RATES OF ANTIBODY SYNTHESIS DURING FIRST, SECOND, AND HYPERIMMUNE RESPONSES OF RABBITS TO BOVINE GAMMA GLOBULIN*, ‡, §

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Knowledge of the rates of antibody synthesis throughout the immune response is of great importance in the evaluation of the various factors contributing to antibody production. Estimations of these rates have been previously possible in systems in which precise day to day measurement of circulating antibody was carried out. However, such estimations, even when taking into account the half-life of antibody molecules (1–3) cannot approach the accuracy and reliability of direct measurements. Tracer studies employing labelled amino acids offer a means of determining rates of antibody synthesis directly, and recently two such studies have been reported (4, 5). These two reports, which dealt with the early phases of antibody synthesis in the secondary response, were in disagreement concerning the amount of antibody formed during the first 3 days after injection of antigen.

In the present investigation, we have obtained data concerning rates of antibody synthesis throughout the first, second, and hyperimmune responses with special emphasis on the rate of decline of antibody production. Bovine gamma globulin was chosen as the antigen because there are available extensive, albeit conflicting, observations of its fate in rabbits. It was hoped that knowledge of the rates of antibody synthesis might throw light on this area of disagreement.

EXPERIMENTAL PROCEDURE

Use was made of the fact that amino acids are incorporated into proteins or catabolized and/or excreted within a few hours after their injection into animals. In rabbits virtually all of the initial incorporation of injected S^{35}-labelled amino acids into proteins occurs within 12 hours (6). We have observed that within 7 hours after injection more than 90 per cent of the original free S^{35}-labelled amino acid is lost from the serum. Proteins once labelled by incorporation of S^{35} amino acids appear

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to retain their label until they are degraded (5). Following degradation of S^{35}-labelled protein the S^{35} amino acid may either be catabolized or become available for reincorporation into another protein.

The specific radioactivity of circulating serum globulins or antibodies following the intravenous injection of S^{35}-labelled amino acids increases rapidly during the first few hours, reaching maximum or near maximum values within 5 to 7 hours (7). Similar observations have been made using C^{14}-labelled amino acids (8). These values remain relatively constant during the rest of the first 24 hours, after which the specific activity falls at a rate which reflects the turnover of the protein (8, 9). Wherever possible in these experiments, bleedings to determine specific activity of antibody were made 24 hours after injection of S^{35} amino acids which assured maximum S^{35} activity. The rapid appearance of S^{35} amino acids in circulating proteins including antibody indicates not only a rapid incorporation of amino acids into protein but also a rapid equilibration between protein at the site of synthesis and protein in circulation.

Since antibodies, like other serum proteins, appear to be synthesized *de novo* from amino acids (4, 9), the amount of antibody synthesis occurring within the first few hours after injection of labelled amino acids will be proportional to the specific activity of the antibody isolated subsequently. Thus, rabbits were given antigen, later were injected with a standard dose of S^{35}-labelled amino acids, and subsequently were bled and the specific activity of antibody and non-antibody globulin was determined. Appropriate time intervals between injections of antigen and S^{35} amino acids afforded a measure of antibody synthesis throughout the immune response.

First Response.—Each of 40 white male rabbits weighing between 2.2 and 2.6 kg. received a single intravenous injection of 100 mg. bovine gamma globulin (BGG), Armour and Co. lot No. C904. The sera of all rabbits were tested for BGG by ring test with high titre anti-BGG 7, 8, and 9 days after injection of BGG, and all rabbits were bled for determination of maximum serum antibody concentration 3 days after the elimination of detectable circulating antigen. Serum antibody concentration was measured by ability of the sera to precipitate I^{131}-labelled BGG (I*BGG) (10) and also by quantitative precipitin determinations (11). Eleven rabbits having serum antibody concentrations more than two standard deviations from the mean of the group were not used for the present calculations since we were trying to observe typical primary responses.

In addition to the BGG, each rabbit received a single i.v. injection of S^{35}-labelled amino acids. These labelled amino acids were prepared by Abbott Laboratories and consisted of an acid hydrolysate of S^{35} containing yeast to which was added unlabelled tryptophane to replace that lost by hydrolysis. The radioactivity injected per rabbit in this experiment varied from 0.6 to 0.9 mc. and all calculations have been corrected to a standard injection of 0.9 mc./rabbit. The schedule of S^{35} injections in relation to the injection of BGG is shown on Table I. Two rabbits received S^{35} 1 day before BGG, two received S^{35} simultaneously with BGG and the rest received S^{35} at different intervals after BGG.

