GAMMA GLOBULIN AND ANTIBODY FORMATION IN VITRO*

III. Induction of Secondary Response at Different Intervals after the Primary; the Role of Secondary Nodules in the Preparation for the Secondary Response

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It was shown previously (1) that the peak of antibody production in the spleen during the primary response to bovine gamma globulin (BGG) coincides with immature plasma cell proliferation at the border of red and white pulp. The so called "secondary nodules" in the white pulp reach their peak of development after the production of antibodies has subsided. Since the follicular centers are therefore not major sites of antibody production during the primary response, the possibility that they may play another role in the immune process (2) such as preparing for the secondary response, a suggestion also made by White (3), has been investigated in the present study. This has been attempted by studying the secondary response induced at various intervals after a primary injection of BGG. It was felt that reinjection of BGG at the time of secondary nodule proliferation might produce a pattern of response different from that seen in the secondary response induced at 1 month after the first antigen injection.

This study involves the preparation of roller tube cultures of spleen and bone marrow for the determination of antibody and gamma globulin synthesis, serum antibody titrations, and histological examination of the tissues at various days after the second intravenous antigen injection. It will be shown that the time of appearance of antibody production in the spleen during the secondary response, induced at 10 days after a primary injection of antigen, is quite different from that of the primary response, and also differs from that of the secondary response induced at 1 month after the primary. The findings suggest that the secondary nodules which are proliferating at the time of reinjection

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of antigen may provide cells which respond to a booster injection by rapid differentiation to immature plasma cells. Differences between primary and secondary response are indicated both by the nature of the antibody formed (19S *versus* 7S gamma globulin) and by the temporal relationships (appearance and subsidence of plasma cell proliferation and antibody production).

Materials and Methods

Animals[1] *and Immunization.*—New Zealand rabbits of either sex, weighing 2 to 3 kg. were employed. Most of the animals received two intravenous injections of 10 mg bovine gamma globulin (BGG, Pentex Biochemicals, Kankakee, Illinois, Lot B24) at the intervals indicated in the tables. One group of rabbits received 5 or 10 μg of purified endotoxin (*Salmonella abortus equi*[2]) intravenously at the time of the first BGG injection. Four rabbits received two intravenous injections of 25 mg ovalbumin, 4 weeks apart.

The animals were killed by exsanguination at various days after the last antigen injection. A few rabbits were given an intravenous injection of 5 or 10 ml of an anti-BGG serum obtained from hyperimmunized rabbits (hemagglutination titer 1:256,000). The serum was injected 9 days after a primary injection of BGG, and 1 day before a secondary injection. These animals were killed on days 6, 7, and 9 after the last BGG injection (Table II).

The method used for *preparing the roller tube cultures and control extracts* was similar to that described previously (1). The medium consisted of Hanks' balanced salt solution, 10 per cent normal rabbit serum (or calf serum), added glucose (to 22 mM), vitamins (4), amino acids (4), and penicillin (200 U/ml). Cultures were incubated at 37°C. The cultures and controls were first frozen and thawed, and then subjected to sonic oscillation (15 minutes, 9 K cycle, Raytheon magneto striction oscillator) in order to combine the antibody present in the medium, with that in the tissue.

Antibody Titrations.—Hemagglutination titers of anti-BGG in culture fluids and sera were determined as previously described (1). In the experiments when ovalbumin was used as the antigen, a different method for antibody determination was employed. The medium used contained calf serum instead of rabbit serum. Each centrifuged extract and culture fluid was incubated for 30 minutes at 37°C with 6 μg of an I^{131}-labeled ovalbumin preparation[3] containing 250 CPM/μg. Each tube then received 0.1 ml of a 1:10 dilution of normal rabbit serum in order to provide carrier gamma globulin, and an excess of a specific sheep anti-rabbit gamma globulin serum. The precipitates were washed 3 times in ice-cold saline and their radioactivity measured with an end window Geiger Müller counter. If the precipitate from the culture fluid was found to have greater radioactivity, *i.e.* a greater capacity to bind the I^{131}-ovalbumin, than that from the extract fluid, it was attributed to the production of anti-ovalbumin antibody *in vitro*.

Gamma Globulin Formation.—The tissues of some animals were also used for the study of gamma globulin synthesis *in vitro* according to methods previously described (5). Similar roller tube cultures were prepared for this purpose as for the antibody studies. The medium contained 10 per cent calf serum or 0.5 per cent ovalbumin instead of rabbit serum, and a mixture of amino acids (6) from which lysine had been omitted. Uniformly labeled C^{14}-L-lysine

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(Institut Pasteur, Paris, or Nuclear Chicago Corp., Des Plaines, Illinois) was added in a concentration of 1 μc (20 to 25 μg) per tube.

In principle, incorporation of C^{14}-labeled lysine into rabbit gamma globulin (RGG) *in vitro* is estimated by measuring the radioactivity of the precipitates (counted at infinite thinness), obtained from culture fluids with the aid of carrier RGG and a specific sheep anti-RGG serum, after appropriate absorption with unrelated immune precipitates (5).

