EXPERIMENTAL HYPERGLOBULINEMIA

The Effect of Injected Ribonucleotide on Serum Globulin Levels in Rabbits Undergoing Immunization*

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Although hyperglobulinemia is frequently seen in a number of diseases, notably multiple myeloma, lymphogranuloma venereum, kala azar, sarcoidosis, amyloidosis, disseminated lupus erythematosus, and hypersensitive states (1–4), its pathogenesis in these various circumstances remains largely unknown. Furthermore, apart from a few investigations on the hyperglobulinemia associated with immunization and with amyloidosis (5–9), the condition has not been studied experimentally. The phenomenon of hyperglobulinemia is necessarily related to the more fundamental problem of how proteins are formed by the living organism. In recent years numerous cytological and chemical studies have provided evidence that nucleotides are involved in the formation of proteins in diverse animal and plant cells (10–15), and it has been reported that an increase in cytoplasmic ribonucleotides develops in cells which may be actively engaged in the production of antibody globulins (16–18). It should be noted, however, that the part played by nucleotides in proteosynthesis has been inferred from correlations of cytological observations and chemical analyses, not from direct evidence; as pointed out by Brachet, the relation between nucleic acids and the synthesis of proteins is far from being solved (15).

The experiments now to be reported were undertaken to study directly the relationship of ribonucleotides to globulin formation, and hence to learn more about the pathogenesis of hyperglobulinemia. The findings show that repeated injection of a ribonucleotide into rabbits undergoing immunization with horse serum markedly enhances the production of serum globulins; they indicate also that most of the globulin appearing in the blood under such circumstances differs from that comprising the specific antibodies resulting from the immunization.

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Materials and Methods

The general aim of the experiments was to find out whether ribonucleotides influence the globulin production which is stimulated when animals are immunized. For this purpose groups of rabbits were given repeated injections of horse serum and of sodium ribonucleate at separate sites while control animals were given either horse serum and saline or sodium ribonucleate alone. The serum protein and antibody levels of the animals in the various groups were then determined from time to time. Kingsley's method (a modified Howe procedure) and electrophoresis were used to determine the serum protein concentrations, while a standard precipitin test was employed for the determination of the antibody levels.

Rabbits.—Animals of both sexes, most of them hybrid albinos weighing between 1400 gm. and 2500 gm., were used. Rabbits of comparable weight range were used in each experiment. They were kept on a diet of rabbit pellets (Rockland) and water.

Ribonucleate.—Ribose nucleic acid (prepared from yeast) and its sodium salt were procured from Schwarz Laboratories, Inc., New York. These preparations gave a negative biuret test for protein, and they had a nitrogen-phosphorus ratio, as determined by the manufacturer, close to the theoretical (1.69). Solutions of the ribose nucleic acid were prepared in 0.5 per cent NaOH at 90°C., 8 gm. per 100 cc. of solvent. The solutions were cooled and their acid pH adjusted to 7.3 by means of 0.5 per cent NaOH and a phosphate buffer; they were then passed through Seitz filters. The sodium ribonucleate was used as a 5 per cent solution in 0.9 per cent physiological saline, with pH adjusted to 7.3. This solution was also passed through a Seitz filter. All injections were given subcutaneously according to schedules to be described with each experiment.

Horse Serum.—This was obtained from the Public Health Research Institute of the City of New York and passed through a Seitz filter before use. Intravenous as well as subcutaneous injections were given. The procedures followed in each experiment will be described separately.

Bleeding of Animals and Preparation of Sera.—The rabbits were generally bled by cardiac puncture, though occasionally blood was obtained from the ear veins. The amounts removed were approximately the same for all animals at each bleeding and are recorded later. After the blood had clotted, and the clot retracted, the remaining liquid was centrifuged. The sera were then removed. Only sera showing no or negligible hemolysis were used.

Determination of Serum Proteins: (*a*) *Micro-Kjeldahl and Salt Fractionation Method.*—Determinations of serum protein levels were carried out by means of a modified Howe procedure as described by Kolmer and Boerner (19) which makes use of Kingsley's method for the separation of albumin and globulin (20). The procedure was further modified in that the non-protein nitrogen was determined by the micro method of Folin as described by Gradwohl (21).

It was necessary to do repeated determinations in duplicate on a considerable number of sera without undue delay, and the size of the test animals placed a limit on the amount of serum that could be safely obtained by repeated bleeding. Electrophoretic determinations, as described in the next section, were used to check the essential estimations. For these reasons, and because of the comparative nature of the experiments, the indicated salt fractionation procedure was preferred to the more refined and elaborate methods of fractional precipitation recently developed by Cohn and his coworkers.

(*b*) *Electrophoresis.*—Electrophoretic analysis of a considerable number of sera was car-

ried out in an Aminco-Stern apparatus manufactured by American Instrument Company, Silver Spring, Maryland.[1] For these analyses a barbiturate buffer, pH 8.52–8.60 was used. The ionic strength of the solutions was 0.1, temperature generally 1°C. Voltage gradients varied from 5.0 volt/cm. to 9.04 volt/cm. The final protein concentrations were generally between 2 per cent and 3 per cent.

