GAMMA GLOBULIN TURNOVER IN RABBITS BEFORE AND DURING HYPERIMMUNIZATION*

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Gamma globulin in the body is subject to continuous synthesis and breakdown. Synthesis is taking place in the plasma cells of the reticuloendothelial system (1–4). It is possible that catabolism takes place partly by leakage into the gastrointestinal tract and subsequent digestion by the intestinal proteolytic enzymes (5) and partly in the liver (6). Several things are known about the regulation of the synthesis: for example intensive immunization will cause a rise in serum gamma globulin concentration by way of increased production (7), whereas adrenocortical hormones (8, 9), various toxic agents, and pathological processes in the reticuloendothelial system may inhibit the production and lower the serum concentration of gamma globulin. But almost nothing is known about the regulation of the breakdown of gamma globulin.

On the basis of exponential decline of gamma globulin infused into the blood stream of children suffering from agammaglobulinemia, Gitlin (10) concluded that the breakdown follows a first order rate process. In an attempt to elucidate the problem of the regulation of gamma globulin turnover, we have studied the turnover in rabbits with two widely different serum gamma globulin concentrations. The turnover was determined by means of ^{131}I-labeled gamma globulin, first on normal level of serum gamma globulin and then, in the same rabbits, 4 to 8 weeks later, during hyperimmunization by which the serum concentration was raised from 4 to 10 times above normal values.

Methods

Material.—The study was carried out on seven rabbits of the Danish country race, bred in the State Serum Institute, weight 3 to 4 kg. The rabbits, which were 30 months old, had during the previous 24 months been hyperimmunized in five periods by intravenous injection of the same polyvalent pneumococcic vaccine as was used in the present study. They were apparently healthy, and the weight remained constant throughout the study.

Gamma globulin was isolated from serum of normal rabbits of the same race by means of column chromatography on diethylaminoethyl (DEAE) cellulose[1] (11). Serum was dialyzed

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[1] Eastman Kodak Co., Rochester, New York.

overnight against the elution buffer (phosphate 0.01 M, pH 8.2). The gamma globulin peak was located by measurement of the ultraviolet absorption of the eluate at 210 mμ diluted 1:120 in saline (12). The pooled fractions of the peak (about 50 ml) were sterile filtered through a glass filter (Jena 17 G5, pore diameter 1 to 1.5 μ), and the solution was concentrated to 2 to 3 ml by vacuum dialysis. The yield from about 15 ml serum was about 50 mg in each preparation. Each solution of isolated gamma globulin was examined by paper electrophoresis; each yielded one band only, namely gamma globulin.

Immunoelectrophoresis[2] was carried out in some of the preparations and showed precipitation corresponding to gamma globulin only (Fig. 1). Ultracentrifugal analysis[2] showed no content of rapidly sedimenting material, and the protein appeared as a single symmetrical peak with sedimentation constants about 6.5S. The labeled gamma globulin showed the same pattern on column chromatography as the genuine protein; this is considered to be a sensitive physicochemical test for denaturation (13).

Iodination.—The protein was labeled with carrier-free ^{131}I without reducing agent (Radiochemical Center, Amersham, England). Iodine monochloride was used as the inactive carrier solution according to the method of McFarlane (14). Non-protein bound ^{131}I was removed by a resin column (amberlite 400 IRA-cl). 66 to 78 per cent of the initial radioactivity passed through the column, and 0.1 per cent was present in the supernatant after precipitation with 10 per cent trichloroacetic acid. The mean ratio of iodine bound to protein (mol wt 160,000) was from 3.2 to 4.0 atoms/molecule. From 7.6 to 13.2 μc were bound per mg of protein. Inactive human serum albumin (The State Serum Institute, Copenhagen) was added to a concentration of about 20 mg/ml of the final solution, partly to prevent adsorption to glassware (15) and partly to prevent damage of the protein by self-irradiation (16). Three different ^{131}I-labeled gamma globulin preparations were used in the studies.

