THE IMMUNOPHYSIOLOGY OF SERUM SICKNESS. ALTERATIONS IN THE BLOOD VOLUME AND THIOCYANATE SPACE IN RELATION TO THE DEVELOPMENT OF HUMORAL ANTIBODIES IN THE RABBIT[1]

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The development of edema during the course of acute infectious diseases has been observed in patients for many years. The retention of water and salt during the course of untreated pneumococcal pneumonia was described early in the century (1). The loss of chlorides from the blood and their subsequent excretion in the urine during recovery was shown to be associated with retention by the body of sodium and calcium while potassium and magnesium were excreted normally or in excess.

After antipneumococcal horse serum became available for treatment of pneumonia, serum sickness developed frequently in patients during convalescence. The clinical picture of serum disease with edema and fever was shown to be accompanied by the retention of chlorides and water in the body (2). Further investigation of serum disease in human beings revealed that the clinical symptoms are associated with the appearance in the blood of precipitins against the species of animal in which the therapeutic serum was prepared (3). The appearance of precipitins precedes recovery from the disease by a short interval and is coincident with disappearance of the antigen. These studies indicated that antigen-antibody reactions were of clinical significance in the production of disease in human beings, and that the mechanism was in some way related to that of experimental anaphylaxis in animals.

During the course of studies on patients with Rocky Mountain spotted fever, it was observed that alterations in the distribution of body fluids with the development of marked clinical edema reached a maximum just before clinical recovery begins. The alterations in distribution of fluid appear to predispose to peripheral circulatory collapse. In such crises the blood chlorides, blood volume and extra-vascular thiocyanate space are altered transiently but return to normal with recovery (4). Observations in one patient who had been given hyperimmune rabbit antiserum for Rocky Mountain spotted fever showed that the alterations in the blood volume and thiocyanate space, which occurred during classic serum sickness following recovery from the initial disease, resembled strikingly those seen in the same patient during the acute phase of the rickettsial infection. These observations suggested the possibility that an immune reaction may be directly or indirectly responsible for the changes in the permeability of membranes which permit alterations in fluid distribution in rickettsial spotted fever.

The present experiments were undertaken to determine if changes in the blood volume and thiocyanate space occur in an antigen-antibody reaction induced *without infection* and to relate the physiologic alterations to the development of immunity. The anatomic changes occurring with serum sickness have been well studied, but the physiologic alterations induced by the disease are very poorly understood (5). Accordingly, rabbits were injected with human plasma and the changes in distribution of body fluids were followed serially.

MATERIAL

Domestic rabbits of mixed breeds, weighing 2 to 4 kilograms each, were placed in individual cages and fed a stock diet, supplemented once weekly by fresh green vegetables. Water was given without restriction.

The same sample of pooled human plasma was used as the sensitizing antigen and as antigen for the precipitin tests in each individual experiment.

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METHODS

Chemical techniques: The *plasma volume* was determined by the T-1824 (Evans Blue) dye method. Syringes holding 2 ml. were calibrated to deliver 0.5 ml. of a solution containing 3 mg. of the dye per milliliter. The *thiocyanate space* was determined simultaneously. Syringes holding 10 ml. were calibrated, and 2.0 ml. of a solution containing 50 mg. of sodium thiocyanate per milliliter were injected.

The concentrations of the dye in rabbit serum were determined by the Evelyn photoelectric microcolorimeter and the thiocyanate concentrations with the Evelyn photoelectric macrocolorimeter (6). *Hematocrit* readings were made on oxalated blood placed in Wintrobe tubes, centrifuged at 3,000 revolutions per minute for 30 minutes.

The *total blood volume* was calculated from the plasma volume and the hematocrit.

Immunologic techniques: The humoral antibody titer was followed by ring *precipitin tests.* The human plasma was diluted 1:100 in water and used as antigen to overlay rabbit serum serially diluted in 2 × 20 mm. tubes to a maximum of 1:32. In the definitive experiment human plasma was diluted in physiologic saline, but the rabbit serum was undiluted. In the preliminary experiments dilution of human plasma (antigen) as high as 1:100,000 gave positive tests with undiluted rabbit serum (antibody) as definite as those obtained with 1:100 dilution of antigen and 1:32 dilution of antibody.

The dye was diluted as much as 1:50,000 for precipitin tests in group 1; no precipitins were encountered, so the test was not performed in later groups.

