GAMMA GLOBULIN METABOLISM IN RABBITS DURING THE ANAMNESTIC RESPONSE*

By EVANGELOS A. CATSOULIS, M.D., EDWARD C. FRANKLIN,‡ M.D., MURRAY ORATZ, PH.D., AND MARCUS A. ROTHSCHILD, M.D.

(From the Radioisotope Service, Veterans Administration Hospital, the Department of Medicine and Rheumatic Disease Study Group, New York University School of Medicine, and the Department of Biochemistry, New York University College of Dentistry, New York)

(Received for publication, November 20, 1963)

A number of diseases in man are associated with markedly increased gamma globulin levels in the serum and an even greater increase in total gamma globulin pool. The most striking of these is multiple myeloma, but elevations are also seen in cirrhosis of the liver, certain of the collagen diseases such as lupus erythematosus and rheumatoid arthritis, and a variety of chronic infectious processes (1–9).

Studies of the metabolism of gamma globulin with I^{131}-labeled human gamma globulin in conditions with an elevated serum gamma globulin level have indicated an increase in the catabolism of gamma globulin (1–4, 8–11). A similar situation appears to exist in animals with increases in gamma globulin levels (12–14), whether due to plasma cell tumors (12), immunization (13), or the passive administration of gamma globulin (14).

While these findings indicate that synthesis must increase many fold to achieve the high gamma globulin pool size in the face of an increase in breakdown, none of the studies in man or in tumor-bearing animals have given an accurate picture of the potential capacity of the subject to produce gamma globulin under physiological conditions. Similarly, little is known of the effect of a state which may well approach maximal gamma globulin production, on gamma globulin metabolism. To investigate this problem studies of gamma globulin metabolism have been undertaken in rabbits hyperimmunized with polyvalent pneumococcal antigen, a stimulus known to induce very high levels of gamma globulin (15–18).

---

* Presented in part at the Ninth Interim Scientific Session, American Rheumatism Association, December 1962. This investigation was supported in part by United States Public Health Service Grants A-2489, A-2594, and AM-01431 and by the Arthritis and Rheumatism Foundation.

‡ Career Scientist; New York Health Research Council.

Methods

Female rabbits were used in all studies. The animals were injected intravenously with 2 to 3 ml of a polyvalent pneumococcal vaccine prepared from types I, II, VI, IX, XI, and XVD. The preparation used has been described previously (18). The rabbits were kept in metabolism cages and fed a standard rabbit diet. Complete urine and stool collections were made daily. Two to three drops of Lugol's solution were administered daily in the rabbits' drinking water in order to inhibit thyroidal uptake of I^{131}.

The distribution and metabolism of gamma globulin was measured by means of rabbit serum gamma globulin labeled with I^{131}. Rabbit gamma globulin (RGG) was separated from fresh rabbit serum by means of starch block electrophoresis for 22 hours employing barbital buffer pH 8.6, ionic strength 0.05. The gamma globulin migrating under the peak of gamma globulin or more slowly, was eluted with borate buffer pH 8.4, concentrated, and dialyzed against borate buffer for 24 hours. The more rapidly migrating material known to be richer in 19S gamma globulin was discarded. The gamma globulin so obtained sedimented as a rather homogeneous peak with a sedimentation coefficient of about 7S and contained no detectable 19S gamma globulin on analytical ultracentrifugation at concentrations of 10 to 15 mg/ml. Gamma globulin was iodinated as described previously (19), and the material used contained less than 1 iodine atom per protein molecule. Prior to use all lots of I^{131} rabbit gamma globulin were tested in control rabbits to guard against the use of a preparation that contained a significant fraction of denatured material.

Turnover studies of I^{131} rabbit gamma globulin were performed 24 times in 13 control rabbits. Four other rabbits were given a primary course of immunization with pneumococcal vaccine as described (15–18). Gamma globulin metabolism was measured during the resting phase, 3 to 5 months following initial exposure to the vaccine, and then restudied during the rapid development of hypergammaglobulinemia induced by a second course of immunization. Each rabbit received an injection of 15 to 275 μc of rabbit gamma globulin I^{131} into an ear vein. Heparinized blood samples were obtained from the contralateral ear 6 to 10 minutes after the injection to measure the plasma volume and at daily intervals thereafter. Control observations were made daily on the excretion of radioactivity in urine and stools for 10 to 36 days.

The 4 previously immunized animals were given a tracer dose of I^{131} RGG in the resting phase, at which time they had normal serum gamma globulin levels. The fate of the injected protein was studied for 11 to 16 days which may be considered as the control period. Following this, 2 to 3 ml of the vaccine, containing 1×10^9 organisms per ml were injected intravenously every 3 days to induce a rapid anamnestic response, and the fate of the previously injected RGG I^{131} was followed during the initial period of antibody synthesis which was accompanied by the rapid development of hypergammaglobulinemia. Eighteen to 26 days following the beginning of immunization a second injection of gamma globulin I^{131} was given in order to reevaluate gamma globulin distribution, and observations of radioactivity in plasma, urine and, stool were made for an additional 10 to 17 days, while vaccine injections were continued. Thus, gamma globulin metabolism was first studied during what might be considered steady state and again during and following the anamnestic response.

The plasma volume (PV) was determined from the mean space of distribution of rabbit gamma globulin I^{131} at 6 and 10 minutes after injection. Total exchangeable gamma globulin (TEG) was determined from the product of the equilibrium space of distribution of the injected rabbit gamma globulin I^{131} and the serum gamma globulin concentration. Fecal excretion of I^{131}, which amounted to between 5 to 9 per cent of that excreted in the urine was included in the urinary excretion. Plasma, urine, and stool samples were assayed for I^{131} in a well-type scintillation counter with a sensitivity of 9.0×10^5 CPM per μc I^{131} above a background of 160 CPM.