Histological studies were performed on sections prepared from spleen tissue fixed for 5 hours in a mixture of Zenker's solution with formalin 10 per cent (90:10), and stained with methyl green–pyronin according to Brachet (7).

RESULTS

Response to a Secondary Injection of a Protein Antigen, 1 Month after the Primary.—The results obtained with tissues from 4 individual rabbits killed on

TABLE I

Antibody and Gamma Globulin Production by Rabbit Spleen Tissue in Vitro Taken at Various Days Following a Second Intravenous Injection of Ovalbumin, 1 Month after the Primary Injection

Day	Extract* spleen	Culture* spleen	Globulin‡ production	Serum§ titer
2	48	185	175	20
3	69	336	320	
4	72	227	160	1280
7	160	188	120	2560

* Antibody measured in CPM of I^{131}-ovalbumin bound by the gamma globulin of culture and extract fluids.

‡ Gamma globulin production measured as C^{14} radioactivity (CPM) in gamma globulin precipitate less radioactivity of last absorption precipitate.

§ Serum titers expressed as reciprocals of greatest dilutions giving positive hemagglutination patterns.

different days after a booster injection of ovalbumin are listed in Table I. Production of antibodies was high on days 2, 3, and 4 after the booster injection, and absent on day 7. The peak of gamma globulin and of antibody formation, in this small series of animals, occurred on day 3.

The experiments on the secondary response to BGG with an interval of 4 to 5 weeks between primary and booster injections are summarized in Table II. High antibody formation in the spleen was found on days 2, 3, and 4. Much lower production occurred on days 6 and 7. When cultured separately, the red pulp of the spleen showed much higher antibody production than the white pulp. The bone marrow was found to form antibody in most of the animals studied, up to 7 days after the booster injection. The peak of antibody formation in spleen and bone marrow was observed on days 3 and 4.

The sequence of histological changes in the spleen observed following a

booster injection given 1 month after the primary injection, developed more rapidly, but was otherwise similar to that observed in the primary response (1). Quantitative differences from the primary response were noted particularly with respect to the numbers of proliferating hemocytoblasts and immature

TABLE II

Antibody Production by Rabbit Tissues in Vitro Taken at Various Days Following a Second Intravenous Injection of BGG, 10 or 30 Days after the Primary Injection*

Day	30-day interval						10-day interval					
	Spleen			Bone Marrow			Spleen			Bone Marrow		
	Extract titer	Culture titer	Production	Extract titer	Culture titer	Production	Extract titer	Culture titer	Production	Extract titer	Culture titer	Production
2	<1	24	++	<1	1	±	3	12	+	<3	<3	
	<2	32	++	<1	<1		2	32	++	<1	<1	
	<1	1	±	<1	3	+	2	64	++			
	<1	4	+				2	16	++	<1	1	±
3	<2	32	++				2	12	+	<2	<2	
	8	128	++				8	64	++			
							6	128	++			
							32	192	+	1	2	±
4	6	48	++	4	16	+	2	8	+	1	4	+
	6	96	++	6	48	++	<1	2	+	1	4	+
	2	16	++	8	64	++						
6	<2	<2		<1	<1		2	6	+	<2	8	+
	24	48	±	128	384	+	6	6				
‡							<2	<2		<1	1	±
7	2	6	+	<1	<1		<2	<2		1	1	
	<3	8	+	2	6	+	<2	4	+	<2	2	±
	2	4	±	8	8		2	2		1	1	
	1	2	±				2	2		8	16	±
‡							<3	<3		1	1	
9	<2	<2		<2	<2							
‡							<2	<2				
11	<2	<2		2	2		<2	<2		<2	<2	
	<2	<2		2	2							

* Antibody titers are expressed as reciprocals of greatest dilutions giving positive hemagglutination of BGG-sensitized erythrocytes. Production *in vitro* is graded from high (++) to equivocal ±.

‡ Received anti-BGG passively before the booster injection (see text).

plasma cells (Fig. 1). On days 2 and 3 after the injection, these cells could be seen in large numbers, partly inside, but mostly surrounding the sheaths of lymphocytes which make up the white pulp. They showed many mitotic figures. In the period from days 2 to 7, the number of immature plasma cells in the developing plasma cell clusters first increased and then decreased. During maturation these cells were found surrounding penicilli arterioles in the red pulp, further removed from the white pulp than they had been initially. This latter histological feature was quite similar to that described previously for the secondary response to paratyphoid vaccine (8) and for the primary response to BGG (1).

The white pulp of the spleen showed very few reaction centers (secondary nodules) until day 4 after the injection. These nodules appeared later, and showed maximal development around days 6 to 9.

Response to a Secondary Injection of BGG, 10 Days after the Primary.—Table II shows the results obtained with tissues from animals receiving 2 injections of BGG, 10 days apart, while Table III shows the antibody production by tissues from rabbits receiving 5 μg of *S. abortus equi* endotoxin with the primary injection of BGG and a secondary injection of BGG alone, 10 days later.

In the first group (Table II) the spleen (red pulp) showed very high antibody production on days 2 and 3, little on day 4, almost none thereafter. When cultured separately, the white pulp showed very little or no antibody formation. Highest antibody production in the spleen occurred on day 2. As compared with the serum titers of rabbits which had an interval of 1 month between the 2 antigen injections, the titers were higher on day 2, but did not rise to comparable levels on days 6 to 7, indicating a less sustained antibody response.