Serological Tests.—Antibody levels of the sera of rabbits immunized with horse serum were determined by macroscopic precipitin tests. To 0.5 cc. of conventional serial twofold dilutions of the rabbit sera was added 0.5 cc. of a 1:300 dilution of horse serum in 0.9 per cent saline. The mixtures were incubated in a water bath at 37°C. for 1 hour, then kept at room temperature for 18 hours. Final readings were then taken. The amounts of precipitate were recorded as 1 plus to 4 plus, "1 plus" referring to the smallest visible quantity of precipitate and "4 plus" to the estimated largest quantity seen in several potent antisera. Appropriate controls of saline plus antigen and saline plus antiserum were included in each test. An undiminished potency of the horse serum used as antigen in each test was assured by titration against a standard rabbit antiserum.

In addition, tests were done on serially diluted sera of animals which had received treatment with ribonucleate, using as antigen a 4 per cent solution of ribose nucleic acid in 0.3 per cent NaOH, buffered at a pH of 7.3.

Postmortem Examinations.—Animals that died were autopsied as soon after death as possible. The surviving animals were killed and autopsied at the end of each experiment.

The Development of Hyperglobulinemia in Rabbits Receiving Ribonucleate and Horse Serum.—The effects of injected ribonucleate on serum globulin levels were tested in the following four groups of rabbits:

Group 1.—Ten animals that received no injections.

Group 2.—Ten animals that were given 5 cc. of an 8 per cent solution of ribonucleic acid in 0.5 per cent NaOH subcutaneously every day except Sundays, the injections being given in the same flank.

Group 3.—Ten animals that received two subcutaneous and one intravenous injection of horse serum on each of 3 successive days of every week. The doses varied from 5 cc. to 1.5 cc. These animals were also given 10 cc. of 0.9 per cent saline subcutaneously every day except Sundays as a control for the ribonucleate injections given to group 4. The subcutaneous injections of horse serum and saline were always given separately, using right and left flanks respectively.

Group 4.—Twelve animals that were treated with ribonucleate like the animals of group 2, and with horse serum like those of group 3. The subcutaneous injections of ribonucleate and horse serum were given separately and into opposite flanks.

These rabbits were treated for 2 months during which their serum proteins were studied repeatedly. Before the first injections were given, 30 cc. to 35 cc. of blood was removed from their hearts for chemical, electrophoretic, and serological studies. Blood was again taken from each animal on four subsequent occasions: 20 cc. after 2, 30 cc. after 4, 40 cc. after 7, and 45 cc. after 9 weeks of treatment.

The average globulin and total protein concentrations and their ranges, as determined by means of the modified Howe procedure, are shown in Table I, and the changes in the average globulin concentrations are shown graphically

[1] These determinations were done in the laboratory of Dr. K. G. Stern of the Polytechnic Institute of Brooklyn whose help and advice are gratefully acknowledged.

in Fig. 1. The data obtained by means of electrophoresis and the results of precipitin tests will be considered in later sections.

The most striking fact revealed by this experiment is that the rabbits given ribonucleate and horse serum (group 4), in contrast to the others, manifested a very sharp rise in globulin levels within the first 2 weeks, followed by a fur-

TABLE I

Summary of Changes in Serum Globulin and Total Protein Concentrations in Controls and in Rabbits Injected with Ribonucleate and Horse Serum—First Experiment

Group and treatment	Day	No. of rabbits	Globulin concentration,* *gm./100 cc.*		Total protein concentration,* *gm./100 cc.*	
			Average	Range	Average	Range
1. Controls—no injections	1	10	1.74	1.0–2.5	5.46	5.1–5.7
	16	10	1.66	1.1–2.3	5.91	5.5–6.3
	31	9	1.56	1.2–2.1	5.86	5.3–6.2
	51	8	1.30	0.9–2.0	5.89	5.4–6.8
	66	6	1.40	1.0–2.0	6.25	5.7–6.8
2. Controls—ribonucleate	1	10	1.51	1.1–3.2	5.37	5.0–5.9
	16	10	1.46	1.0–3.0	5.30	4.9–5.5
	31	10	2.00	1.1–3.2	5.33	4.2–6.0
	51	10	1.72	1.0–3.2	5.65	5.0–6.4
	66	8	1.91	1.5–3.3	5.77	5.3–6.5
3. Controls—horse serum and saline	1	10	1.84	1.2–2.5	5.43	5.1–6.0
	16	10	1.92	1.1–2.8	5.37	4.4–6.4
	31	10	2.69	1.8–3.9	5.32	4.1–6.4
	51	8	2.06	0.9–3.2	5.29	4.0–6.5
	66	7	2.54	2.0–2.9	5.91	5.1–6.3
4. Horse serum and ribonucleate	1	12	1.48	0.9–2.5	5.83	4.9–7.4
	16	12	3.77	3.0–4.8	5.88	4.8–7.6
	31	11	4.65	4.0–5.6	6.24	5.3–8.4
	51	9	3.87	1.9–5.6	6.03	5.0–8.6
	66	5	4.40	3.9–5.2	6.80	6.2–7.7