Skin tests to the dye were never positive in group 1; so they were not performed in later groups. No skin tests were done with plasma in any group.

Experimental procedure: In the morning before feeding the animals were weighed and strapped on their backs to wooden boards. In groups 1 and 2 blood was withdrawn without anesthesia in an oiled syringe by cardiac puncture.

Comparison of data indicated that reliable results could be obtained without deaths from hemopericardium by nicking an ear vein with a knife and collecting the blood in dry test tubes. Accordingly in group 3, 2 ml. were collected and allowed to clot; 1 ml. was then collected in another dry tube containing 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate. The dye was injected into a vein in the opposite ear, the syringe changed, and the sodium thiocyanate injected through the same needle within one minute. Ten minutes following completion of the injection, 2 ml. of blood were collected from the first ear.

Preliminary experiments: No data were available on the reliability of the technical methods in small animals, on the effect of repeated bleedings at short intervals, or on the normal values for plasma volume and thiocyanate space in rabbits. It was not known whether rabbits might become sensitized after repeated injections of the dye and hence develop a picture analogous to serum sickness.

Accordingly, six animals (group 1) were used as a control group on the technical procedures. Determinations of the blood volume, thiocyanate space and precipitin titers, as well as a skin test, were done simultaneously every second or third day. The animals received no plasma. They were kept under observation for a minimum of 30 and a maximum of 46 days. A minimum of nine and a maximum of 16 determinations were done on each individual animal.

A second experiment was performed in order to study the variability of the physiologic changes in individual animals ill with serum sickness. It was also necessary to determine the optimum route of sensitization, the required dose of plasma and the time relationship of any observed alterations to the development of humoral immunity.

Accordingly, 11 animals (group 2) were injected with plasma. The two groups were treated in the same fashion except for the sensitizing injections of plasma. After a baseline for the individual animal had been established, the rabbits in group 2 received injections of plasma ranging from 2.2 to 5.0 ml. per kilogram of body weight. Eight animals received the sensitizing dose intravenously and three were injected subcutaneously in the intrascapular region. Three of the former animals received a second injection intravenously on the day following the initial injection.

During the clinical peak of the disease, determinations of the blood volume and thiocyanate space were done on two successive days. All animals in group 2 were followed at least 24 days following the injection of plasma, unless accidental death from hemopericardium supervened. A minimum of seven determinations were done on each animal in group 2 after the baseline was established.

In order to interpret the data critically a satisfactory statistical method of analysis had to be devised. It was necessary to determine whether groups could be compared or whether each individual animal must serve as its own control. The data from groups 1 and 2 were analyzed by several techniques and the results obtained were submitted to an impartial arbiter for criticism.[2] A satisfactory statistical method was devised and the definitive experiment designed to improve the statistical significance of the data obtained in the preliminary experiments.

Definitive experiment: Twelve rabbits (group 3) of more uniform size, weighing approximately 2 kilograms each, were injected with human plasma. The chemical and immunologic techniques and experimental procedure as described above were followed. At least two baseline determinations were made every second day on each animal. After the second determination, each animal was injected intravenously with a single dose of 3 ml. of plasma per kilogram of weight. Precipitin tests were performed daily on each rabbit, but no further fluid vol-

[2] We are deeply indebted to the Institute of Statistics of the University of North Carolina, Raleigh, N. C., for critical review of the data and for suggesting the method subsequently used.

ume determinations were done until the day that a positive precipitin reaction was first obtained. The measurements were repeated on that day, on two consecutive days thereafter, and at varying intervals subsequently until the chemical values had returned approximately to the baseline.

Statistical analysis: The total blood volume, plasma volume, hematocrit and thiocyanate space were recorded in absolute values. The mean of two successive baseline determinations on each animal was compared with the mean of the two successive determinations made on the day the precipitins became positive and on the following day. The mean differences between the baseline values and those obtained after the precipitin test became positive were calculated. The significance of the mean difference was tested by the following formulae in which $\bar{d}$ = mean difference, $s_{\bar{d}}$ = standard error of the mean difference, S = summation of, n = number of animals:

$$t = \frac{\bar{d}}{s_{\bar{d}}} \qquad s_{\bar{d}} = \sqrt{\frac{Sd^2 - \frac{(Sd)^2}{n}}{n(n-1)}}.$$

The calculated value for t was compared with the value at $n-1$ degrees of freedom as obtained from standard tables.