When endotoxin was given with the first BGG injection (Table III), the response to a second injection (after 10 days) was markedly different. On days 2 and 3 there was, as expected, high antibody production in the spleen. However, on days 7 and 9 the antibody production was still high, and the serum titers were higher than during the secondary response in animals which had not received endotoxin. The white pulp formed antibody in most of these rabbits but it was usually less active than the red pulp. Both white and red pulp showed significantly elevated levels of gamma globulin production compared with non-immunized control rabbits (Table III). A few of the cultures from this group of rabbits showed significant differences between the extract and culture titers only when tested with cells sensitized with crude BGG, but not with cells sensitized with purified BGG. These spleen cultures probably formed antibody against a contaminant antigen (1). All the other cultures showed antibody formation when tested with either purified or crude BGG, indicating antibody production to BGG itself.

Antibody production was also found in some of the bone marrows cultured from these groups of rabbits (Tables II and III). Although the exact day of the

TABLE III

Antibody and Gamma Globulin‡ Production by Rabbit Tissues in Vitro Taken at Various Days Following a Second Intravenous Injection of BGG, 5 to 10 Days after a Primary Injection of BGG with Endotoxin*

Day	Red pulp of spleen				White pulp of spleen				Bone marrow		
	Extract titer	Culture titer	Production Ab.	Production γ-Glob.	Extract titer	Culture titer	Production Ab.	Production γ-Glob.	Extract titer	Culture titer	Production Ab.
5 + 2	<1	3	+		1	1					
	8	32	+		<4	32	++				
	8	64	++		<2	4	+				
	12	48	+		6	48	++				
7 + 1	<2	<2			<2	4	+				
8 + 1	1	<1			1	6	+				
7 + 2	<1	24	++		<3	12	++				
	<2	32	++		<1	24	++				
10 + 2	12	96	++		8	16	±		<2	<2	
	2	48	++		2	8	+		2	4	±
	1	24	++	650	<1	1	±	50			
	1	48	++§						2	8	+
10 + 3	8	64	++	350	16	64	+	100			
10 + 6	4	32	++‖		<2	<2			<2	<2	
	2	4	±	80	<3	<3		15	<1	<1	
10 + 7	4	16	+	430	<2	16	++	340			
	3	32	++	400	1	6	+	45			
	3	24	++‖	210	2	2			2	8	+
10 + 9	2	16	++	200	4	6	±	90	2	8	+
	2	64	++	490	4	16	+	260	2	6	+
	<1	<1			<1	1	±		8	8	
	16	64	+‖	105	16	64	+		8	16	±
10 + 10	1	3	+‖	40	1	1			1	1	
Control				80				30			
Rabbits				100				−10			

* Antibody titers and production are expressed as in Table II.

‡ Gamma globulin production measured as in Table I.

§ Only whole spleen cultured.

‖ The antibody production observed in the spleen of these animals could not be demonstrated with sheep cells sensitized with the purified BGG preparation. All the other animals showed production equally well when tested against purified or crude BGG-sensitized cells.

peak of antibody production in the bone marrow was not determined, it seemed clear that the peak of antibody production by bone marrow tissue came later than that by splenic tissue. This may be due to the factors mentioned (1) when the complete lack of antibody formation in the bone marrow during the primary response was discussed. In view of the observed proximity of immature antibody-forming cells to lymphoid tissue in the spleen, the fact that bone marrow lacks lymphoid tissue accumulations lends support to the hypothesis that bone marrow receives immunologically competent cells from other sites.

In the spleens of animals receiving two injections of BGG 10 days apart, the development of blast cells and plasma cells was similar to that seen in the secondary response 1 month after the primary. Secondary nodules in the white pulp, presumably persisting from the primary response, were present throughout the period of plasma cell proliferation. They showed a gradual loss of mitotic activity. In some animals, mature plasma cells were seen inside pale centers of the white pulp around small blood vessels.

The histological response in the spleens of animals that had received endotoxin with the primary injection was different. The beginning of the plasma cellular reaction was characterized by an unusually large number of hemocytoblasts and immature plasma cells (Fig. 2). Some of the large blast cells were also located inside the centers of secondary nodules. The reaction diminished, but did not subside completely as in the previous group. Large numbers of immature plasma cells were present as late as day 9, sometimes combined with the typical granulomatous lesions described by Germuth (9) (Fig. 3). The secondary nodules were initially larger (days 2 and 3 after booster), than in spleens of animals not given endotoxin. The white pulp was so large and poorly demarcated that a good separation of "white" from "red" pulp under the dissecting microscope was often impossible. Many plasma cell aggregates were found next to blood vessels within areas that appeared to belong to the periarteriolar sheaths of the white pulp.

The histological changes seen in spleens of animals given two injections of BGG, and antibody a day before the second injection, were similar to those seen in the spleens of animals that had received two injections of antigen alone.

