GAMMA GLOBULIN AND ANTIBODY FORMATION IN VITRO

I. GAMMA GLOBULIN FORMATION IN TISSUES FROM IMMATURE AND NORMAL ADULT RABBITS*, ‡

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Studies on antibody formation "in vitro" have supplied information concerning the site of antibody production (1-5), the rates of antibody synthesis per unit weight of tissue (6-7) and the percentage of active cells in lymphoid tissue during the primary and secondary antibody response (8-9). In vitro experiments have also been used in attempts to elucidate the mechanism of induction of antibody formation by antigen (10, 11).

Askonas et al. (12) studied the synthesis of gamma globulin by tissues from non-immunized and immunized rabbits. In their studies incorporation of labeled amino acid by rabbit tissue in vitro was shown to occur in protein which upon column chromatographic and electrophoretic fractionation behaved like gamma globulin. In tissues from immunized rabbits an enhanced synthesis of gamma globulin was shown to occur (13, 14).

In the present study, the in vitro synthesis of gamma globulin was investigated using immunological techniques to isolate the gamma globulin rather than biochemical fractionation, because it was felt that such methods provide a better characterization of a protein, particularly in the presence of other tissue proteins. This method involves the measurement of the incorporation by tissue in vitro of labeled amino acid into a protein precipitable with an antiserum specifically directed against rabbit gamma globulin.

With this method an attempt has been made to study further the relationship between gamma globulin and antibody formation in various tissues. The present paper presents evidence that, in immature rabbits, gamma globulin synthesis occurs predominantly in the lymphoid tissue of the intestinal wall. At this age rabbits show very low or negligible gamma globulin formation in spleen and thymus. The results on gamma globulin formation are correlated with the histological appearance of lymphoid organs of newborn and immature rabbits.

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† Presented in part at the 1959 meetings of the American Association of Immunologists.
Materials and Methods

Preparation of Anti Rabbit Gamma Globulin Sheep Serum.—Two sheep were immunized\(^1\) at weekly intervals with 2 intramuscular injections of a washed immune precipitate of Type III pneumococcus polysaccharide\(^2\) with the corresponding rabbit antibody in Freund's adjuvant. A total of 90 mg. protein was used per sheep. The sheep were bled 3 weeks after the last injection. The antisera used was absorbed for antibodies against other rabbit serum components with rabbit serum from which gamma globulin had been precipitated at 17 per cent Na\(_2\) SO\(_4\) (15).

Text-fig. 1 shows the results of immune electrophoresis (16) using the unabsorbed and absorbed sheep anti rabbit gamma globulin against rabbit serum as the antigen. The only antibody left after absorption is shown to be the anti rabbit gamma globulin. In Ouchterlony

![Text-fig. 1. Immune electrophoresis of rabbit serum as the antigen with unabsorbed and absorbed sheep anti rabbit gamma globulin as the two antisera.](image)

plates (17) and Preer tubes (18) the absorbed antiserum also showed only one precipitation line with rabbit serum. Micro immune electrophoresis (19) in which an antigen was used consisting mainly of 19 S rabbit gamma globulin and about 5 per cent of the 7 S component,\(^3\) showed that this sheep antiserum had antibody against both components of rabbit gamma globulin. Maximum precipitation was observed with 200 \(\mu\)g. of rabbit gamma globulin protein\(^4\) per ml. of antiserum.

\(^1\) Dr. M. B. McGinness at the Antitoxin Laboratory, Otisville, New York, was so kind as to immunize the sheep for us.

\(^2\) We are grateful to Dr. M. Heidelberger, Institute of Microbiology, New Brunswick, for supplying the pneumococcus polysaccharide and the rabbit antiserum.

\(^3\) The 19 S rabbit gamma globulin was kindly supplied by Dr. E. C. Franklin, Department of Medicine, New York University–Bellevue Medical Center. A more detailed description of the reaction of these antisera with 7 S and 19 S rabbit gamma globulin will be published elsewhere.

\(^4\) The gamma globulin used in the precipitation tests was obtained commercially from Pentex Biochemicals, Bourbonnais, Illinois. Lot 65 F05.
Three intramuscular booster injections of incomplete Freund's adjuvant and rabbit gamma globulin, isolated by anion exchange column chromatography (total of 30 mg), 8 months after the first series of injections, further enhanced the anti gamma globulin titers to about 2 times the strength after absorption.

