STUDIES ON ANTIBODY PRODUCTION

III. The Alum Granuloma*, ‡

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Plates 14 and 15

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The aim of the present study was to determine the nature and situation of antibody-producing cells following the subcutaneous injection of diphtheria toxoid and ovalbumin adsorbed to aluminum phosphate.

In 1926 Glenny, Pope, Waddington, and Wallace (1) showed that when a solution of potassium aluminum sulfate, colloidal iron, or tungstic acid was added to crude formol toxoid a precipitate formed and that when guinea pigs were inoculated with such preparations appreciably greater antitoxin responses followed, compared with those following the injection of the untreated formol toxoids.

In 1931 Glenny, Buttle, and Stevens (2) published investigations designed to account for this increased antigenic potency of alum-precipitated toxoid over its parent formol toxoid. They stated that this enhancement was due to the delayed absorption from the site of inoculation. Moreover, they considered that the release of toxoid from the injected alum precipitate was prolonged so that antigen liberated subsequently could act as a secondary type stimulus.

The hypothesis of the delayed release of antigen was put on a sound basis by this and subsequent work (3, 4) which demonstrated that a persistent deposit of antigen is in fact locally created. Experiments were described by Glenny (2) in which 3-day-old alum nodules from guinea pigs were excised, macerated, and tested for their capacity to elicit a secondary response in animals. The recipients developed antitoxin. Harrison (3) found that 7-week-old alum granulomata were still antigenically active when injected. Faragó (4) carried out similar estimations *in vivo* of the antigenic activity of macerated nodules; he found a declining potency over a period of 48 days, although antigen was detectable by flocculation tests for 3 days only.

Holt (5) compared the antitoxin responses of groups of animals with and without the excision of the alum-toxoid nodules and concluded that the effective stimulus from them had disappeared by about the 14th day. In view of the known persistence of antigen in such nodules, which he also confirmed, he concluded that the antigen was

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excluded from the physiological interior of the animal because of its gradual encapsulation. He therefore criticized the hypothesis of Glenny that the increased activity conferred by alum was due to the elicitation of a secondary response.

It was suggested by Landsteiner (6) that many factors might play a role in immunization with alum-precipitated antigens. He included slow absorption, delayed removal of antigen, the particulate state, and consequent phagocytosis of the particles by antibody-producing cells, and the stimulation of cell activity.

Materials and Methods

Animals.—Rabbits weighing 2 to 3 kg. and aged 6 months were fed on a pellet diet plus water. Guinea pigs of 350 to 500 gm. were fed on a pellet diet with supplementary cabbage *ad lib.*

Antigens and Dosage.—Aluminum phosphate-precipitated diphtheria toxoid was generously supplied by the Massachusetts Biologic Laboratories, Boston, through the courtesy of Dr. Harry Bowen. This contained 20 Lf of toxoid per ml., with mineral carrier equivalent to 0.9 mg. Al (metal) per ml.

Crystalline hen's ovalbumin (Armour and Company) was used as an alternative antigen and prepared as a precipitate with aluminum phosphate according to the details of Holt (5) modified so as to contain 10 mg. of ovalbumin per ml., with mineral carrier equivalent to 1.5 mg. Al (metal) per ml.

As a routine 0.5 ml. of both antigen mixtures were used. Aluminum phosphate diphtheria toxoid was injected subcutaneously into the right hind foot-pad, and 0.5 ml. of aluminum phosphate-precipitated ovalbumin was injected into the left hind foot-pad.

Estimation of Serum Antibody Levels.—The estimation of diphtheria antitoxin was performed by titration of mixtures of diphtheria toxin and antitoxin in the rabbit's skin, as described in reference 7.

