INVESTIGATIONS CONCERNING THE CHANGES IN SERUM PROTEINS DURING IMMUNIZATION

The Cause of Hypoalbuminemia with High Gamma Globulin Values

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(Received for publication, April 22, 1959)

Following hyperimmunization of rabbits a marked increase in the gamma globulin and fall in the albumin in the blood may be demonstrated (1). The increase in globulin (measured by precipitation with ammonium sulfate according to Henriques and Klausen's method (2)) is identical with the increase in the antibody protein. The fall in the albumin concentration is probably due to a regulative mechanism which attempts to maintain a normal colloid-osmotic pressure. This regulation may act in various ways of which the following may be concerned: (*a*) inhibition of the production of albumin or increase in the rate of its catabolism, (*b*) displacement of albumin from intra- to extravascular spaces, or (*c*) simple dilution with increase of the plasma volume.

The object of this paper is to investigate which of these three possible mechanisms is concerned.

Methods

White rabbits of the Danish country race bred in the State Serum Institute were employed in the experiment. At the commencement of the experiment the animals were 6 to 7 months old and weighed 2,800 to 3,000 gm. The vaccine employed consisted of formalin-killed pneumococci of eight different types (9N, 11B, 14, 22L, 23 Mac, 28A, 33A, 41A) in equal quantities of each. The concentration of the vaccine was 10^9 pneumococci per ml. The rabbits were immunized during two periods: Dec. 17, 1957, to Apr. 12, 1958, and July 14, 1958, to Sept. 30, 1958. During the first period 4 ml. of vaccine was administered thrice weekly from Dec. 17, 1957, to Jan. 24, 1958, and thereafter 1 ml. thrice weekly. During the second period 2 ml. of vaccine was administered thrice weekly from July 14, 1958, to Sept. 30, 1958.

The serum protein analyses were undertaken by paper electrophoresis according to the method of Laurell *et al.* (3) and the total protein determinations by the method given by Lowry *et al.* (4).

The plasma volume, albumin degradation, and the distribution of albumin intra- and extravascularly were determined according to Sterling's method (5) with rabbit albumin labelled with I^{131}. For determination of the plasma volume the 15 minute blood sample was

* Supported by a grant from P. Carl Petersens Fond, Denmark

employed. The rabbit albumin was prepared by the Department of Physical Chemistry, The State Serum Institute, Copenhagen, employing precipitation with ammonium sulfate, dialysation and lyophilisation. Labelling of the albumin was undertaken according to the method of Veall, Pearson, and Hanley (6). All of the samples were counted on 0.5 to 1.0 ml. heparin-plasma in a well-type scintillation counter. Four investigations with injection of isotope-labelled albumin were performed on Nov. 11, 1957, Jan. 27, 1958, Aug. 18, and Dec. 19, 1958.

These investigations were thus undertaken prior to immunization, twice during the periods of immunization and approximately 2½ months after the conclusion of immunization. In the first and fourth investigations, however, only plasma volume was determined.

The investigations were performed in the following manner: After withdrawal of a sample of blood, 5 ml. labelled albumin was injected intravenously. A blood sample was withdrawn 15 minutes after injection and every 2nd day for 18 days. Further, in the second and third investigations, blood samples for electrophoresis were withdrawn on three occasions during the period in which the albumin degradation curve was determined. Thus, in this period a total of 35 to 40 ml. of blood was withdrawn from each of the animals. No correction for this loss, which was uniform for all of the animals, was made in determination of the degradation curve.

During the experimental periods the rabbits received supplementary iodine in the diet.

The rabbits were weighed every week or every 2nd week during the entire experiment.

Some animals died during the experiment, mostly during the periods of immunization. The experiment commenced with *20* rabbits and concluded with *12*.

EXPERIMENTAL

The weight of the rabbits increased during the experiment. After the first immunization period the average increase was about 500 gm. and after the second about 750 gm. The scatter of the weights increased with age and the immunized animals weighed about 100 gm. less than the controls.

The serum protein values for the various experimental periods appear in Tables I *a* and I *b* and Fig. 1.

The normal values for albumin were 5.06 per cent ± 0.32 (standard deviation); and the normal gamma globulin values were 0.75 per cent ± 0.22 (standard deviation).