During the control period when steady state conditions were present (at least in terms of gamma globulin metabolism), gamma globulin degradation and synthesis can be assumed to be equal. Gamma globulin degradation was calculated from the product of the total exchangeable gamma globulin and the rate of decline of the plasma radioactivity after distribution equilibrium. However, during the experimental period when gamma globulin levels and gamma globulin metabolism were changing, steady state conditions were not present and this method is not valid. Therefore the rate of gamma globulin degradation during both the control and experimental periods was determined also by the metabolic clearance procedure which has been shown to be valid in the non-steady state (20, 21). The amount of gamma globulin degraded per day represents the product of the renal clearance of I^{131} and the serum gamma globulin concentration. Firstly the renal clearance of I^{131} (derived from degraded protein) was determined by dividing the μc of I^{131} excreted/day by the mean plasma concentration of RGG-I^{131} for that day. The product of this clearance and the mean value for the serum gamma globulin concentration resulted in daily values for gamma globulin degradation. In the non-steady state it is not possible to estimate daily gamma globulin synthesis. However, gamma globulin synthesis for the whole period of study was determined from the difference between the amount of gamma globulin degraded during the experimental and control periods and the change in the total exchangeable gamma globulin pool calculated at the beginning and end of the study. Some of the limitations of this type of study will be pointed out below.

Total serum protein was determined by the Folin method modified by Lowry *et al.* (22). Protein partition was determined either by boundary electrophoresis employing a Kern microelectrophoretic unit, as described previously (23), or by quantitative elution of stained paper strips following serum paper electrophoresis (24, 25). The stained strips were cut into the desired segments and suspended in 0.5 per cent sodium carbonate for 30 minutes, and the dye liberated into the supernatant fluid was read within 1 hour in a Beckman model spectrophotometer at 590 $m\mu$ in 1 cm cuvettes. Results were interpreted on standard curves of known quantities of rabbit serum gamma globulin and albumin which were subjected to electrophoresis and stained under similar conditions. In general the 2 techniques agree quite well. Urine was checked for protein by precipitation with cold 10 per cent trichloracetic acid.

TABLE I

Control

*Gamma Globulin Distribution**

	Weight	Plasma volume	TEGS	γ-Globulin, intravascular
	kg	*ml/kg*	*ml/kg*	*per cent*
Range	3.1–5.7	25–44	55–108	34–51
Mean	4.1	33	75	44
± SE of mean		1	1	1

TEGS, total exchangeable gamma globulin space.

* $\frac{\text{No. of studies}}{\text{No. of rabbits}} = \frac{24}{13}$.

RESULTS

The data for the distribution and metabolism of gamma globulin in the control rabbits are shown in Table I. During the control studies the individual

rabbits' weights did not change significantly. The plasma volume averaged 33 ± 1 ml/kg, and the total apparent space for gamma globulin distribution was 75 ± 1 ml/kg. Forty-four ± 1 per cent of the total gamma globulin pool

TABLE II

Control

*Gamma Globulin Metabolism**

	Total protein	Albumin	γ-Globulin	TEG	γ-Globulin degradation		
					From plasma T ½	From clearance	
	gm/100 ml	*gm/100 ml*	*gm/100 ml*	*gm/kg*	*gm/kg/day*	*gm/kg/day*	*day, per cent*
Range	6.0–7.3	3.1–4.5	0.6–1.4	0.4–1	0.03–0.07	0.02–0.08	5.7–10.0
Mean	6.5	3.8	0.9	0.6	0.05	0.05	8.0
± SE of mean	0.1	0.1	0.1	0.1	0.01	0.01	0.3

TEG, total exchangeable gamma globulin.

* $\frac{\text{No. of studies}}{\text{No. of rabbits}} = \frac{24}{13}$.

TABLE III

*γ-Globulin Distribution Before and Following the Anamnestic Response**

	Weight		Plasma volume		TEGS		γ-Globulin, intravascular	
	C	E	C	E	C	E	C	E
	kg	*kg*	*ml/kg*	*ml/kg*	*ml/kg*	*ml/kg*	*per cent*	*per cent*
Range	4.1–5.2	3.6–5.6	29–34	34–46	59–70	66–93	41–50	46–53
Mean	4.4	4.5	31	38	66	78	46	48
± SE of mean			1	3	2	5	2	2
Change, *per cent*				+23		+18		

C, control studies.

E, experimental

TEGS, total exchangeable gamma globulin space.

* $\frac{\text{No. of studies}}{\text{No. of rabbits}} = \frac{4}{4}$.

was located intravascularly. The data for gamma globulin metabolism are shown in Table II. The mean control value for total protein was 6.5 ± 0.1 gm/100 ml, and values for plasma albumin and gamma globulin concentration were 3.8 ± 0.1 gm/100 ml and 0.9 ± 0.1 gm/100 ml respectively. Total exchangeable gamma globulin averaged 0.6 ± 0.1 gm/kg. Gamma globulin degradation derived by both the plasma T½ and metabolic clearance methods

