Agglutination of Bacteria by Lymphoid Cells *in Vitro*. (18030)

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While some investigators still believe that the lymphocytes are cellular sources of antibodies(1), others have come to the conclusion that it is the plasma cell rather than the lymphocyte which elaborates these globulins (2-5). As there has been no direct evidence for either theory, we have continued to search for a crucial experiment. We believe that this has been found. It was observed that certain lymphoid cells of antibody forming lymph nodes exhibited on their surface an antigen-antibody reaction when exposed to antigen *in vitro*. Polymorphonuclear leucocytes, monocytes and macrophages failed to show this phenomenon.

Method. Antibody production was induced in the popliteal lymph nodes of rabbits through injection into the footpads of 0.5 ml of a commercial typhoid H or brucella antigen ("febrile antigen," Lederle). After intervals varying from 2 to 14 days, the nodes were removed and their cells suspended in physiological saline solution. The antibody contained in the lymph plasma was washed out by twice repeated centrifugation and resuspension in saline. Of the final suspension, a drop was placed on a slide and covered with a drop of a similarly washed and suitably diluted portion of the antigen. The preparation was then sealed and studied microscopically.

Results. During the first 10 to 15 minutes following the addition of bacteria to the cells, both the bacteria and the cells were evenly distributed throughout the microscopic fields. Thereafter some bacteria began to stick to the surface of certain lymphoid cells. After 30 to 45 minutes this phenomenon was at its peak. The agglutination of the bacteria on the surface of the cells was associated with loss of Brownian movement.

The number of cells showing this phenomenon was greatest 5 to 7 days following the injection of antigen. It was smallest on the third and fourteenth days, and there was none on the second. The percentage of cells showing the phenomenon was not determined. During the height of the reaction we often found 2 to 3 such cells in one microscopic field.

The nature of the agglutinating cells was difficult to determine when unstained or supravitally stained preparations were used. The addition of Giemsa, Wright or some other stains caused separation of cells and bacteria. However, when the drops were placed on dried films of an alcoholic Janus Green solution, satisfactory results were obtained. Fig. 1 and 2 were made from unstained preparations, all others from preparations with Janus Green.

Fig. 1, 4, 6, 7, 9, 10 and 11 leave no doubt that many of the agglutinating cells were plasma cells. However, only a certain number of the identifiable plasma cells showed this phenomenon. Fully mature plasma cells were more often negative than positive.

Other agglutinating cells had the appearance of immature lymphoid cells (Fig. 2, 3, 4, and 5). Many of these were obviously plasmablasts as shown by the coarseness of their mitochondria and the abundance and deep basophilia of their cytoplasm. These immature elements agglutinated bacteria more often and more strongly than the mature cells indicating a loss of this function during maturation (Fig. 4). Immature lymphocytes which were present in large numbers during

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the second week of the experiment were not observed to agglutinate.

The cell which seemed to exhibit the phenomenon most often when present was comparatively small, though larger than a small lymphocyte (Fig. 11). It had a round, rather diffusely stained nucleus and usually a good deal of cytoplasm. Because it was so poorly differentiated structurally, it is felt that this cell was close to mitotic division either arising from it or about to divide. The nature of this cell could not be established. In view of all other findings, however, it may be assumed that it belonged to the plasma cell series.

Typical small lymphocytes never showed the phenomenon (Fig. 1 to 8). The only agglutinating cell found in our preparations that resembled a small lymphocyte is illustrated in Fig. 8. It can be seen that this cell is somewhat larger than a typical small lymphocyte. It also contains much more cytoplasm. It may well be that this was a plasma cell which had lost some cytoplasm as the result of the centrifugation or for some other reason. That plasma cells through loss of cytoplasm may approach the appearance of lymphocytes can be seen in Fig. 12.

Discussion. It has thus been demonstrated that certain lymphoid cells of antibody forming lymph nodes exhibit agglutination on their surface when exposed to antigen *in vitro*. That this was not an artefact, but true agglutination is apparent from the facts that it occurred only between cells of immunized rabbits and the bacteria with which this immunization was achieved, that it was at its peak when the antibody concentration in the lymph nodes was highest, and that there were no cross reactions when typhoid antigen was added to cells from rabbits immunized with brucella organisms and vice versa.

