metous fluids(2). Thus, rate of phagocytosis of the granulocytes, as well as rate of migration of the mononuclear cells from the inflammatory area, account to a great extent for the sequence of cell types seen in the inflammatory exudate.

The number of each cell type responding to an injection of antigen varies depending, in part, upon sensitivity of the animal to that antigen. The neutrophil response appears to be somewhat greater in animals injected with antigen for the first time than in reinjected animals. In contrast to neutrophils, eosinophils are found in greater numbers when the antigen is reinjected. The local increase in eosinophils is a consistent finding whenever an antigen is repeatedly injected into an animal(4). This has recently been confirmed by Litt(11) who observed a quantitative local eosinophilia in the guinea pig following repeated intraperitoneal injections of an antigen. Archer(12) has also found local increases in eosinophils at the site of reinjection of antigen in the skin of the horse. The relation of these cells to adrenal cortical hormone secretions and to antibody formation has been presented earlier(8,9,10).

The mononuclear cells of the sensitized animals showed marked changes in morphological appearance during various periods of the inflammation. Intense basophilic staining of the cytoplasm was observed between the 4th and 10th days of inflammation. It is considered significant that these changes occur in macrophages which have engulfed eosinophils(2,3).

Summary. This paper compares procedures for qualitatively and quantitatively studying cellular responses to inflammatory substances. It was noted that estimates of total cells in the aspirated exudate suggested conclusions not obvious from data obtained from differential counts. These data suggest that the observed sequence of inflammatory cells is due not only to the initial migration of cells into the inflamed area, but to the rate of phagocytosis of the granulocytes by the macrophages. The inflammatory exudate of sensitized animals contained fewer neutrophils and greater numbers of eosinophils. Plasma cells and mononuclear cells with intense basophilic cytoplasm were frequently observed following the disappearance of eosinophils.

1. Harris, H., *Physiol. Rev.*, 1954, v34, 529.
2. Speirs, R. S., *Reticulo-Endothelial Structure and Function*, Chap. 7, 1960. Ed. by J. H. Heller, Ronald Press, N. Y.
3. ———, *Ann. N. Y. Acad. Sci.*, 1958, v73, 283.
4. ———, *ibid.*, 1955, v59, 706.
5. Speirs, R. S., Dreisbach, M. E., *Blood*, 1956, v11, 44.
6. Speirs, R. S., Wenck, U., Dreisbach, M. E., *ibid.*, 1956, v11, 56.
7. Carpentier, P. J., *Nature*, 1955, v175, 1128.
8. Speirs, R. S., *ibid.*, 1958, v181, 681.
9. Dreisbach, M. E., Snell, G. D., Speirs, R. S., *J. Nat. Cancer Inst.*, 1956, v17, 297.
10. Speirs, R. S., Wenck, U., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 571.
11. Litt, M., *Blood*, 1960, v16, 1318.
12. Archer, R., personal communication.

Received July 13, 1960. P.S.E.B.M., 1961, v106.

DNA Synthesis in Inflammatory Cells of Immunized and Non-Immunized Mice.* (26301)

R. S. SPEIRS, Y. OSADA† AND V. JANSEN
Department of Anatomy, State University of New York, Downstate Medical Center, Brooklyn, N. Y.

This study utilizes quantitative technics developed earlier(1) to determine the number of cells synthesizing DNA in the inflam-

* This work was supported by research grants from Nat. Inst. Health, PHS, and from Atomic Energy Comm. The experiments reported were carried out in part by students attending a course of Experimental Hematology.

† Recipient of a Research Fellowship from Rockefeller Foundation. Present address: Inst. of Public Health, Tokyo, Japan.

matory exudate of immunized and non-immunized mice. Recent experiments indicate that thymidine incorporation occurs during interphase in cells which are doubling their nuclear DNA preparatory to mitosis(2,3,4, 5). Cells during various stages of inflammation were incubated *in vitro* with tritiated thymidine, and incorporation of radioactivity into nuclei was determined by autoradiography.