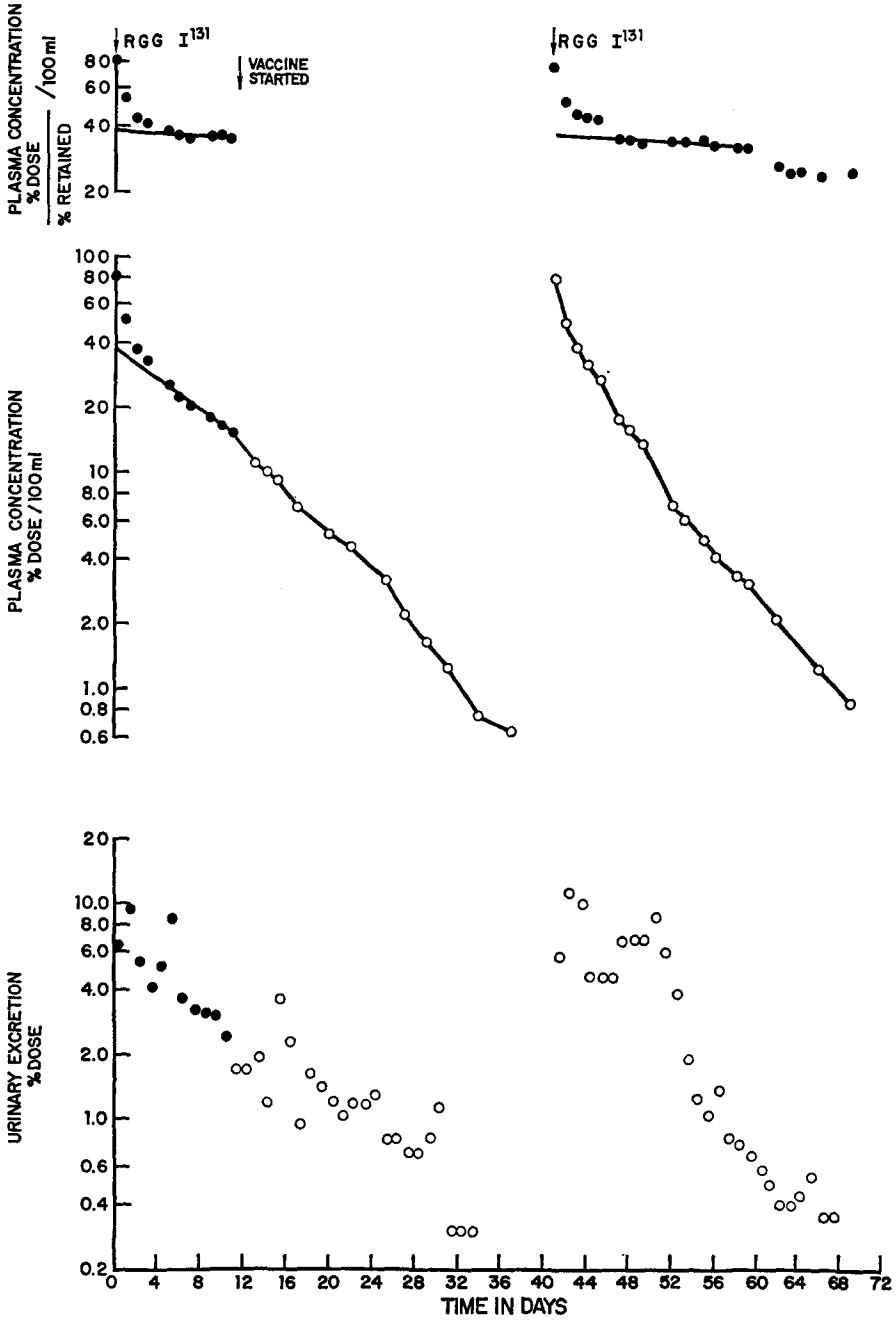

FIG. 1. Gamma globulin distribution and degradation in rabbit 1-13 during the control and experimental period. The distribution curves remained steady during the control period and during the first 12 days of the reinjection study (upper curves). There was a slight increase in the slope of the plasma decay curves following the vaccine (center curves). The urinary excretion is shown in the lower curves.

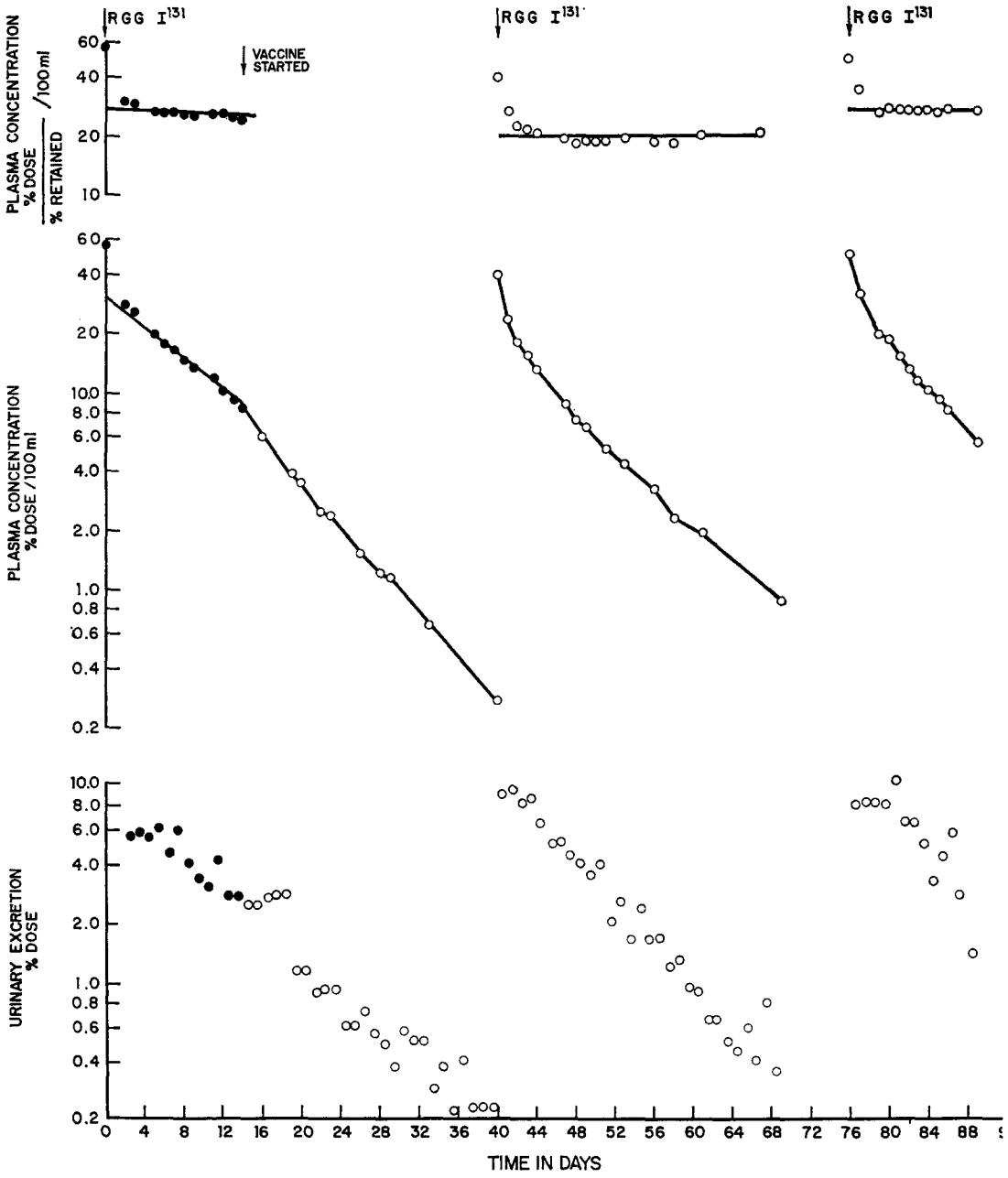

FIG. 2. Gamma globulin distribution and degradation of rabbit 1-14 during the control and experimental period. Tl distribution curves (upper graphs) remained steady during all three injections of RGG–I^{131}. Following the start of tl vaccine, there was a slight increase in the rate of loss of plasma I^{131} which persisted during the subsequent studies (cent curves). The urinary excretion is shown in the lower curves.

averaged 0.05 gm/kg/day. The mean fractional turnover rate was 8.0 ± 0.3 per cent/day.