Paper electrophoresis was carried out by the method of Laurell *et al.* (17). Protein fractions are determined spectrophotometrically after elution of paper electrophoretic strips stained with bromophenol blue. Since the stainability of gamma globulin is lower than that of albumin by a factor 0.59 (18), the extinction of the gamma globulin band was corrected by a factor 1.69 (= 1/0.59) before calculation of the protein fractions.

Gamma globulin Turnover.—The rabbits received potassium iodide (25 to 50 mg/day) for 2 days before and subsequently throughout the period of blood sampling. 1 to 2 ml of labeled gamma globulin (20 to 50 μc) was injected intravenously in a marginal ear vein; the dose injected was determined by weighing the syringe before and after injection. Blood samples were collected into tubes containing dried heparin. The first plasma sample was taken after 10 minutes, and daily plasma samples (about 1 ml) were taken for the next 2 weeks at least. Radioactivity was measured in a well-type scintillation counter (tracerlab versamatic). No correction for the loss of radioactivity due to the daily blood sampling was made. It amounted to about 1 per cent of the plasma pool per day; this seems to be insignificant compared with a fractional turnover rate of about 35 per cent.

The turnover of gamma globulin was calculated by mathematical analysis of the plasma curve (19). This method is based on the assumption that degradation and synthesis occur intravascularly or in a compartment in rapid exchange with the plasma pool. Pool masses, exchange rates, synthesis, and rate of catabolism are assumed to be constant throughout the experiment. The following calculations were made:

$$\text{Plasma volume } (PV) = \frac{\text{(injected dose)} \times 0.98}{\text{plasma activity/ml after 15 min.}}$$

[2] Kindly carried out by B. Mansa, Biophysical Department, The State Serum Institute, Copenhagen.

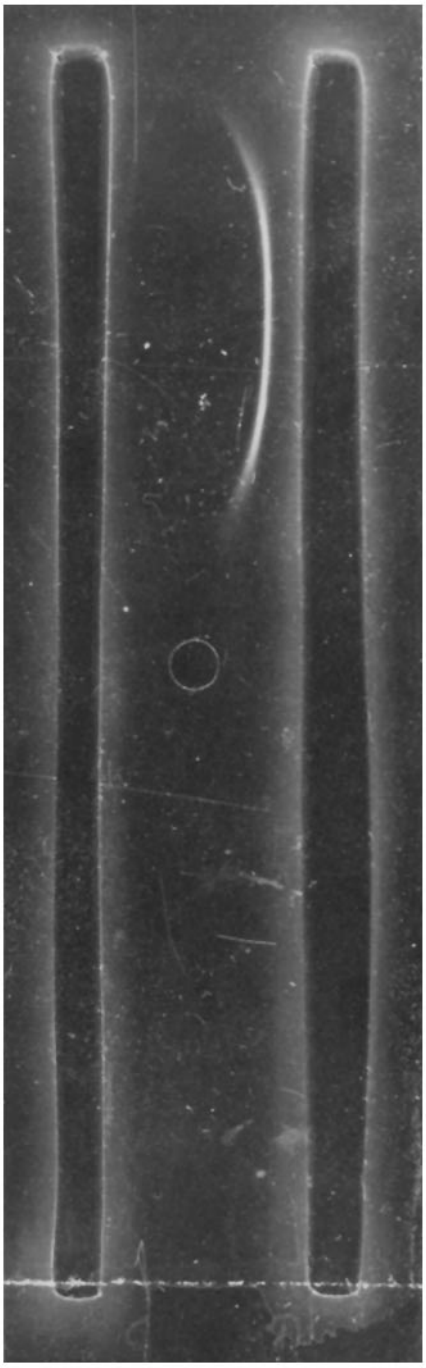

FIG. 1. Immunoelectrophoresis of rabbit gamma globulin isolated by chromatography on DEAE cellulose. Two different samples of goat anti-rabbit serum are used in the troughs.

assuming an elimination to extravascular compartments of 2 per cent in 10 minutes.

