

IMMUNOPHYSIOLOGY OF IMMUNE REACTIONS

ALTERATION IN THE THIOCYANATE SPACE IN QUANTITATIVE PASSIVE TRANSFER EXPERIMENTS IN RABBITS

JERRY K. AIKAWA, M.D.,* AND ELOISE L. RHOADES, B.S.
WINSTON-SALEM, N. C.

PREVIOUS studies have suggested that certain generalized physiologic disturbances are related to the host's response to the parenteral introduction of an antigenic substance.¹ In rabbits injected with human plasma and followed serially with determinations of the blood volume and extravascular fluid space, significant changes were noted at the time humoral antibodies appeared in the blood. The increase in thiocyanate space and decrease in blood volume, which were interpreted as being due to alterations in the permeability of capillary and cell membranes, appeared to be causally related to in vivo antigen-antibody reactions.

In order further to establish such a relationship, the passive transfer method, an example of the anaphylactic type of antigen-antibody reaction, has been employed. The human plasma used in the previous experiments was a mixture of many fractions, each of which could have induced a slightly different antibody response at different time intervals.² In the present experiment, a refined but not pure antigen, gamma globulin, was used.

MATERIAL

Animals.—Fourteen domestic albino rabbits weighing 2.1 to 4.4 kilograms were used in these experiments. The animals were kept in individual cages and were fed a stock diet. Water was given without restriction.

Concentrated Human Gamma Globulin, which contained 19.7 mg. of nitrogen per milliliter, was used without dilution as the immunizing specific antigen for the preparation of the immune serum.

Fraction II of Lyophilized Bovine Plasma, which is predominantly gamma globulin, was employed as a species-specific control antigen. The desired weight of this material was dissolved in 5 ml. of physiologic saline solution immediately prior to use.

Normal Rabbit Serum, obtained by bleeding rabbits from the marginal ear vein, was stored at 0° C. until used. No preservative was added.

Antihuman Gamma Globulin Rabbit Serum was prepared by repeatedly injecting 2 ml. of human gamma globulin into the interscapular space of normal

*Research Fellow of the American Heart Association.

From the Department of Internal Medicine, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, North Carolina.

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The human gamma globulin used was supplied by the American Red Cross. The bovine fraction II was supplied by Armour Laboratories.

TABLE I. SUMMARY OF DATA ON RABBITS INJECTED INTERSCAPULARLY WITH HUMAN GAMMA GLOBULIN AFTER THE INTRAVENOUS INJECTION OF SPECIFIC ANTISERUM (GROUP I)

RABBIT NUMBER	ANTISERUM (MG. N/KG.)	ANTIGEN (MG. N)	BASE LINE			RESPONSE					
			WEIGHT (KG.)	SCN (ML./KG.)	PV (ML./KG.)	6 HRS.		24 HRS.			
						WEIGHT (KG.)	SCN (ML./KG.)	WEIGHT (KG.)	SCN (ML./KG.)	PV (ML./KG.)	PV (ML./KG.)
1	3.5	40	3.15	207	28	3.24	351	3.18	214	32	75
2	3.5	40	3.75	194	31	3.77	174	3.67	305	31	70
3	3.5	40	3.56	211	37	3.56	251	3.44	251	40	44
4	3.5	40	2.49	206	38	2.56	208	2.56	253	44	33
5	4.9	80	3.07	196	37	3.24	211	3.13	243	44	43
6	4.9	80	2.84	195	37	3.01	229	2.89	232	45	48
7	3.5	40	2.13	226	45	2.34	260	2.33	247	41	43
Mean			3.00	205	36	3.10	241	3.03	249	40	51

RESULTS OF STATISTICAL ANALYSES

	d	s _d	t
Body weight	.069	.110	.62
Thiocyanate space	40.0	6.4	6.25*
Plasma volume	8.7	3.9	2.22

*Statistically significant ($P = < .01$).

rabbits at weekly intervals for a minimum of six injections. Blood was collected from the ear veins of 4 animals 8 to 10 days after the injection of the last sensitizing dose. The pool of immune serum used contained 0.70 mg. of antibody nitrogen per milliliter.³

METHODS

Each animal served as its own control. The following sequence of procedures was followed in each rabbit: After a single base line determination of the fluid compartments was made, the immune antiserum or the control (normal rabbit) serum was injected intravenously. Usually the injection was made on the day following the base line determination; in a few animals, however, several days elapsed between the base line determination and the injection of antibody. Approximately 24 hours later, the immunizing antigen or the species-specific control antigen (bovine globulin) was injected into the interscapular space. Six hours following this injection, the initial experimental plasma volume and thiocyanate space studies were done. The second experimental determinations were performed 24 hours after the interscapular injection. Each animal was weighed immediately prior to each fluid volume determination.