Those rabbits injected with S^{35} amino acids during the first 9 days after BGG were exsanguinated 10 days after BGG, at the time of maximum serum antibody concentration. Those rabbits receiving S^{35} 10 or more days after BGG were exsanguinated 24 hours after injection of S^{35}. Sera from all exsanguination bleedings were analyzed for μg. anti-BGG N/ml., S^{35}/μg. anti-BGG N, and S^{35}/μg. non-antibody globulin N. Prior to these determinations the sera were decomplemented by adding 100 μg. N of a preformed specific Ea-anti-Ea precipitate per ml. of serum (12). The serum-precipitate mixtures were incubated at

TABLE I

Primary response

Rabbit No.	S^{35} injection time before or after BGG	Maximum serum antibody	S^{35} counts/μg. globulin N	S^{35} counts/μg. antibody N	Antibody S^{35}/ml.*
	days	*μg.N/ml.*			
24-31	−1	38.3	10.4	12.1	1160.0
24-32	−1	50.6	9.6	9.5	989.6
22-13	0	62.0	10.82	12.5	1155.3
22-14	0	28.5	11.8	10.7	906.8
22-15	1	49.3	17.6	20.0	1136.4
22-16	2	95.8	13.5	16.2	1200.0
22-17	2	56.4	21.4	27.6	1290.0
22-19	4	54.5	22.7	23.8	1048.4
22-20	4	61.2	26.6	25.1	943.6
22-23	6	47.1	35.4	71.4	2017.0
22-24	6	51.7	19.7	30.0	1522.8
24-21	6	70.2	26.6	61.6	2315.8
24-23	6	40.4	25.7	68.4	2661.5
22-26	7	56.2	28.2	60.0	2127.6
22-27	8	62.5	28.6	34.8	1216.8
22-28	8	28.6	30.0	68.0	2266.7
22-29	9	52.0	29.4	60.6	2061.0
22-30	9	30.8	19.7	36.7	1862.9
22-33	10	39.2	32.1	13.7	196.0
22-36	10	73.3	26.2	24.8	359.0
26-25	10	42.0	23.6	46.8	1716.0
22-32	12	29.9	26.0	16.1	335.0
22-34	12	108.0	29.4	16.2	249.0
24-29	12	29.2	38.8	7.4	90.0
26-28	12	59.5	48.3	18.6	210.0
26-36	12	55.0	26.2	5.5	142.0
22-31	15	41.8	24.2	6.2	48.0
24-25	15	82.5	24.9	4.6	76.4
26-41	16	84.7	44.4	6.8	24.6

* S^{35} counts/μg. antibody N × μg. antibody N/ml. × $\frac{\text{average } S^{35} \text{ counts/μg. globulin N}}{\text{individual } S^{35} \text{ counts/μg. globulin N}}$ × $\frac{\text{average maximum serum antibody N/ml.}}{\text{individual maximum serum antibody N/ml.}}$.

37°C. for 30 minutes and then placed in the icebox at 0–3°C. for 24 to 48 hours. In the early stage of this study some sera were decomplemented by heating at 56°C. for 1 hour; but this procedure was discontinued because it increased the time necessary for complete precipitation of antigen and antibody in some sera. After sera were decomplemented, they were clarified by centrifugation at 4,000 R.P.M. at 0°C. for several hours.

Preliminary analyses of the antibody content of the sera were made using I*BGG (10). In order to determine the equivalence zone and the exact amount of antibody which was precipitated by I*BGG, the quantitative immunochemical methods of Heidelberger (11) were modified as previously described (13). Then exact amounts of non-labelled BGG were added, at or near equivalence, to various volumes of antisera so that 300 to 400 μg. of total nitrogen were precipitated. Tubes containing the antibody-antigen mixtures were incubated at 37°C. for 30 minutes and then placed in the icebox at 0–3°C. for 4 to 6 days with daily mixing. These precipitates were centrifuged at 2,200 R.P.M. at 0°C. for 30 minutes and washed twice with chilled 0.15 M NaCl. The 0.15 M NaCl washings were followed by a final washing in distilled water (pH 7.0). The washed precipitates were then dissolved in 2 ml. of 0.01 M NaOH and duplicate aliquots of 0.5 ml. and 0.3 ml. were removed for determinations of total nitrogen and S^{35} activity respectively. Nitrogen analyses were done by the Markham modification of the micro-Kjeldahl technique (14). Antibody nitrogen was calculated by subtracting the antigen nitrogen added per 0.5 ml. of dissolved precipitate from the total nitrogen content of the same aliquot.