Plasma pool of gamma globulin (P) = $PV \times$ gamma globulin concentration (G).

Fractional turnover rate (F) = per cent of plasma pool catabolized per day.

Rate of catabolism (C) = mass of gamma globulin catabolized per day. ($C = F \times P$).

Distribution ratio (D) = ratio between extravascular pools (E) and plasma pool $\left(D = \frac{E}{P}\right)$.

Exchange rate = per cent of plasma pool transferred to extravascular compartments daily.

Experimental Procedure.—The gamma globulin turnover was determined in each rabbit by means of intravenous injection of labeled gamma globulin. Following that the rabbits were hyperimmunized by intravenous injections of a polyvalent pneumococcic vaccine three times weekly as previously described (20). After 7½ weeks when the serum gamma globulin was high a new gamma globulin turnover determination was carried out. At the same time the rabbits were continuously immunized by intravenous injection of the pneumococcic vaccine.

TABLE I

Gamma Globulin Turnover in Rabbits before Immunization

Rabbit No.	Weight	Plasma volume	Serum gamma globulin	Plasma pool	Fractional turnover rate	Rate of catabolism	Distribution ratio	Exchange rate	Half-life of plasma curve
	gm	*ml*	*gm/100 ml*	*gm/kg*	*per cent*	*mg/kg/day*		*per cent*	*days*
56-55	4210	101	1.15	0.28	32	88	0.73	45	4.2
56-56	3320	98	1.62	0.48	42	204	0.84	75	4.8
56-60	3370	97.	1.06	0.30	38	114	1.33	110	5.7
56-66	3480	121	1.15	0.40	36	144	1.04	96	4.9
56-70	4120	149	1.25	0.45	35	158	0.83	75	5.6
56-71	3710	108	0.97	0.27	43	115	1.20	93	6.1
56-74	3540	107	1.29	0.39	28	110	1.03	50	7.3
Mean.....	3680	112	1.21	0 37	36	133	1.00	78	5.5
SE.....	135	7.0	0.079	0.032	2.0	14.7	0.081	8.3	0.38

RESULTS

Gamma Globulin Turnover before Immunization.—The data of turnover are presented in Table I. Plasma volume varied from 24 to 36 ml/kg (mean 30 ml), serum gamma globulin from 0.97 to 1.62 gm/100 ml (mean 1.21), and plasma pool of gamma globulin from 0.27 to 0.48 gm/kg (mean 0.37).

Plasma radioactivity plotted on a semilogarithmic scale showed initially the usual rather rapid decrease associated with mixing of labeled molecules with extravascular protein. After 5 to 9 days this phase was followed by a linear rate of decrease. Plasma samples were taken for at least 8 days after the curves had become linear. Half-lives calculated from this portion of plasma curve varied from 4.2 to 7.3 days (mean 5.5 days). The curve could in all rabbits by analyzed into three exponential components. Fractional turnover rate varied from 28 to 43 per cent of the plasma pool per day (mean 36 per cent).

The rate of synthesis varied from 88 to 204 mg/kg/day (mean 133 mg). The distribution ratio of extra- to intravascular gamma globulin was 0.73 to 1.33 (mean 1.00 which means that the gamma globulin on an average was distributed evenly between plasma and extravascular compartments. The exchange rate of gamma globulin from plasma to extravascular pools varied from 45 to 110 per cent of the plasma pool per day (mean 78 per cent).

Gamma Globulin Turnover during Immunization.—Turnover data are shown in Table II. Plasma volume varied from 32 to 71 ml/kg (mean 40 ml). Serum gamma globulin at the beginning of the turnover study varied from 5.3 to 10.9 gm/100 ml (mean 7.8) a rise by a factor of 6.5. Plasma pool of gamma globulin varied from 1.7 to 6.9 gm/kg (mean 3.3), a rise by a factor of 8.9.