The data as obtained in absolute values were then calculated in terms of milliliters per kilogram of body weight on the day the measurement was made. The statistical significance was determined by the same formulae.

In view of the fact that the experiment was planned to evaluate changes in a febrile illness occurring in a growing animal the difference in the amount of food and water ingested by the animals unavoidably introduced variables which were difficult to control. Accordingly, the data were re-calculated in terms of milliliters per kilogram of *initial* body weight (the day of the first baseline determination) in order to avoid consideration of differences in food and water intake and in order to permit ready comparison with the data in group 1. The statistical significance was then determined by the same method.

It should be noted that in the definitive experiment no attempt was made statistically to compare the experimental group as a whole with the control group. Each animal served as its own control.

RESULTS

Control on methods: None of the animals in group 1 showed any symptoms or signs suggesting serum sickness. No reaction was noted in any rabbit to the repeated intravenous injection of dye and no evidence of sensitization was detected by skin or precipitin tests. The fatal dose of T-1824 dye was found to be 3 mg. per kilogram of body weight given intravenously; death occurred within 15 minutes.

Moderate variations in *plasma volume, thiocyanate space* and *total blood volume* per kilogram of body weight and in the *hematocrit* were noted among the different animals. In serial determinations on a single rabbit, however, the results were much more uniform. When the data obtained near the end of the experiment were examined, a slight decrease in the thiocyanate space in relation to

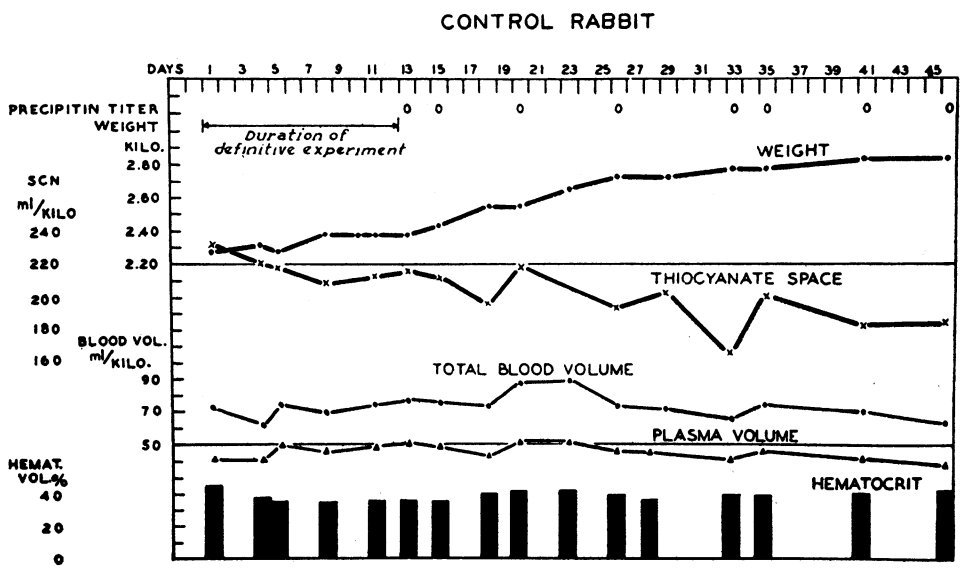

FIG. 1. FLUID SPACES IN A CONTROL RABBIT (GROUP 1) NOT INJECTED WITH PLASMA

All of the serial determinations are plotted in this graph; only four values which fall within the indicated duration of the definitive experiment are used in the calculations in Tables I and II.

TABLE I

Control of technical methods (group 1)

Rabbit number	Baseline					Time of expected response				
	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit
	Kilo.	*ml./Kilo.*	*ml./Kilo.*	*ml./Kilo.*	*Vol. %*	*Kilo.*	*ml./Kilo.*	*ml./Kilo.*	*ml./Kilo.*	*Vol. %*
1	2.30	219.6	44.2	68.8	36.0	2.39	216.1	49.4	75.5	34.5
2	3.04	197.8	47.0	68.8	31.3	3.04	197.0	55.8	87.2	35.7
3	2.53	210.3	51.3	73.5	30.0	2.78	193.8	49.8	72.8	31.5
4	3.27	177.5	43.0	63.5	33.3	3.24	162.5	40.0	62.1	35.5
5	2.76	207.7	49.7	77.3	35.8	2.93	198.1	47.9	73.3	34.5
6	2.73	155.3	42.7	67.0	36.3	2.92	162.2	45.0	62.7	28.0
Mean for group	2.77	194.7	46.3	69.8	33.8	2.88	188.3	48.0	72.3	33.3
s	3.05	24.00	3.62	4.90	2.68	0.29	21.50	5.27	9.27	2.99
s_x	0.14	4.69	1.47	2.00	1.08	0.12	8.80	1.02	3.78	1.22