Morphological Techniques.—At autopsy small samples of tissue were quick-frozen and stored for subsequent preparation of frozen sections by methods previously described (8, 9). Portions were also fixed in Bouin's fluid. Immunohistochemical staining for diphtheria toxoid, egg albumin, diphtheria antitoxin, and anti-egg albumin antibody was carried out by methods described in detail in earlier and accompanying papers (9, 10). Briefly, staining for *antigen* was carried out by allowing the fixed tissue section to react with homologous, fluorescein-labelled antibody, washing, and examining the section under the fluorescence microscope for fluorescent deposits. The specificity of such reactions was controlled by the method of specific inhibition (11), that is, inhibition of the reaction by pre-treatment of the section with unlabelled homologous antibody. *Antibody* was detected by first treating the section with homologous antigen solution. In the case of diphtheria toxoid, the solution contained about 100 Lf/ml. in buffered saline, in the case of egg albumin, 1 mg./ml. After a 30 minute exposure, the section was washed in buffered saline for 10 minutes, and then treated with homologous-labelled antibody. Cells which had been unremarkable in the background when stained for antigen became brightly fluorescent after this two-step procedure, and their content of antibody thus revealed. Most of the animals studied were injected with two antigens, ovalbumin–aluminum phosphate into the left hind foot-pad, and diphtheria toxoid aluminum phosphate into the right. For this reason, it was possible by staining different sections of both popliteal nodes for each kind of antibody to establish the specificity of the reaction on tissues of the same animal.

Non-Specific Staining.—The conjugates used in the studies reported in this paper were absorbed with mouse liver powder (*cf.* reference 10). In rabbit tissues, there was non-specific staining of certain cell types. Although this phenomenon has been described in another paper (10), it is advantageous to report some of its appearances here in somewhat different material and through the eyes of another observer. The cells which reacted non-specifically with

fluorescein-serum conjugates included polymorphonuclear leucocytes and eosinophils. These were easily distinguished from antibody-containing plasma cells by the negative images of their multilobed nuclei. Non-specifically stained eosinophils and eosinophil myelocytes could be recognized with experience; their cytoplasmic fluorescence was bright and sharply serrated in outline, and made up of large fluorescent granules. The non-specifically stained neutrophil myelocyte is a cell which could be confused with an immature plasma cell owing to its round or oval nucleus. In practice, however, the distinction was usually easy since its outlines were not sharp and its fluorescence not bright, whereas the antibody-containing plasma cell had a sharp outline and a bright fluorescence.

In the guinea pig these cell types did not stain non-specifically.

The sections stained by these techniques were visualized under the fluorescence microscope as described (12), with the modifications described subsequently (13). In order to localize the staining by the above techniques to structures stained by more conventional methods, adjacent sections were stained by Giemsa's method after fixation in Bouin's solution.

Nomenclature of Cell Types.—The nomenclature adopted in the descriptions of histological material follows the definitions and illustrations of cells in Marshall and White (14), for the terms primitive reticular cell, activated reticular cell, plasmablast (hemocytoblast), immature plasma cell, mature plasma cell, reticulo-endothelial system.

The cell which is described as a plasmablast is indistinguishable when stained by hematoxylin and eosin, methyl green–pyronine, or Romanowsky stains, from the cells which are the precursors of the normal erythrocyte or leucocyte series. On these criteria, therefore, the term hemocytoblast can be used to include all these cells. There are, however, additional reasons for the use of the term plasmablast for such immature cells; first, there is evidence that antibody is produced by these cells in addition to more mature plasma cells (14, 15), and secondly, they can be stained to show a cytoplasmic content of antibody (10).

DESCRIPTION OF FINDINGS

Rabbits

When a 10 Lf dose of aluminum phosphate–precipitated diphtheria toxoid was deposited subcutaneously in the foot-pad of the rabbit, the first cellular event locally was the exudation of large numbers of polymorphonuclear leucocytes. Sections taken at 24 hours following antigen injection showed lakes of aluminum salt lying between the separated collagen fibres of the dermis with a dense infiltration of polymorphonuclear leucocytes. In sections stained for diphtheria antigen, the aluminum phosphate deposits had an intense yellow-green fluorescence due to the specific staining of this antigen. Control sections which were unstained or stained with a heterologous antibody-fluorescein conjugate showed that the aluminum salt possessed a pale blue natural fluorescence. All sections which were stained with a fluorescein conjugate, whether of homologous or heterologous antibody, showed a green non-specific staining of polymorphonuclear leucocytes and pseudo-eosinophils.

Fig. 1 shows a lake of subcutaneous antigen-staining material. The faint negative shadows of the contained polymorphonuclear leucocytes can be distinguished within this mass of bright fluorescence.