The data for gamma globulin distribution during the "rest period" and the anamnestic response in the 4 immunized rabbits are shown in Table III and Figs. 1 and 2. There was no significant change in weight during immunization. The plasma volume increased 23 per cent while the exchangeable gamma

TABLE IV

Gamma Globulin Metabolism during the Anamnestic Response

Rabbit No.	Total protein		Albumin		γ-Globulin		TEG		γ-Globulin degradation			Study	γ-Globulin synthesis	
									A $T_{1/2}$ × TEG	B Clearance				
	C	E	C	E	C	E	C	E	C	C	E		C	E
	gm/100 ml		*gm/100 ml*		*gm/100 ml*		*gm/kg*		*gm/kg/day*	*gm/kg/day*		*days*	*gm/kg/day*	
1-13	6.8	13.2	4.2	3.1	0.8	6.0	0.5	4.5	0.04	0.04	0.35	36	0.04	0.46
1-14	7.8	13.2	4.1	1.7	1.5	7.5	1.0	7.2	0.10	0.10	0.54	33	0.10	0.74
1-19	5.8	11.3	3.3	3.0	0.8	5.5	0.5	4.0	0.05	0.07	0.22	21	0.07	0.38
1-96	6.1	10.6	3.3	2.9	0.9	5.0	0.8	3.2	0.05	0.04	0.19	21	0.04	0 31
Mean value...	6.6	12.1	3.7	2.7	1.0	6.0	0.7	4.7	0.06	0.06	0.33	28	0.06	0.47
± SE...	0.4	0.7	0.2	0.3	0.5	0.5	0.1	0.9	0.01	0.01	0.08		0.01	0.09
Change, *per cent*.....		+83		−27		+500		+570			+450			+685

C, control.
E, experimental.
TEG, total exchangeable gamma globulin.
A, determined from the product of the plasma T ½ and TEG.
B, determined from the metabolic clearance procedure.

globulin space increased 18 per cent. The fraction of the gamma globulin pool located intravascularly showed no significant change.

The anamnestic response was associated with striking changes in gamma globulin metabolism as shown in Table IV and Figs. 3 and 4. There was an 83 per cent increase in total serum protein due largely to a 6-fold rise in the gamma globulin level. In contrast, as previously demonstrated, there was a 27 per cent decrease in the concentration of serum albumin (15–18). During the secondary response the mean value for gamma globulin degradation rose 450 per cent. In the presence of the significant increase in gamma globulin degradation, there was a marked rise in gamma globulin pool size from 0.7

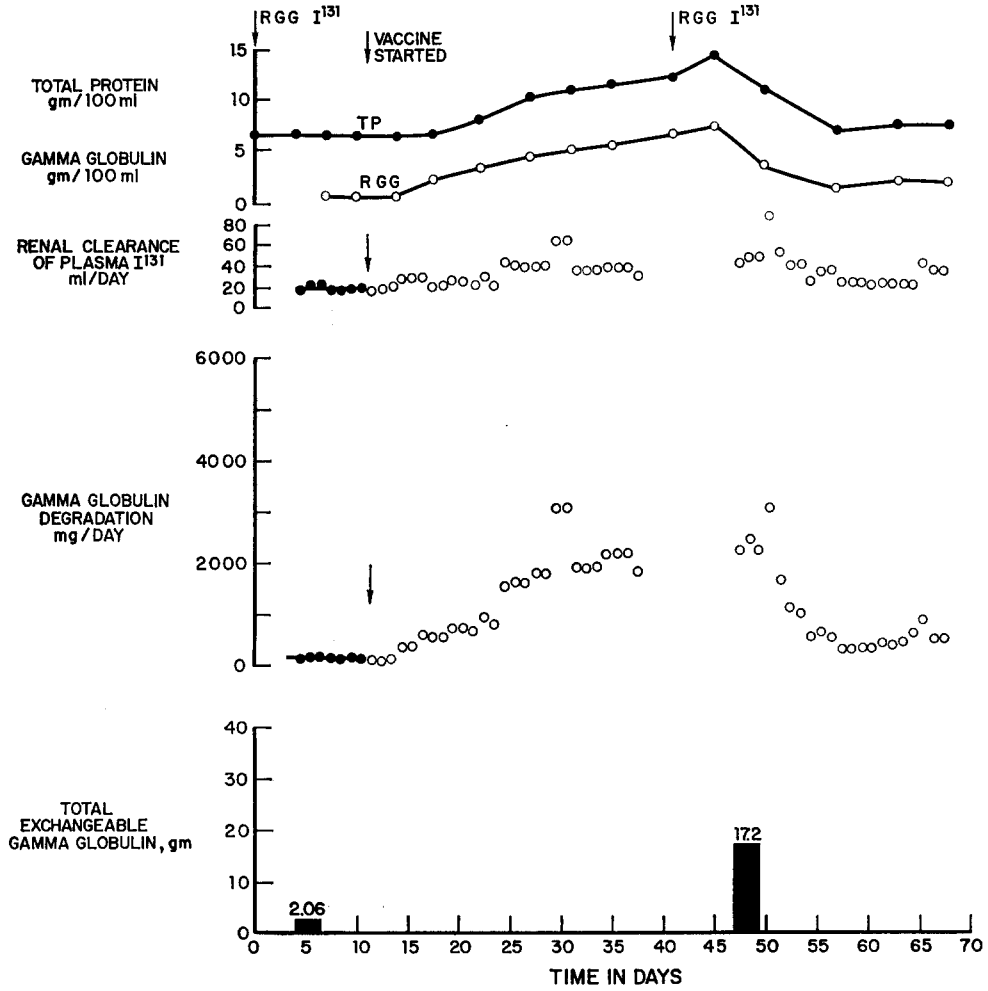

FIG. 3. Gamma globulin metabolism in rabbit 1-13 during the control and experimental period. The total protein and gamma globulin levels rose rapidly following the start of the vaccine, associated with a marked increase in gamma globulin degradation which seemed to plateau between the end of the first study and the early part of the reinjection period (days 25 to 50). Total gamma globulin pool size increased while degradation was increasing. Following day 47 there was a decrease in the serum gamma globulin levels and gamma globulin degradation decreased.