The mean of two consecutive determinations at the start of the experiment is compared with the mean of two consecutive determinations in the same animal six to eight days after the baseline was established. No injections of plasma were made.

s = Standard deviation

s_x = Standard error of the mean

weight was observed; this finding would be expected as the animals increased in size from normal growth. Figure 1 illustrates the serial values in a representative control rabbit.

The mean of two consecutive baseline determinations is recorded in Table I and compared with the mean for two consecutive determinations done between six and eight days after the second determination, the time interval at which a positive precipitin test would be expected were the animals injected with plasma. The statistical analysis is summarized in Table II and indicates that, in absolute values or in milliliters per kilogram of body weight on the day of the determination, no significant difference was found. This statement is true for plasma volume, thiocyanate space, total blood volume, hematocrit, and weight.

These data have subsequently been extended in a series of 45 animals in conjunction with other studies on normal values in rabbits (7).

General observations: Clinical serum sickness, manifested by erythema, edema, or both at the base of the ears, generalized subcutaneous edema and hyperirritability developed in 10 of the 11 animals in group 2, and in 11 of the 12 animals in group 3. Clinical signs usually began on the fifth or sixth day following the initial injection of plasma and usually lasted two or three days. The edema was most striking in the skin over the abdomen, but was not evident unless the fur had been clipped; the skin appeared pale and felt soggy, hot, and moist. Fever (rectal temperature over 40 C.) was not a constant feature of clinical serum sickness. The clinical severity of serum sickness bore no relation to the size of the sensitizing dose. Multiple injections produced no more severe reactions than did single injections. The subcutaneous route of sensitization was as effective as the intravenous route, but variations in absorption could not be detected or controlled.

TABLE II

Statistical significance of controls on methods (group 1)

	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit
	Kilo.	*ml.*	*ml.*	*ml.*	*Vol. %*
$\bar{d}$	+.11	+4.33	+10.00	+15.33	+.50
$S\bar{d}$	.05	13.60	5.30	9.35	1.81
t	2.20	.32	1.89	1.64	.28
		ml./Kilo.	*ml./Kilo.*	*ml./Kilo.*	
$\bar{d}$		−6.42	+1.67	+2.45	
$S\bar{d}$		3.67	1.90	3.58	
t		1.75	.88	.68	

No significant difference is noted between the baseline values and those obtained at the time of the expected response. The data are calculated in absolute values and in terms of weight on the day of the determination. The value of t was not considered significant if P was greater than .01.

Preliminary experiments: The alterations in plasma volume, thiocyanate space, total blood volume, and hematocrit which were observed in a representative rabbit sensitized with human plasma are illustrated in Figure 2. The changes in fluid distribution bore no direct relationship to the sensitizing dose of plasma, but paralleled the clinical severity of the disease.

In 10 of the 11 animals in group 2 increases in the *thiocyanate space* developed at some time during the period of observation. In eight animals two rises occurred, the first usually between the third and sixth days, and the second between the 11th and 20th days. The first rise usually coincided with the onset of the clinical manifestations; the second was usually marked by an increase in the subcutaneous edema. The changes in the thiocyanate space usually paralleled the weight changes, especially with the second reaction. Certain discrepancies were noted, however.

The most profound drop in *plasma volume* was noted in those animals which developed the severest clinical signs of serum sickness and the greatest and most prolonged increases in thiocyanate space. In seven animals there was no significant decrease in plasma volume, however.

In the four animals which showed a definite drop in plasma volume an appreciable decrease in the *total blood volume* also occurred. In four animals no changes in the total blood volume occurred during the experimental period.