* As determined by the salt fractionation and micro-Kjeldahl procedures explained in the text.

ther rise in the next 2 weeks, the values reaching peaks which averaged almost 2 gm./100 cc. above those of the control animals treated with saline and horse serum (group 3). The rise in globulin concentrations of the individual rabbits of group 3 ranged approximately 20 per cent to 100 per cent above the original concentrations. The animals treated with ribonucleate alone (group 2) also manifested a slight rise in the globulin levels during the course of the experiment, as Table I and Fig. 1 show. There were no appreciable alterations

in the untreated control rabbits (group 1). It is apparent that the increase in the average globulin concentrations of group 4 (Fig. 1) which resulted from injections of ribonucleate and horse serum is more than a summation of the effects produced by injections of either of these agents alone.

The data show further that the globulin concentrations of all groups diminished somewhat between the 4th and 7th weeks of the experiment, and rose again thereafter, particularly in the animals that received horse serum (groups 3 and 4). It seems possible that this temporary decline may have resulted from the repeated bleedings. In this connection it should be noted that all animals, though not yet fully grown, either lost weight or failed to gain weight during the experiment. They also manifested a decrease in the serum albumin

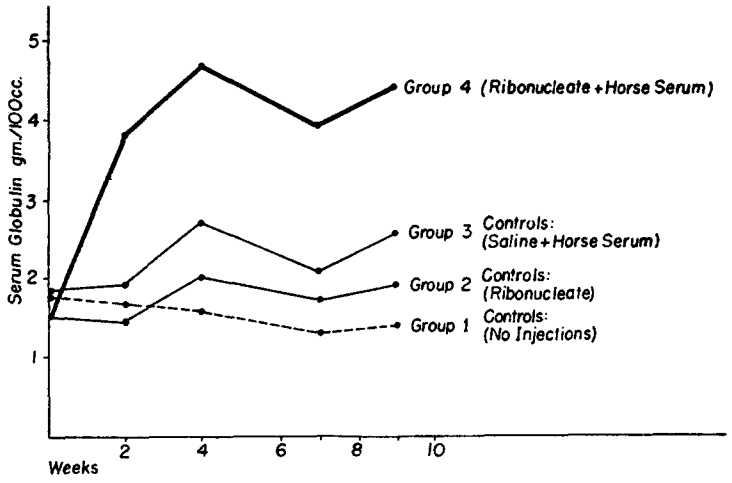

FIG. 1. Average serum globulin levels of the four groups of rabbits in Table I.

concentrations as will be discussed further on. These changes were especially noteworthy in the animals of groups 3 and 4, perhaps owing to the injections of horse serum, which may have produced serum sickness with fever, and hence may have resulted in an increased protein metabolism.

The findings were confirmed in a second experiment in which the procedure followed was somewhat different.

As in the preceding experiment four groups of rabbits were used. There were five animals in each group. The ribonucleate solution was prepared by dissolving sodium ribonucleate in physiological saline to make a 5 per cent solution. Horse serum was always injected intravenously, and only once a week. On the 1st day, before any injections were given, all animals were subjected to plasmapheresis in order to deplete them of protein while minimizing the effects of anemia. 40 cc. of blood was drawn from each animal. 10 cc. was allowed to clot, and serum was obtained for protein determinations. The remaining 30 cc. was citrated, and following removal of the plasma the cells were suspended in saline and the suspensions

injected intravenously into the appropriate rabbits. After 1 month, and again after 2 months, 30 cc. of blood was taken for serum protein determinations and serological tests. Plasmapheresis was not carried out on these occasions. The animals were weighed before each bleeding.

TABLE II

Globulin and Total Protein Concentrations in Sera of Controls and Rabbits Injected with Ribonucleate and Horse Serum—Second Experiment

Group and treatment	Rabbit No.	Weights		Serum protein concentration, *gm./100 cc.*					
				Day 1		32		62	
		Initial	Final	G*	TP*	G	TP	G	TP
		kg.	*kg.*						
1. Controls—no injections	11	2.2	2.3	2.0	6.6	1.8	6.5	1.9	6.7
	12	1.9	2.2	1.8	6.3	2.0	6.2	2.0	6.5
	13	2.3	2.4	2.3	5.8	2.4	6.1	2.2	6.3
	14	1.7	1.9	2.4	6.9	2.3	6.7	2.4	6.4
	15	1.8	2.0	2.2	6.1	2.0	5.9	2.1	5.9
2. Controls—ribonucleate	16	2.3	2.6	2.3	6.5	2.5	6.9	2.6	6.8
	17	1.7	2.0	2.5	5.8	2.3	5.5	2.3	5.9
	18	1.5	1.6	2.0	5.7	†	†		
	19	2.0	2.1	2.4	6.0	2.5	5.9	2.3	6.4
	20	1.7	1.8	1.9	5.9	2.3	6.4	†	†
3. Controls—horse serum and saline	21	2.5	1.9	2.1	6.2	2.6	6.6	2.6	6.4‡
	22	2.2	2.7	2.5	6.6	2.9	7.2	2.7	7.6
	23	2.4	2.3	2.5	6.3	3.0	7.2	2.3	6.3
	24	2.1	2.5	2.3	6.9	2.7	7.3	2.4	6.8
	25	2.0	2.4	2.2	6.2	2.7	6.9	2.7	7.3
4. Horse serum and ribonucleate	26	2.1	1.8	2.3	5.9	5.2	9.1	4.2	8.3
	27	1.8	2.0	2.8	6.6	4.2	8.5	3.5	8.3
	28	1.7	1.5	1.8	6.0	4.2	8.7	4.5	8.9‡
	29	1.7	1.5	2.3	6.1	4.6	7.4	5.9	9.9‡
	30	1.9	1.7	2.5	6.2	4.4	9.2	3.5	7.3