During the first period of immunization an increase in gamma globulin and a slight fall in the albumin values were observed. During the second period of immunization, a greater increase in gamma globulin occurred and a correspondingly greater fall in the albumin values. The reciprocal relationship between the albumin and gamma globulin values appears in Fig. 2 which gives the protein concentrations in the experimental animals on Aug. 14, 1958.

The values for alpha and beta globulins also showed changes during the experiments. In the control animals the values for alpha globulins varied from 0.40 to 0.87 per cent with an average of 0.64 per cent while the values for the beta globulins varied from 0.36 to 1.05 per cent with an average value of 0.58 per cent. In the immunized animals, the alpha globulins varied from 0.68 to 1.13 per cent with an average of 0.88 per cent and the beta globulins from 0.47 to 1.04 per cent with an average of 0.75 per cent. The concentrations of these

TABLES I *a* and I *b*

Total Serum Protein, Albumin, and Gamma Globulin before, during and about 3 Months after Immunization

Table I a

Rabbit No.	Serum protein	Before immunization	During 1. Immunization period		
		No. 28–31: Dec. 7, 1957 No. 38–45: Dec. 17, 1957	Jan. 31, 1958	Feb. 14, 1958	Feb. 17, 1958
Control rabbits					
		per cent	*per cent*	*per cent*	*per cent*
52–28	Total protein	7.20	7.20	7.80	7.50
	Albumin	5.01	5.58	5.96	5.74
	Gamma globulin	0.68	0.53	0.67	0.53
52–29	Total protein	7.21	7.10	7.50	7.50
	Albumin	4.89	5.27	5.56	5.90
	Gamma globulin	0.82	0.77	0.80	0.56
Immunized rabbits					
52–30	Total protein	7.49	10.00	9.20	9.50
	Albumin	4.27	4.69	4.71	4.99
	Gamma globulin	1.31	3.35	2.80	2.61
52–31	Total protein	7.60	7.80	7.60	7.40
	Albumin	5.32	4.82	4.65	4.84
	Gamma globulin	0.86	1.61	1.49	1.14
52–38	Total protein	7.00	8.40	7.60	7.50
	Albumin	5.36	5.42	4.80	5.10
	Gamma globulin	0.47	1.60	1.25	0.91
52–39	Total protein	7.21	8.10	7.80	8.20
	Albumin	5.24	5.31	4.65	5.29
	Gamma globulin	0.70	1.49	1.49	1.15
52–41	Total protein	6.79	9.20	8.10	7.50
	Albumin	5.03	4.56	4.06	3.96
	Gamma globulin	0.61	3.01	2.29	1.88
52–42	Total protein	7.40	8.00	8.30	9.90
	Albumin	5.54	4.10	3.91	4.95
	Gamma globulin	0.59	2.28	2.90	3.24
52–43	Total protein	6.91	6.90	7.50	7.50
	Albumin	5.34	4.09	4.64	4.73
	Gamma globulin	0.45	1.49	1.34	1.16
52–45	Total protein	7.60	8.10	7.40	7.40
	Albumin	4.98	4.64	4.41	4.76
	Gamma globulin	0.76	1.94	1.47	1.12

TABLE I b

Rabbit No.	Serum protein	During 2. Immunization period			3 mos. after immunization
		Aug. 14, 1958	Aug. 26,1958	Sept. 3, 1958	Dec. 19, 1958
Control rabbits					
		per cent	*per cent*	*per cent*	*per cent*
52–28	Total protein	6.60	6.80	6.80	7.10
	Albumin	4.99	4.87	4.75	5.06
	Gamma globulin	0.56	0.68	0.74	0.76
52–29	Total protein	6.80	6.10	7.20	7.20
	Albumin	4.76	4.08	3.97	4.94
	Gamma globulin	0.76	0.81	1.08	0.98
52–33	Total protein	7.10	6.40	7.00	6.80
	Albumin	4.90	4.40	4.95	4.96
	Gamma globulin	0.83	0.73	0.68	0.74
52–48	Total protein	7.10			
	Albumin	4.84			
	Gamma globulin	0.92			
Immunized rabbits					
52–30	Total protein	10.50	9.40	9.60	7.20
	Albumin	4.43	4.14	4.33	4.48
	Gamma globulin	4.23	3.21	2.74	1.28
52–31	Total protein	11.40	9.70	8.80	7.10
	Albumin	3.15	2.90	3.64	5.15
	Gamma globulin	6.94	5.20	3.64	0.83
52–35	Total protein	10.90	9.90	9.60	6.40
	Albumin	4.35	4.30	4.02	4.46
	Gamma globulin	4.84	3.47	3.59	0.70
52–38	Total protein	9.10	7.50	8.80	6.40
	Albumin	4.35	3.61	3.93	4.56
	Gamma globulin	3.14	2.40	2.92	0.66
52–41	Total protein	11.50	10.60	9.70	7.10
	Albumin	4.13	4.36	4.19	4.80
	Gamma globulin	5.75	4.23	3.55	0.96
52–42	Total protein	14.20	12.90	12.40	7.30
	Albumin	2.44	3.21	3.52	5.28
	Gamma globulin	10.10	7.99	6.64	0.80
52–43	Total protein	9.10	8.10	9.00	6.80
	Albumin	4.41	3.62	4.20	4.79
	Gamma globulin	2.90	2.74	3.05	0.80
52–47	Total protein	9.50	9.10	9.80	6.50
	Albumin	4.32	3.95	4.35	3.69
	Gamma globulin	3.43	3.35	3.46	0.70