7 days after injection, there was a considerable diminution in the brightness of the antigen staining. Many polymorphonuclear leucocytes were present,

largely necrotic. The main feature at this time as revealed both by Giemsa-stained sections and by sections under the fluorescence microscope was the invasion of the periphery of the aluminum salt–antigen mass by macrophages. The particles of mineral carrier phagocytosed by these cells were segregated within their cytoplasm as fine discrete granules. Sections stained for antigen showed a few cells within the alum mass with a faintly green fluorescent cytoplasm, suggesting that they contained diphtheria toxoid antigen. The peripherally situated macrophages had their cytoplasm outlined by their content of brilliant whitish blue fine granules of aluminum salt, without any trace of green, and their nucleus was visible as an oval or reniform negative shadow. At 7 days following antigen injection the appropriately stained sections revealed no evidence of antibody-containing cells in the local nodule.

However, in sections from the popliteal lymph glands of rabbits autopsied at the same time interval after injection and stained for antibody, numerous solitary antibody-containing cells could be distinguished. Almost invariably the cells were isolated and single, with 5 to 10 cell diameters between stained cells. The whole section of the rabbit's popliteal gland contained 200 to 400 cells. It was possible to recognize several morphological types of cell. Fig. 2 illustrates a cell type which was common at 7 days. The cells were large, up to 16 μ in diameter, with a narrow rim of fluorescent cytoplasm around a large, oval, or almost round nucleus. The cytoplasmic outline of this was clear cut, rounded, and free from any dendritic extensions. The cytoplasm was not always uniformly fluorescent but often included a clear area. The cell illustrated in Fig. 2 shows a fluorescent area within the nucleus. Fluorescence within the nuclei of antibody-containing cells was often observed but was of very diverse outline—dots, rings, and irregular strands. The localization of this fluorescence within the nucleus was not constant and could not be associated with the nucleolus. The size, and outline, of these cells were thought to correspond with the hemocytoblast (plasmablast) when compared with the cells occurring in corresponding areas of adjacent frozen sections stained with Giemsa.

Other cell types present in the medullary strands of the popliteal glands at 7 days corresponded to more mature plasma cells and resembled those illustrated in Fig. 3 (3 weeks after injection). Fig. 3 (*B*) illustrates a cell of a type which possessed a nucleus which was a circle or a broad oval of 8 to 10 μ diameter, surrounded by a rim of cytoplasm which was wider and more brightly fluorescent than that of the plasmablast. Since the nucleus was smaller, the ratio of area of nucleus to area of cytoplasm was much decreased in these cells as compared with the plasmablasts. They were thought to correspond with immature plasma cells.

Fig. 3 (*A*) illustrates a type of cell in which the nucleus was smaller still (6 to 8 μ diameter) with an eccentric position within cytoplasm which was extensive and very brightly fluorescent. Often it was easy to see an oval clear area abutting on the nucleus at the side corresponding to the more extensive

cytoplasm. These cells were considered to represent mature plasma cells. The clear area corresponds with the very prominent Golgi element of these cells. Fig. 4 shows the progressive increase in intensity of cytoplasmic fluorescence with increasing maturity of plasma cell type.

Sections of the lymph glands of rabbits also sometimes contained small amounts of masses of bright whitish blue fluorescent material. This was present as aggregations of granules free in the cortical and medullary sinuses and also in the cytoplasm of macrophages within and lining these sinuses. This material was not seen in the lymphatic glands of uninjected control animals and was regarded as particles of the aluminum phosphate conveyed to the gland by the lymph channels. No adsorbed antigen was demonstrable.

In summary, at 7 days after injection the local nodule contained antigen, but no demonstrable antibody-containing cells, while the regional lymphatic gland (popliteal) contained scattered antibody-containing cells in its medulla, corresponding in morphology with the various members of the plasma cell series.

Tissue sections of the local granuloma in the foot-pad of animals sacrificed at longer intervals after injection, showed at 14 days the beginnings of a neat organization. In the center of the nodule was the mass of aluminum phosphate infiltrated with large numbers of macrophages. This central mass when appropriately stained was shown to contain antigen. A few of the macrophages within or at the edge of this mass also had faint green fluorescence indicating a cytoplasmic content of antigen.