In all the animals with severe early clinical manifestations of serum sickness a drop in *hematocrit* was associated with a rise in thiocyanate space and a decrease in plasma volume. In six animals no striking variations in the hematocrit occurred

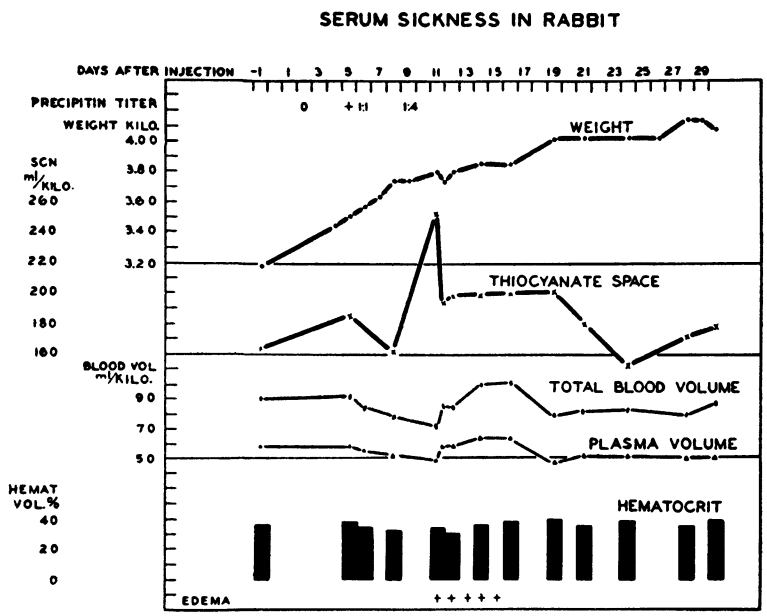

FIG. 2. FLUID SPACES IN A RABBIT (GROUP 2) GIVEN AN INTRAVENOUS INJECTION OF HUMAN PLASMA

Note the increase in thiocyanate space, which is out of proportion to the slight increase in weight, on the 11th day and the associated decrease in the plasma and total blood volume. On the following day, there was a marked decrease in the thiocyanate space, which was out of proportion to the decrease in weight. All the thiocyanate values from the 11th to the 21st day were elevated. After the 21st day, the variations in the thiocyanate space paralleled the weight changes and were not significant.

In the statistical calculations in the definitive experiment (group 3), Tables III and IV, two baseline values, two values obtained at the time precipitins appeared, and two values obtained at the termination of the experiment were used.

TABLE III

Alterations in fluid spaces in rabbits ill with serum sickness after an injection of human plasma (group 3)

Rabbit number	Baseline					Response*				
	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit
	Kilo.	*ml./Kilo.*	*ml./Kilo.*	*ml./Kilo.*	*Vol. %*	*Kilo.*	*ml./Kilo.*	*ml./Kilo.*	*ml./Kilo.*	*Vol. %*
18	2.10	228.5	46.0	75.5	39.0	2.10	322.5	48.0	74.0	35.0
19	2.14	235.5	48.5	80.0	40.0	2.14	242.5	47.0	75.5	37.5
20	1.99	246.5	48.5	79.5	39.0	2.14	282.5	39.5	61.5	35.7
21	1.87	245.5	45.0	75.5	40.5	1.86	296.0	39.0	62.5	37.5
22	1.74	289.5	48.5	78.5	37.5	1.96	273.0	44.0	68.5	35.2
23	1.87	266.0	45.5	74.0	38.5	2.04	313.5	40.0	62.0	36.0
24	1.76	229.5	50.0	80.0	37.0	2.05	281.5	35.0	54.5	36.0
25	2.10	212.5	51.0	84.0	39.5	2.30	265.0	39.0	62.0	36.7
26	2.22	183.0	54.0	88.5	39.0	2.24	225.5	40.5	62.5	35.7
27	1.76	215.0	63.0	108.0	42.0	1.93	280.0	47.0	78.0	40.0
28	1.96	202.5	52.0	85.5	39.5	2.07	307.5	40.5	63.5	36.0
29	1.85	220.5	54.0	92.0	41.0	1.90	311.0	48.5	78.0	38.5
Mean for group	1.95	231.2	50.5	83.4	39.4	2.06	283.4	42.3	66.9	36.7
s	0.17	28.60	4.95	9.47	1.38	0.13	28.00	4.40	8.44	1.48
s_x	0.05	8.21	1.45	2.73	0.40	0.04	8.42	1.27	2.54	0.42

* The mean of two consecutive determinations at the start of the experiment is compared with the mean of two consecutive determinations in the same rabbit done on the day precipitins to human plasma appeared and on the next day.
s = Standard deviation
s_x = Standard error of the mean

with the initial increase in thiocyanate space. None of the animals revealed a decrease in hematocrit in association with the second rise in thiocyanate space.