Antibody S^{35} activity was determined in the following manner. Steel planchets were flamed to lower the surface tension between the metal and the solution (15). A ring was made with a wax pencil on the outer edge of the horizontal surface to prevent the solution from creeping up the sides of the planchets. The 0.3 ml. aliquots of the dissolved precipitates were pipetted into the planchets and spread evenly over the surface. The planchets were dried and then counted for a total of 4,000 counts. All counts were corrected for self-absorption and S^{35} decay. Control tubes containing antisera and no BGG were analyzed for nonspecific precipitation of S^{35} activity, and the values obtained were subtracted from the S^{35} activity of aliquots of the dissolved precipitates formed with BGG. The specific S^{35} activity of the antibody (S^{35} counts per μg. antibody nitrogen) was then determined by dividing the corrected S^{35} counts obtained per 0.3 ml. by the respective antibody nitrogen content.

Globulins were fractionated from sera by 50 per cent saturation at 0°C. with neutralized ammonium sulfate. The antibody was absorbed from the sera prior to fractionation. The precipitates were washed twice with 10 ml. of 50 per cent ammonium sulfate and then dissolved in 10 ml. of 0.15 M NaCl. The dissolved precipitates were dialyzed against running tap water for 2 days, and against agitated distilled water for 1 day. These samples were then diluted to 20 ml. with 0.02 M NaOH which gave a final NaOH concentration of 0.01 M. Duplicate aliquots of 0.5 and 0.3 ml. were removed for nitrogen and S^{35} activity determinations respectively. The specific S^{35} activity of non-antibody globulin (S^{35} counts/μg. globulin nitrogen) was calculated by dividing the S^{35} counts/0.5 ml. of the globulin solution by the globulin nitrogen per 0.5 ml.

Second Response.—Each of 36 white male rabbits weighing between 2.2 and 2.6 kg. received i.v. 100 mg. BGG and was bled 12 days later and serum anti-BGG concentrations determined. The 30 rabbits with more than 40 μg. anti-BGG/N ml. serum were given a second i.v. injection of 100 mg. BGG 5½ weeks after the first. 4 and 5 days after the second injection, the sera of all rabbits were tested for the presence of BGG and all were bled for determination of maximum serum antibody concentration 3 days after elimination of antigen, 8 days after injection of BGG.

These rabbits all received 0.6 to 0.7 mc. S^{35} amino acids with calculations corrected to 0.9 mc./rabbit as for the primary response. The schedule of S^{35} injections in relation to the second injection of BGG is shown in Table II.

TABLE II

Second response

Rabbit No.	S^{35} injection time after BGG	Maximum serum antibody	S^{35} counts/μg. globulin N	S^{35} counts/μg. antibody N	Antibody S^{35}/ml.*
	days	*μg. N/ml.*			
23-67	0	291.2	14.4	12.6	3504
23-68	1	164.0	17.2	17.6	4100
23-69	2	664.8	21.1	25.8	4892
23-71	3	225.4	21.6	25.8	4768
23-72	3	198.2	23.8	36.5	6136
23-73	4	61.5	25.3	34.8	5504
23-74	4	222.4	19.5	46.0	9436
23-76	5	116.2	24.2	58.3	9636
27-16	5	280.0	26.0	41.2	6184
27-18	5	118.0	27.0	54.2	8028
27-22	5	109.1	26.0	43.5	6692
23-77	6	138.7	29.0	60.7	8372
23-78	6	89.0	17.9	37.9	8468
23-79	7	157.0	17.9	16.2	3620
23-84	9	92.1	17.9	6.1	1312
27-21	10	84.8	17.6	3.8	782
27-24	10	374.0	16.7	5.6	489
27-26	10	222.8	14.3	4.8	975
23-85	15	351.2	22.6	4.6	352
27-23	15	108.6	16.6	3.3	532
27-25	15	97.0	20.5	3.6	365
27-20	20	275.0	20.5	2.9	177
27-27	20	454.0	16.6	5.5	436
27-31	20	502.0	19.9	3.1	148
23-87	21	120.9	20.1	4.3	287
23-89	21	124.4	17.3	5.9	479
23-94	25	251.0	22.1	1.8	53
27-15	25	450.0	19.7	3.0	140
27-29	25	301.0	18.0	3.9	230
27-30	25	572.0	14.1	3.0	183

* S^{35} counts/μg. antibody N × μg. antibody N/ml. × $\frac{\text{average } S^{35} \text{ counts/μg. globulin N}}{\text{individual } S^{35} \text{ counts/μg. gloublin N}}$ × $\frac{\text{average maximum serum antibody N/ml.}}{\text{individual maximum serum antibody N/ml.}}$.