More peripherally was a broad zone of macrophages packed with alum granules. Under the fluorescence microscope these fluoresced a bright, whitish blue. They were large cells, up to 35 μ in diameter, with extensive cytoplasm of wavy outline. The nucleus, usually reniform, could be seen as a negative shadow amid the blue granules of the cytoplasm. A smaller number of polymorphonuclear leucocytes was also present. Surrounding the thick macrophage layer at 14 days was a narrow zone of young fibrous granulation tissue (Fig. 7). Collagen fibres made up the periphery of this outer zone, with spindle-shaped basophilic fibroblasts between the individual fibres. At the inner margin of this fibrous tissue were groups of mature and immature plasma cells. These were more numerous at 3 weeks (Fig. 8 and 9), at which time the concentric zones of macrophages, plasma cells, and collagen fibres with fibroblasts, were very distinct. The sections stained for antibody showed that in the zone corresponding with the plasma cells of the Giemsa-stained sections (Fig. 8), there existed collections of antibody-containing cells (Fig. 9). Comparison of adjacent frozen sections stained with the two techniques left no doubt that the only cells which possessed a cytoplasmic content of antibody were the cells with the morphology of plasma cells. The macrophages were completely devoid of antibody content. Also the fibroblasts failed to stain for antibody.

Inspection of the local nodules at 4 weeks showed that the outer zone of

fibrous tissue was now broad and prominent. The numbers of antibody-containing cells within these nodules appeared to decrease after the 3rd week. However, nodules inspected at 7 weeks after injection of antigen still showed small groups of plasma cells with a cytoplasmic content of antibody, isolated in the dense collagenous tissue (Fig. 6).

Sections of the regional (popliteal) glands at 14 days showed that the number of antibody-containing cells had increased over that at 7 days. In most instances these were scattered as single cells. All the morphological types of plasmablasts, immature and mature plasma cells, were represented throughout the medulla of the gland. The lymphoid nodules of the cortex were devoid of staining for antibody. In one case (out of four) groups of 3 to 5 adjacent cells

TABLE I

Serum Diphtheria Antitoxin Levels (in Units per Ml.) at Various Time Intervals Following Injection of 10 Lf of Diphtheria Toxoid Adsorbed to Aluminum Phosphate into the Foot-pad of Rabbits

Animal No.	Time after injection	Antitoxin
	wks.	*units/ml.*
7-77	1	0.01
7-81	2	1.8
8-58	2	0.80
8-59	2	1.0
7-39	3	3.2
7-58	3	3.2
8-17	4	1.8
7-50	7	1.0
7-60	7	0.60

of immature plasma cell form were present as well as isolated single cells. The popliteal glands from animals sacrificed at 3 weeks (Fig. 5) showed numbers of antibody-containing cells which were rather less than at 14 days. The cells were mainly immature and mature plasma cells, scattered singly throughout the medullary strands of the node (Figs. 4 and 5). Sections from popliteal glands of animals autopsied at longer time intervals, *i.e.* at 4, 5, and 7 weeks, showed a decreasing number of cells so that at 5 weeks they were very rare and, at 7 weeks, only an occasional antibody-containing cell could be found (Fig. 6).

The antitoxin titers in the serum of these rabbits are tabulated in Table I.

Staining of the popliteal lymph gland of the opposite side with the technique for revealing antibody (as was performed, of course, in the controls) showed that no antibody-containing cells appeared here at any of the time intervals used in this study. Also, no antibody-containing cells were detected in the spleen. The spleen of rabbits often had groups of non-specifically staining neutrophils and neutrophilic myelocytes.

A surprising finding was the localization of a fluorescence characteristic for antibody on the surface of the collagen fibres which enclosed the granuloma at the site of injection. This staining was first faintly visible at 2 weeks after injection but became much more intense at 3 weeks and later. This is faintly visible in Fig. 9, in which the green fluorescence outlining the collagen fibres was specific for diphtheria antitoxin. Thus this staining was absent from sections stained for diphtheria antigen. It was also absent from sections stained for heterologous antibody; *e.g.*, ovalbumin antigen and anti-ovalbumin antibody conjugate. This staining of collagen fibres was confined to the immediate neighborhood of the local nodule and decreased peripherally, so that collagen of the normal dermis at the edge of the section was unstained.