Response to a Secondary Injection of BGG, 5 to 8 Days after the Primary.— Table III lists the results obtained with spleen tissue from rabbits receiving 2 intravenous injections of BGG with a 5 to 8 day interval between the two injections and 5 μg of endotoxin with the first injection. It has been shown that spleens taken 9 or 10 days after a single injection of BGG and endotoxin did not show much antibody production, and that at 7 days production was found in the red pulp but not in the white pulp (1). However, 2 days after a second injection given on days 5 to 8 after the primary, a high production of antibodies was found. 1 day after the booster injection, given on day 7, the white pulp showed some antibody production, and 1 day later both white and red

pulp were found to form anti-BGG. This indicates that a rapid response to a booster injection could already be elicited 5 to 7 days after the primary, approximately at the same time as the first appearance of antibody in the serum (1).

The spleens of these animals[4] showed a histologic response similar to the preceding group. However, the response induced after such a short time interval following the primary, showed fewer hemocytoblasts at the periphery of the white pulp, and more in the lymphocyte mantle of the secondary nodules and periarteriolar sheaths (Figs. 4 to 6).

Serum Antibody Titers during the Secondary Response to BGG.—Results have been presented elsewhere (1) concerning the inactivation by 0.1 M mercaptoethanol of antibody present in the sera taken early during the primary response to BGG. With the sera from animals after a second injection of BGG such inactivation by mercaptoethanol treatment was not observed, indicating that most of the antibody produced was 7S rather than 19S gamma globulin (10).

Most of the sera of these animals showed 2 precipitation lines with BGG (5 mg/ml) upon double diffusion in agar. Since the sera of animals which received endotoxin with the first antigen injection already showed two lines upon double diffusion in agar with crude BGG during the primary response, the secondary response was obviously a mixture of reactions to at least 2 antigens.

The rabbits receiving 2 injections of BGG, 10 days apart, showed peak serum titers on days 4 to 7, varying from 1:160 to 1:10,000, whereas the rabbits which had received endotoxin with the first injection had serum titers between 1:1,000 to 1:100,000. Serum titers similar to those observed in the latter group were found in rabbits when there was an interval of 4 weeks between the primary and secondary BGG injection.

It was found that with a short interval between the antigen injections, the demonstration of antibody production by the spleen *in vitro* often allowed a more reliable evaluation of the booster response than did the serum titrations.

DISCUSSION

The present studies show that a secondary response can be induced by an antigen injection given as early as 5 to 10 days after the primary injection. Since the earliest appearance of antibody in the serum is on day 5 of the primary response (1), the effect of a second injection can be shown after a period of only 1 to 2 days without antigen in the circulation. Apparently, cells which have proliferated in response to the primary injection are now capable of giving a very rapid response upon further exposure to antigen. This may also indicate that the decrease of antibody production during the primary response after the 7th day (1) is due to the lack of a sufficient amount of the protein antigen to stimulate the "sensitized" cells.

[4] Some of these rabbits were operated as described previously (1) in order to remove a small portion of the spleen before the booster injection of antigen was given.

The secondary response at 1 month after the primary is somewhat more prolonged than that elicited at 10 days. Higher serum titers are obtained confirming the observations of others on the influence of the length of time between primary and booster injection (11). This may be due to the presence, after the longer period, of many more sensitized cells distributed over various lymphoid organs.

Antibody production in the spleen of rabbits after a secondary injection of BGG is higher and appears much more rapidly than after a primary injection (see also reference 1). A more rapid and elevated production of antibody upon secondary exposure to a protein antigen, given 1 month or more after a primary injection has been repeatedly demonstrated (12–16). The increase in the rate of antibody formation *in vitro* on day 2 after the booster injection, observed by Dutton *et al.* (17), is in agreement with the results reported here. Immature plasma cell proliferation with a high mitotic activity was observed in the red pulp of the spleen during the secondary response, as has also been described previously (18–21). The mitotic activity of these cells has been emphasized by several authors (22–24).

Gamma globulin formation was shown to be enhanced in the spleen during the peak of antibody formation. The histological observations and studies on the serum antibody during primary (1) and secondary response indicate that both 19S and 7S antibodies to BGG in the rabbit are formed by cells derived from large blast cells of lymphoid tissue, presumably immature plasma cells and possibly also lymphocytes (25).

The transformation of the precursor blast cells into immature plasma cells occurs later in the primary than in the secondary response to BGG, and the presence of the immature plasma cells coincides with the peak of antibody production. Antibody production and immature plasma cell proliferation in the red pulp of the spleen also diminish later in the primary response (day 7) (1) than in the secondary (day 4), when the booster injection is given 10 days after the primary injection. There is, thus, an apparent difference between the response to a simple protein antigen, as used here, and to bacteriophage antigens, since it has recently been shown (26) that rates of antibody production in primary and secondary response to a bacteriophage antigen are quite similar. An interesting aspect of the differences between primary and secondary responses is that the antibody formed in the primary response, both to bacteriophage (26) and to other antigens (27, 28), is 19S gamma globulin, while in the secondary it is 7S gamma globulin.