An interval of 24 hours was allowed to elapse between the injection of antibody and the administration of antigen in order to permit the fixation of antibody to tissue cells. The antigen was injected subcutaneously, since it was found in preliminary experiments that its intravenous injection resulted in anaphylactic death. The interscapular area was chosen for subcutaneous injection because Fleisher and Jones,⁴ in their extensive investigations on serum sickness in rabbits, have shown that absorption of the antigen by this route was rapid.

Group 1 (Experimental Group).—The 7 rabbits in the experimental group each received intravenously 3.5 to 4.9 mg. of antibody nitrogen per kilogram. Approximately 24 hours later, each animal was injected interscapularly with 40 to 80 mg. of antigen nitrogen. The amount of gamma globulin administered to each rabbit was such that, when the antigen-antibody ratio was calculated by the method of optimal proportions, the antigen was in the zone of considerable excess.⁵

Group 2 (Control Group).—Of the 7 animals in the control group 2 were given, intravenously, 5 ml. of normal rabbit serum per kilogram. This procedure was followed after 24 hours by the interscapular injection of 40 mg. of human globulin nitrogen into each animal. Each of the remaining 5 animals received intravenously 2.8 to 3.5 mg. per kilogram of antihuman globulin antibody nitrogen, followed in 24 hours by 0.025 to 0.20 Gm. of bovine Fraction II given interscapularly.

The difference, then, between Groups 1 and 2 was the following: Each of the animals in Group 1 received both antibody and specific antigen, whereas those in Group 2 were given either the immune serum or the antigen, but not both. In the latter group, the injection of these substances was preceded or followed by the administration of nonspecific serum or antigen.

TABLE II. SUMMARY OF DATA ON RABBITS GIVEN NONSPECIFIC ANTIGEN OR SERUM (GROUP 2)

RABBIT NUMBER	ANTISERUM (MG. N/KG.)	ANTIGEN	BASE LINE				RESPONSE							
			WEIGHT (KG.)	SCN (ML./KG.)	PV (ML./KG.)	WEIGHT (KG.)	6 HRS.				24 HRS.			
							WEIGHT (KG.)	SCN (ML./KG.)	PV (ML./KG.)	WEIGHT (KG.)	SCN (ML./KG.)	PV (ML./KG.)	WEIGHT (KG.)	PV (ML./KG.)
8	3.5	BG .1 Gm.	3.41	203	33	3.31	3.31	233	40	3.33	232	40	3.33	232
9	3.5	BG .1 Gm.	3.79	206	31	3.79	3.79	216	37	3.80	184	34	3.80	184
10	3.5	BG .025 Gm.	2.55	238	44	2.62	2.62	224	45	2.66	231	45	2.66	231
11	3.5	BG .03 Gm.	2.19	239	46	2.35	2.35	220	45	2.55	209	43	2.55	209
12	2.8	BG .2 Gm.	2.67	227	58	2.67	2.67	177	57	2.73	225	34	2.73	225
13	Normal Rabbit Serum (5 ml.)	Human Gamma Globulin (40 mg.)	4.35	221	32	4.43	4.43	226	33	4.40	268	39	4.40	268
14	Normal Rabbit Serum (5 ml.)	Human Gamma Globulin (40 mg.)	2.94	222	34	2.96	2.96	206	34	2.79	238	36	2.79	238
Mean			3.13	222	40	3.16	3.16	215	42	3.18	227	39	3.18	227

RESULTS OF STATISTICAL ANALYSES

	d	sd	t
Body weight	.043	.044	.98
Thiocyanate space	1.6	8.3	.19
Plasma volume	1.7	1.8	.94

Physiologic Methods.—Determinations of the plasma volume and the thiocyanate space were done simultaneously. Three to 4 ml. of blood was obtained from the ear veins and collected in an oiled test tube. Bleeding was done without anesthesia. Two milliliters of a solution containing 1 mg. of Evans Blue and 50 mg. of sodium thiocyanate per milliliter was then injected into a marginal vein. Ten minutes following completion of the injection, 3 to 4 ml. of blood was obtained from the opposite ear and placed in oiled test tubes. The plasma volume was determined by the T-1824 (Evans Blue) dye method, the thiocyanate space by the dilution of sodium thiocyanate.⁶ Both methods were adapted to the Coleman Jr. spectrophotometer. The former was read at a wave length of 620 m μ and the latter at 490 m μ .