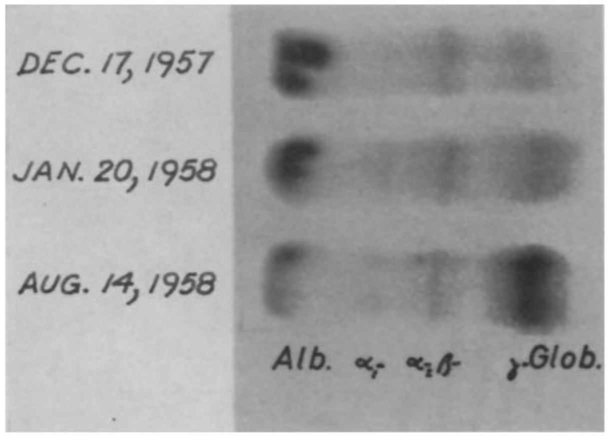

FIG. 1. Three electrophoretic strips from the same rabbit before, during the first and during the second immunization period. The increase in gamma globulin and decrease in albumin can be seen directly.

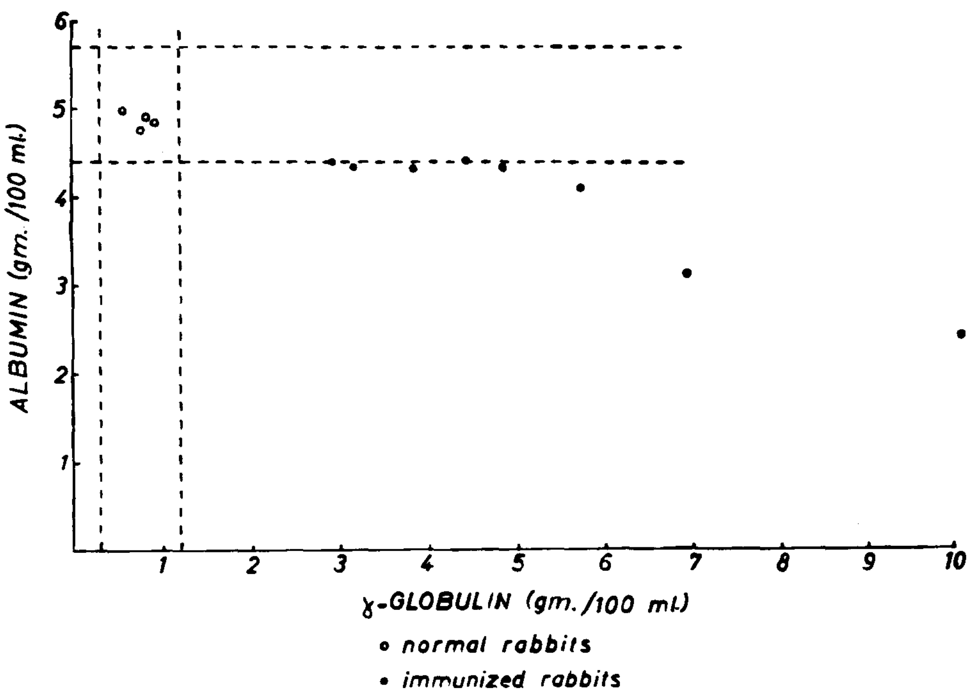

FIG. 2. The relationship between serum albumin and gamma globulin in 12 rabbits on Aug. 14, 1958.

globulins are thus somewhat higher in the immunized animals but the differences are not great.