The fate and distribution of antigen after both primary and secondary exposure may be a decisive factor in determining the duration of the immune response. It is difficult to evaluate the influence of high serum antibody levels on the immune response to a booster injection, since the response to antigen-antibody complexes has been shown to depend greatly on the antigen-antibody ratio of the aggregates formed (29, 30). The influence of antigen-antibody

complexes on the spleens of some of the animals studied here was evident from the typical granulomatous lesions observed.

Endotoxin greatly stimulates the formation of secondary nodules, as has been previously described (31, 1). This effect is the only striking histological change noticeable at day 10 after the primary injection. The sustained and enhanced secondary response observed after a 10 day interval in endotoxin-injected rabbits thus seems correlated to the spectacular proliferation of secondary nodule cells in the white pulp. This leads to the possibility that the secondary nodules are involved in the production of "sensitized cells" which can respond to the booster injection.

It has been proposed (31, 32), that secondary nodule cells ("medium-sized" lymphocytes) produce antibody. The present observations as well as those of others (33) show that antibody production is clearly correlated with immature plasma cell proliferation both in the primary (1) and in the secondary response, and that, as a rule, much less antibody is produced by the "white" pulp than by the "red" pulp of the spleen. This demonstrates that immature plasma cells, rather than the typical secondary nodule cells, are responsible for most of the antibody production.

The results of the experiments of White (3), using the fluorescent antibody technique, as well as some of the data presented here, particularly those demonstrating antibody production in the white pulp early after a booster injection might suggest that repeated exposure to antigen stimulates the secondary nodule cells to form antibody. However, the large increase in the numbers of hemocytoblasts and immature plasma cells both at the periphery of, and in, the white pulp in response to an early booster injection indicates that these cells are the antibody producers. It appears more likely, therefore, that the proliferating secondary nodule cells are precursors of cells which respond to a booster injection, rather than the antibody-forming cells, themselves.

The histological distribution of the cells which respond to the booster injection at 7 to 10 days after the primary is probably influenced by the fate of the antigen which has been injected at the time of high antibody titers in the serum. It is also possible that not all the secondary nodule cells react to the booster injection, because they have to attain a certain maturity (perhaps become small lymphocytes) before they can respond to a booster injection. It should be noted, however, that a direct transformation of secondary nodule cells to lymphocytes or hemocytoblasts has not yet been demonstrated, and that the experiments of Nossal and Mäkelä (34) indicate that the cells which will respond to a booster injection are actively dividing cells rather than small lymphocytes. Experiments are in progress to further study the fate and function of the secondary nodule cells.

A marked tendency of the developing plasma cells to be further removed from the white pulp than their precursors, the hemocytoblasts, can be observed both during primary and secondary responses. Similarly, in lymph nodes, the

localization of the large blast cells is predominantly in the cortex, while immature and mature plasma cells proliferate in the medullary cords. In view of the recent demonstration (35, 36) that small lymphocytes can become transformed into the immunologically competent large blast cells, the close relationship of these plasma cell precursors with lymphoid tissue is not surprising. It appears likely that during the primary response such a transformation of small lymphocytes to hemocytoblasts occurs (37), but the role of the small lymphocyte in the secondary response is less clearly established.

As was mentioned above, a striking histological difference between secondary and primary responses was noted in the localization, the number, and the rapidity of appearance of the hemocytoblasts. In the secondary response to BGG a large number of these cells arise within 1 day, almost immediately followed by proliferation and differentiation into immature plasma cells. In the primary response the number of these cells increases without much further differentiation into immature plasma cells until days 3 to 4, when they are located at the periphery of the white pulp (1). The earliest stages of secondary nodule development (days 4 to 5) consist of small groups of blast cells. If both the plasma cellular response and the secondary nodule proliferation are induced by, and are specific reactions to, the same antigen, it seems possible that the earlier response (plasma cells) is induced by a higher dosage of antigen than the later (secondary nodules). It is also possible that the later phase is induced by a special kind of antigen-antibody complex.

In keeping with the impression that quantity and distribution of antigen are of major importance in the process of the primary and secondary antibody response, the following hypothesis can be formulated.

During the primary response, transformation of lymphocytes of the white pulp into large blast cells (with ribosomes in their cytoplasm but without endoplasmic reticulum, reference 38) may be induced by exposure of lymphocytes to antigen or to a nucleic acid intermediate. As Gowans (39) has recently shown, such a transformation can occur without intervening mitoses. These large blast cells then would slowly lose the antigen, and follow one of two pathways:

(*a*) The cells which migrate towards the periphery of the periarteriolar lymphoid tissue reach regions in the spleen where more antigen is present (perifollicular zone, reference 40). These cells proliferate and the daughter cells have antigen at their disposal so that they are able to form antibody (immature plasma cells with rough endoplasmic reticulum, reference 38).

(*b*) The cells which remain in the white pulp also start proliferating, but because of a relative lack of antigen do not form antibody, although the information for antibody production is retained (secondary nodules—"sensitized cells").

This concept is consistent with the hypothesis of Sercatz (41) that a cell has to encounter antigen twice before it can produce antibody.