* G, globulin; TP, total protein; determined by means of salt fractionation and micro-Kjeldahl procedures as explained in the text.

† Died.

‡ These determinations were made on the 50th day.

The changes in serum protein levels are shown in Table II and Fig. 2. The animals treated with ribonucleate and horse serum (group 4) again manifested elevations of serum globulin levels which were strikingly higher than were those of animals given saline and horse serum or ribonucleate alone (groups 2 and 3), the findings being generally similar to those of the previous experiment.

Antibody Levels in Relation to the Hyperglobulinemia.—To learn whether the hyperglobulinemia of the rabbits treated with ribonucleate and horse serum was due to an increased production of antibodies against horse serum, precipitin tests were done on the serum specimens of the two experiments described in the previous section.

The results of these tests, shown in Tables III and IV, failed to disclose significant differences between the antibody levels of animals treated with saline and horse serum (group 3) and those of animals treated with ribonucleate and horse serum (group 4). Comparison of the data in Tables I and II with those in Tables III and IV shows that there were, in fact, animals in group 3 of both experiments which developed higher antibody levels (more than one dilution) than did some of the animals in group 4 although the serum

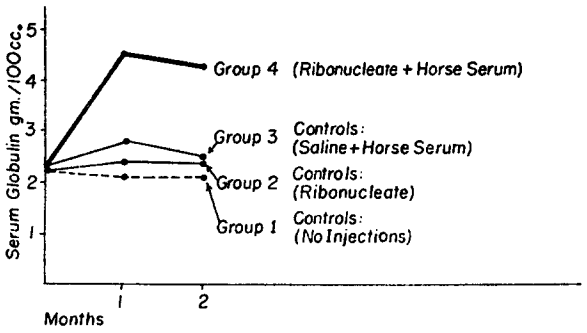

FIG. 2. Average serum globulin levels of the four groups of rabbits in Table II.

globulin levels of the latter were much higher. For example, it may be seen in Tables II and IV that rabbit 25, treated with saline and horse serum, had a globulin level of 2.7 gm./100 cc. and an antibody titer of 1:256 after 32 days, while at the same time rabbit 28, treated with ribonucleate and horse serum, had a globulin level of 4.2 gm./100 cc. and a titer of 1:64.

These findings indicate plainly that most of the new globulin which appeared in the blood of the rabbits treated with ribonucleate and horse serum differed from that comprising the specific antibodies to horse serum.

In an attempt to find out whether this new globulin might have resulted from the production of antibodies specific for the ribonucleate, precipitin tests were done on appropriate sera using solutions of ribonucleate as antigen. However, repeated tests with varying dilutions of antigen and of the rabbit sera were negative.

Failure of Abscesses to Promote Hyperglobulinemia; Absence of Hepatic Lesions.—Many years ago Letterer (7, 8) found that subcutaneous injections of

alkaline solutions of ribonucleic acid into mice often gave rise to transitory elevations in serum globulin levels similar to those observed in rabbits treated with ribonucleate alone in the present experiments (Fig. 1). He ascribed this phenomenon to destruction of subcutaneous tissues by the injected material since the animals frequently developed abscesses. Hence it may be argued that the observed effects of ribonucleate in rabbits undergoing immunization

TABLE III

Titers of Antibodies against Horse Serum in Rabbits of Tables I and VI

Group	Rabbit No.	Serum antibody titers*		
		Day.....31	51	66
3	44	1:64	1:128	1:512
	47	1:64	1:256	1:512
	48	1:64	—	—
	49	1:64	—	—
	50	1:64	1:256	1:512
	51	1:64	1:128	1:512
	52	1:64	1:128	1:256
	53	1:128	1:128	—
	73	1:256	1:256	1:512
	75	1:128	1:256	1:512
4	32	1:64	1:128	—
	33	1:64	1:128	1:512
	34	1:64	1:256	—
	40	1:128	—	—
	41	1:128	1:128	1:512
	42	1:64	1:128	—
	43	1:64	1:128	—
	45	1:256	1:64	1:512
	78	1:64	1:256	1:512
	79	1:64	1:64	1:512

* The titers indicate the highest dilution of rabbit serum in which there was definite, visible precipitation. They represent a reading of "one plus" or higher as given in Table IV

(Figs. 1 and 2) may also have resulted from irritation or damage of tissues at the sites of injection. To learn about this, a highly irritating agent, capable of producing extensive necrosis, *viz.* oil of turpentine (U.S.P.), was used in another experiment.