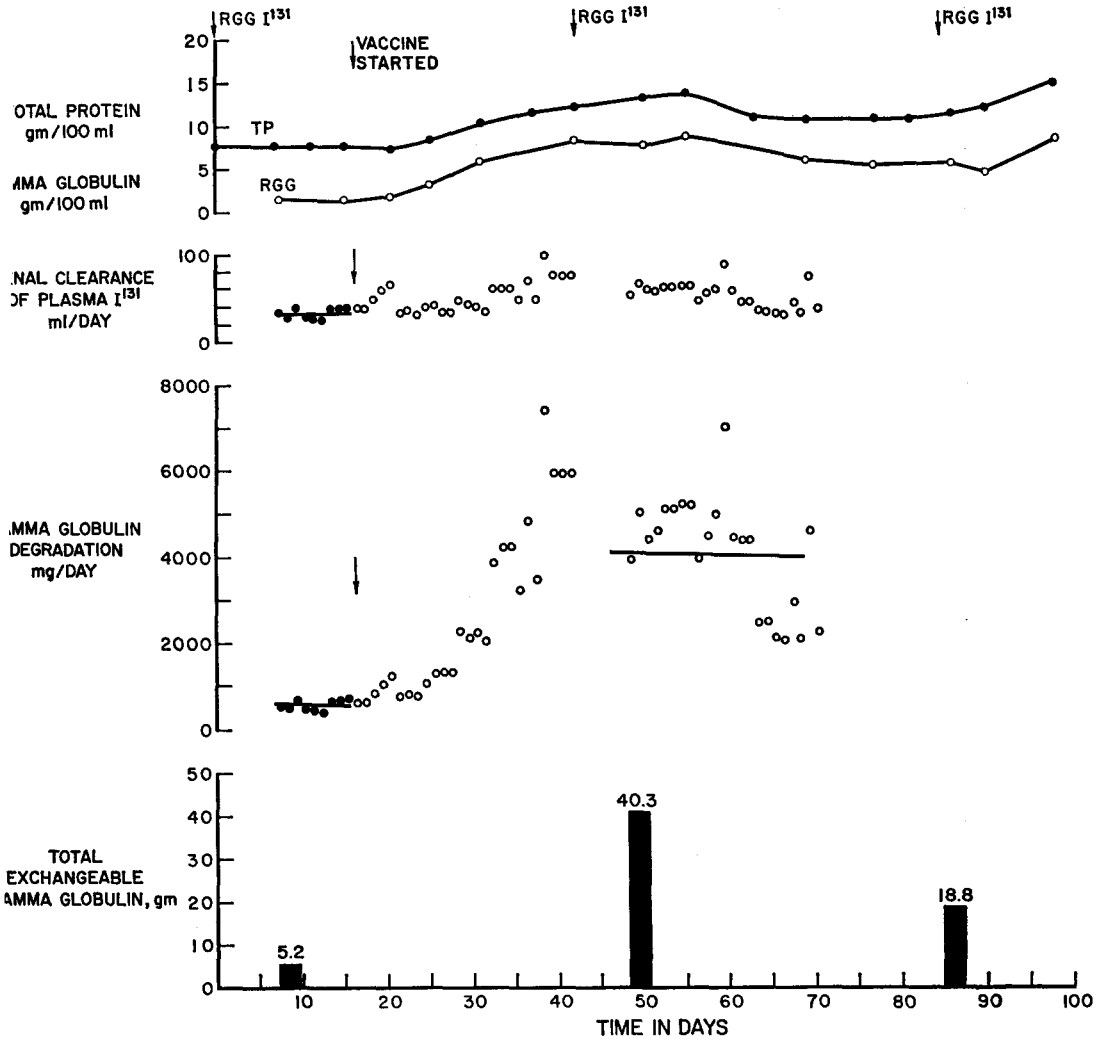

FIG. 4. Gamma globulin metabolism in rabbit 1-14. The total protein and gamma globulin levels increased rapidly lowing the start of vaccine; gamma globulin degradation increased markedly and plateaued between days 35 and 70. .e gamma globulin pool size rose during the first 40 days of vaccine treatment. Thereafter, associated with a fall in : serum gamma globulin levels and in gamma globulin degradation, the total gamma globulin pool size decreased to out ½ of its peak value.

± 0.1 gm/kg to 4.7 ± 0.9 gm/kg during a mean period of observation of 28 days. Thus, while degradation rose nearly 5-fold, gamma globulin synthesis increased approximately 8-fold to a mean value of 0.47 ± 0.09 gm/kg/day to account for the increment in gamma globulin pool size. After a period of sustained increase in synthesis, a transient decline in gamma globulin pool size, secondary to a decrease in synthesis, was noted in 2 rabbits (1–13, 1–14) (Figs. 3 and 4), but a similar phenomenon was not noted in 2 other rabbits. This lasted for only a relatively short period of time (Fig. 4), following which gamma globulin levels returned towards the previous high levels.