Statistical Methods.—The data were analyzed statistically by the following method: The absolute values for plasma volume and thiocyanate space were calculated in terms of milliliter per kilogram of body weight. The base line values for body weight, plasma volume, and thiocyanate space in each animal were then compared with the mean of the 6 and 24 hour values. The average difference for each group was obtained by adding the differences between the base line and the experimental values in all the animals in the group and dividing by the total number of animals. Each group was assumed to be normal at the start of the experiment, and the control and experimental groups were analyzed separately. The two groups as a whole were not compared with each other, since we were primarily interested in the magnitude of the change in the individual animals within each group.

To test the significance of the results the following formulas were used:

$$t = \frac{\bar{d}}{S_{\bar{d}}} \quad S_{\bar{d}} = \sqrt{\frac{\sum d^2 - \frac{(\sum d)^2}{n}}{n(n-1)}}$$

The calculated value of t was compared with the tabled value at $(n-1)$ degrees of freedom.

A significant increase in the thiocyanate space was noted in the experimental group when the average base line value was compared with the average mean of the 6 and 24 hour values. In the control group, comparison of the average base line value with the average mean of the 6 and 24 hour values showed no significant difference.

No significant changes in body weight were found in either group, and in neither of the two groups was there a significant alteration in the plasma volume.

COMMENT

The present experiments were devised in an attempt to study, under more physiologic conditions than are afforded by the classic methods of anaphylaxis, the changes which might be related to in vivo antigen-antibody reactions. The experiments were designed to determine whether or not the injection of both specific antigen and antibody into a normal animal would produce any changes in the volume of fluid in which T-1824 and the thiocyanate ion were distributed.

The present experiment afforded no immunologic method for detection of the instant when the maximal physiologic changes appeared as did the serum sickness experiments. No positive clinical signs developed to serve as guides. The only alternative for statistical evaluation was to compare the base line values with the physiologic measurements observed at various arbitrary time intervals after the injection of both antigen and antibody. Statistical analyses were therefore conducted in which the base line values were compared with the mean of the response values obtained 6 and 24 hours after the injection of these substances.

In the experimental group these analyses have shown a statistically significant increase in the thiocyanate space when the mean of the 6 and 24 hour determinations is compared with the base line values. Such changes were not observed in the control group.

The observed increase in the thiocyanate space may have been due to one or more of the following causes: interstitial edema, a shift of water from the intracellular to the extracellular compartment, a liberation of cellular water as a result of cell destruction, or an increase in the permeability of membranes to the thiocyanate ion. However, the rapidity with which the alterations in the thiocyanate space occurred and the lack of correlation with the fluctuations in body weight fit best with the interpretation that an abnormal increase in the permeability of cell membranes permitted a wider distribution of the thiocyanate ion. Since no changes in plasma volume were observed, there were no evidences to suggest any alteration in capillary permeability.

The results of the present experiment may be interpreted as providing additional evidence for the view that physiologic alterations which are measurable quantitatively, and which are not clinically obvious, can occur when *in vivo* antigen-antibody reactions take place. The most logical explanation of the results is provided by the assumption that an increase in the permeability of cellular membranes had occurred. Such changes cannot be detected by following the body weight, since the fluid is simply redistributed between two compartments. The alteration in the hydration of cells, with resultant ionic interchange, may cause significant changes in the cellular milieu, and hence in the function of intracellular enzyme systems.

SUMMARY

In a further study of immunophysiology, the passive transfer method was utilized in rabbits in an effort to determine the possible relationship between *in vivo* antigen-antibody reactions and certain alterations in the plasma volume and thiocyanate space. In animals given intravenous injections of antibody, followed by the interscapular administration of specific antigen, an increase in the thiocyanate space was detected within 24 hours after injection of the antigen. Such a change was not observed in control animals given either specific antigen or antibody. This increase in the thiocyanate space has been interpreted as indicative of an abnormal increase in the permeability of cell membranes which was directly or indirectly due to the antigen-antibody reaction.

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