The rabbits receiving S^{35} during the first 7 days after BGG were exsanguinated on day 8 at time of maximum circulating antibody. Those receiving S^{35} after day 8 were exsanguinated 24 hours after injection of S^{35}. Sera from all exsanguinations were analyzed as in the primary response.

Hyperimmune Response.—Thirteen white male rabbits weighing between 2.2 and 2.6 kg. and demonstrating a good primary response were given 5 to 6 courses of BGG injections during a 3 month period. Each course consisted of 5 injections totalling 150 mg. of BGG. 3 months after the beginning of the immunization and 3 weeks after the last injection of BGG, ten of these rabbits received 100 mg. BGG intravenously. Tests for circulating BGG

TABLE III

Hyperimmune response

Rabbit No.	S^{35} injection time after BGG	Maximum serum antibody	S^{35} counts/μg. globulin N	S^{35} counts/μg. antibody N	Antibody S^{35}/ml.*
	days	*μg. N/ml.*			
26-63	5	1044	27.4	40.3	29400
26-64	5	1128	23.9	37.4	31200
26-78	10	511	19.0	10.5	5029
26-79	10	774	21.8	14.0	6475
26-67	20	1592	21.0	9.4	2168
26-68	20	934	31.4	11.9	3841
26-69	20	1454	18.4	8.6	4909
26-70	30	954	20.0	6.8	2011
26-71	30	988	16.9	6.1	1964
26-76	30	1351	20.7	6.9	1377

$$* S^{35}\text{ counts/}\mu\text{g. antibody N} \times \mu\text{g. antibody N/ml.} \times \frac{\text{average } S^{35}\text{ counts/}\mu\text{g. globulin N}}{\text{individual } S^{35}\text{ counts/}\mu\text{g. globulin N}} \times \frac{\text{average maximum serum antibody N/ml.}}{\text{individual maximum serum antibody N/ml.}}.$$

were made 4 and 5 days after injection and all rabbits were bled for determination of maximum serum antibody concentration 3 days after elimination of antigen, 8 days after injection of BGG.

The schedule of S^{35} amino acid injections in relation to injection of BGG is given in Table III. The rabbits receiving S^{35} on day 5 were exsanguinated on day 8; all others were exsanguinated 24 hours after injection of S^{35}. Sera from exsanguinations were analyzed as in the primary response.

RESULTS

All observations are presented on the accompanying tables and figures. The incorporation of S^{35} into antibody expressed as antibody S^{35}/ml. serum is considered to be proportional to the rate of antibody synthesis at the time of S^{35} injection. Thus, these antibody S^{35}/ml. figures express relative rates of

antibody synthesis at different times throughout the immune response. In expressing S^{35} incorporated into antibody as antibody S^{35}/ml. serum certain experimental variations are eliminated. In this calculation the amount of S^{35} incorporated into the antibody in 1 ml. of serum of the terminal bleeding is multiplied by

$$\frac{\text{average } S^{35}/\mu\text{g. globulin}}{\text{individual } S^{35}/\mu\text{g. globulin}}$$

and also by

$$\frac{\text{average maximum serum antibody concentration}}{\text{individual maximum serum antibody concentration}}.$$

Using the individual rabbit's S^{35}/μg. non-antibody globulin in the denominator corrects for individual variations in the amino acid pools in which the injected S^{35} was diluted. If the amino acid pool of an animal was small at the time of injection, the S^{35} specific activity of the pool would be high and both antibody and globulin would be heavily labelled. If the pool was large, both proteins would be lightly labelled. Thus, dividing the specific activity of antibody by the specific activity of the non-antibody globulin eliminated this variable. A similar correction was used by Fineberg and Greenberg in their study of the incorporation of labelled amino acids into ferritin (16). In this case the specific activity of the ferritin was compared to the specific activity of the liver proteins. Dividing the antibody S^{35} by the non-antibody globulin S^{35} also corrects for non-immune catabolism of antibody between time of injection of S^{35} and final bleeding. Since antibody and non-antibody globulin are normally turned over at the same rate, the loss of S^{35} from the two should be comparable. This correction is unimportant for those rabbits receiving S^{35} after elimination of antigen when they were bled within 24 hours of S^{35} injection, but for those rabbits injected early in the response with a considerable delay before bleeding this correction is sizable. Using the maximum serum antibody concentration for each rabbit in the calculations serves to eliminate individual variations in magnitude of the immune response as a source of error. If the amount of antibody formed was great, the antibody S^{35}/ml. would be high, but dividing by the individual's maximum serum antibody concentration would compensate for this. If the antibody response were below average, dividing by the serum antibody concentration would offset the low antibody S^{35} value.