In the presence of the rapidly increasing serum gamma globulin levels, the amount of gamma globulin degraded each day also rose very rapidly and achieved the highest values within 2 to 3 weeks following the start of reimmunization (Figs. 3 and 4). Thereafter the rate of degradation of gamma globulin remained relatively constant and approached the mean rate of gamma globulin synthesis determined for the whole experimental period (Figs. 3 and 4). While an exact sequential analysis of alterations in gamma globulin metabolism under such conditions of flux is impossible, it appears that a new equilibrium between gamma globulin synthesis and degradation had been achieved. Consequently it seems probable that the increment in the gamma globulin pool had occurred early during the anamnestic response and that thereafter, when the gamma globulin pool reached its peak value, a new equilibrium was achieved. At this time the increase in gamma globulin synthesis would again be compensated for by an equivalent increase in degradation, thus resulting in a new steady state. If this interpretation is valid, then the average fractional rate of gamma globulin degradation during the anamnestic response, determined from the ratio, gamma globulin degradation/TEG, should be based upon the maximum TEG with only a small error introduced by the lower TEG during the early development of the hypergammaglobulinemia. This calculation would result in a minimal mean value for the fractional rate of degradation of 7 per cent/day compared to a mean control value of 8 per cent/day. The true value would be slightly greater if corrected for the early days where the TEG is still rising. On the other hand, if gamma globulin synthesis did not parallel degradation but increased steadily, then the TEG pool would be increasing throughout the period of study. Under these circumstances, the fractional rate should be calculated from the average value for TEG during the control and experimental periods. This calculation would result in an average fractional turnover rate of 12 per cent/day. It is probable that during the development of hypergammaglobulinemia the true degradative rate was somewhere between these two values and consequently exceeded the control value by only a moderate degree. However after the levels of gamma globulin had been elevated for some time the fractional degradative rate determined from the second dose of I^{131} RGG was elevated to a value of 12.5

± 1.2 per cent/day agreeing with the higher estimate obtained from the first dose of RGG I^{131}.

Certain technical aspects of this and similar studies would be greatly facilitated if the same preparation of gamma globulin could be used in a number of different animals. For this reason studies of the turnover of gamma globulin

TABLE V

Turnover of Autologous and Homologous Gamma Globulin

Recipient rabbit No.	Phenotype recipient	Donor rabbit No.	Phenotype donor	Date	Plasma T½
					days
1-13	A1, 2	1-13	A1, 2	1/23/62	9.4
1-55	A2, 3	1-13		1/30/62	9.3
2-10	A1, 2	1-13		1/23/62	14.8
1-40	A1, 3	1-13		1/23/62	10.3
1-55	A2, 3	1-26	A1, 2	5/7/62	12.4
1-55		1-55	A2, 3	8/7/62	9.7
1-55		1-13(I)	A1, 2	3/5/62	12.4
1-55		1-13	A1, 2	1/30/62	9.3
3-08	A1	3-08	A1	10/18/62	8.6
3-08		3-09	A1	12/17/62	9.6
3-08		3-34	A1, 2	1/21/63	9.0
3-09	A1	3-08	A1	10/18/62	7.0
3-09		3-09	A1	12/17/62	7.0
3-09		3-34	A1, 2	1/21/63	7.2
3-34	A1, 2	3-09	A1	12/17/62	8.6
3-34		3-34	A1, 2	1/21/63	8.2

I, immunized gamma globulin.

TEG, total exchangeable gamma globulin ranged from 0.5 to 0.8 gm/kg and plasma gamma globulin from 0.8 to 1.0 gm/100 ml.

Allotypes differed only in the "a" locus; the "b" locus was identical in all instances with a formula of A4 and all rabbits were P+ (26, 27).

I^{131} were performed in a group of rabbits with varying gamma globulin allotypic specificities (26, 27), each of which received its own protein and sequentially the protein of several others.[1] The data for these studies are shown in Table V. The rate of plasma disappearance of the various I^{131}-labeled autologous and homologous gamma globulin preparations showed only minor variations, and there was no constant reproducible difference between autologous or

[1] We would like to thank Dr. Sheldon Dray and Dr. E. Lichter for determining the allotypic specificities of these sera for us.

isologous preparation, indicating that either could be used. In one instance gamma globulin was obtained from the serum of an hyperimmunized rabbit, and the data obtained with this preparation were likewise not significantly different from those observed with control gamma globulin preparations. Likewise allotypic variations in gamma globulin between donor and recipient rabbit were not associated with any constant change in degradative rate (Table V).

Bjørneboe (15, 28), has previously shown that the antibody titers tested by agglutination with any one of the pneumococci used in similarly immunized animals ranged between 640 and 1280, yet that all but approximately 1.3 gm/100 ml gamma globulin could be removed by absorption with pneumococci. The results of antibody titrations and absorption experiments in the present study are entirely in agreement with these findings, and consequently they will not be discussed in detail.

DISCUSSION

Polyvalent pneumococcal vaccine in rabbits is a potent physiological stimulus resulting in a rapid and possibly even maximal elevation of gamma globulin synthesis for short periods of time (15–18). The present studies clearly show that normal animals, subjected to intensive antigenic stimulation, possess an enormous reserve capacity to synthesize gamma globulin. They further demonstrate that for a short period of time at least, gamma globulin synthesis can exceed 1.0 gm/kg body weight/day, a value which agrees with the maximal catabolic rate of gamma globulin I^{131} observed in mice (13). The potential magnitude of this phenomenon can be estimated only from metabolic studies of this kind since simple measurements of gamma globulin levels would take into account only the increase in gamma globulin pool size but would completely ignore the increase in degradation in the face of which the rise in gamma globulin pool size occurred.

The marked increase in degradation associated with hypergammaglobulinemia is not unique to this situation but also has been shown to be present in a variety of other hypergammaglobulinemic states (1–5, 8–13), and implies that the amount of gamma globulin degraded is probably related to the gamma globulin levels or pool size (13). That the mechanisms controlling degradation may be very specific is suggested by the finding that in subjects with multiple myeloma there is an increased breakdown of gamma globulin in patients with gamma myeloma proteins and beta globulin in those with beta type myelomas while the metabolism of the other proteins is only little affected or reduced (1, 2, 13). Similarly, the passive administration of 7S gamma globulin but not of 19S gamma globulin, gamma 1A globulin or albumin increases the breakdown of 7S gamma globulin in mice (13). It seems possible that a similar feed back mechanism may be operative also in the regulation of degradation of other proteins.