The rate of albumin degradation appears in Table II in which the half-life is recorded. From the total quantity of albumin, the quantity of albumin degraded per day is calculated. It will be observed that the half-life is slightly longer for the control animals than for the immunized animals which, on the other hand, have a slightly lower content of albumin so that the quantity of albumin broken down (and synthesized) per day is more or less the same in both groups. This is seen most clearly in the experiment in January-February while the values in August-September are somewhat scattered in both groups.

TABLE II

Serum Albumin, Total Albumin, Proportion Intravascular to Extravascular Albumin (ia/ea), Plasma Volume per Kg., Albumin Degraded per Day, and Half-Life of Labelled Albumin in the Experiments Started on Jan. 27, 1958, and August 18, 1958

Plasma volume per kg. is also given before (Nov. 11, 1957) and after (Dec. 19, 1958) immunization. In the calculations of the total albumin, a serum albumin concentration is used that in the experiment started Jan. 27, 1958, is an average of the values of Jan. 31, Feb. 14, and Feb. 17 (Table I *a*), in the experiment started on Aug. 18, 1958, is an average of the values of Aug. 14, Aug. 26, and Sept. 3 (Table I *b*).

Rabbit No.	Nov. 11, 1957	Jan. 27, 1958					Aug. 18, 1958					Dec. 19, 1958
	Plasma volume/kg.	Total albumin	ia/ea	Plasma volume/kg.	Albumin degraded/day	t/2	Total albumin	ia/ea	Plasma volume/kg.	Albumin degraded/day	t/2	Plasma volume/kg.
						Control rabbits						
	ml.	*gm.*		*ml.*	*gm.*	*days*	*gm.*		*ml.*	*gm.*	*days*	*ml.*
52–28	35.0	22.0	0.40	31.7	2.42	6.3	17.9	0.38	30.5	1.57	7.9	26.4
52–29	31.6	23.2	0.33	29.9	2.33	6.9	15.7	0.37	26.8	1.73	6.3	24.0
52–32	33.6											
52–33							23.2	0.46	41.4	2.01	8.1	27.0
52–48									30.2			
						Immunized rabbits						
52–30	33.7	16.0	0.51	38.8	1.91	5.8	24.4	0.63	49.0	3.13	5.4	
52–31	32.9	19.8	0.50	43.4	2.36	5.8	16.3	0.49	49.0	1.75	6.5	30.2
52–35							17.2	0.40	43.8	1.98	6.1	27.5
52–38		17.7	0.44	29.5	2.45	5.0	18.2	0.38	34.8	2.22	5.7	25.0
52–39		18.2	0.46	32.2	2.04	6.2						
52–41		19.2	0.55	49.4	2.30	5.8	21.4	0.42	39.5	2.21	6.7	
52–42		17.3	0.53	41.2	2.22	5.4	17.7	0.61	65.3	2.22	5.5	30.4
52–43		18.5	0.45	32.5	2.07	6.2	20.6	0.43	37.5	2.27	6.3	28.2
52–45		18.0	0.42	39.6	1.98	6.3						
52–47							18.2	0.49	39.9	2.95	5.5	27.5

The proportion, intravascular/extravascular albumin will, similarly, be seen in Table II. This proportion is, on the whole, less (average 0.39) in the control animals than in the immunized animals (average 0.48 in both experiments); these animals thus keep a slightly larger fraction of the albumin in circulation.