So far, the results which have been cited were obtained with aluminum phosphate–precipitated diphtheria toxoid (dose 10 Lf) injected into the foot-pad of rabbits. These experiments were duplicated with similar injections of aluminum phosphate–precipitated egg albumin, dose 5 mg. The results were so similar to those with diphtheria toxoid that little is to be gained by repeating them in detail.

Guinea Pigs

Injections of aluminum phosphate–precipitated diphtheria toxoid and egg albumin were also made into the foot-pads of guinea pigs. Certain constant differences were noted from the results of the same investigations in rabbits. First, the popliteal lymph glands of guinea pigs showed very prominent masses of pale blue brightly fluorescent material present, free as granular masses in the sinuses of the gland and also within the cytoplasm of macrophages within these sinuses. This material was absent from the lymphatic glands of uninjected control guinea pigs and was regarded as mineral carrier which had reached the gland *via* the lymph stream from the foot-pad. As mentioned above, identical fluorescent material was found in the popliteal glands of rabbits, but infrequently and in inconspicuous amounts. With guinea pigs the finding was constant and conspicuous. This material was present in the glands of animals autopsied 24 hours after antigen injection. Secondly, more antibody-containing cells were seen in the popliteal glands of guinea pigs than in a comparable area of the section of a rabbit gland. (This was particularly so at 14 and 21 days after injection, at which times small groups of cells were present). Thirdly, repeated experiments failed to demonstrate any antibody-containing cells in the granulomata at the sites of injection at 7, 14, and 21 days after injection. Antibody-containing cells were first present in the local granuloma at 4 weeks after injection. As in the case of the rabbit, they were situated on the inner side of the zone of fibrous granulation tissue surrounding the macrophage mass. They possessed the size and cytoplasmic outline of immature and mature plasma cells and were localized in adjacent frozen sections stained by Giemsa to groups of typical mature and immature plasma cells.

DISCUSSION

When the morphological effects of the injection of 10 Lf of aluminum phosphate–precipitated diphtheria toxoid are compared with those produced by injection of 150 Lf of soluble toxoid (both similarly injected into the foot-pad of the rabbit) (7) the following facts emerge. First, the cellular response at 3 weeks after injection of soluble diphtheria toxoid had almost completely disappeared from the popliteal node as judged by the numbers of demonstrable antibody-containing cells. After injection of aluminum phosphate–precipitated toxoid, the cellular response in the popliteal gland is still very evident at 3 weeks and 4 weeks after injection implying that the antigenic stimulus reaching these glands persists longer in this case. Such a prolongation of antigenic stimulation was postulated by Glenny, Buttle, and Stevens (2). Secondly, the cellular response at 7 days was greater in the popliteal nodes of animals receiving 10 Lf of aluminum phosphate–precipitated toxoid than in animals receiving 150 Lf of soluble toxoid. This observation would imply that some adjuvant effect has already been exerted at this time interval.

The diminishing numbers of antibody-containing cells within the regional glands from 14 days onwards and the finding in rabbits, at least, that at 3 weeks and onwards only single isolated cells are demonstrable in the popliteal glands, imply that the antigen stimulation is having little effect. This is in spite of the fact that Harrison (3) and Faragó (4) have conclusively demonstrated that antigen persists at the local site of injection and is moreover active in causing antibody production when injected into other already sensitized animals. The results of staining of antigen in this investigation also demonstrated such a persistence for at least 3 weeks.

In the rabbit experiments were made to test this point. 3 weeks after sensitization with 10 Lf of aluminum phosphate–precipitated diphtheria toxoid, injected sometimes into the back and sometimes into one foot-pad, 1/10 Lf and 10 Lf doses of soluble diphtheria toxoid were injected into both foot-pads. The results showed that with both doses there resulted, after 4 days, in the popliteal glands of both sides, responses which had all the morphological characteristics of secondary type responses with the formation of groups of adjacent cells (7). These results reinforce the conclusion that the antigen which demonstrably persists at the local site is escaping in very small amounts after 14 days; and would appear to accord with the shape of the curve of antitoxin production in the serum following subcutaneous stimulation with aluminum–precipitated toxoid. The serological results of this investigation and the published reports of others indicate that the serum levels fall after 3 weeks.