Materials and methods. A total of 80 adult C57 BL/6 mice were used in these experiments. One-half of the animals were sensitized (immunized) by a series of 7 subcutaneous injections of 5 Lf Diphtheria toxoid (Lederle). Thirty days after the last sensitizing injections, 30 mice from each group were injected intraperitoneally with 5 Lf of the toxoid in 0.4 ml isotonic saline. The remaining mice were used as uninjected controls. Each day, autopsies were performed on 3 animals from each injected group, and one animal from each uninjected group. Data were omitted for "day 8" because of technical difficulties in preparation of the autoradiographs.

At time of autopsy, 0.2 ml of a suspending fluid, consisting of polyvinylpyrrolidone (Schenley) and Hank's balanced salt solution, was injected intraperitoneally. The inflammatory exudate was immediately removed by aspiration, samples were taken for counting, and the remaining cell suspension (approximately 0.20 ml) added to 0.05 ml pyrogen-free saline containing 1 μc tritiated thymidine (360 μc/mM Schwarz). The cells were incubated for 1 hour, cooled to 5°C, then painted on gelatine-subbed glass slides, using the brush technic of Burke, *et al.* (6). The slides were then air dried, fixed in methyl alcohol, washed for 15 minutes in distilled water, and dipped into NTB[3] liquid nuclear emulsion (Kodak) according to procedures outlined(7,8). After exposure for 7 days at 5°C, the slides were developed in D-19 for 2 minutes, fixed, washed and stained for 3½ minutes in May-Gruenwald Blood stain.

The proportion of inflammatory cells incorporating tritiated thymidine was estimated by counting approximately 5,000 cells on the autoradiograms of each animal. Only cells containing 6 or more silver grains over the nucleus were recorded as labeled cells. Number and types of cells at each stage of inflammation were calculated(1) and total number of cells incorporating thymidine estimated.

Results. Examination of the autoradiograms indicated that only mononuclear cells were capable of *in vitro* incorporation of the tritiated thymidine. In no case was a label obtained over neutrophils, eosinophils or mast cells. The number of mononuclear cells capable of incorporating thymidine in the uninjected (0 day) animals averaged 36/10,000 (0.36%) cells in the normal, and 9/10,000 (0.09%) cells in the immunized animals. (Table I and Fig. 1 Top).

In the non-immunized animals injected with diphtheria toxoid, a total of 839 labeled cells were found among 130,000 inflammatory cells. Average number of labeled cells for the period from 1 to 10 days was 64/10,000. A gradual increase in proportion of cells incorporating thymidine was observed, beginning on the 3rd day, and reaching a peak on the 7th day (181/10,000).

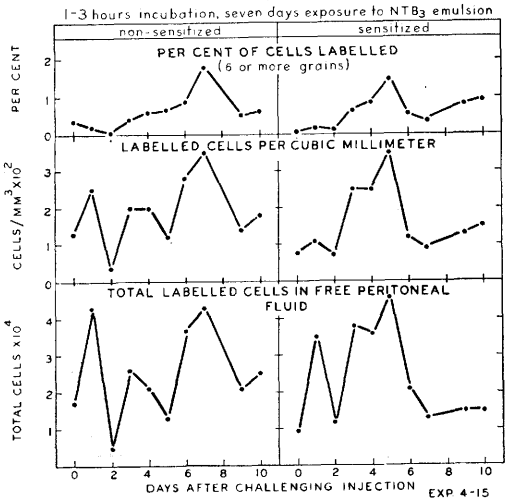

FIG. 1. *In vitro* incorporation of tritiated thymidine in inflammatory mononuclear cells of non-sensitized and sensitized mice. Calculations were made of No. of labeled cells per cmm, and total No. which could be aspirated in the inflammatory exudate. Labeling was obtained by incubating the cells with thymidine, and exposing them to NTB_3 emulsion, for 7 days.

TABLE I. *In Vitro* Incorporation of Tritiated Thymidine by Mononuclear Cells in an Inflammatory Exudate.

