these were regarded as possibly infected and are omitted here. If a one plus difference in degree of induration between experimental and control points is taken as significant, the addition of living macrophages to lymph node cells enhanced the sensitization process in the chamber significantly in 5 of 11 individual experiments but had little effect in 4½ additional experiments (Table II). In 1½ experiments, the control points were significantly greater than the experimental points. If reactions graded as + and ≧ 5 mm in diameter are accepted as positive, lymph node cells alone (or with added testicular cells) gave positive reactions in 5 of 17 instances, while with addition of living macrophages to the lymph node cells, positive reactions were obtained in 14 of 19 instances. Two experiments, in which heat killed macrophages were mixed with lymph node cells in the chambers, resulted in sensitization. Macrophages alone, in the small numbers used, failed to produce significant lesions. There was no evidence that treatment with glass wool or with iron particles diminished the ability of lymph node cells alone to develop specific reactivity.

*Discussion.* The experimental data confirm our earlier report(1) that suspensions of lymph node cells, exposed in a diffusion chamber to homologous skin, develop less intense immunity than intact fragments of lymph node similarly exposed. In approximately half the experiments, addition of living macrophages to the chamber resulted in a significant increase in degree of immunity obtained, as judged by the size of transfer reactions produced by the immunized cells. We were unable to inoculate chambers with numbers of macrophages alone comparable to the total number of lymph node cells usually employed. However, macrophages, in numbers sufficient to enhance immunization of lymph node cells, showed no sign of immunization after incubation alone with skin. It should be noted that peritoneal exudate cells of previously immunized animals are able to induce transfer reactions(3).

These data are compatible with the theory that both macrophages and lymphocytes are necessary for the primary response to homograft antigen and appear consistent with the data of Fishman(14,15). He has shown that interaction of macrophages with antigen and the transfer of specific information to other cells of the lymph node may be requisite steps in the primary antibody response. The fact that lymph node cells alone frequently showed some degree of immunization, which in one or two instances exceeded that developed by the same cells with added macro-

phages, may depend on the continued presence in our lymph node cell suspensions of phagocytic reticulo-endothelial cells (about 3%). Fishman's data support this conjecture; he found that rabbit lymph node cells, unlike those of the rat, respond to direct addition of antigen with appreciable antibody titers.

*Summary.* Primary immunization of lymphoid cells by exposure to homologous skin within diffusion chambers was demonstrated by the ability of these cells to produce a transfer reaction in the donor's skin. Addition of peritoneal exudate or alveolar macrophages to lymph node cell suspensions resulted in significant enhancement of sensitization in 5 of 11 experiments. There was no enhancement in 4½ of 11 experiments, and in 1½ sensitization was greater in the absence of macrophages.

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## Diarrhea in Cecectomized Rabbits Induced by Staphylococcal Enterotoxin.* (29510)

T. HAYAMA† AND H. SUGIYAMA

*Food Research Institute and Department of Microbiology, University of Chicago, Chicago, Ill.*

The ingestion of preformed staphylococcal enterotoxin is the cause of staphylococcal food poisoning. The essential features of this illness are nausea, vomiting, abdominal cramping and diarrhea with shock-like collapse in severe cases. The emetic response is used for laboratory detection of enterotoxin (1) but the diarrheal aspects have not been extensively studied. Diarrhea can be observed in monkeys fed highly purified enterotoxin near the threshold emetic dose and in cats given intravenous challenges, but the response is not consistent. Similarly, mice and rabbits injected parenterally have proved not to be satisfactory in the study of enterotoxin-induced diarrhea.

Nausea, vomiting and gastrointestinal re-

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† Permanent address: Laboratory of Pharmacology and Toxicology, Inst. of Food Microbiology, Chiba Univ., Japan.