In all cases, the cells both of the local granuloma in the foot-pad and of the regional lymphatic glands which showed a content of antibody appeared to correspond with plasma cells of varying grades of maturity. This was checked by reference to Giemsa-stained adjacent frozen sections. Thus, in all the lymph glands which were inspected in this series of experiments the antibody-con-

taining cells were confined to the medulla of the gland, and no antibody-containing cells were present in the lymphoid nodules. However, a striking exception to this was encountered. In the lymphatic glands of the animals described above, which were restimulated at 3 weeks following a primary sensitization by injection of aluminum phosphate–precipitated diphtheria toxoid, antibody-containing cells were observed to occur in the centers of some of the lymphoid nodules (Fig. 10). These were present only on the side which had received the primary sensitizing injection. Inspection of the Giemsa-stained adjacent sections showed that the areas corresponded to germinal centers of the lymphoid nodules and that a proportion of the cells in these centers possessed basophilic cytoplasm and the appearances of activated reticular cells mainly, with a few hemocytoblasts. Similar results were also obtained with ovalbumin as antigen. The presence of activated reticular cells and hemocytoblasts in the germinal centers of lymphoid nodules following antigenic stimulation has been previously described (14). No mature plasma cells were seen.

The finding that the mineral carrier is conveyed by the lymphatics to lodge in the sinuses of the regional glands particularly of the guinea pig may, in this species at least, have an important bearing on the adjuvant effect. Certainly the finding is sufficient to confuse the analysis of the results of experiments in which the intent is to remove antigenic deposits by surgical removal of the local nodule.

CONCLUSIONS

After subcutaneous injection of hen's ovalbumin or diphtheria toxoid precipitated with aluminum phosphate, the production of antibody, as judged by the presence in the tissues of antibody-containing cells, proceeds partly within the regional lymphatic glands and partly in the granulation tissue surrounding the nodule which develops at the site of injection. The first production of antibody takes place in the regional lymphatic gland and antibody production in the local granuloma becomes apparent only from 14 days onwards (rabbit).

Antibody-containing plasma cells were demonstrated in the local granuloma up to 7 weeks. Antibody-containing cells in the regional lymphatic glands reach maximum numbers at 2 weeks following injection and decrease thereafter to few cells at 5 weeks.

The adjuvant effect of the aluminum phosphate is interpreted as due partly to the delay in absorption of antigen from the local site of its injection which results in prolongation of stimulation of cells within the regional lymphatic glands, and partly to the production of a local granuloma which contains antibody-producing plasma cells.

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EXPLANATION OF PLATES

All photomicrographs are of organs of rabbits which were injected with 10 Lf of aluminum phosphate–precipitated diphtheria toxoid subcutaneously into the foot-pad. All figures, except Figs. 7 and 8, are fluorescence micrographs of frozen sections treated with fluorescein conjugates. The brightest areas (except when otherwise stated) represent the yellow-green fluorescence of the deposited fluorescein antibody; the topography of the tissues is made visible by the faint blue autofluorescence of the normal tissue. Fig. 1 was stained for content of *antigen. All other* fluorescence micrographs were stained for content of *antibody.*

PLATE 14

FIG. 1. Foot-pad. Subcutaneous tissue at site of injection. 24 hours. Note mass of aluminum salt with intense antigen staining. Negative shadows of polymorphonuclear leucocytes visible within the fluorescent mass. × 150.