Another example of the importance of the amount of antigen in the immune response can be found in the phenomenon of immunological unresponsiveness (42). If immunological unresponsiveness is regarded as an imbalance between the number of available reactive lymphocytes and the amount of antigen injected, resulting in inhibition of antibody production by excess antigen inside the cells, relatively high dosages of easily diffusible antigens in the primary response might result in lengthening the time interval before antibody is produced ("induction period"). Such factors may have to be considered when differences between primary and secondary antibody responses are evaluated. Thus, each of the three immunological phenomena: unresponsiveness, antibody production, and preparation for a booster response may be different expressions of the influence of dose of antigen per immunologically competent cell in the immune response.

SUMMARY

Antibody formation *in vitro* by red and white pulp of the spleen and by bone marrow tissue was studied at various days after an intravenous booster injection of soluble antigens such as ovalbumin and bovine gamma globulin (BGG). When the booster injection of antigen was given early (10 days) after an intravenous primary injection, high antibody formation could be demonstrated in the spleen primarily 2 to 3 days after the injection, but much less afterwards. When the booster injection was given later (1 month) after the primary, the antibody production by the spleen lasted longer and higher serum titers were obtained. The bone marrow formed antibody in both cases but, particularly with the short interval between injections, its response was delayed as compared to the spleen. It was also shown that during antibody formation the production of gamma globulin *in vitro* was enhanced. Histologically the antibody production was always correlated to immature plasma cell proliferation, located at the border of red and white pulp and in the red pulp of the spleen.

When endotoxin had been injected at the time of a primary BGG injection, and a second antigen injection was given 5 to 10 days later, a booster response could be elicited which was sometimes limited to the white pulp on day 1, and on day 2 was divided between "red" and "white" pulp. The response induced at day 10, at the peak of secondary nodule proliferation, lasted very long and was accompanied by an enormous plasma cellular proliferation in and around the periarteriolar lymphoid areas of the spleen. The possible importance of the secondary nodules of the white pulp in the preparation for a secondary response is discussed.

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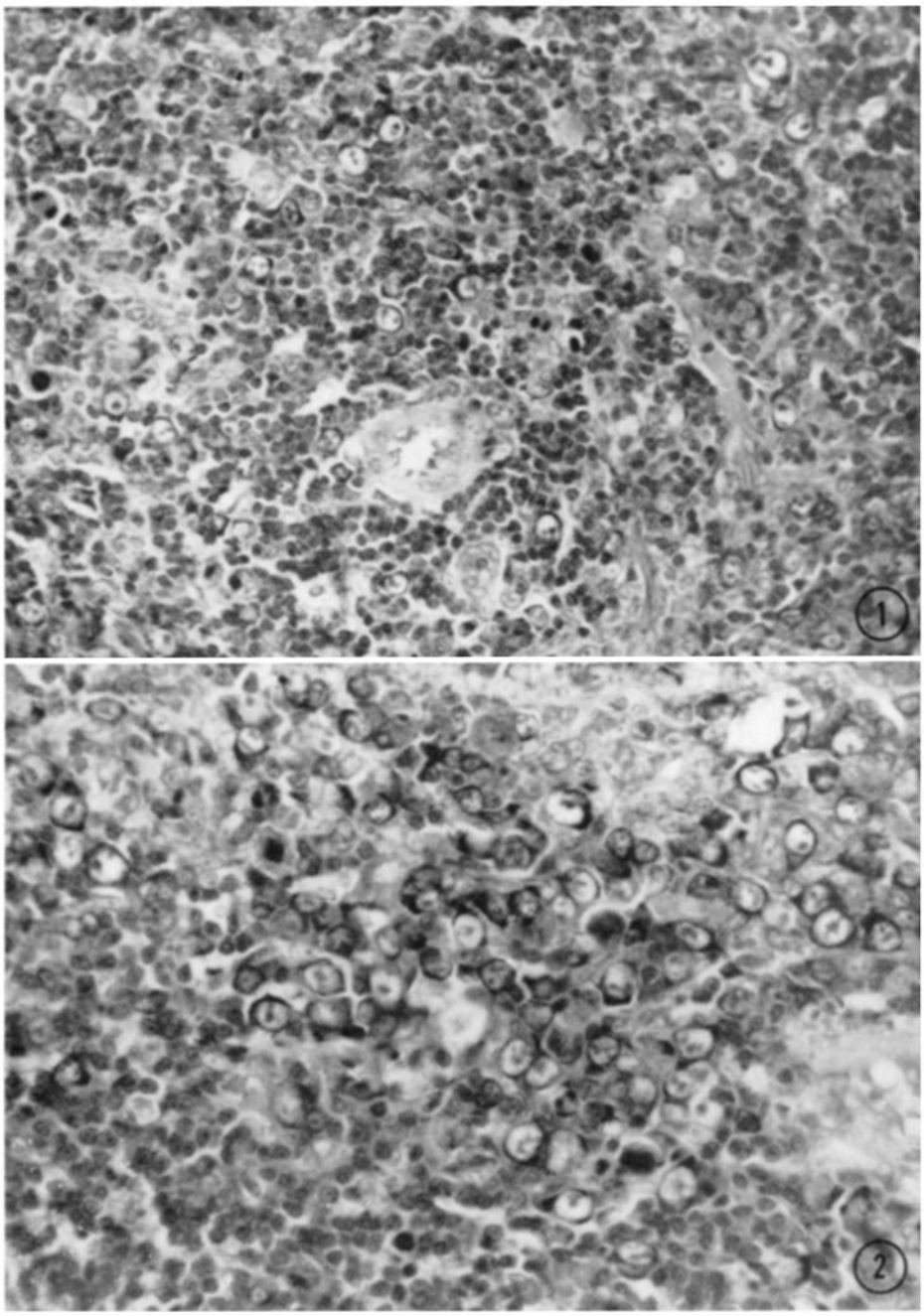
EXPLANATION OF PLATES