		Days after intraper. inj.									
	Animal	0	1	2	3	4	5	6	7	9	10
Total mononuclear cells in aspirate ($\times 10^6$)	Normal	4.8	21.7	7.4	6.3	3.5	2.0	4.3	2.4	4.0	4.0
	Sens.	11.0	17.7	9.9	5.8	4.0	3.2	3.8	3.6	1.8	1.6
Labeled cells per 10^4 mononuclear cells	Normal	36	20	6	40	64	68	87	181	52	78
	Sens.	9	20	11	65	87	145	65	37	83	90
Total No. of labeled cells in aspirate ($\times 10^3$)	Normal	17.1	43.0	4.5	26.0	21.0	13.0	37.0	43.0	21.0	25.0
	Sens.	9.5	35.4	12.0	38.0	36.0	46.0	21.0	13.0	15.0	15.0

In the immunized animals a total of 1170 labeled cells were found among approximately 156,000 cells. Average number of labeled cells for the period from 1 to 10 days was 67/10,000. A gradual increase in proportion of labeled cells also occurred beginning on the 3rd day, and reaching a peak on the 5th day (145/10,000).

Calculations were also made of number of labeled cells per cubic millimeter, and total number which could be aspirated from the peritoneal cavity (Table I, Fig. 1). An in-

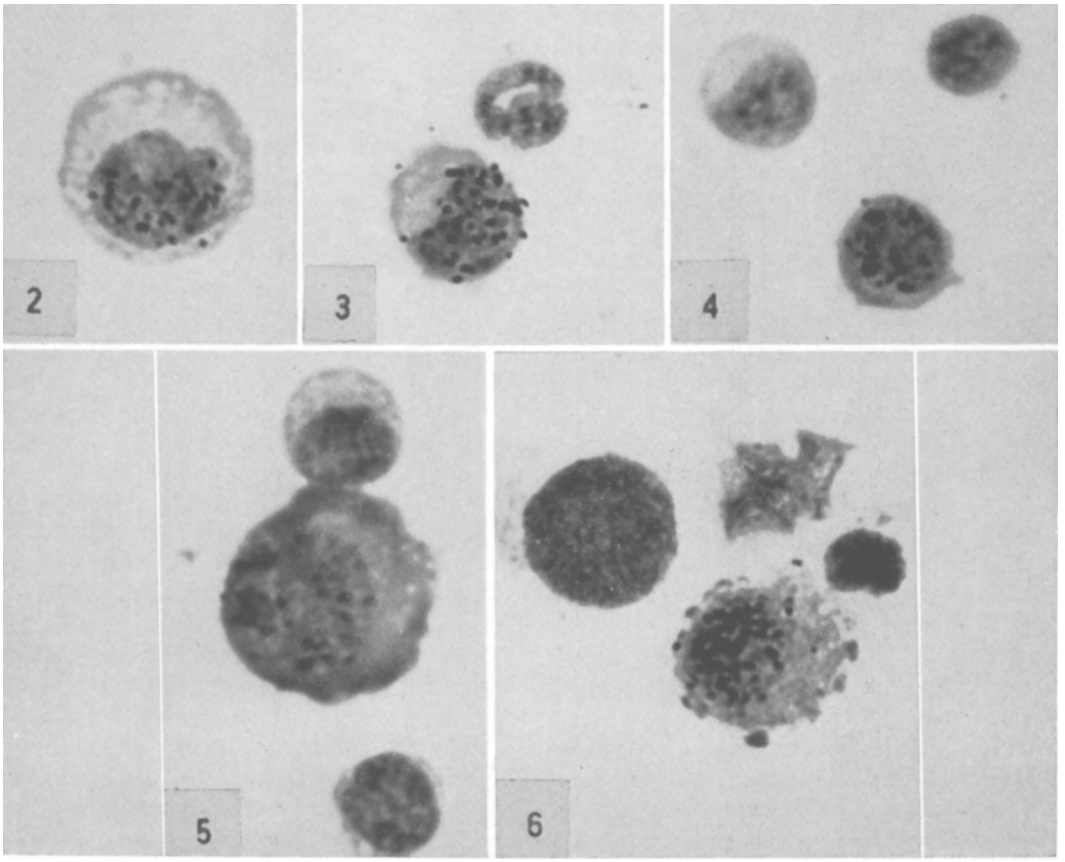

FIG. 2-6. Inflammatory cells incorporating tritiated thymidine *in vitro*.