TABLE II

Gamma Globulin Turnover in Rabbits during Hyperimmunization

Rabbit No.	Weight	Plasma volume	Serum gamma globulin	Plasma pool	Fractional turnover rate	Rate of catabolism	Distribution ratio	Exchange rate	Half-life of plasma curve
	gm	*ml*	*gm/100 ml*	*gm/kg*	*per cent*	*mg/kg/day*		*per cent*	*days*
56-55	4220	133	5.29	1.67	35	590	0.86	107	3.3
56-57	3710	140	5.32	2.01	34	680	0.80	125	3.2
56-60	3580	117	7.14	2.34	39	920	0.32	63	3 4
56-66	3290	149	10.91	4.95	33	1630	0.29	46	3.7
56-70	3790	268	9.72	6.88	29	2020	0.28	20	3.8
56-71	3930	124	8.33	2.62	49	1280	0.47	53	3.2
56-74	3490	110	7.97	2.51	39	980	0.47	54	3.8
Mean.....	3720	149	7.81	3 28	37	1160	0 50	67	3.5
SE.....	115	20.5	0.795	0.721	2.4	196	0.091	13.8	0.09

The plasma curve became linear after 4 to 7 days. The half-lives varied from 3.2 to 3.8 days (mean 3.5 days). The curve could be analyzed into three exponentials in all rabbits except one (No. 56-70). Fractional turnover rate varied from 29 to 49 per cent of the plasma pool per day (mean 37 per cent). The rate of synthesis varied from 590 to 2020 mg/kg/day (mean 1160 mg), a rise by a factor of 8.7. The distribution ratio of extra- to intravascular gamma globulin varied from 0.23 to 0.86 (mean 0.49), indicating that about ⅔ of the total pool of gamma globulin was localized intravascularly. The exchange rate from plasma to extravascular pools varied from 20 to 125 per cent of the plasma pool per day (mean 67 per cent).

DISCUSSION

Turnover data of plasma proteins from various laboratories have been described by a number of different parameters. Frequently the turnover has

been described only by the plasma disappearance half-life, an unsatisfactory parameter because no information is given about the pool size or the distribution of the protein. Furthermore the plasma half-life will not correctly reflect the turnover rate in a system as complicated as the mammalian body; the resultant error will depend partly on the slope of the plasma curve and partly on the rate of exchange between extra- and intravascular protein pools (21, 22). Therefore a mathematical model, simulating the conditions in the body, has to be applied. The model used in the present study (19) is based on the assumption that synthesis and degradation of the protein occur either intravascularly or in a compartment in rapid equilibrium with plasma. These assumptions are probably justified in man (23, 24), but not much is known about the conditions in rabbits, except that synthesis of gamma globulin may occur near the plasma pool (25). However, the assumptions have been assumed to be fulfilled, and the model of Matthews (19) was used for the calculations.

Previous studies of gamma globulin turnover in rabbits are few. Dixon and coworkers (26) found by means of gamma globulin isolated by cold ethanol that the plasma disappearance half-life was 4.6 days. With gamma globulin isolated by ammonium sulphate the half-life was 5.7 days, a value identical with the one found in the present study. Cohen and coworkers (27) studied the turnover of ^{131}I-labeled globulin isolated by Na_2SO_4 and found that the plasma disappearance half-life was 4.9 days and that the fractional turnover rate was 30 per cent of the plasma pool per day, values almost identical with our results in rabbits before immunization. The ratio of extra- to intravascular globulin, however, was found to be 1.67 while in the present study it was 1.00.

Our turnover studies during immunization showed that the fractional turnover rate was unaltered from the value obtained before immunization in spite of the fact that serum concentration, plasma pool, rate of synthesis, and degradation of the protein had increased 5 to 10 times. The exchange rate of gamma globulin from plasma to extravascular compartments was the same, but the half-life of the plasma curve was lowered from 5.5 days before, to 3.5 days after immunization. The distribution of the protein was changed so that two-thirds of the total pool was localized intravascularly instead of half of the total pool. As in previous studies (20, 28, 29) a rise in plasma volume was found.