The sheep antiserum also contained a little antibody against the pneumococcal polysaccharide used in the immunizing precipitate. Absorptions of this antibody did not influence the results of these experiments and was, therefore, omitted in most of the experiments.

Animals.—Male and female rabbits of various ages were used. The animals were exsanguinated and the organs to be cultured removed aseptically. Small pieces of each tissue were fixed in a mixture of Zenker's solution (9 parts) and formaldehyde 10 per cent (1 part) for 5 to 6 hours. Sections were stained according to a modified Wright procedure (20) and with methylgreen-pyronin Y (Allied Dye Corporation, New York).

Preparation of Cultures.—Roller tube tissue cultures were prepared with 50 to 70 mg of minced tissue from various organs of normal adult and immature rabbits, and incubated for 20 hours at 37°C. The culture medium consisted of 2 ml of Hanks balanced salt solution with 10 per cent calf serum, to which had been added glucose (up to 22 mEq/liter), para-aminobenzoic acid (2 mg/liter), and mixtures of antibiotics, vitamins (21), and of amino acids (22) from which lysine had been omitted. In later experiments inositol (2 mg/liter) was also added to the medium.

Uniformly labeled C14-lysine (Nuclear-Chicago Corp., Des Plaines, Illinois) was added in a concentration of 1 μc (20 to 25 μg) per culture tube. This concentration was shown to provide a sufficient excess of labeled lysine so that it was not a limiting factor in any of the experiments.

Rates of gamma globulin synthesis in vitro were studied in 1 to 8 hour cultures of 10⁷ spleen cells suspended in 1 ml of the same culture medium containing 0.5 μc C14-lysine.

Preparation, Washing, and Counting of Precipitates.—At the end of the incubation period the cultures were frozen and thawed, centrifuged at 15,000 g and the supernatant culture fluids dialyzed for 24 hours at 4°C against saline.

In order to correct for non-specific absorption of radioactive material on gamma globulin precipitates, the culture fluids were absorbed 2 times with precipitates of diphtheria toxoid and horse antitoxin at equivalence. The precipitates were formed in the fluids, incubated for 30 minutes at 37°C and left 24 to 48 hours at 4°C. The first absorption precipitate contained about 2 mg of protein, the second was of similar size as the final gamma globulin precipitate (25 to 1 mg). The gamma globulin precipitates were prepared by adding to the culture fluids a small amount of non-labeled gamma globulin in the form of diluted rabbit serum (0.05 to 0.1 ml of a 1:10 dilution) and an excess of the absorbed sheep antiserum (1 ml) in order to precipitate virtually all the rabbit gamma globulin present. Preimmunization sheep serum did not precipitate any radioactivity from the culture fluids.

The second absorption and the gamma globulin precipitates were washed in cold saline (3 times), treated with 5 per cent trichloroacetic acid at 90°C for 15 minutes (23), washed in 60 per cent ethanol, 100 per cent ethanol and acetone before being transferred to planchettes. Counting was performed at infinite thinness (self-absorption <5 per cent) with an end-window Geiger-Müller counter. Biuret reactions (24) were done on all precipitates after counting. Similar protein contents were found in all planchettes, usually within the range of 500 to 1000 μg protein, depending upon the amount of carrier gamma globulin used.

Both before and after treatment with trichloroacetic acid the precipitates contained no measurable phosphorus (25) and gave ultraviolet absorption spectra consistent with protein without significant nucleic acid contamination.

* The chromatographically isolated gamma globulin was kindly supplied by Dr. N. Cooper, Department of Pathology, New York University School of Medicine, New York.
Results of in Vitro Studies

Precipitates formed in culture medium with C14-lysine, which has not been incubated with tissue, do not carry down any measurable radioactivity. However, immune precipitates formed in C14-labeled culture fluids which have been incubated at 37°C with various tissues, particularly with fast growing tissues, carry down some radioactivity non-specifically (26, 27). Sodium ethylenediaminetetraacetate in an amount sufficient to inhibit complement activity, if present, does not decrease the absorption of these substances to immune precipitates as it does in S35-labeled serum (28).

Assuming that immune precipitates of similar size would carry down approximately equal amounts of radioactivity non-specifically, it was decided to subtract the counts in the last absorption precipitate from the counts in the gamma globulin precipitates and let the difference be a measure of gamma globulin synthesis. The absorption precipitates usually contained 10 per cent or less of the counts of the gamma globulin precipitates in cases in which much gamma globulin synthesis was present. However, in some of the tissue culture fluids from immature rabbits the last absorption precipitates showed amounts of radioactivity which were as high or somewhat higher than that of the gamma globulin precipitates, illustrating the non-specific character of this radioactivity.