FIG. 2. Histiocytes. Cells which most frequently incorporate thymidine.
FIG. 3. Histiocyte and an unlabeled eosinophil.
FIG. 4. Medium-sized lymphocyte.
FIG. 5 & 6. Basophilic transitional cells which appeared between 4 and 7 days in immunized group but were occasionally seen in non-immunized animals.

crease in actual number of cells capable of synthesizing thymidine occurred during the first day of inflammation in both groups. This increase was not evident when percentage data alone were considered.

The types of labeled mononuclear cells varied, depending upon stage of inflammation. The majority of labeled cells at all stages consisted of large macrophages or histiocytes (Fig. 2,3), and medium sized lymphoid cells (Fig. 4). Beginning on the 4th day, cells with varying amounts of cytoplasmic basophilia became labeled (Fig. 5 and 6). In immunized mice these basophilic cells averaged 13% of all the labeled cells found between 4th and 10th days. In the non-immunized mice approximately 2.5% of the labeled cells were basophilic during this same period.

All stages of transition between macrophages and plasma cells were observed, and it was impractical to classify the cells into distinct morphological groups.

Discussion. These experiments demonstrate a procedure for study of metabolic activity of inflammatory cells. A comparison was made of number of cells capable of *in vitro* incorporation of thymidine in the inflammatory exudates of immunized and non-immunized mice injected with Diphtheria toxoid. The cells which were found to incorporate thymidine are mononuclear cells—a heterogeneous group including lymphoid cells, histiocytes, macrophages and cells with varying amounts of basophilic-staining RNA in their cytoplasm. The granulocytes found in the inflammatory exudate are end cells which, since they are incapable of cell division, do not incorporate thymidine.

The number of inflammatory cells capable of incorporating tritiated thymidine was small compared with the number of such cells found in bone marrow or spleen. During the first 2 days of inflammation, total number of peritoneal exudate cells incorporating thymidine never exceeded 46,000, although, within the first 24 hours, there was an increase of more than 10 million mononuclear cells in the exudate of the non-sensitized animals. These additional cells must arise by migration of mononuclear cells into the exudate, since the DNA synthesizing cells were too few in number to account for the increase. These data do not indicate whether the migrating cells originate in blood vessels, omentum, mesenteries or adjoining tissues. Other experiments, however, have demonstrated that at least some of the mononuclear cells must migrate from the blood vessels along with the granulocytes(9,10,11).

Following the first day of inflammation, there is a disappearance of mononuclear cells due, in part, to necrosis and disintegration of the cells, and, in part, to migration of the cells out of the inflammatory area and into the lymphatic and blood vessels(1,12). There is, thus, a large turn-over of cells moving both in and out of an inflammatory exudate, and a true picture of this turn-over can be obtained only by considering quantitative data.

Greater numbers of mononuclear cells with intense basophilic cytoplasm (due to RNA synthesis) were observed in the sensitized animals(12). These cells also appear to incorporate amino acids(13) and are probably involved in the synthesis of antibody(12,14, 15). Although differences in morphological appearance were apparent in the sensitized mice, total number of cells present, and number synthesizing DNA, were identical to that of the non-sensitized animals. The sensitized animals appeared to synthesize DNA somewhat sooner than the non-sensitized animals, possibly because they are more efficient in rate of phagocytosis and removal of antigen (16). There was no indication that these cells were dividing to form plasma cells. Evidence available in our laboratory, and from others(17,18), does not support the concept that antibody-producing cells arise by mitosis, but rather suggests that there is a morphological transformation of macrophages, or similar mononuclear cells.

Additional experiments are in progress in which cells labeled with tritiated thymidine are traced from the inflammatory exudate into various organs and tissues of the body.