The results of the turnover studies before and after immunization are only compatible, if it is assumed that the exchange rate of protein from extravascular compartments to the plasma has increased almost two times. On the other hand the results of the turnover determinations during immunization are not completely reliable, because the rabbits were in a metabolically unsteady state during the study. The concentration of serum gamma globulin fell during the study by 0 to 40 per cent (mean 26 per cent) of the initial value (Table III). Therefore the amount of gamma globulin catabolized has been calculated

by means of the initial plasma concentration, since the labeled protein was originally mixed with this concentration. The unsteady state will invalidate the results to some degree, but compared to a fractional turnover rate of 37 per cent per day a decrease of the gamma globulin concentration of 26 per cent in 14 days (or about 2 per cent/day) appears to be negligible. Nonetheless it cannot be excluded, that exchange rates and distribution ratio might be influenced by the error involved in these studies, on account of unsteady state conditions. The true values of these parameters might then be different from those determined experimentally. The results concerning the fractional turnover rates must however be regarded as well founded. If we accept that gamma globulin is broken down in a compartment in rapid exchange with plasma then

TABLE III

Variations in the Concentration of Serum Gamma Globulin during the Study of Gamma Globulin Turnover in Rabbits during Hyperimmunization

Rabbit No.	Date of serum gamma globulin determination		
	Jan. 26, 1962	Feb. 2, 1962	Feb. 9, 1962
	gm/100 ml	*gm/100 ml*	*gm/100 ml*
56-55	5.29	4.26	4.00
56-57	5.32	3.83	3.39
56-60	7.14	5.13	4.47
56-66	10.91	7.82	7.82
56-70	9.72	7.01	5.77
56-71	8.33	6.94	7.01
56-74	7.97	7.41	8.06

the fractional turnover rate appears to be independent of serum gamma globulin concentration. This indicates that the rate of breakdown is governed by a first order rate process (30).

In theory this requires that the rate of breakdown should be controlled by the rate at which the protein enters the breakdown compartment (30). Now the question arises: Is it possible to combine these theoretical considerations with what is known of the physiological gamma globulin metabolism and of the pathways of gamma globulin metabolism during immunization? It is thought that normally a substantial fraction of gamma globulin breakdown takes place in the gut, the size of this fraction depending on the exchange rate of the protein (5). In that case it would follow a first order process. It has been suggested that the remaining part of gamma globulin is catabolized during phagocytosis of antigen-antibody complexes in the reticuloendothelial system (24 *a*), a route of degradation which is likely, at least in hyperimmunized animals. The rise in serum gamma globulin is due mainly to specific antibodies

(7, 31), the magnitude of the rise being reflected roughly by the amount and number of antigens used, so that a high concentration of gamma globulin corresponds to a high concentration of antigens. Therefore, if gamma globulin is catabolized during reactions with the specific antigen then the rate of breakdown would be independent of the concentration, because a high concentration of gamma globulin depends on a high concentration of antigens which at the same time will be available for the catabolism of gamma globulin. Therefore also under these conditions the rate of breakdown would follow a first order process.

SUMMARY

The turnover of ^{131}I-labeled gamma globulin has been determined in rabbits before and during (8 weeks later) hyperimmunization with pneumococcic vaccine, which increased the gamma globulin concentration 5 to 10 times. Before immunization fractional turnover rate was an average of 36 per cent of the plasma pool per day, and the rate of catabolism was 133 mg/kg/day. During hyperimmunization fractional turnover rate was an average of 37 per cent per day, and the rate of catabolism was 1160 mg/kg/day. The observation that the fractional turnover rate is independent of the concentration suggests that the rate of breakdown should have the characteristic of a first order process.

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