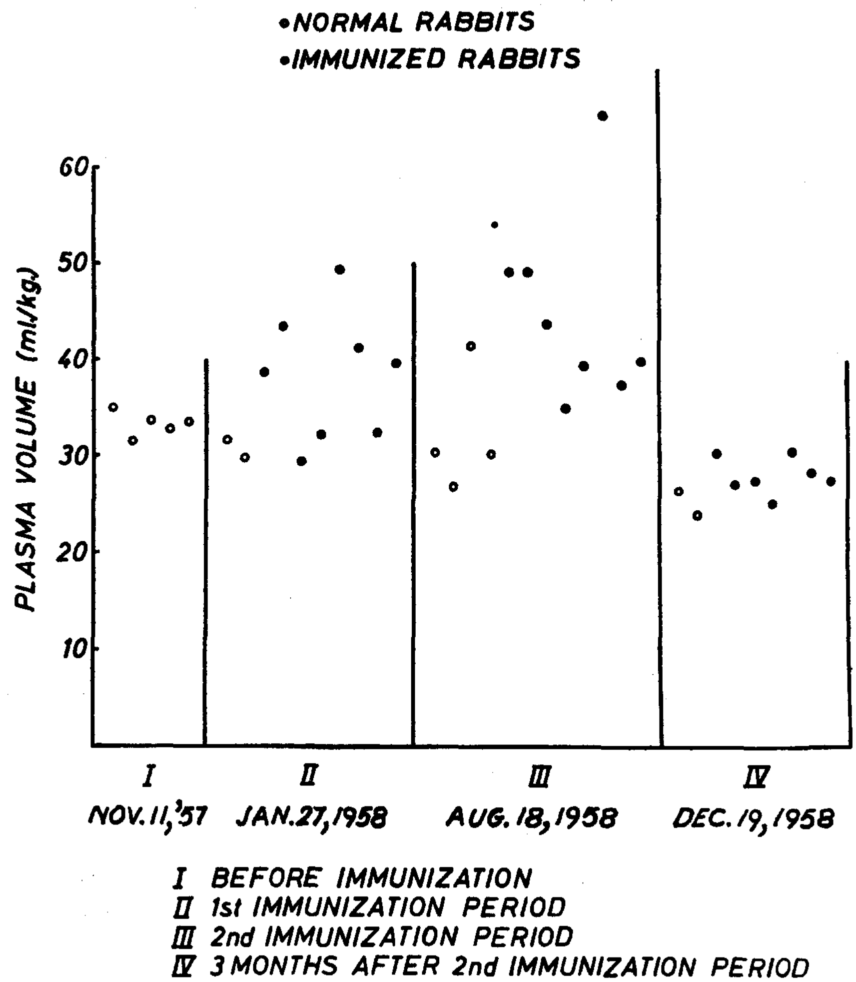

FIG. 3. Plasma volume per kg. in immunized animals and in control animals before, during, and after immunization.

It must be emphasized in this connection that the quantity of albumin degraded per day in the immunized and the non-immunized animals is more or less uniform. The experiments do not appear to indicate any significant change in the degradation of albumin.

The plasma volume calculated per kg. appears in Table II and Fig. 3 and will be observed to be between 24.0 and 65.3 ml. It will be noted that all of the non-immunized animals had plasma volumes which, with one exception (No. 52-33 Aug. 18, 1958) were between 24.0 and 35.0 ml, and that the plasma volume decreases with time (because of a higher fat content in the older animals?). The plasma volumes of the immunized animals were higher, from 29.5