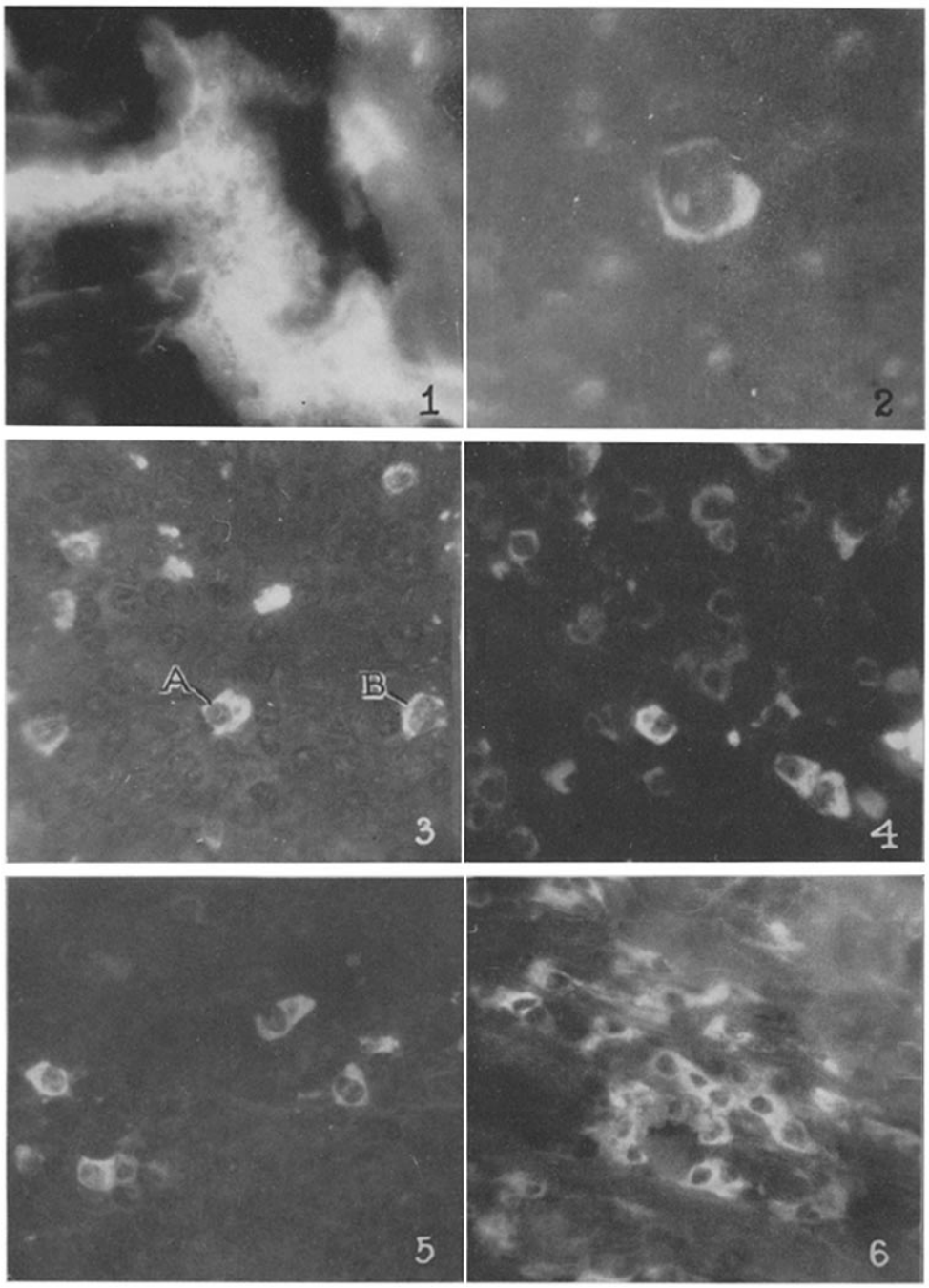
FIG. 2. Popliteal lymphatic gland. Hemocytoblast (plasmablast) in medulla at 7 days after injection. × 1360.

FIG. 3. Popliteal lymphatic gland. (*A*) Mature plasma cell. (*B*) Immature plasma cell. Medulla of gland at 3 weeks after injection. × 600.

FIG. 4. Popliteal lymphatic gland. 14 days after injection. Cells of medulla showing varying grades of maturity. × 600.

FIG. 5. Popliteal lymphatic gland. 3 weeks after injection. Isolated antibody-containing cells. × 600.

FIG. 6. Foot-pad 7 weeks after injection. Fibrous tissue at edge of alum granuloma enclosing group of antibody-containing cells. × 600.



(White *et al.*: Studies on antibody production. III)