Two groups of four rabbits were given a series of injections of horse serum comparable to those given in the previous experiments. One of these groups was also given ten subcutaneous injections of 0.5 cc. of oil of turpentine during a period of 3 weeks. A third group of four rabbits received treatment with turpentine only. The injections of turpentine resulted in the formation of subcutaneous abscesses, several of which ruptured and drained to the outside.

TABLE IV

Results of Precipitin Tests with Rabbit Sera of Table II Using Horse Serum as Antigen

Group	Rabbit No.	Serum titers*																		
		Day.....32									Day.....62									
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	C‡	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	C‡
1.	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2.	16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3.	21	++++	++++	+++	++	++	+	+	0	0										
	22	++++	++++	+++	++++	+++	++	+	0	0	++++	++++	+++	+++	++	+	+	+	0	0
	23	++++	++++	+++	+++	++	++	+	0	0	++++	++++	++++	++++	+++	++	+	+	0	0
	24	++++	++++	++++	++++	+++	++	+	+	0	++++	++++	++++	+++	+++	++	+	+	0	0
	25	++++	++++	++++	+++	+++	++	++	+	0	++++	++++	+++	+++	+++	++	++	+	+	0
4.	26	++++	++++	++++	+++	++	++	+	+	0	++++	++++	++++	+++	++	+	+	0	0	0
	27	++++	++++	+++	+++	++	++	+	0	0	++++	++++	+++	++	+	+	+	+	0	0
	28	++++	++++	++++	+++	++	+	0	0	0										
	29	++++	++++	++++	++++	+++	++	+	+	0										
	30	++++	++++	++++	+++	+++	++	0	0	0	++++	++++	+++	+++	++	+	+	0	0	0

* See text for explanation of procedure.

‡ C, control (a 1:2 dilution of rabbit serum to which no antigen was added, but 0.5 cc. of 0.9 per cent saline).

The serum globulin and total protein concentrations of all animals were determined before treatment, after 2 weeks, and after 4 weeks. The results, given in Table V, showed that there were no significant differences attributable to the turpentine treatment.

It is clear, therefore, that under the stated conditions irritation or damage of tissues at the site of injection probably did not cause the hyperglobulinemia produced with the ribonucleate treatment.

The question also arises whether the hyperglobulinemia that developed in animals of group 4 may have been due in part to liver damage. Gross and

TABLE V

Failure of Turpentine Injections to Induce Hyperglobulinemia during Immunization

Rabbit No.	Treatment	Serum globulin, *gm./100 cc.*			Total serum protein, *gm./100 cc.*		
		Date...3/30	4/14	5/1	Date...3/30	4/14	5/1
1	Oil of turpentine*	1.7	1.8	2.3	6.6	6.2	6.0
2	" " "	2.6	2.4	2.9	6.3	6.5	6.7
3	" " "	1.7	2.0	3.2	5.8	6.2	6.3
4	" " "	1.9	1.8	2.1	6.1	6.0	6.0
5	Horse Serum‡	1.6	1.7	1.8	6.2	6.3	6.1
6	" "	2.1	2.0	2.1	5.9	5.9	6.3
7	" "	2.2	2.4	2.3	6.5	6.4	6.2
8	" "	1.8	1.9	1.9	6.2	6.1	6.2
9	Horse serum‡	1.7	1.8	1.9	6.6	6.5	5.7
10	and	2.0	2.2	2.0	6.0	5.9	5.7
11	Oil of turpentine*	1.9	2.0	2.6	6.2	6.4	6.7
12	" " "	2.5	2.4	2.8	6.5	6.3	6.0

* Ten subcutaneous injections of 0.5 cc. of oil of turpentine.

‡ 2 cc. of horse serum were administered intravenously on 3 successive days, followed by a subcutaneous injection of 2 cc. once a week.

histologic examinations of the livers of these animals, however, failed to reveal any abnormalities.

Character of the Hyperglobulinemia as Determined by Electrophoresis.—Electrophoretic studies were made of the sera of a number of animals in each of the groups in Table I, both before treatment and again after 4 weeks. The changes are illustrated by the representative patterns and the values obtained from them which are shown in Figs. 3, 4, and 5. Table VI gives the averages and ranges of concentrations of each of the electrophoretic components in a number of sera from untreated and treated animals.

Detailed consideration of the findings showed that there were no significant changes in the sera from the untreated animals (group 1) with the exception of somewhat lower albu-

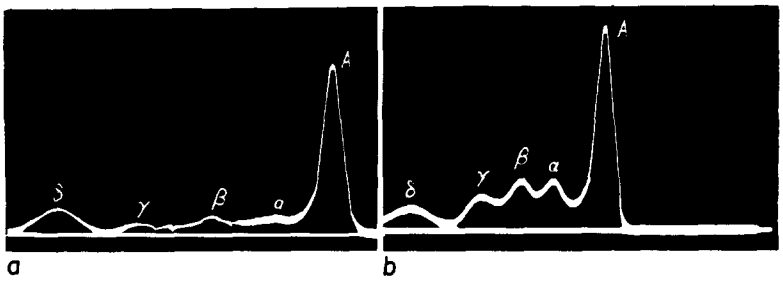

FIG. 3. Electrophoretic patterns of a rabbit's serum (*a*) before and (*b*) after 4 weeks of treatment with ribonucleate solution. The percentages of the serum components in Figs. 3 to 5 were obtained by planimetry on enlargements of the patterns. These percentages were applied to the Kjeldahl total protein values to obtain the absolute concentration of each component. The concentrations of the components in Fig. 3 are as follows:—