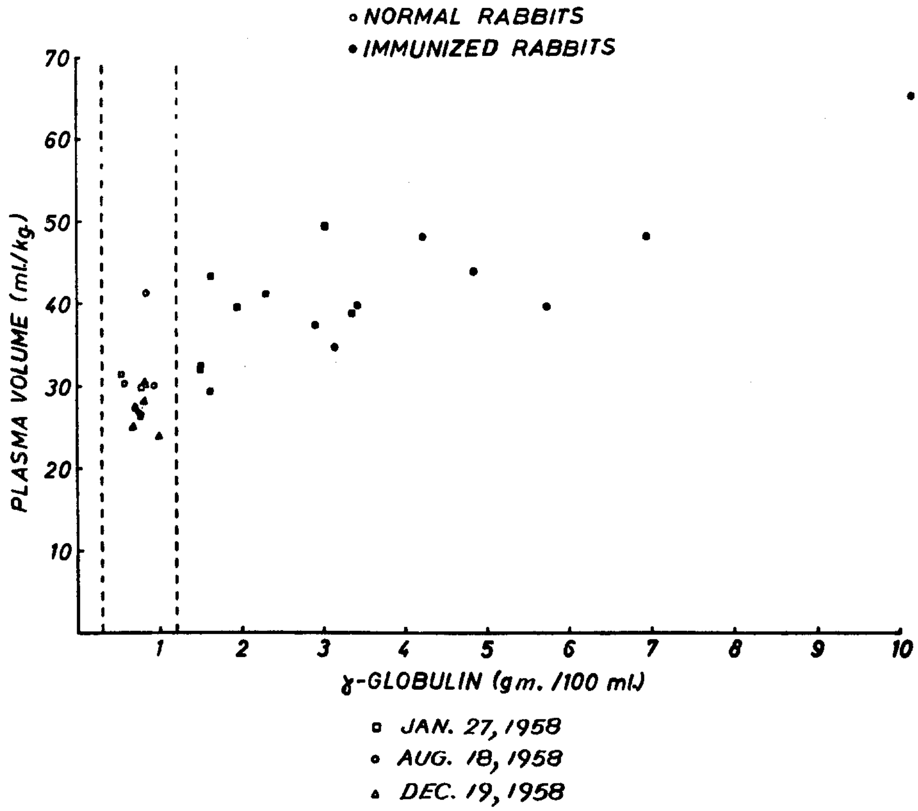

FIG. 4. Relationship between plasma volume and gamma globulin. Gamma globulin values of Jan. 31, Aug. 14, and Dec. 19: see Table I. Plasma volumes of Jan. 27, Aug. 18, and Dec. 19: see Table II.

to 65.3 ml. per kg. It appears from Fig. 4 that the plasma volume increases with increase of gamma globulin and simultaneously with the increase in plasma volume, the percentage of albumin shows a tendency to fall (Fig. 5).

DISCUSSION

As stated previously, the problem to be solved by these experiments consisted of the following: Is the fall in the concentration of albumin on intensive immunization due to: (*a*) changes in the synthesis or destruction of albumin, (*b*) alteration in the distribution of intra- and extravascular albumin, (*c*) increase in the plasma volume.

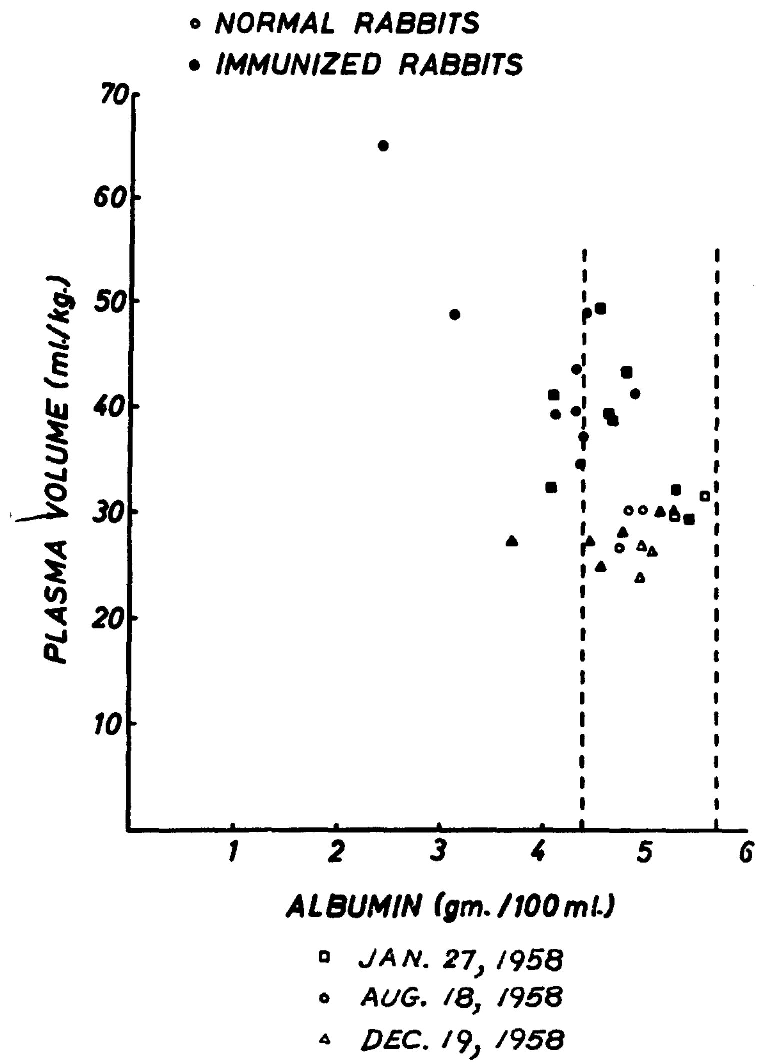

FIG. 5. Relationship between plasma volume and serum albumin. Serum albumin values of Jan. 31, Aug. 14, and Dec. 19: see Table I. Plasma volumes of Jan. 27, Aug. 18, and Dec. 19: see Table II.