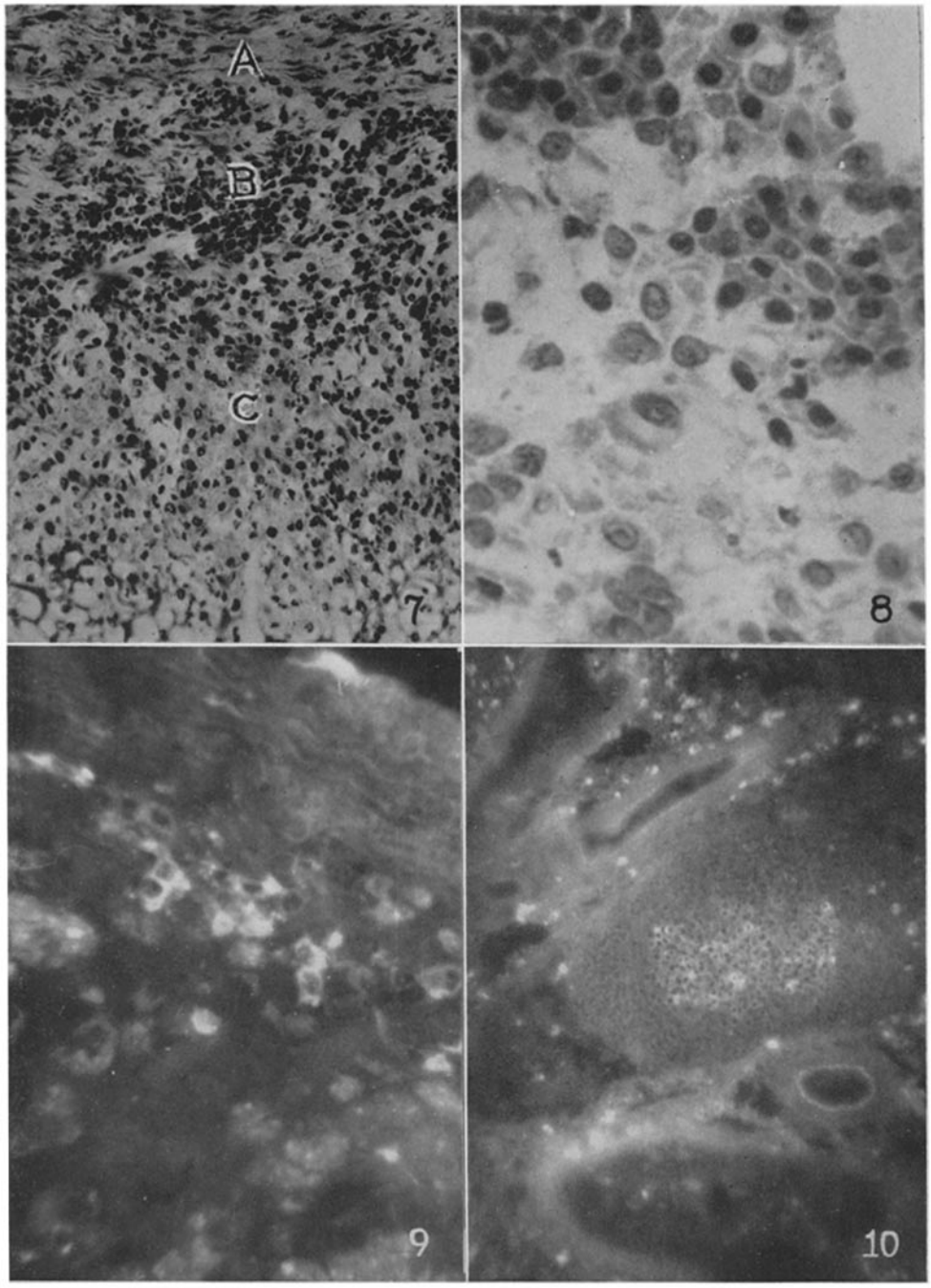
PLATE 15

FIG. 7. Foot-pad. 14 days after injection. Periphery of alum granuloma. Lower zone of macrophages (*C*) with basophilic reticulum of aluminum salt precipitate along lower edge of photograph. Upper zone of fibrous tissue (*A*) and fibroblasts separated from macrophage zone below by narrow zone of plasma cells (*B*). Frozen section. Giemsa. × 138.

FIG. 8. Foot-pad. 21 days after injection. Periphery of alum granuloma. Frozen section. Giemsa. × 800.

FIG. 9. Foot-pad. 21 days after injection. Periphery of alum granuloma. Note at top of picture fluorescent staining which outlines collagen fibres. Below is zone of antibody-containing cells. At bottom, macrophages. These cells contain cytoplasmic content of aluminum salt granules which fluoresce whitish blue. × 600.

FIG. 10. Popliteal gland. Antibody-containing cells in germinal center of a lymphoid nodule (see Discussion). × 150.



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