The most meaningful part of these results deals with the decline of antibody synthesis which begins about the time of elimination of detectable circulating antigen in each response. After the elimination of circulating antigen, the interval between injection of S^{35} and the final bleeding was brief, usually 1 day, so that there is little or no experimental error due to catabolism of antibody or reincorporation of S^{35}. In the primary response, the maximum rate of synthesis

appears to be reached between 6 and 8 days after injection of antigen (Fig. 1). During the decline there is considerable scatter of points, but the straight line of best fit would indicate a rate of decline of antibody synthesis with a half-life of approximately 1.3 days. Antibody synthesis declines within 7 to 8 days to a level approximately 1 per cent of the maximum. Whether or not these points are best characterized by a straight line cannot be determined certainly from these data but it does not seem unlikely that such may be the case.

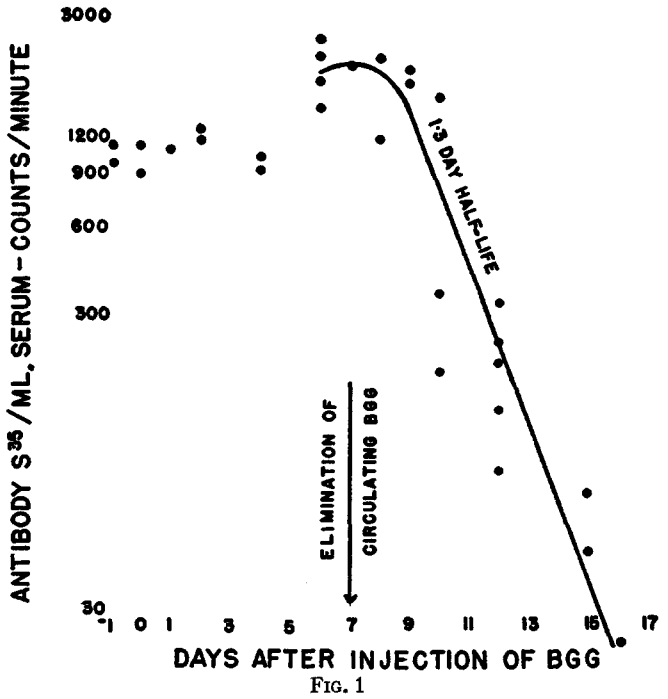

FIG. 1

FIGS. 1 to 3. S^{35} incorporation into antibody is expressed as S^{35} counts/minute present in the antibody in 1 ml. serum in each response. The S^{35} value is plotted on a log scale according to the day of S^{35} administration. Thus, each point indicates the relative rate of antibody synthesis occurring on the day of S^{35} administration.

In the second response the maximum rate of antibody synthesis is found between 4 and 6 days after injection of antigen, and the decline of antibody synthesis appears to have two distinct phases (Fig. 2). The first rapid rate of decline is similar to that seen in the primary response but this steep decline changes at about the time antibody synthesis falls to 8 to 9 per cent of the maximum and thereafter the decline is much slower. There is considerable scatter in the day 20 and 25 observations making a precise estimate of this second rate of decline difficult.