As regards the first possibility the experimental results appear to show that there is no definite alteration in the catabolism or synthesis of albumin during the process of immunization. It should, however, be noticed that in several of the animals, the serum albumin during the second period of immunization did not remain constant in the weeks during which the degradation curve of the albumin was investigated (See Tables I *a* and I *b*). We do not consider, however, that these deviations from ideal experimental conditions (constant albumin concentration) invalidate the results obtained. In any case, it should be emphasized that the quantity of albumin degraded per day for immunized and non-immunized animals is more or less uniform.

As regards the second possibility suggested, *i.e.* that albumin might exhibit another distribution into extra- and intravascular space during immunization, the experiments recorded here appear to indicate that the distribution is almost the same in the control animals and in the immunized animals and that rather a greater proportion of the albumin is in the circulation in the immunized animals than in the control animals. The albumin does not appear to be forced out of the circulating blood.

The third possibility that the fall in albumin during immunization is due to dilution on account of an increase in the plasma volume appears to be strongly supported by the results of these experiments. It appears clearly from Fig. 3 that the plasma volume of the immunized animals is greater than that of the control animals and Fig. 5 shows that a rough correlation exists between the albumin concentration and the plasma volume so that the albumin percentage falls simultaneously with the increase of plasma volume.

The conclusion to be drawn from this investigation is, thus, that the fall in the albumin concentration observed on immunization leading to high gamma globulin concentrations is due to increased plasma volume. This increase in plasma volume is probably due to a mechanism regulating colloid-osmotic pressure of the blood.

The literature concerning these problems is extremely sparse and investigations such as those recorded here have probably not been published previously.

Bjørneboe (1) demonstrated, in hyperimmunized rabbits, that the relationship between the serum globulin and serum albumin could be explained by the assumption of a regulatory mechanism which attempts to maintain the colloid-osmotic pressure constant.

In a subsequent work Bjørneboe (7) was of the opinion that he could demonstrate that this regulation consisted in either that albumin was eliminated from the blood or that the rate of production was reduced. It should be noted that in these investigations ammonium sulfate precipitation was employed for determination of albumin and globulin and that the plasma volume was not measured directly. Wuhrmann and Wunderly (8) presented a similar hypothesis based upon clinical observations in man. Sussman and Freed (9) produced hypoalbuminemia and hyperglobulinemia in rabbits by means of intraperitoneal injections of massive doses of rabbit globulin. The mechanism of the fall in the albumin percentage was compared with the hypoalbuminemia observed in multiple myeloma, kala-azar, and malaria but no further comments were made. Waldenstrøm (10) considered that the fall in the albumin level in myelomatosis and similar conditions in which hyperglobulinemia is found is produced because the synthesis of the pathological globulin fractions, under these circumstances, have higher priority as regards the available amino acids. Ott (11) in an investigation of the serum proteins in Bennhold's patients with analbuminemia considered that the regulation of the colloid-osmotic pressure may be conceived to function so that certain globulin fractions increase when albumin is absent. The demonstration of the reduction of these globulin fractions during infusion of albumin supports this theory.

In summarizing, it may be said that several theoretical hypotheses concerning the regulation of the colloid-osmotic pressure in the blood are to be found in the literature, but there are only isolated experimental studies, none of which permits further discussion of the experimental work recorded here.

SUMMARY

On hyperimmunization of rabbits with pneumococcal vaccine, increase in the content of gamma globulin and fall of albumin in the serum are produced. In such hyperimmunized rabbits, investigations of the plasma volume, albumin degradation, and the distribution of the albumin intra- and extravascularly were undertaken by Sterling's method employing rabbit albumin labelled with I^{131}.

The investigations do not suggest any significant alteration in the albumin degradation nor in the distribution between the intra- and extravascular albumin. It was demonstrated that the plasma volume increases simultaneously with increase in the gamma globulin and fall in albumin.

It is concluded that the fall in serum albumin observed is due to a regulative mechanism which attempts to maintain the colloid-osmotic pressure by increasing the plasma volume.

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