Many of the agglutinating cells were identified as plasma cells or their predecessors. Polymorphonuclear leukocytes, monocytes and macrophages as well as typical small lymphocytes were ineffective. The observation that immature plasma cells agglutinated better than mature elements, and cells which were interpreted as close to mitotic division were particularly effective, could be construed into a confirmation of the theory of Fagraeus(3) that the production and release of antibody by these cells was a function of their youth. It is equally conceivable, however, that the loss of agglutinating power of the more mature cells was due to maturation of the cell membrane. It may be assumed that the regenerating cuticula after mitotic division should at first be delicate enough to allow contact between antibody within the cell and antigen without, whereas in the mature cells it may be so thick as to prevent antigen-antibody union.

Experiments with cortisone and similar substances* which are believed to cause shedding of cytoplasm(6) did not distinctly enhance the phenomenon. Cortisone in doses of 50 γ per ml caused considerable acceleration of intracellular vacuolization especially in the plasma cells and budding of cytoplasm especially in the lymphocytes, but these phenomena occurred also in the absence of such substances though less rapidly, and therefore are interpreted as agonal phenomena. As this acceleration was effected only with large doses of cortisone, we suspect that it was due to the solvent contained in the commercial preparation rather than the hormone. It is interesting that the plasma cells differed from the lymphocytes by less budding. This is in keeping with our impression that mature plasma cells have a thicker cell membrane than small lymphocytes.

Testicular hyaluronidase, desoxycorticosterone acetate and Artisone (21-acetoxypregnenolone) in doses up to 125 γ per ml had no appreciable effect on the cells or the agglutination phenomenon.

Summary. It has been demonstrated that certain lymphoid cells of antibody forming lymph nodes agglutinate on their surface *in vitro* the bacteria with which the animals were immunized. The agglutinating cells which could be identified belonged to the

* We are indebted to Dr. Joseph Seifter of the Wyeth Institute of Applied Biochemistry for supplying us with these materials.

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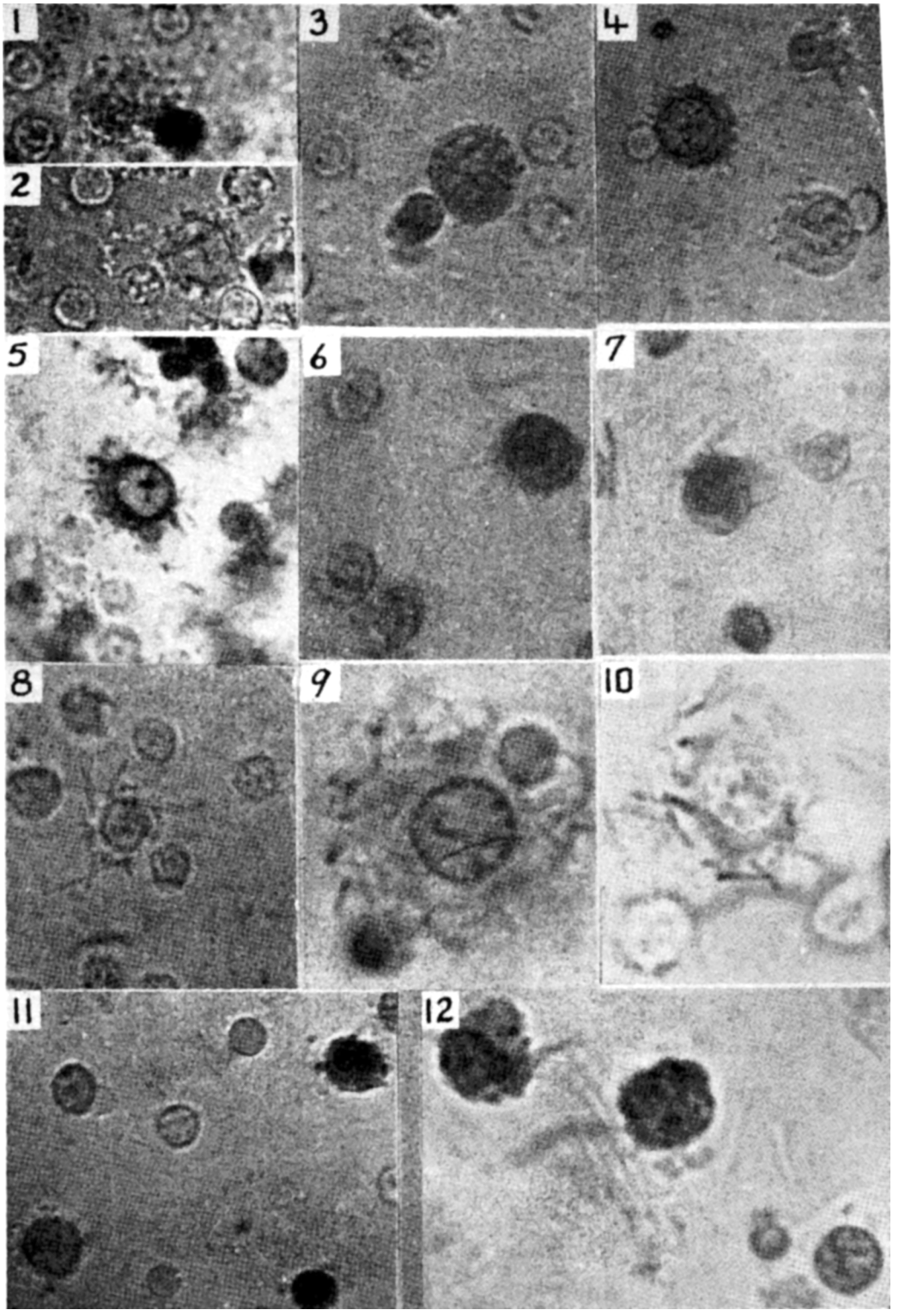
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Fig. 1 to 4 and 11 show agglutination of brucella, Fig. 5 to 10 that of typhoid H. The large rods in Fig. 12 are Cortisone rods. Fig. 1 and 2 were made from unstained preparations, all others from preparations stained with Janus Green. Fig. 9, 10, and 12 were magnified × 2000, all others × 1000.