In the hyperimmune response it was assumed that the maximum rate of

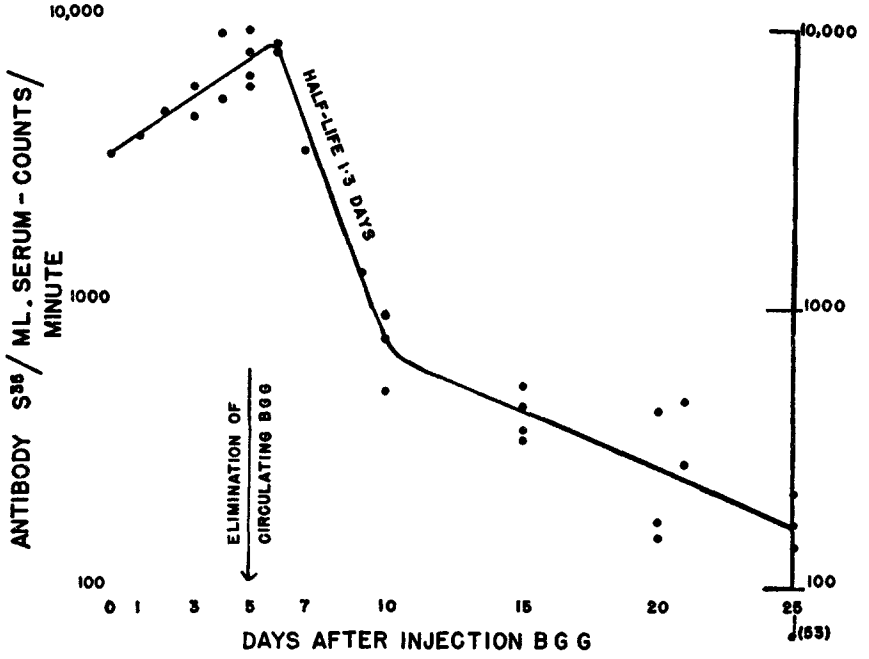
10,000
1000
100
ANTIBODY S35/ML. SERUM - COUNTS/MINUTE
HALF-LIFE 1·3 DAYS
ELIMINATION OF CIRCULATING BGG
0 1 3 5 7 10 15 20 25
DAYS AFTER INJECTION BGG
10,000
1000
100
(53)

FIG. 2

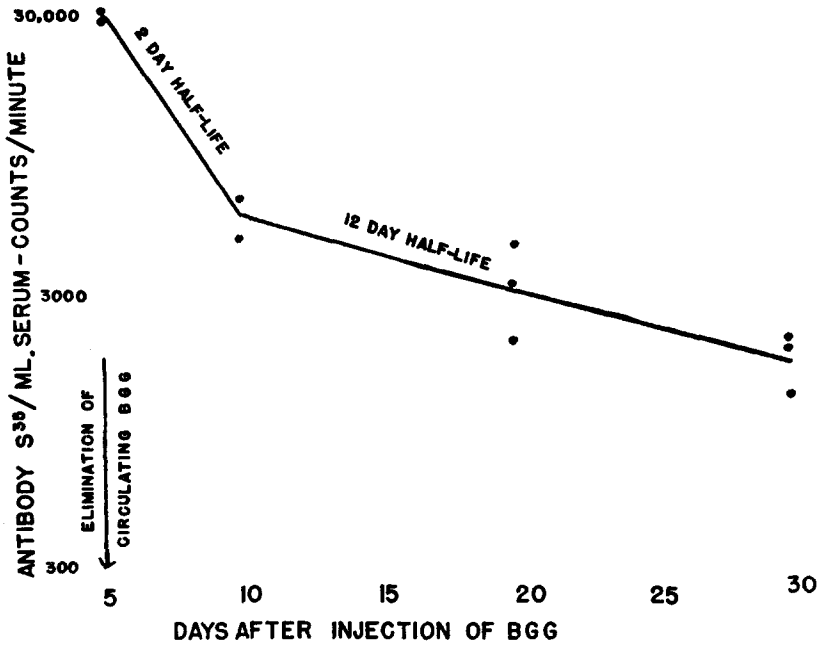
30,000
3000
300
ANTIBODY S35/ML. SERUM - COUNTS/MINUTE
2 DAY HALF-LIFE
12 DAY HALF-LIFE
ELIMINATION OF CIRCULATING BGG
5 10 15 20 25 30
DAYS AFTER INJECTION OF BGG

FIG. 3

antibody synthesis would coincide approximately with the disappearance of antigen and that the change in rates of decline would occur about day 10 as was seen in the second response. The decline of antibody synthesis in the hyperimmune response also has two distinct phases. The initial rapid decline with a 2-day half-life stops at or before the time antibody synthesis falls to approximately 20 per cent of the maximum rate. The second rate of decline has a half-life of about 12 days. If the initial observed decline in this response is resolved into two components by extrapolating the decline with a half-life of 12 days, the initial rapid component would be virtually the same as the initial decline in the first and second responses.