TABLE IV

Statistical significance of alterations in fluid spaces in rabbits ill with serum sickness (group 3)

	Weight	Thiocyanate space	Plasma volume	Total blood volume	Hematocrit
	Kilo.	*ml.*	*ml.*	*ml.*	*Vol. %*
$\bar{d}$	+.11	+126.00	−11.08	−24.67	−2.73
$S\bar{d}$	.03	21.40	2.81	4.57	.23
t	3.70	5.89	3.94	5.40	11.87
		*ml./Kilo.**	*ml./Kilo.*	*ml./Kilo.*	
$\bar{d}$		+51.30	−8.50	−16.50	
$S\bar{d}$		9.85	1.49	2.60	
t		5.21	5.70	6.35	
		ml./Kilo.†	*ml./Kilo.*	*ml./Kilo.*	
$\bar{d}$		+74.16	−6.25	−12.25	
$S\bar{d}$		9.98	1.23	2.35	
t		7.43	5.08	5.21	

* Calculated in terms of weight on the day of determination.

† Calculated in terms of weight on the day of the first baseline.

The data all show statistically significant differences between the baseline values and those obtained at the time of the humoral antibody response. The value of t was considered significant if P was <.01.

In all 11 animals positive *precipitin tests* to human plasma developed between the third and the 12th days following the initial injection of plasma. In eight animals the thiocyanate space decreased and the clinical symptoms and signs subsided as the precipitin tests became positive. In all animals except one the precipitin tests remained positive throughout the period of observation. In this animal, which was followed longer than the others, the precipitin test was negative after the 28th day.

Definitive experiment: The alterations in plasma volume, thiocyanate space, total blood volume, hematocrit and body weight are summarized in Table III.

The statistical analyses are summarized in Table IV.

A statistically significant increase in *thiocyanate space* was noted in each animal at the time the humoral antibody was first detected. In all instances a return to baseline values had occurred by the 20th day after injection of the sensitizing dose. A significant increase in body *weight* oc-

curred during the period of alteration in thiocyanate space. The body weight continued to increase from normal growth after the animal recovered from serum disease.

In contrast to the increase in thiocyanate space and weight, a statistically significant decrease in *plasma volume, total blood volume* and *hematocrit* occurred in all animals.

All of the data are statistically significant, whether the calculations are based on the comparison of *1*) absolute values; *2*) values based on milliliters per kilogram of weight on the day of the determination; or *3*) values based on milliliters per kilogram of weight on the day of the first baseline measurement.

In order to determine whether these changes were reversible, the mean of two consecutive determinations at the termination of the experiment was compared with the mean baseline values in each animal. The mean differences for the body weight, plasma volume, total blood volume, and the thiocyanate space were not significant. The decrease in the hematocrit at this time was significant. These data are not included in the tables.

In all 12 animals positive *precipitin tests* to human plasma developed between the fourth to the eighth day after injection. The distribution was as follows:

	Onset of positive precipitin tests				
Days after injection	4	5	6	7	8
Number of rabbits	2	1	2	4	3

DISCUSSION

The experiments indicate that immune reactions may be associated with alterations in the blood volume and the fluid space which is available for the diffusion of thiocyanate ion. The maximum alterations in rabbits sensitized with pooled human plasma occurred at the time circulating humoral antibodies (precipitins) appeared in the serum. This finding correlates well with the previous clinical observation that the symptoms of serum sickness begin to disappear with the appearance of precipitins (3).

The greatest change was observed in the thiocyanate space. Alterations in the plasma volume and the hematocrit were less pronounced in magnitude and were more transient, lasting only one to three days as compared with the changes in thiocyanate space which occasionally persisted into the second week. The weight of the animals tended to increase with the increase in thiocyanate space, though the changes could not always be correlated closely. Apparently the normal distribution of fluid in the body was altered as the result of an increase in permeability of membranes which was completely reversible.

Methods: The chemical methods were reliable since the results in the control animals (group 1) were consistently reproducible. The possibility that the dye might act as an antigen, producing serum sickness, or as a haptene, forming a new compound which might induce an auto-antibody response, was ruled out by the preliminary experiments.