	Before		After	
	gm./100 cc.	*per cent*	*gm./100 cc.*	*per cent*
Albumin	4.40	74.5	3.28	54.6
α-Globulin	0.67	11.4	0.98	16.4
β-Globulin	0.55	9.3	1.02	16.9
γ-Globulin	0.28	4.8	0.72	12.1

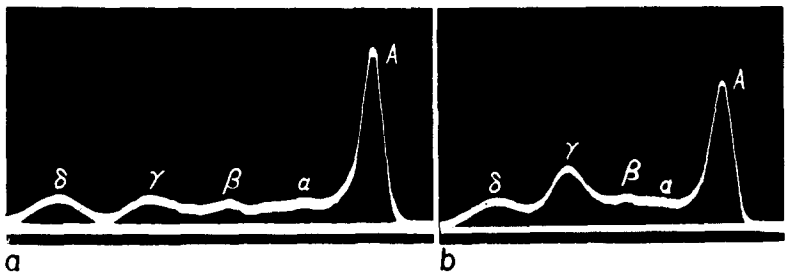

FIG. 4. Electrophoretic patterns of a rabbit's serum (*a*) before and (*b*) after 4 weeks of treatment with horse serum and saline. The concentrations of the components are as follows:—

	Before		After	
	gm./100 cc.	*per cent*	*gm./100 cc.*	*per cent*
Albumin	3.11	57.6	2.34	41.6
α-Globulin	0.96	17.8	0.93	16.7
β-Globulin	0.43	7.9	0.56	10.1
γ-Globulin	0.90	16.7	1.77	31.6

min components; this decrease may well have resulted from the repeated bleedings since it was also noted in comparable specimens from animals of other groups. The animals treated with ribonucleate alone (group 2) had slight elevations of alpha and beta globulin levels, and occasionally of the gamma globulins also; these will be referred to again further on. In

the animals injected with saline and horse serum (group 3) there were moderate elevations in the gamma globulin levels; these ranged from 1.52 to 1.77 gm./100 cc. with an average of 1.63, whereas the average level in five of the uninjected controls was 0.46 (Table VI). By contrast, the rabbits treated with ribonucleate and horse serum (group 4) developed much greater increases in the gamma globulins together with slight to moderate increases in alpha and beta globulins. The gamma globulin values of four animals in this group (group 4), for example, ranged from 2.56 to 3.20 and averaged 2.75 gm./100 cc. (Table VI); the increases in the alpha and beta components were generally comparable to those noted in the animals injected with ribonucleate alone, as will also be mentioned again further on.

It is noteworthy that the total serum globulin concentrations, as calculated from the electrophoretic patterns, paralleled the values obtained by means of the salt fractionation

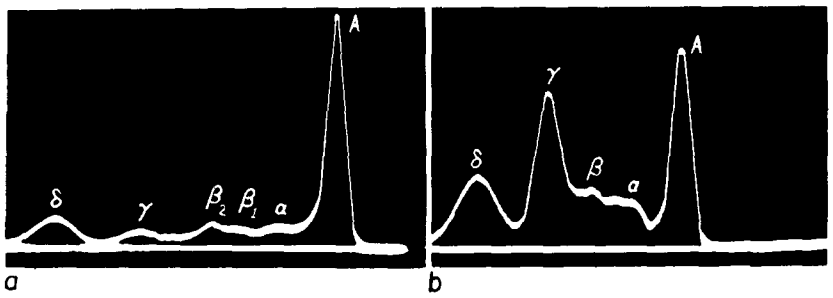

FIG. 5. Electrophoretic patterns of a rabbit's serum (*a*) before and (*b*) after 4 weeks of treatment with ribonucleate solution and horse serum. The concentrations of the components are as follows:—

	Before		After	
	gm./100 cc.	*per cent*	*gm./100 cc.*	*per cent*
Albumin	4.91	66.3	2.19	34.3
α-Globulin	0.92	12.4	0.91	14.2
β_1-Globulin	0.49	6.6	0.73	11.4
β_2-Globulin	0.58	7.9		
γ-Globulin	0.50	6.8	2.57	40.1

method. For example, the average total globulin level of four animals in group 4, as determined by electrophoresis, was 4.5 after 4 weeks of treatment, while that of four animals in group 3 was 2.9 gm./100 cc. (Table VI). At approximately the same time salt fractionation gave an average of 4.65 for eleven animals in group 4, and of 2.69 gm./100 cc. for ten animals in group 3 (see Fig. 1 and Table I).