PLATE 35

FIG. 1. Spleen of rabbit, 2 days after a booster injection of BGG, given 1 month after a primary. White pulp area around central artery. Hemocytoblasts arising throughout the white pulp, no secondary nodules. × 500.

FIG. 2. Spleen of rabbit, 2 days after a booster injection of BGG, given 10 days after a primary (with endotoxin). Large area of hemocytoblasts arising at the periphery of the white pulp. × 750.



(Thorbecke *et al.*: Antibody formation in secondary response)

PLATE 36

FIG. 3. Spleen of rabbit, 7 days after a booster injection of BGG, given 10 days after a primary (with endotoxin). On the right, part of the center of a secondary nodule with its zone of lymphocytes, and on the left the red pulp with immature plasma cells. The light areas contain epitheloid cells (serum sickness lesions). Note a few hemocytoblasts in reaction center, zone of lymphocytes and red pulp. × 500.

FIG. 4. Spleen of rabbit, 2 days after a booster injection of BGG, given 5 days after a primary injection (with endotoxin). On the right is the center of a follicle, and on the left its zone of lymphocytes. The artery is located eccentric to the reaction center. Many hemocytoblasts are seen in the reaction center and in the zone of lymphocytes. × 500.

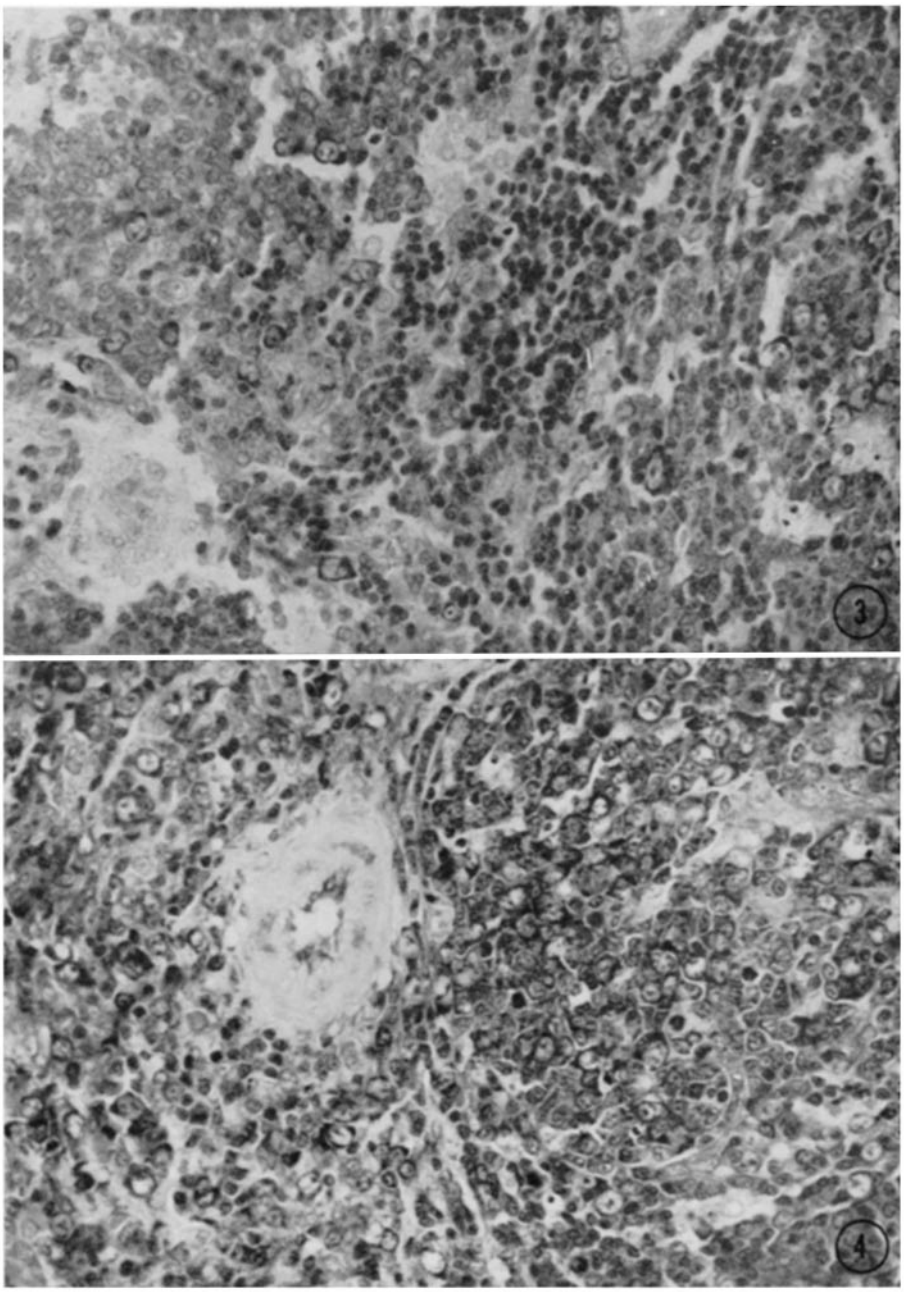


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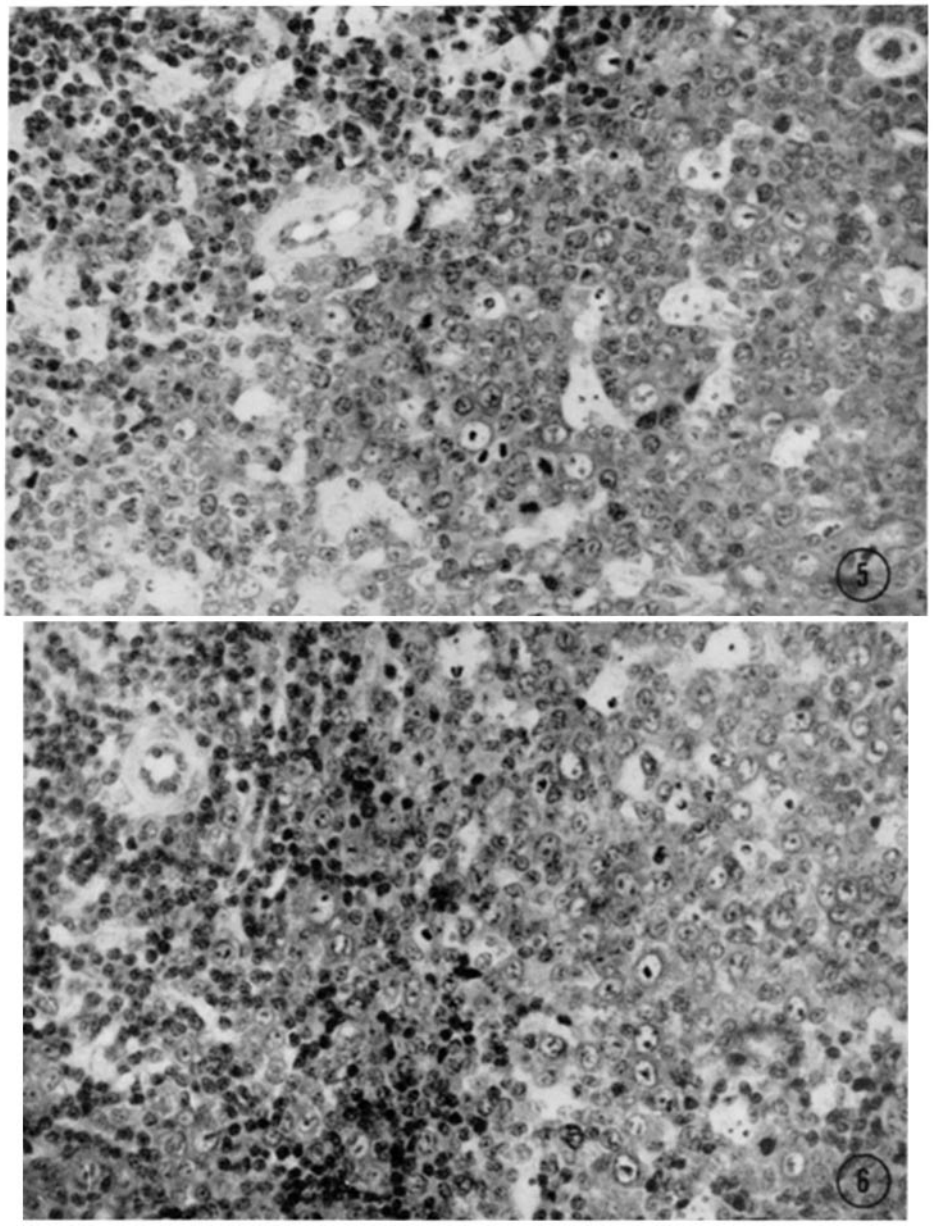
PLATE 37

FIG. 5. Spleen of rabbit, 10 days after a primary injection of BGG with endotoxin. A large part of a secondary nodule is depicted here; the zone of lymphocytes is on the upper left, and the artery is located at the border of the reaction center. Note the well defined border between this center and its zone of lymphocytes, and the absence of hemocytoblasts from the zone of lymphocytes. × 500.

FIG. 6. Spleen of the same rabbit as seen in Fig. 5, but taken 1 day following a booster injection. On the right, part of the center of a follicle, and on the left, its zone of lymphocytes and artery. Several hemocytoblasts are present in the zone of lymphocytes. Note that the border between reaction center and zone of lymphocytes is much less defined than before the booster injection. × 500.



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