The observations made prior to the elimination of circulating antigen in the first and second responses are subject to several inherent errors and are, therefore, of limited value. First, antibody formed while considerable amounts of antigen are present may combine with the antigen and be rapidly catabolized (17), and, therefore, not available for analysis at the time of bleeding 3 days after disappearance of antigen. This factor would tend to make the early observations falsely low. Second, when S^{35} is injected early in the response, with the turnover of the initially labelled proteins, it may become available for reincorporation into antibody prior to the antibody bleeding. This would result in reincorporation of S^{35} into antibody after the period of initial uptake and make the values falsely high.

In the primary response it would appear that the reincorporation factor is sizable, since the S^{35} content of antibody obtained 10 days after injection of antigen is considerable even when the S^{35} was given 1 day prior to the antigen. A specific labelling of nearly ½ of the maximum found in the primary response resulted from this reincorporation. There is no detectable uptake of S^{35} into antibody above this reincorporation level when S^{35} is given during the first 4 days after antigen. In the second response, there appears to be an increase in S^{35} incorporation into antibody with time after injection of antigen. The day 0 value again probably primarily reflects reincorporation.

The amounts of S^{35} incorporated/μg. antibody N in the three responses were very similar since antibody synthesis in each instance did not significantly increase the total protein synthesis. Since serum protein synthesis represents only a small part of total protein synthesis it would not be expected that even large antibody responses would make appreciable increases in total protein synthesis. On the basis of the present data, it appears that on the day of maximum antibody synthesis the approximate ratio of serum globulin production to specific antibody production was as follows: primary response, 30:1; second response, 8:1; and hyperimmune response, 2:1. Looked at another way, at the time of maximum rate of antibody synthesis, the per cent of total S^{35} injected which was incorporated into antibody was as follows: primary, 0.1 per cent; second, 0.4 per cent; and hyperimmune, 1.5 per cent.

DISCUSSION

The finding of a single rapid rate of decline of antibody synthesis in the primary response and a similar, initial rapid rate of decline followed by a slower rate in the second and hyperimmune responses is not unexpected in the light of estimates of rates of antibody synthesis based on levels of circulating antibody alone. However, the more precise definition of these rates of decline and their duration throw light on several aspects of the antibody response. First, the fact that in all three responses the rate of antibody synthesis begins to decline rapidly after the elimination of detectable circulating antigen suggests that the circulating antigen plays a major role in stimulating antibody synthesis. Circulating antigen may provide a source of continuing antigenic stimulation to antibody producing cells. Such a concept would be in accord with the present observations. Second, the rates of initial rapid decline of antibody synthesis are approximately the same in the three responses, suggesting that they reflect the loss of a substance or process of fundamental importance in all three situations. Third, there is a relatively persistent source of antibody production which appears after repeated stimulation and increases in proportion to the number of repeated stimuli.

It may be worthwhile to review the conflicting reports on the persistence of BGG in rabbits in the light of the observed rates of antibody synthesis. Using I^{131} tracer techniques (2), fluorescent antibody techniques (18), or ordinary precipitin tests on serum or tissue extracts, neither BGG nor human gamma globulin has been found to persist in significant amounts in the blood or tissues of rabbits after the appearance of circulating antibody, 7 days after the initial injection of antigen and 4 to 5 days after a secondary injection. However, studies based on soluble antigen-antibody complexes (19), S^{35}-labelled antigens (20) and mouse anaphylaxis tests of organ extracts (21), have indicated the persistence of BGG in rabbits for many weeks. The rapid decline of antibody synthesis after the 7th or 8th day of the primary response and 5th or 6th day of secondary response would seem to indicate the loss of the principal antigenic stimulus, in agreement with the former findings.

While the present observations on antibody synthesis cannot answer the question of persistence of antigen directly, they do indicate the duration of effective antigenic stimulation and impose definite conditions on any persisting antigen. If, in the primary response, BGG or some recognizable fraction thereof persisted in rabbits after the appearance of circulating antibody, the present observations would ascribe little antigenicity to it. Certainly there is no parallel between the rapid decline of antibody synthesis in the primary response and the reported long persistence of BGG. If it is assumed that BGG in an active antigenic form persists in rabbits for several weeks after its primary injection, one must then postulate that there is a mechanism for abruptly terminating antibody synthesis in the presence of persisting antigen. While suppression of

antibody formation may result from overdoses of antigen (22), there is no evidence that effective doses of antigens which are known to persist for long periods, such as pneumococcal polysaccharides, lose their ability to stimulate antibody formation with time (23). In addition, the persistent antibody synthesis of the second and hyperimmune responses cannot be correlated with an observed increased retention of BGG after repeated injections (2). Even those techniques showing persistence of antigens in the host after primary injection indicate less retention of antigen after repeated stimulation (24, 25). If the studies based on antigen-antibody complexes, S^{35}-labelled antigens, and mouse anaphylaxis do detect persisting BGG or some of its products which escape the I^{131} tracer and fluorescent antibody techniques, it might be more desirable to consider this material as merely foreign rather than antigenic, especially in the primary response. Until the above measures of persisting BGG can be correlated with observed rates of antibody synthesis in primary and secondary responses they must be accepted with considerable reservation.

