THE CHANGES IN SERUM PROTEINS AND BLOOD VOLUME DURING IMMUNIZATION*

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(Received for publication, February 27, 1961)

A marked increase in the gamma globulin and a fall in the albumin has been demonstrated following hyperimmunization of rabbits. In an earlier study it was shown that the plasma volume increases simultaneously with increase in gamma globulin and fall in albumin. The gamma globulin apparently has the effect of a "plasma expander" (1).

The object of the present paper has been to try to confirm this observation of a relationship between gamma globulin and plasma volume. At the same time the red cell volume was studied in order to see how the change in plasma volume affected the red cell volume.

Methods

The same race of rabbits and the same pneumococcal vaccine as used in earlier experiments were employed. 2 ml. vaccine were given intravenously 3 times a week during 3 periods: the 1st immunization period being November 10, 1959, to December 5, 1959, the 2nd period January 5, 1960, to April 11, 1960, the 3rd period July 13, 1960, to September 21, 1960. The study of plasma proteins, plasma volume, and red cell volume was done during the 3rd immunization period in 5 immunized animals and 4 control animals (August 27, 1960: rabbits 56-70 (immunized) and 56-79 (control), September 10, 1960: rabbits 56-74, 56-66 (immunized), and 56-77, 56-78 (controls), September 17, 1960: rabbits 56-56, 56-64 (immunized) and 56-76 (control)).

Serum protein analyses were undertaken by paper electrophoresis according to the method of Laurell *et al.* (2) and the total protein determinations by the method of Lowry *et al.* (3). Several protein analyses were performed during the immunization periods and the study of plasma volume and red cell volume was done during the 3rd immunization period when several of the rabbits had rather high gamma globulin values.

Hematocrit values (duplicates) were measured in modified Winthrobe tubes spun 30 minutes at 3000 R.P.M. (effective radius 15 cm.).

Plasma volume (PV) was determined as the 10 minutes distribution volume of intravenously injected ^{131}I-labelled rabbit serum albumin. Rabbit albumin was labelled according to the method of Veall, Pearson, and Hanley (1955) (4). More than 99 per cent of total activity was protein-bound. 2 to 5 μc. were applied in each animal.

Red cell volume (RCV) was similarly calculated from the dilution of intravenously injected ^{51}Cr-tagged autologous erythrocytes 10 minutes after the injection and the hematocrit reading of the blood sample. The labelling procedure was essentially the same as that given by

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

Mollison (1959) (5). Well below 1 per cent of the activity was extracellular. The amount of ^{51}Cr-activity was 5 to 10 μc. in each experiment. A high fragility of rabbit erythrocytes necessitated separate injections of ^{131}I-albumin and ^{51}Cr-erythrocytes which was done successively in the same ear vein. Blood was obtained after 10 minutes from the opposite ear. The amount supplied was determined by weighing the syringe before and after the injection. Aliquots (2 ml.) of heparinized blood were pipetted into vials designed for a well type NaI-thallium-activated scintillation counter. Red cells contained in 2 ml. blood were washed 3 or 4 times with saline until the activity of the saline was less than 1 per cent of red cell activity. Measurements on whole blood and red cells were made for a period sufficient to ascertain a standard error of about 1 per cent of the net counting rate. The difference between their rates indicated the contents of ^{131}I-albumin in 2 ml. blood or in $\left[2 \times \frac{(100 - Htc)}{100}\right]$ ml. plasma (Htc = hematocrit).

Hence, the volumes of plasma (PV), red cells (RCV), and blood (BV) could be calculated knowing the injected dose of ^{131}I and ^{51}Cr-activity, and the ^{131}I- and ^{51}Cr-activity contained in 1 ml. of RC and plasma respectively.

BV was taken as the sum of plasma volume and RCV or it was calculated from RCV and hematocrit of blood sample.

Body hematocrit was calculated as: $\frac{RCV \times 100.}{RCV+PV}$

The rabbits were weighed every week or every 2nd week during the entire experiment. 20 rabbits were immunized and 5 were kept as controls. Only one immunized rabbit was lost during the experiment, 6 weeks after the end of the 2nd immunization period, of an unrelated disease (encephalitis).

RESULTS

The weight of the rabbits increased during the experiment from about 2800 to about 3500 gm. in the immunized as well as in the control animals. The hyperimmunized animals were clinically healthy.

The serum protein values for the 9 rabbits studied appear in Table I. The gamma globulin values for the 4 control animals vary from 0.17 to 0.70 gm. per cent and for the immunized animals from 3.57 to 10.4 gm. per cent. Three of the albumin values in the immunized animals are lower than the albumin values in the control group.

The plasma volumes calculated per kg. appear in Table I and are between 28.8 and 32.1 ml. for the control animals and between 39.3 and 72.8 ml. for the immunized animals.