Text-Fig. 2. Rates of gamma globulin synthesis in cell suspensions prepared from a normal and from an immune spleen (see text).
Text-fig. 2 illustrates the results obtained from the studies on rates of gamma globulin formation in vitro. During the first 6 hours of incubation a linear relationship was observed between the radioactivity of the gamma globulin precipitates and time. The upper curve represents the rate of formation of gamma globulin by a spleen cell suspension from an immunized rabbit, taken 4 days after an intravenous booster injection of $3 \times 10^8$ paratyphoid A bacteria (formalin-killed). The lower curve shows the gamma globulin formation by spleen cells from a non-immunized control rabbit. It is obvious that the rate of synthesis in the immune spleen is much higher than that in the normal spleen.

Table I shows the dilution effect of the addition of non-labeled l-lysine to cultures of equal amounts of minced spleen tissue from an immunized rabbit. The addition of 25 $\mu$g. or of 100 $\mu$g. non-labeled lysine per culture tube reduced the specific radioactivity of the synthesized gamma globulin to different degrees. From these data it could be calculated that usually about 50 to 70 $\mu$g. of readily available l-lysine was present in a culture tube containing 1 $\mu$c. C$^{14}$-l-lysine. A concentration of 0.5 mM dinitrophenol in the medium completely inhibited gamma globulin synthesis in vitro.

In young adult rabbits gamma globulin production occurred mainly in peripheral lymph nodes, bone marrow, spleen, and lung. Mesenteric lymph nodes, appendix, and thymus showed irregular activity, while kidney cortex and liver did not seem to participate at all in gamma globulin production (Table II).

When the same technique was applied to tissues taken from newborn and immature rabbits different results were obtained (Table III). In general, it was found that tissues like spleen, lung, bone marrow, and thymus, which formed gamma globulin in vitro when taken from adult rabbits, did not do so when taken from rabbits before the age of 4 weeks. The appendix was the only exception: it formed gamma globulin already at 1 week of age. No significant gamma globulin formation was detected in any of the organs cultured from rabbits younger than 1 week.

Dates of experiments were included in the tables because it was observed that the yields of gamma globulin formation in the experiments performed after
August, 1959, were larger than those of earlier dates. Small changes in the medium are probably responsible for this finding.

TABLE II
Gamma Globulin Formation in 20-Hour Cultures by 50 Mg. of Tissue from Organs of Young Adult Rabbits

<table>
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<td>Weight of rabbit, gm.</td>
<td>2000</td>
<td>1300</td>
<td>1700</td>
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<tr>
<td>Bone marrow</td>
<td>124</td>
<td>39, 27</td>
<td>113</td>
<td>154</td>
<td>172</td>
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<td>Spleen</td>
<td>76</td>
<td>55</td>
<td>107</td>
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<td>Thymus</td>
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<tr>
<td>Liver</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>39</td>
<td>94</td>
<td>48</td>
<td></td>
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<tr>
<td>Popliteal lymph node</td>
<td>175</td>
<td>173</td>
<td></td>
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<td></td>
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<td>Appendix</td>
<td>23</td>
<td>22</td>
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</tr>
<tr>
<td>Kidney</td>
<td>1</td>
<td>3</td>
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* Expressed as total counts per minute in gamma globulin precipitates less counts per minute in last absorption toxoid immune precipitates.

TABLE III
Gamma Globulin Formation in 20-Hour Cultures by 50 Mg. of Tissue from Organs of Immature Rabbits

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<td>52</td>
<td>145</td>
<td>120</td>
<td>110</td>
<td>150</td>
<td>165</td>
<td>260</td>
<td>900</td>
<td>1020</td>
<td></td>
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<tr>
<td>Age, days</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>14</td>
<td>21</td>
<td>22</td>
<td>28</td>
<td>31</td>
<td>44</td>
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<td>10</td>
<td>0</td>
<td>2</td>
<td>11, 11</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>225</td>
<td>330</td>
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<td>0</td>
<td>0</td>
<td>10, 13</td>
<td>20</td>
<td>11</td>
<td>0</td>
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<td>27</td>
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<td>120, 100</td>
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<td>46</td>
<td>53, 33</td>
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<td>46</td>
<td>85</td>
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<td>174</td>
<td>70</td>
<td>200</td>
<td>450, 230</td>
<td>150</td>
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<tr>
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<td>0, 12</td>
<td>0, 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td></td>
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<td></td>
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* Expressed as in Table II.