The variability between individual animals in the experimental group (group 3) was controlled by determining baseline values on each rabbit and comparing these values with the response in the same animal for the statistical analysis. The effect of growth and of variations in food and water intake was apparently of little significance in this experiment, since the data were found to be statistically significant whether calculated in terms of absolute values or in relation to weight at the time of the first baseline determination or on the day of the immunologic and clinical response.

Though whole plasma is a complex of many protein fractions, each of which might induce its own slightly different immunologic response, it was felt that a mixed antigen might more closely simulate a natural infection in which multiple antigens are liberated by the infecting organism. Anatomic studies have indicated that the body responds at different times after the injection of albumin or globulin fractions derived from the same plasma (5). The two peaks in the thiocyanate space observed in the preliminary experiments may be due to this difference.

Permeability of membranes: The present experiment offers a logical explanation for the phenomenon of retention of salt and water; if the permeability of membranes to ions is increased, these ions could diffuse from blood into interstitial spaces and from interstitial fluid spaces into cells. The

resultant hypochloremia would decrease the renal excretion of salt and water. The administration of saline would increase the edema, until restoration of the integrity of membranes corrected the alterations in the internal environment. It is not clear whether the physiologic alteration is confined solely to vascular walls, as pathologic experiments might suggest, or whether cellular membranes are also affected. Von Pirquet suggested that the edema was interstitial, but the data in the present experiments may indicate that some of the change occurs within cells (8).

Capillary permeability: The alterations in the vascular tree can be visualized as a series of pores in the capillary wall whose size is related to the clinical severity of the disease. In mild instances of the disease water and ions which normally traverse the membrane would leave the blood more readily. Larger molecules such as protein would be lost in more severe instances and red blood cells would pass out of the vascular tree only in the most severe reactions. Petechial hemorrhages in organs have been recognized for years as a manifestation of anaphylactic reactions.

The loss of water and ions from the vascular tree into tissues is compatible with the observations on retention of water, sodium and chloride during pneumonia and serum disease (1, 2). The permeability of the vascular tree to some colloids must have been increased because the decrease in plasma volume was concomitant with the increase in thiocyanate space. The colloids lost from the circulation to the interstitial spaces would tend to retain water extravascularly until they had been reabsorbed, presumably through the lymphatics, broken down in the tissues, or until other homeostatic mechanisms had been operative.

It is probable that the amount of protein lost from the vascular tree during serum disease is not great. The dye is known to be bound to albumin, one of the smaller protein molecules of the blood. Any great loss of albumin into interstitial spaces would reduce the amount of circulating dye and hence lead to a larger value for plasma volume by the chemical method; a loss in blood volume of any magnitude would thus be obscured. If protein was lost from the vascular tree, the defect was detected only with the first rise in thiocyanate space and was transient.

The drop in hematocrit though slight may indicate a loss of red blood cells from the vascular compartment. The drop was apparently not due to repeated bleeding since the hematocrit remained constant in group 1 in which more determinations were done in the same interval of time. No jaundice was noted clinically and the serum was never icteric, hence it seems unlikely that the red cells were hemolyzed. It is possible that the red cells were reduced in size or that they were pooled in some portion of the vascular tree which was not measured by the techniques used.

Cellular permeability: It is not known whether the thiocyanate ion normally diffuses into cells or not. The increase in thiocyanate space therefore could be due to *1*) interstitial edema, *2*) increase in permeability of cells to the thiocyanate ion, *3*) destruction of tissue cells with liberation of cellular water, or *4*) dehydration of cells with a shift of intracellular fluid into the interstitial spaces. The fact that some animals showed a sudden increase in thiocyanate space without an increase in body weight would favor the thesis that some redistribution of fluid had occurred whether or not additional fluid ingested in food or water was retained. The hypothesis that tissue cells were destroyed, liberating additional water and making it available for dilution of thiocyanate ion, does not seem likely. The rapidity of the increase in the thiocyanate space and its reversibility makes this explanation appear highly improbable. Such a sudden increase in thiocyanate space without an increase in body weight would have to mean that the associated tissue destruction exceeded the amount of interstitial edema. Furthermore, preliminary nitrogen balance studies in other rabbits have disclosed no marked increase in the excretion of urinary nitrogen, and presumably in cell destruction, during clinical serum sickness.