It appears from Fig. 1 that the plasma volume increases with the increase of gamma globulin. In this figure are also plotted results from experiments performed in 1958 (1) (2 control animals and 7 immunized rabbits from the 2nd immunization period) and it is apparent that the same relationship exists for both groups of experiments. Hemoglobin concentration, venous hematocrit, and red cell volume appear in Table I and Fig. 2. It will be seen that hemoglobin concentration and hematocrit are lower in the immunized animals but

TABLE I

Hemoglobin, Hematocrit, Serum Protein, Albumin, Gamma Globulin, Plasma Volume, Erythrocyte Volume, Blood Volume Calculated as Sum of Plasma Volume and Erythrocyte Volume, Blood Volume Calculated from ^{51}Cr Erythrocyte Dilution, Body Hematocrit, Body Hematocrit/Venous Hematocrit and Circulating Albumin in 5 Immunized and 4 Control Rabbits

Rabbit	Hb	Hemato-crit	Serum protein	Albumin	Gamma globulin	Plasma volume ^{131}I albumin	Erythro-cyte Volume ^{51}Cr	Blood volume ^{131}I albumin + ^{51}Cr	Blood volume calculated from ^{51}Cr erythro-cyte dilution	Body hemato-crit	Body htc. / Ven. htc.	Circ. albumin
	gm./100 ml.	*per cent*	*gm. per cent*	*gm. per cent*	*gm. per cent*	*ml./kg.*	*ml./kg.*	*ml./kg.*	*ml./kg.*	*per cent*		*gm./kg.*
						Controls						
56-79	14.6	42.9	8.4	6.13	0.70	28.8	17.5	46.3	40.9	37.8	0.882	1.77
56-78	13.6	39.2	6.2	4.51	0.54	29.1	14.4	43.5	36.7	33.1	0.844	1.31
56-77	14.5	39.8	6.2	4.67	0.17	30.8	16.8	47.6	42.2	35.4	0.890	1.44
56-76	13.5	38.4	6.1	4.56	0.58	32.1	14.9	47.0	38.8	31.7	0.826	1.46
Average....	14.1	40.1				30.2	15.9	46.1	39.7		0.861	1.50
						Immunized						
56-74	11.0	31.6	10.6	3.59	5.68	39.4	14.2	53.6	44.8	26.4	0.835	1.41
56-70	7.4	25.4	15.6	3.27	10.4	72.8	18.3	91.1	71.9	20.0	0.788	2.38
56-66	9.0	28.3	11.4	3.41	6.44	47.5	15.6	63.1	55.2	24.8	0.876	1.62
56-64	11.5	33.7	12.2	4.55	6.36	42.8	16.5	59.3	49.0	27.8	0.825	1.95
56-56	11.8	35.0	10.2	5.19	3.57	39.3	14.4	53.7	41.1	26.8	0.766	2.04
Average....							15.8				0.818	

red cell volumes are the same in the two groups. Total blood volume determined as the sum of plasma volume and red cell volume is higher in the immunized animals. Blood volume calculated from the dilution of the injected labelled red cells is also higher in the immunized animals than in the controls. Blood volumes calculated in these two different ways are not identical, blood volume calculated as the sum of plasma and red cell volume being the greatest. The difference must be due to a difference in distribution volume of labelled

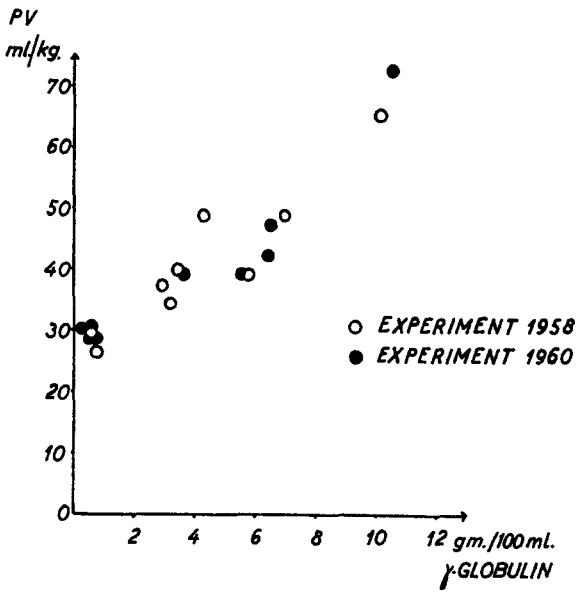

FIG. 1. The relation between plasma volume and gamma globulin in 6 control rabbits and 12 immunized rabbits.

Black dots indicate present series of experiments. Open circles indicate experiments of August, 1958, (see references 1).

cells and labelled serum albumin. Body hematocrit is lower than venous hematocrit. The ratio body/venous hematocrit was below unity in all animals, in 4 controls it averaged 0.86, in 5 immunized animals 0.82, but scattering was considerable. The total circulating albumin (calculated from plasma volume and albumin concentration) is slightly higher in the immunized animals than in the control animals (Table I).