Results of Histological Studies

Appendix.—At the age of 1 to 3 days after birth the rabbits show only a few small accumulations of lymphocytes and some scattered lymphocytes in the submucosa of the appendix. However, at this site, just like the gamma globulin
formation, the lymphoid tissue develops very rapidly. Already at the age of 1 week, when gamma globulin formation is first demonstrable, definite lymphoid nodules are present, consisting mainly of large lymphoid blast cells (hemocytoblasts) and medium-sized lymphocytes. A zone or cap of small lymphocytes is not yet present at this age, but becomes visible when the nodules enlarge.

Soon after the appearance of these nodules (Fig. 1), it can be observed that the hemocytoblasts tend to be located primarily in the part of the follicles closest to the mucosal lining and that similar cells are present scattered in the submucosal tissue around the nodules. Already in rabbits 1 to 2 weeks old, these cells seem to differentiate into immature plasma cells in the connective tissue of the submucosa, and seem to at least partly supply the enormous number of plasma cells found in the submucosa of the appendix of older rabbits. In the appendix of rabbits of 4 to 6 weeks old, many mature plasma cells are found. They are not located as yet throughout the stromal tissue of the villi as they are in older rabbits, but seem to be present primarily at the base and in the stem of the villi, often mixed with some immature plasma cells. The number of lymphoid nodules at this age has also increased considerably.

**Spleen.**—The development of the lymphoid tissue in the spleen is much slower and very different from that in the appendix of immature rabbits. Although the white pulp enlarges during the first few weeks after birth, it does not show the drastic changes that are observed in the appendix. In a newborn rabbit the white pulp shows a rather basophilic dense reticulum which surrounds the branching arterioles and contains some scattered small lymphocytes (Fig. 2). During the first weeks postnatally the white pulp differentiates into a perivascular accumulation of lymphocytes surrounded by similar dense and basophilic reticulum, which begins to look like the so-called perifollicular zone of lymphoid nodules in adult rabbits. The basophilic reticulum cells often round off and acquire the appearance of medium-sized lymphocytes with basophilic rim of cytoplasm and nucleoli.

The appearance of hemocytoblasts, plasma cells, and of germinal centers in the accumulations of lymphocytes around the arterioles occurs, in normal rabbits, only around the 4th week after birth. This development of the lymphoid tissue seems to take place at about the same age as when the first definite gamma globulin production in the spleen can be demonstrated.

However, in preliminary experiments, when newborn rabbits had been injected with 1 mg. of living tubercle bacilli (BCG, Phipps strain), immature plasma cells were definitely present in the spleen of 2-week-old rabbits (Fig. 3). Besides this, hemocytoblasts and immature plasma cells were observed in the mesenteric lymph node of a BCG-treated 1-week-old rabbit. Experiments with the aim of modifying the development of the lymphoid tissue in the spleen of newborn animals are in progress.

**Thymus.**—No apparent explanation has been found histologically for the
fact that thymuses of rabbits of 4 weeks old and older formed some gamma globulin, whereas no significant gamma globulin was formed in thymus tissue taken from younger animals.

Plasma ceil aggregates are observed regularly in thymus tissue from adult mice, guinea pigs, and chickens. In adult rabbit thymus they are much more scarce but can sometimes be observed, particularly around blood vessels in the cortex, although they seem hardly numerous enough to account for the gamma globulin formation in vitro. Without further expedients, it may be too difficult to distinguish histologically the immature cells capable of gamma globulin formation from the other thymus cells.

DISCUSSION

The optimal conditions for the synthesis of serum proteins in vitro have not been determined as yet. This is evidenced by the fact that synthesis of albumin by tissues in vitro (29, 30) is always lower than by isolated perfused organs (31).

Antibody formation in vitro is frequently low or impossible to detect depending on the time after antigenic stimulation and on the sensitivity of antibody detection methods. Steiner and Anker (6) were the most successful in obtaining a high yield of antibody formation in vitro. They observed rates of up to 0.75 mg. of antibody per hour per whole spleen, but only in spleens taken from rabbits at the height of antibody formation.