As already mentioned, the alpha and beta globulin components were slightly or moderately elevated in the sera of some of the animals treated with ribonucleate alone (group 2), and there were, in some animals of this group, moderate elevations of gamma globulin also (Fig. 3, Table VI), though these latter were much less marked than were the corresponding values of animals given ribonucleate and horse serum (group 4; Fig. 5, Table VI). Some animals of the latter group, however, manifested elevations of the alpha and beta components which were quite comparable to those encountered in the animals of group 2. Previous work provides further indication that the elevations in alpha and beta components

resulted from the effects of the ribonucleate alone. For recently Bohle, Hartmann, and Pola (24), extending the older observations of Letterer (7, 8, 25), have observed slight or moderate elevations of the alpha and beta globulins in mice given repeated injections of ribonucleate. These elevations of alpha and beta globulins were quite similar to those noted in the present work, though they generally proved transitory even when the injections of ribonucleate were continued over long periods. As a rule they were not accompanied, but were frequently followed by increases in gamma globulins. The latter increases were usually associated with the development of amyloidosis, and they occurred most often in mice with abscesses that had resulted from the repeated subcutaneous injections.

Further observations, not recorded here in detail, showed that the electrophoretic patterns of sera procured from animals of the present experiments after 2 months of treatment were essentially similar to those observed after 4 weeks; it was evident that the differences

TABLE VI

Summary of Electrophoretic Findings on Sera of Table III

Treatment	No. of sera examined	Serum protein concentrations, *gm./100 cc.**							
		Albumin		α-Globulin		β-Globulin		γ-Globulin	
		Average	Range	Average	Range	Average	Range	Average	Range
None	5	4.10	3.11–4.91	0.76	0.62–0.96	0.62	0.41–1.07	0.46	0.28–0.90
Horse serum and saline—4 wks.	4	2.33	2.18–2.48	0.62	0.34–0.93	0.65	0.56–0.79	1.63	1.52–1.77
Horse serum and ribonucleate—4 wks.	4	2.73	1.97–3.46	0.80	0.53–1.03	0.94	0.64–1.39	2.75	2.56–3.20
Ribonucleate—4 wks.	4	3.30	2.92–3.58	0.88	0.79–0.98	0.98	0.95–1.01	0.89	0.72–1.04

* See text for explanation of methods used.

in electrophoretic patterns of sera from rabbits of the various groups remained relatively constant.

In sum, the electrophoretic findings confirm and extend those obtained by means of the salt fractionation procedure. They show that the hyperglobulinemia of rabbits treated with ribonucleate and horse serum was due to a marked and sustained increase in the gamma globulins, as well as a much smaller increase in the alpha and beta globulins. A similar increase in alpha and beta globulins also developed in the sera of control animals given ribonucleate alone, and some of these animals also had moderate increases in gamma globulins.

DISCUSSION

The essential observation here recorded—that injected ribonucleate enhanced the production of serum globulins in animals undergoing immuniza-

tion—is in accord with the hypothesis formulated by Brachet (13, 15) and by Caspersson (10–12, 14) that nucleotides of animal and plant cells play an important part in the synthesis of proteins. According to these investigators the cytoplasmic ribonucleotides are involved in the production of such proteins as normally pass out of the cells. This inference is supported by the studies of Bjoerneboe and Gormsen (22), Bing, Fagraeus, and Thorell (23), Fagraeus (16), Ehrich *et al.* (17), and Harris and Harris (18) on the relationship of plasma cells and other lymphoid cells to antibody production. Yet, as already stated, the previous work is based upon cytological-chemical correlations, and it has provided only indirect evidence for the hypothesis.

Did the injected ribonucleate or components of it enter directly into and stimulate the cells involved in globulin synthesis in the present experiments? Such a phenomenon would not be without parallel. For example, it is well known that the excessive administration of iodine to human beings may stimulate an overproduction of thyroid hormone. It may be recalled in this connection that, according to a number of reports, injections of nucleotides may stimulate leukopoietic tissues (26), and that some nucleotides promote the growth or activity of other tissues (27–30). Moreover, recent evidence based on the use of isotopes indicates that injected nucleotides as such can actually enter cellular metabolism (31). In the animals treated with horse serum the cells concerned with the production of antibody globulins were obviously active. The work of others indicates that such cellular activity probably involves an increased capacity to utilize ribonucleotides (17, 18, 23), and the injections of ribonucleate furnished a plentiful and usable source for this material in the present experiments. The overproduction of globulins in animals treated with horse serum and ribonucleate may therefore have been a result of an abnormally increased accumulation or mobilization of nucleotides in cells that were stimulated by horse serum to produce globulins.[2]

It remains to be learned whether the excess globulin in the foregoing experiments originated entirely in cells concerned with the production of antibodies or in others as well (for example, those of the liver), and whether nucleic acid compounds or derivatives other than the ribonucleate used will also promote hyperglobulinemia in animals. The findings here set forth provide reasons for studying further the possibility that the hyperglobulinemias of man may result from an abnormally increased utilization of ribonucleotides by those cells that produce globulins.