The initial rate of decline of antibody synthesis has a half-life of approximately 1.3 days in the first and second responses and also in the hyperimmune response if the observed 2 day half-life is corrected to eliminate the slow component. The significance of this rate of decline is not apparent, but it could reflect several things: first, it could parallel the loss of antigenic material; second, it could reflect the loss of a synthetic process, such as an adaptive enzyme (26), or inducer, in the absence of antigenic stimulation; or, third, it could be dependent upon the loss of a short lived cell type involved in antibody production. These alternatives are not mutually exclusive and their roles, if any, in the decline cannot be determined on the basis of the present observations.

The second slow phase of decline of antibody synthesis which increases in importance with repeated antigenic stimulation suggests a more lasting modification or adaptation of globulin synthesis. It could reflect the rate of loss of a different, more permanent cell type involved in the antibody response after repeated stimuli or the loss of an adapted globulin synthesizing mechanism.

In discussing the rates of hemolysin production in rabbits, Taliaferro and Taliaferro postulated that the initial, short lived, rapid synthesis took place in the spleen and the prolonged synthesis occurred in non-splenic sites (1). This postulation was based on observations of hemolysin production in intact and splenectomized rabbits. However, in contrast to our findings there was no evidence that the prolonged non-splenic hemolysin synthesis increased with repeated immunization (27). It is likely that the sites and nature of the persistent antibody synthesis in response to foreign red cells and foreign serum proteins are puite different, probably because of differences in physical and chemical characteristics of the antigens.

Our observations on the early part of these responses are difficult to interpret. The lack of evidence of incorporation of S^{35} into antibody during the first

4 days of the primary response may reflect an initial induction period during which little antibody is formed. In the secondary response, however, there appears to be a steady rise in the rate of antibody beginning with the injection of antigen. The early part of the curve in the second response resembles the plot of Green and Anker's results (4). However, our early values, and perhaps those of Green and Anker, are probably much too high. A considerable part of the S^{35} found in antibody following S^{35} injection early in the secondary response is probably the result of reincorporation as demonstrated in the primary response. Our observations on the second response do not agree completely either with the work of Green and Anker who concluded that, of the antibody present 6 days after the second injection of antigen, 31 per cent was formed in the first 60 hours, before the appearance of circulating antibody; or with that of Taliaferro and Talmage (5) who found virtually no incorporation of amino acid into antibody during the first 3 days of the secondary response. If it is assumed that the day 0 S^{35} value of the secondary response is caused by reincorporation and this value is subtracted from the other early second response observations, the increase of antibody synthesis would be nearly logarithmic beginning at a very low level. A logarithmic increase of antibody synthesis in the secondary response of rabbits has been postulated by Burnet and Fenner (26). In such a situation the amount of antibody synthesized in the first 3 days would amount to only a few per cent of the total synthesized in the first 6 days, which would be close to those values obtained by Taliaferro and Talmage.

The data on the specific activity of antibody and other serum proteins would corroborate at least for antibody, the conclusions of others working with bacterial (28, 29) and mammalian (16, 30) systems; namely that protein biosynthesis is *de novo* from amino acids. Antibody synthesis probably involves the induced formation (in the presence of antigen) of new gamma globulin rather than the conversion of a preexisting serum globulin. This system resembles closely the induced synthesis of proteins as studied by Spiegelman and Monod and their coworkers.

SUMMARY

Determinations of the rates of antibody synthesis during first, second, and hyperimmune responses to bovine gamma globulin using S^{35}-labelle aminod acids indicate the following:

1. In all three responses the rate of antibody synthesis increases while antigen is circulating and then begins to decline rapidly after elimination of detectable circulating antigen.

2. The initial rates of decline of antibody synthesis are approximately the same for all three responses.

3. There is a relatively persistent source of antibody production which ap-

pears after repeated stimulation and increases in proportion to the number of repeated stimuli.

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