In studies on perfusion of isolated lungs from hyperimmunized rabbits Askonas and Humphrey (7) obtained a rate of gamma globulin synthesis as high as 6 mg. per hour. Martin et al. (32) have reported that lymph nodes from an immunized human donor, transplanted into an agammaglobulinemic recipient, could form up to 0.5 mg. of gamma globulin per gm. per hour.

If in the present studies it is assumed that gamma globulin contains 7 gm. of lysine per 100 gm. and that all the label in gamma globulin is still present in the form of lysine, it can be calculated that an incorporation of 100 counts per minute into gamma globulin by 50 mg. of spleen tissue during the incubation period amounts to synthesis of about 1 μg. of gamma globulin. This calculation indicates also the level of sensitivity of measurement of gamma globulin synthesis in the present studies.

Differentiation between the formation of 19 S and of 7 S gamma globulin was not attempted. Both types of gamma globulin could be precipitated by the antisera used, not only because of the cross-reactivity between them, but also because both antisera were demonstrated to have antibody specific for 19 S as well as for 7 S gamma globulin.

The main points of interest in the appearance of the lymphoid organs from the immature rabbits in these studies were:

1. The early development of the lymphoid tissue in the wall of the appendix occurs a few weeks before any differentiation of lymphoid cells can be observed.
in the spleen. Concomitantly with the observed proliferation of lymphoid nodules and immature lymphoid blast cells, gamma globulin formation can be found in the lymphoid tissue of the appendix as early as 1 week after birth. Observations by others (33, 34) suggest that both the lymphoid nodules and plasma cells may synthesize gamma globulin. The sequence of development which was outlined above indicates that the lymphoid nodules with their peculiar localization of hemocytoblasts actually supply the cells which migrate into the stroma of the mucosal lining and of the villi while differentiating into typical immature and mature plasma cells. During the booster response to an intravenous antigen injection a similar sequence of plasma cell maturation, combined with a migration away from the lymphoid tissue of the white pulp, is observed in the spleen. Such cells start as hemocytoblasts in the so called perifollicular zone, and end as plasma cell aggregates in the red pulp around the small arterioles which emerge from the white pulp (13).

2. During the first 3 weeks of postnatal life in the rabbit, under normal conditions, the lymphoid tissue of the spleen does not develop secondary nodules or plasma cells, nor does it show significant gamma globulin synthesis in vitro. Even upon stimulation with antigens, the lymphoid tissue of such young rabbits seems to be reluctant to form antibodies. Bridges et al. (35), using Freund's adjuvant and bovine serum albumin as the antigenic stimulant in newborn rabbits, found no evidence of plasma cell and secondary nodule formation in regional lymph nodes, nor of any antibody formation before the 16th day of life, and concluded that some form of "physiologic maturation" must occur before rabbits can respond with antibody formation to an antigenic stimulant. Šterzl and Trnka (36) reported that antibodies against a bacterial antigen could be formed in rabbits as early as the 9th postnatal day, but only if very large doses of bacteria were injected on the 5th postnatal day. The usual immunizing dose did not result in antibody formation before the 30th day.

On the other hand, Dixon and Weigle (37) observed that neonatal lymphoid cells formed antibodies readily if transferred to adult x-irradiated recipient rabbits, whereas adult cells transferred to neonatal rabbits formed antibody only poorly. These results have been confirmed, as far as the primary response is concerned, by Nossal (38) using non-inbred rats and mice.

One may conclude from such data that there is in neonatal animals, at least in some species, an inhibitor of antibody synthesis, particularly of the primary response, or else that the newborn lacks some nutritional or growth factors which are necessary for the induction of antibody formation. The fact that, in the present experiments, development of lymphoid tissue and of gamma globulin formation in the intestinal wall occurred early after birth may indicate, that the intestinal bacterial flora provides the lymphoid tissue with the required antigenic stimulation or with a growth factor necessary for lymphoid tissue development. The results of Hrubešova et al. (39) showing a stimulatory effect of
ribonucleoprotein injections on gamma globulin synthesis in 9 day old rabbits may have bearing on this. The absence of lymphoid tissue development in the intestinal wall in germfree animals (40) speaks in favor of a strong influence of bacterial (antigenic) products on the maturation of lymphoid tissue.

Trnka and Říha (41) have shown that adult cells in neonatal recipients form antibody if they are allowed to survive long enough to enable them to make a primary response, in other words if they are not destroyed by homograft rejection. The fact that Nossal (38) found neonatal mice of an inbred strain equally good as x-irradiated adult recipients in supporting the primary response by transferred isologous cells from adult donors can be explained on the same basis.