DISCUSSION

The experiments confirm earlier observations that during immunization increase in serum gamma globulin concentration (caused by increase in antibody protein) is followed by increase in plasma volume. The antibody protein

acts as a "plasma expander" when it is produced in such high concentrations as here. During this expansion of plasma volume total red cell volume is constant. Consequently hemoglobin concentration falls.

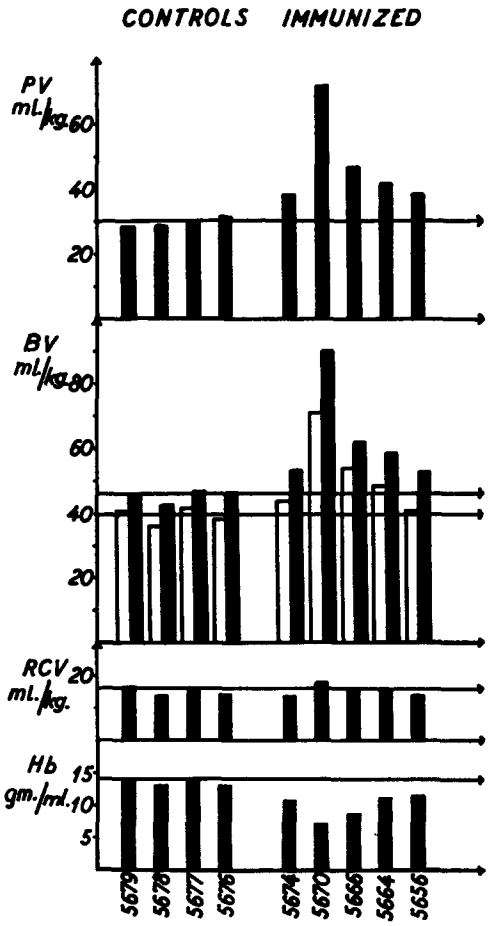

FIG. 2. Plasma volume (PV), blood volume (BV), red cell volume (RCV) and hemoglinob (Hb) in 5 immunized and 4 control rabbits.

Horizontal arrows indicate the mean values for the four control rabbits.

Blood volume is calculated in two ways *i.e.*: (*a*) as the sum of plasma volume and red cell volume (black columns) and (*b*) from the dilution of labelled red cells (white columns).

Plasma volume determined by aid of labelled albumin (dye or ^{131}I) may be erroneously high in infectious states (6) and in hypervolemia (7). Theoretically this might be part of the explanation why a marked increase of PV was previously found in immunized animals. Thus, if labelled albumin escaped from the vascular bed at an increased rate during mixing time due to abnormal capillary permeability, then its

distribution volume might be appreciably greater than the plasma volume. This source of error could largely be ruled out in our experiments because the apparent anemia was associated with a normal red cell volume as determined by labelled cells, which implies that the increase in plasma volume was a real one. Further evidence of the reliability of the ^{131}I-plasma volume was given by the body/venous hematocrit ratio. Normally this is about 0.9 (8). The low body hematocrit is ascribed to an uneven distribution of cells and plasma within the vascular bed and to the occurrence of an extravascular red blood cell-free plasma compartment which almost momentarily equilibrates with circulating plasma. If the ^{131}I-albumin-plasma volume was erroneously high in immunized rabbits one would expect a body/venous-hematocrit ratio much lower than normal, but the mean value of 5 animals was only 5 per cent lower than that of 4 controls. On the other hand this may reflect that the over-all endothelial permeability to albumin was somewhat higher than normal.

A similar demonstration of the action of gamma globulin as a "plasma expander" is published by McCance and Widdowson (9). These authors have shown that newborn piglets getting colostrum exhibit an increase in serum gamma globulin of about 3 grams per cent and at the same time an increase in plasma volume of about 30%. This increase in plasma volume explains the anemia observed in sucking piglets.

Our results fit quite well those of McCance and Widdowson: an increase of about 9 gm. per cent gamma globulin corresponds in our experiments to an increase in plasma volume of about 100 per cent, whereas in their experiments an increase in gamma globulin of about 3 gm. per cent corresponds to an increase in plasma volume of about 30 per cent.

The increase in plasma volume observed in these experiments is probably due to a regulative mechanism, which attempts to maintain the colloid-osmotic pressure. This regulative mechanism seems to have priority over volume regulation.

SUMMARY

In rabbits hyperimmunized with pneumococcal vaccine high concentrations of gamma globulin are produced. In such rabbits, plasma volume was determined with ^{131}I-labelled rabbit albumin and red cell volume with ^{51}-Cr-labelled autologous red cells. It was found that the plasma volume increased with increasing gamma globulin concentration, the highest values observed being about 10 gm. per cent gamma globulin and about 70 ml. plasma per kg as against normal values of 0.6 to 0.7 gm. per cent gamma globulin and 30 ml. plasma per kg. The red cell volume was the same in immunized and in normal rabbits and consequently hemoglobin concentration fell with increasing gamma globulin.

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