SUMMARY

It was shown by means of salt fractionation procedures and electrophoresis that a marked and sustained hyperglobulinemia regularly resulted when so-

[2] The apparent lack of antigenicity of the ribonucleate, and the fact that when injected alone it produced little or no rise in gamma globulin levels, make it unlikely that its effects in animals undergoing immunization can be accounted for by an anamnestic reaction.

dium ribonucleate was injected subcutaneously at frequent intervals into rabbits undergoing immunization with horse serum. The hyperglobulinemia was characterized by a large increase in the gamma globulin levels, and a slight increase in the alpha and beta globulin levels. In control experiments done concurrently, the immunization of rabbits with horse serum, accompanied by subcutaneous injections of saline instead of ribonucleate, resulted in only moderate elevations in gamma globulin levels, while injections of ribonucleate alone brought about slight elevations in all three globulin components in some of the animals.

Precipitin tests showed that the rabbits immunized with horse serum and simultaneously treated with ribonucleate developed antibody titers against horse serum that were no higher than those of the immunized controls given saline instead of ribonucleate. Indeed, some of the animals treated with horse serum and ribonucleate had globulin levels that were much higher and had antibody titers that were significantly lower than were those of several rabbits receiving horse serum and saline. Injections of ribonucleate alone did not result in the formation of specific antibodies detectable by means of precipitin tests. The results made it plain that the hyperglobulinemia of the animals treated with horse serum and ribonucleate was not due to an excessive production of specific antibodies.

The findings as a whole provide further evidence that nucleotides play an important role in the formation of proteins in animals, and they indicate that an abnormally increased utilization of ribonucleotides by cells capable of producing globulins may be a causative factor in the pathogenesis of hyperglobulinemia.

BIBLIOGRAPHY

1. Bing, J., *Acta med. scand.*, 1940, **103,** 547.
2. Luetscher, J. A., Jr., *Physiol. Rev.*, 1947, **27,** 621.
3. Gutman, A. B., *in* Advances in Protein Chemistry, (M. L. Anson and J. T. Edsall, editors), New York, Academic Press, Inc., 1948, **4,** 155.
4. Robertson, T., *Am. J. Med.*, 1950, **9,** 315.
5. Bjoerneboe, M., *Z. Immunitätsforsch.*, 1941, **99,** 245.
6. Bjoerneboe, M., *Acta path. et micr. scand.*, 1943, **20,** 221.
7. Letterer, E., *Beitr. path. Anat. u. allg. Path.*, 1926, **75,** 486.
8. Letterer, E., *Virchows Arch. path. Anat.*, 1934, **293,** 34.
9. Schneider, G., *Virchows Arch. path. Anat.*, 1949, **317,** 26.
10. Caspersson, T., *Naturwissenschaften*, 1941, **29,** 33.
11. Caspersson, T., Landström-Hydén, H., and Aquilonius, L., *Chromosoma*, 1941, **2,** 111.
12. Caspersson, T., and Thorell, B., *Chromosoma*, 1941, **2,** 132.
13. Brachet, J., *Enzymologia*, 1941, **10,** 87.
14. Caspersson, T., Cell Growth and Cell Function, New York, W. W. Norton and Company, Inc., 1950.

15. Brachet, J., Chemical Embryology, translated by Barth, L. G., New York, Interscience Publishers, Inc., 1950, 189, 245.
16. Fagraeus, A., *Acta med. scand.*, 1948, suppl., 204.
17. Ehrich, W. E., Drabkin, D. L., and Forman, C., *J. Exp. Med.*, 1949, **90,** 157.
18. Harris, T. N., and Harris, S., *J. Exp. Med.*, 1949, **90,** 169.
19. Kolmer, J. A., and Boerner, F., Approved Laboratory Technic, New York, D. Appleton-Century Company, 4th edition, 1945, 833.
20. Kingsley, G. R., *J. Biol. Chem.*, 1940, **133,** 731.
21. Gradwohl, R. B. H., Clinical Laboratory Methods and Diagnosis, St. Louis, The C. V. Mosby Co., 3rd edition, 1943, **1,** 191.
22. Bjoerneboe, M., and Gormsen, H., *Acta path. et microbiol. scand.*, 1943, **20,** 649.
23. Bing, J., Fagraeus, A., and Thorell, B., *Acta physiol. scand.*, 1945, **10,** 282.
24. Bohle, A., Hartmann, F., and Pola, W., *Virchows Arch. path. Anat.*, 1950, **319,** 231.
25. Letterer, E., *J. Path. and Bact.*, 1949, **61,** 496.
26. Drury, A. N., *Physiol. Rev.*, 1936, **16,** 292.
27. Fischer, A., *Acta physiol. scand.*, 1941, **3,** 54.
28. Davidson, J. N., and Waymouth, C., *Nutrition Abstr. and Rev.*, 1944, **14** (1), 1.
29. Tennant, R., Stern, K. G., and Liebow, A. A., *Cancer Research*, 1942, **2,** 218.
30. Villee, C. A., and Bissell, H. B., *J. Biol. Chem.*, 1948, **172,** 59.
31. Roll, P. M., Brown, G. B., DiCarlo, F. J., and Schultz, A. S., *J. Biol. Chem.*, 1949, **180,** 333.