Mechanisms: It is not known whether the antigen in serum disease attaches solely to the vascular wall or to tissue cells outside the vascular system as well. As far as the effect on the circulation is concerned, it would be immaterial whether the loss of substances from the vascular tree is attributable to an abnormal passage of fluid between the endothelial cells, as would occur if the permeability of the intercellular cement substance were decreased, or whether the permeability of the

cells themselves is abnormally increased, so that the fluids pass through the cells into the interstitial spaces.

The participation of cells in immune reactions is recognized clinically in allergic states; the deleterious antigen-antibody reaction is assumed to occur on the cell surface or perhaps within the cell. It has been postulated that histamine or some similar substance is released during the interaction of antigens with antibodies. Histamine will alter the local permeability of capillary walls. The liberation at the cell wall of some substance which would increase the permeability of the cell membrane would enhance the transmission of molecules, including the thiocyanate ion, across the surface. Such a hypothesis is of more than academic interest, since it is known that intracellular enzyme systems are affected by the extent of hydration of cells and by the concentration of ions. An increased cellular content of ions could be proved by isotopic techniques which could measure not only the diffusion of labelled thiocyanate but the relative movement of radioactive sodium, potassium, and chloride ions across cell membranes.

Relation to infections: The present findings indicate that the physiologic alterations which may result from antigen-antibody reactions are more profound than has been recognized in the past. The physiologic changes deserve more attention in the therapy of infectious diseases. In infections, the invading micro-organisms multiply within the host and release or secrete multiple antigens. With organisms known to locate intracellularly during some phase of the disease, as is true with the viruses and rickettsias, antigens are produced within the cell. Those antigens which could readily diffuse out would produce predominantly humoral antigen-antibody reactions; those antigens which would be retained within cells would be more likely to induce intracellular antigen-antibody reactions and hence increase the extent of the permeability of cellular walls. A most profound alteration in functions of the body could thus be produced. The possible application of antihistaminic drugs or adrenal cortical hormones to therapy of infections with an allergic element in the host response is based on such alterations in function.

SUMMARY

1. Rabbits sensitized by the intravenous injection of pooled human plasma developed clinical edema with significant increases in weight and in the fluid space available for dilution of thiocyanate ions.

2. Significant decreases in the plasma volume, total blood volume and hematocrit were observed transiently during the most marked alteration of thiocyanate space.

3. The changes were interpreted as indicating an increase in permeability of the vascular tree, probably in the capillary wall, so that crystalloids, colloids, and red blood cells are progressively lost from the circulation as the magnitude of the defect increases with increase in the clinical severity of the disease.

4. Occasional discrepancies observed between the changes in thiocyanate space and in weight suggest that cellular permeability is probably also increased to water and ions.

5. The physiologic changes occurred at the time humoral antibodies (precipitins) appeared, suggesting that the alterations may have been due directly or indirectly to an antigen-antibody reaction.

REFERENCES

1. Peabody, F. W., Studies of the inorganic metabolism in pneumonia with especial reference to calcium and magnesium. J. Exper. Med., 1913, **17**, 71.
2. Rackemann, F. M., Longcope, W. T., and Peters, J. P., The excretion of chlorids and water and the renal function in serum disease. Arch. Int. Med., 1916, **18**, 496.
3. Longcope, W. T., and Rackemann, F. M., The relation of circulating antibodies to serum disease. J. Exper. Med., 1918, **27**, 341.
4. Harrell, G. T., and Aikawa, J. K., Pathogenesis of circulatory failure in Rocky Mountain spotted fever. Arch. Int. Med., 1949, **83**, 331.
5. Hawn, C. V., and Janeway, C. A., Histological and serological sequences in experimental hypersensitivity. J. Exper. Med., 1947, **85**, 571.
6. Rutstein, D. D., Thomson, K. J., Tolmach, D. M., Walker, W. H., and Floody, R. J., Plasma volume and "extravascular thiocyanate space" in pneumococcus pneumonia. J. Clin. Invest., 1945, **24**, 11.
7. Aikawa, J. K., Fluid volumes and electrolyte concentrations in normal rabbits. Am. J. Physiol., 1950, **162**, 695.
8. Von Pirquet, C. E., Allergy. Arch. Int. Med., 1911, **7**, 259.