plasma cell series, whereas typical small lymphocytes failed to show the phenomenon. These observations seem to show that it is the plasma cell rather than the lymphocyte which elaborates agglutinins.

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Prevention of Chemotherapeutic Effects of 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid on Mouse Leukemia by Citrovorum Factor.* (18031)

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4-Amino-N^{10}-methyl-pteroylglutamic acid has previously been shown to be effective in prolonging the survival time of mice with transplanted leukemia AK4(1). This prolongation of survival time can be prevented by prior administration of massive doses of pteroylglutamic acid (PGA) or pteroyltriglutamic acid (PTGA)(2). Since the citrovorum factor (C. F.)(3) has been shown to be more effective than PGA in preventing the toxicity of 4-amino-PGA in the bacterium, *Leuconostoc citrovorum*(4), and in mice(5) attempts were made to prevent the antileukemic effect of 4-amino-N^{10}-methyl-PGA by C. F. (6). The detailed results of these studies are herewith reported.

Method. Experimental. The procedure for inoculating mice of the AKm stock with AK4 leukemia was similar to that reported previously(2). 0.1 cc of a saline suspension of leukemic spleen containing 1,000,000 cells was injected intraperitoneally. Forty-eight hours later, treatment with 4-amino-N^{10}-methyl-PGA was started at a dose of 2 mg/kg three times weekly for ten doses by the intraperitoneal route. Intraperitoneal injections of citrovorum factor were started twenty-four hours after the inoculation of the leukemia and then given fifteen minutes before each dose of the antimetabolite. Various dosage levels in units(3) of C. F. were studied against the standard dose of 4-amino-N^{10}-methyl-PGA. In one experiment various levels of C. F. and PGA were given one hour after each injection of 4-amino-N^{10}-methyl-PGA. In another experiment the C. F. was allowed to stand at pH 2.0 for 24 hours at room temperature before injection in order to destroy the C. F. activity(5). After this procedure all the folic acid activity but only 10% of the C. F. activity remained as shown by microbiological assay against *S. fecalis* and *L. citrovorum* respectively.

Discussion. The preparations of citrovorum

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