An explanation for the difference in results observed by various authors might be that, for some reason, it takes donor cells longer to form a primary response in newborn than in adult x-irradiated recipients, either because of nutritional factors or because of the lack of organization in the structure of lymphoid tissue in the newborn. Therefore, it would not be surprising that the homotransplantation reaction can destroy donor cells before they form antibody only in newborn and not in adult recipients. The results of Harris et al. (42), with x-irradiated newborn recipients, and those of Spärck (43), with transfer of donor cells taken at various intervals after an antigen injection, are also in favor of this interpretation.

While the immunological inadequacy of the newborn rabbit has been extended to newborn humans (35, 44), instances have been reported of human neonates with signs of immunological competence. Human stillborns with congenital syphilis or toxoplasmosis can show plasma cells (45, 46) in their organs and higher titers of antibodies in their serum than are present in the maternal blood (47, 48). Observations made by pathologists many years ago, and more recently by Silverstein (49) and by Thorbecke (50) indicate that the development of lymphoid tissue with plasma cell proliferation can occur in human embryos weighing as little as 1250 gm. (49). While in such cases, the possibility that cells from the mother have invaded the fetus has to be considered, it seems much more likely, also in view of serological data in congenital syphilis (47) and toxoplasmosis (48), that the fetus forms the plasma cells itself. Perhaps, under such conditions as occur in these congenital infections, the parasite presents an adequately strong and continuous stimulant to call forth an immunologic response on the part of the human fetal tissues.

3. In the present studies the thymus was found to produce some gamma globulin in 4-week-old and older animals. Previous studies showed that antibody formation could not be detected in cultures of thymus taken from rabbits after intravenous or subcutaneous immunization (3). On the other hand, it has been shown that antibody formation can be transferred with thymus tissue in mice (51), in guinea pigs (52), and in rabbits (53), although it is usually found that, quantitatively, thymus cells are inferior to lymph node cells in
this respect. It has also been found (54, 55) that isologous thymus cells, although again less efficiently than isologous lymph node cells, can reject homologous bone marrow transplants in x-irradiated mice.

SUMMARY

Gamma globulin formation in vitro by various tissues was studied using the incorporation of C¹⁴-L-lysine into a protein precipitable by a specific anti rabbit gamma globulin serum prepared in a sheep.

It was demonstrated that the rate of gamma globulin formation in similar numbers of spleen cells is much higher if taken from an immunized rabbit at the height of antibody formation than that in normal spleen cells. Besides spleen, other tissues shown to form gamma globulin in normal adult rabbits were: peripheral lymph nodes, bone marrow, lung, mesenteric lymph nodes, appendix, and thymus.

In tissues from newborn rabbits gamma globulin formation could not be demonstrated. Rabbits 1 week old already showed a beginning of significant gamma globulin formation in appendix tissue, followed approximately 3 weeks later by gamma globulin synthesis in spleen and thymus. Histological observations on these tissues were described and correlated with findings on gamma globulin formation.

In the discussion an attempt has been made to relate these observations on newborn and immature rabbits to those available in the literature on antibody formation in newborn animals.

I am very grateful to Dr. B. Benacerraf for his helpful suggestions and criticisms. I also wish to express my thanks to Miss Ened Foster for her assistance in preparing the histological sections.

The competent technical assistance of Mr. H. Meyers is gratefully acknowledged.

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EXPLANATION OF PLATES

PLATE 13

Fig. 1 a. Appendix of a 9-day-old rabbit, showing hemocytoblasts in lymphoid nodules and, together with immature plasma cells, in the connective tissue around the nodules. × 175.

Fig. 1 b. Larger magnification of small area of Fig. 1 a, showing the early stages of plasma cell formation near a lymphoid nodule. Methylgreen pyronin. × 700.
(Thorbecke: Gamma globulin and antibody formation in vitro)
Plate 14

Fig. 2. Spleen of a 2-week-old rabbit, showing a small area of white pulp containing lymphocytes and modified reticulum cells. Methylgreen pyronin. \( \times 700 \).

Fig. 3. Spleen of a 2-week-old rabbit, which received 1 mg BCG (wet weight) intravenously at birth, showing a few hemocytoblasts and immature plasma cells. Notice the difference in size between the hemocytoblasts and modified reticulum cells. Methylgreen pyronin. \( \times 700 \).
(Thorbecke: Gamma globulin and antibody formation in vitro)