Summary. Inflammatory cells aspirated from the peritoneal cavity of mice injected with Diphtheria toxoid were studied by autoradiography for *in vitro* incorporation of

tritiated thymidine. The proportion of labeled cells was low prior to and for the first 2 days following initiation of inflammation. Beginning on the 3rd day the proportion of labeled cells gradually increased reaching a maximum on or about the 7th day in the non-sensitized mice. In animals previously sensitized to the Diphtheria toxoid, the increase in labeled cells occurred more rapidly, reaching a maximum on or about 5th day of inflammation. However, number of cells capable of DNA synthesis in both groups appeared to be identical. The origin of inflammatory mononuclear cells was discussed. Only a small proportion of the cells originate by division of cells suspended in the inflammatory exudate. The majority of cells, both granulocytes and mononuclear cells, migrate into the inflammatory area from the blood vessels and possibly from adjoining tissues.

1. Speirs, R. S., Speirs, E. E., Jansen, V., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 248.

2. Hughes, W. L., Bond, V. P., Brecher, G., Cronkite, E. P., Painter, R. B., Quastler, H., Sherman, F. G., *Proc. Nat. Acad. Sci.*, 1958, v44, 476.

3. Taylor, J. H., Woods, P. S., Hughes, W. L., *ibid.*, 1957, v43, 122.

4. Hughes, W. L., *Kinetics of Cellular Proliferation*. Ed. by F. Stohlman, 1959, Grune & Stratton.

5. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., Brecher, G., Schork, P., *Acta. Haemat.*, 1959, v21, 1.

6. Burke, W. T., Brotherston, G., Harris, C., *Am. J. Clin. Path.*, 1955, v25, 1226.

7. Joftes, D. L., *Lab. Invest.*, 1958, v8, 1931.

8. Boyd, G. A., *Autoradiography in Biology and Medicine*, 1955, Academic Press, N. Y.

9. Speirs, R. S., Dreisbach, M. E., *Blood*, 1956, v11, 44.

10. Speirs, R. S., Wenck, U., Dreisbach, M. E., *ibid.*, 1956, v11, 56.

11. Sieracki, J. C., Rebuck, J. W., *The Lymphocyte and Lymphocytic Tissue*, 1960. Ed. J. W. Rebuck. Paul B. Hoeber.

12. Speirs, R. S., *Reticulo-Endothelial Structure and Function*, Chap. 7, 1960. Ed. by J. H. Heller, Ronald Press, N. Y.

13. Spriggs, A. I., Lajtha, L. G., *Brit. J. Cancer*, 1959, v13, 132.

14. Roberts, J. C., Dixon, F. J., Weigle, W. O., *A.M.A. Arch. Path.*, 1957, v64, 324.

15. Speirs, R. S., *Ann. N. Y. Acad. Sci.*, 1958, v73, 283.

16. Dixon, F. J., Burkantz, S. C., Dammin, G. J., Talmage, D. W., *The Nature and Significance of Antibody Response*, Chap. 11, 1951. Ed. by A. M. Pappenheimer, Columbia Univ. Press, N. Y.

17. Hill, M., *Nature*, 1959, v183, 1059.

18. Roberts, J. C., *The Lymphocyte and Lymphatic Tissue*, Chap. 7, 1960. Ed. by J. W. Rebuck. Paul B. Hoeber.

Received October 10, 1960. P.S.E.B.M., 1961, v106.

In vitro Histamine Release from Rat Mast Cells by Chemical and Physical Agents.* (26302)

RONALD E. BRAY† AND PAUL P. VANARSDEL, JR. (Introduced by R. H. Williams)
(With the technical assistance of Miss Eliza Mooers)
Department of Medicine, University of Washington, School of Medicine, Seattle

A variety of simple chemical compounds capable of *in vivo* and *in vitro* histamine release from cells has been investigated during the last 13 years(1). Most studies, particularly with compound 48/80, have utilized disruption and degranulation of tissue mast cells as an index of histamine release(1-6). Observations on mast cell degranulation may not be adequate for quantitative study of 48/80 and histamine release; indeed, histamine release may occur without degranulation(7). Furthermore, there is almost no quantitative information concerning other chemical releasers. Accordingly, we made direct measurements of histamine released after addition of various concentrations of compound 48/80 to rat peritoneal mast cells, and compared these effects with those of sev-

* Supported by grant of Nat. Inst. of Allergy and Infect. Dis.

† Student Summer Research Fellow, Allergy Foundation of America, 1958, 1959.