The site of degradation of gamma globulin is not known nor can it be said whether the increase in the observed fractional degradative rate is due solely to normal degradative routes or due to other mechanisms in addition. If the fractional rate of degradation can be used as an indirect measure, it seems likely that accelerated normal processes were primarily responsible for the increase in gamma globulin degradation during the hypergammaglobulinemic state. The metabolism of RGG I^{131} during hyperimmunization showed fractional rates of degradation which were somewhat higher than those observed during the control periods, but quite unlike those seen if an immune mechanism were called forth as an additional means of removal of excess gamma globulin (29). While no antibodies to human or rabbit gamma globulin could be detected in any of the sera when tested with sensitized sheep cells coated with rabbit gamma globulin or human cells coated with incomplete human antibodies (30, 31), the existence of small amounts of such substances can not be excluded with absolute certainty. Even if present, however, these substances did not significantly affect the metabolism of autologous gamma globulin. In studies to be reported separately, animals immunized with autologous denatured RGG and high titers of such antibodies showed fractional rates of degradation similar to those seen in the animals in this study and to those in control animals having no antibodies but equivalent changes in gamma globulin pool size (32). In the rabbits with very high gamma globulin turnover rates there was no increase in the amount of radioactivity released from degraded protein, appearing in the stool, a factor which points against the gastrointestinal tract as a major site for gamma globulin degradation.

It would appear that with the type of stimulus under investigation the very rapid rate of production of gamma globulin can only be maintained for a relatively short period since following 3 to 3½ weeks of antigen administration, 2 of the rabbits demonstrated a rather rapid, though transient, decline in serum gamma globulin levels in spite of continued immunization. In rabbits 1-13 and 1-14 it is clear that there was a marked decrease in the concentration of circulating gamma globulin without an equivalent increase in gamma globulin degradation. The observation that gamma globulin degradation did not change for a few days while the pool size was falling indicates a transient increase in the fractional rate of gamma globulin degradation initially (Figs. 3 and 4). However, the fall in gamma globulin pool size must have been due primarily to a decrease in gamma globulin synthesis rather than increased catabolism. The exact reason for this remains to be determined, but several mechanisms appear worthy of consideration. One possibility is that the reserve of amino acids necessary for protein synthesis was rapidly depleted. At peak production, gamma globulin synthesis would require a utilization of nitrogen which exceeds ⅓ of a normal rabbit's total daily nitrogen intake. It is possible that this increment in nitrogen utilization for gamma globulin anabolism might limit further synthesis of gamma globulin; it may be of sufficient degree to

alter also the rate of production of other serum proteins by exhausting the available amino acid pool as has been suggested before (1, 10, 18). The mean calculated red cell mass decreased by about 15 per cent from 18 to 15 ml/kg during the 28 days of immunization. While this change may have been due to a shift in the intravascular distribution of red cells and plasma as the blood volume expanded, decreased red cell production could have also been present. Another possibility is that immune paralysis has set in causing a failure of antibody synthesis. However, the transient nature of this phenomenon speaks against this. Finally, it seems possible that other mechanisms, as for example, the need to maintain a constant colloid osmotic pressure may play an important, if non-specific role in regulating the synthesis of various serum proteins. That this may be of some importance is suggested by the finding that the marked increase in circulating gamma globulin levels was observed to be associated with only a relatively small increase in the plasma volume and no significant change in intra- and extravascular protein partition. The prevention of a significant increase in plasma volume was due to the compensatory decrease in serum albumin levels, a result of lowered albumin synthesis (18). This reciprocal relationship between serum albumin and globulin levels has been shown in a variety of clinical states and has been felt to be the result of an osmotic regulatory system effecting control by changing albumin synthesis (18, 33). The experimental design of this sort of study does not permit a definite conclusion as to the relative temporal onset of changes in synthesis or degradation. However, the nature of the stimulus and the findings during the transient period of exhaustion make it probable that changes in synthesis occurred first and that alterations in catabolism were called forth only as the gamma globulin levels rose. In view of this finding and the repeated observation in a variety of disease states that increased gamma globulin levels are generally accompanied by an increase in degradation (2, 3, 8–10), it seems possible that gamma globulin synthesis and degradation may be mutually self-regulatory, an opinion also voiced recently by Fahey and Robinson (13).

The degree of protein synthesis induced by these physiological stimuli is comparable to that found in certain induced neoplasms in animals (12) and some of the paraproteinemias in man. However, in the latter, prolonged synthesis of protein continues in the absence of the usual regulatory systems which appear to come into play under normal circumstances to limit the perpetuation of these processes. The exact nature of these controls and factors inducing malignant plasma cell proliferation in these conditions offer intriguing avenues for study or speculation.

SUMMARY

1. Gamma globulin metabolism and distribution were studied employing rabbit gamma globulin (RGG)I^{131} 24 times in 13 control rabbits. Similar studies

were performed before and during the anamnestic response in 4 rabbits previously sensitized with a polyvalent pneumococcal vaccine.

2. During the anamnestic response, gamma-globulin levels increased from 1.0 to 6.0 gm/100 ml, and the gamma-globulin pool increased from 0.7 to 4.7 gm/kg. There was no change in the intravascular-extravascular partition of gamma globulin.

3. Gamma globulin degradation increased from 0.06 to 0.33 gm/kg/day during the 28 days of the immunization period while gamma globulin synthesis increased even further to average 0.47 gm/kg/day. Following the attainment of elevated gamma globulin levels the fractional rate of RGG-I^{131} turnover increased from 8.0 to 12.5 per cent/day.

4. No differences were noted in the metabolism of homologous or autologous gamma globulin regardless of the allotypic specificities.

The authors wish to express appreciation to Miss Margaret Tobin and Mrs. Marion Jones for secretarial assistance; to Miss Carole Evans, Mr. Douglas Faison, Mr. Albert Fiorello, Miss Frances Prelli, and Mr. Carl Walker for technical assistance; and to Miss Melanie Bouton and Miss Josephine Neglia for the illustrations.

BIBLIOGRAPHY

1. Berson, S. A., and Yalow, R. S., Serum protein turnover in multiple myeloma, *J. Lab. and Clin. Med.*, 1957, **49**, 386.
2. Lippincott, S. W., Korman, S., Fong, C., Stickley, E., Wolins, W., and Hughes, W. L., Turnover of labeled normal γ-globulin in multiple myeloma, *J. Clin. Inv.*, 1960, **39**, 565.
3. Korman, S., Corcoran, C., Fine, S., and Lippincott, S. W., Comparison of labeled beta and gamma globulin metabolism in multiple myeloma, *J. Lab. and Clin. Med.*, 1962, **59**, 371.
4. Gabusda, G. T., The turnover and distribution of I^{131}-labeled myeloma and macroglobulin proteins, *J. Lab. and Clin. Med.*, 1962, **59**, 65.
5. Grey, J. S., and Guzman-Barron, E. S., The electrophoretic analysis of the serum proteins in diseases of the liver, *J. Clin. Inv.*, 1942, **22**, 191.
6. Ogryzlo, M. A., Machlachlan, M., Dauphinee, J. A., and Fletcher, A. A., The serum proteins in health and disease. Filter paper electrophoresis, *Am. J. Med.*, 1959, **27**, 596.
7. Bonomo, L., Hyperglobulinemia in rheumatoid arthritis, its relationship with disease activity and its changes under adrenocortical treatment, *Ann. Rheumatic Dis.*, 1957, **16**, 340.
8. Mills, J. A., Calkins, E., and Cohen, A. S., The plasma disappearance time and catabolic half-life of I^{131}-labeled normal human gamma globulin in amyloidosis and in rheumatoid arthritis, *J. Clin. Inv.*, 1961, **40**, 1926.
9. Havens, W. P., Jr., Dichensheets, J., Bierly, J. N., and Eberhard, T. P., The half-life of I^{131}-labeled normal human gamma globulin in patients with hepatic cirrhosis, *J. Immunol.*, 1954, **73**, 250.

10. Cohen, S., and Hansen, J. D. L., Metabolism of albumin and gamma globulin in Kwashiorkor, *Clin. Sc.*, 1962, **23,** 351.
11. Birke, G., Lilyedahl, S. O., Olhagen, B., Plantin, L. O., and Ahlinder, S., Catabolism and distribution of gamma globulin. A preliminary study with I^{131}-labeled gamma globulin, *Acta Med. Scand.*, 1963, **173,** 589.
12. Humphrey, J. H., and Fahey, L. J., The metabolism of normal plasma proteins in rabbits and gamma myeloma protein in mice bearing plasma cell tumors, *J. Clin. Inv.*, 1961, **40,** 1696.
13. Fahey, J. L., and Robinson, A. G., Factors controlling serum gamma globulin concentration, *J. Exp. Med.*, 1963, **118,** 843.
14. Humphrey, J. H., and McFarlane, A. S., Rate of elimination of homologous globulins (including antibody) from the circulation, *Biochem. J.*, 1957, **54,** 186.
15. Bjørneboe, M., Serum proteins during immunization, *Acta Path. et Microbiol. Scand.*, 1943, **20,** 221.
16. Bjørneboe, M., and Schwartz, M., Investigations concerning the changes in serum proteins during immunization. The cause of hypoalbuminemia with high gamma globulin values, *J. Exp. Med.*, 1959, **110,** 259.
17. Bjørneboe, M., and Jarnum, S., The changes in serum proteins and blood volume during immunization, *J. Exp. Med.*, 1961, **113,** 1005.
18. Rothschild, M. A., Oratz, M., Franklin, E. C., and Schreiber, S. S., The effect of hypergammaglobulinemia on albumin metabolism in hyperimmunized rabbits studied with albumin I^{131}, *J. Clin. Inv.*, 1962, **41,** 1564.
19. Berson, S. A., Yalow, R. S., Schreiber, S. S., and Post, J., Tracer experiments with I^{131} labeled human serum albumin: Distribution and degradation studies, *J. Clin. Inv.*, 1953, **32,** 746.
20. Berson, S. A., and Yalow, R. S., Quantitative aspects of iodine metabolism. The exchangeable organic iodine pool, and the rates of thyroidal secretion, peripheral degradation and fecal excretion of endogenously synthetized organically bound iodine, *J. Clin. Inv.*, 1954, **33,** 1533.
21. Berson, S. A., and Yalow, R. S., Distribution and metabolism of I^{131}-labeled proteins in man, *Fed. Proc.*, 1957, **16,** suppl. 1, 13S.
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, J., Protein measurements with the Folin phenol reagent, *J. Biol. Chem.*, 1951, **193,** 265.
23. Rothschild, M. A., Oratz, M., Wimer, E., and Schreiber, S. S., Studies on albumin synthesis: The effect of dextran and cortisone on albumin metabolism in rabbits studied with albumin I^{131}, *J. Clin. Inv.*, 1961, **40,** 546.
24. Jencke, W. P., Jetton, M. R., and Durrum, E. L., Paper electrophoresis as a quantitative method, *Biochem. J.*, 1955, **60,** 205.
25. Franglen, G. T., and Martin, N. H., The interaction of dyes with proteins on paper with special reference to paper electrophoresis, *Biochem. J.*, 1954, **57,** 626.
26. Oudin, J., Allotypy of rabbit serum proteins, *J. Exp. Med.*, 1960, **112,** 107; 125.
27. Dray, S., Young, G., and Gerald, L., Immuno chemical identification and genetics of rabbit gamma globulin allotype, *J. Immunol.*, 1963, **91,** 403.
28. Bjørneboe, M., and Gormsen, H., Experimental studies on the role of plasma cells as antibody producers, *Acta Path. et Microbiol. Scand.*, 1943, **20,** 649.

29. Talmage, D. W., Dixon, F. J., Buckantz, S. C., and Dammin, G. J., Antigen elimination from the blood as an early manifestation of the immune response, *J. Immunol.*, 1951, **67,** 243.
30. Milgrom, F., and Witebsky, E., Studies on rheumatoid and related serum factors, *J. Am. Med. Assn.*, 1960, **56,** 138.
31. McCluskey, R. T., Miller, F., and Benacerraf, B., Sensitization to denatured autologous gamma globulin, *J. Exp. Med.*, 1962, **115,** 253.
32. Catsoulis, E. A., Franklin, E. C., Oratz, M., and Rothschild, M. A., γ-Globulin metabolism in rabbits immunized with autologous denatured γ-globulin, *Arthritis and Rheumatism*, 1963, **6**, 766.
33. Bjørneboe, M., Serum albumin and serum globulin after intravenous injection of large amounts of globulin and albumin. A hypothesis about the regulations of the colloid osmotic pressure of the blood, *Acta Path. et Microbiol. Scand.* 1945, **22,** 323.