THE EFFECTS OF VARIOUS HORSE SERUM FRACTIONS IN PRODUCING CARDIOVASCULAR AND RENAL LESIONS IN RABBITS*

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PLATES 40 AND 41

(Received for publication, July 29, 1949)

The clinical and pathological similarities between rheumatic fever in man and serum sickness in man and the rabbit have been observed by many (1–8). But the value of serum disease of rabbits as an experimental tool has been limited by our ignorance of the pathogenesis of the cardiac and arterial lesions which accompany it. Some of the difficulty in understanding the factors responsible for these rheumatic-like (4, 9) lesions may be due to the multiple antigenic components in horse serum which may obscure cause and effect relationships. In recent experiments from this laboratory the lack of correlation between certain antemortem observations (Arthus reaction, humoral antibody levels, etc.) and the development of arterial and cardiac lesions was emphasized and it was suggested that more information might be gained by studying the effects of injecting large quantities of *purified* proteins (10).

Meanwhile Hawn and Janeway (11) have investigated the effects of single large injections of highly purified bovine albumin and gamma globulin into rabbits. Their results indicate that the lesions resulting from the injection of purified beef gamma globulin tend to be localized to the renal glomeruli and to develop relatively quickly, while those following the injection of beef albumin are largely confined to the arteries and heart valves and are delayed. Further investigations of this type seem justified since an understanding of these selective local reactions might add much to our knowledge of tissue hypersensitivity and its relationship to rheumatic fever and glomerulonephritis in man.

The experiments which are reported here represent an extension of this type of study in which the antemortem and pathological responses of rabbits to several partially purified fractions of horse serum protein were observed. Inasmuch as previous reports of the lesions of rabbit serum disease have been largely descriptive, we propose to present our pathological data so that the comparative frequency and relative proportions of the lesions in the various organs and tissues are apparent.

* This study was aided by the Grace M. Closssen Johnson Memorial Fund of the La Rabida Jackson Park Sanitarium.

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These experiments differ considerably from those of Hawn and Janeway (11). Each rabbit received 2 injections of the same antigen, 4 horse serum fractions were employed instead of the 2 beef serum fractions, and the Arthus reaction, sedimentation rate, and rectal temperature were determined in addition to the serological studies.

Plan of Experiments

Two identical experiments were performed about 6 months apart. In each experiment 5 groups, each composed of 5 rabbits, were selected so that an equal sex and approximately equal weight distribution was present in each group. After obtaining 2 pre-injection sedimentation rate determinations and 2 temperature measurements on alternate days, the animals of each group were injected in the lateral ear vein with 0.65 gm. per kg. of horse serum protein or one of the serum fractions. Following this first injection, sedimentation rates were obtained on days 4, 7, 10, and 14 and rectal temperatures on days 3, 6, 9, and 13. Skin tests were performed on days 7 and 14. Blood for serology was obtained on days 0, 7, and 14. The second injection was equal in quantity to the first and was given on the 15th day, following which temperatures were taken on days 16, 18, and 21 and sedimentation rates on days 17, 19, and 22. Skin tests were performed on the 21st day. Sera for antibody titers were obtained on the 22nd day and the rabbits were then sacrificed and autopsied. Five rabbits were included as controls (3 in the first experiment and 2 in the second). They were bled with the experimental animals but received no injections or skin tests. Thus we planned at the completion of the experiments to have for histological examination 10 rabbits which had received 2 injections of whole horse serum and 4 groups of 10 rabbits each of which had received 2 injections of one of the fractions of horse serum (II, III, IV-3,4, or V) and a group of 5 rabbits which had received no injections.

Materials and Methods

Horse Serum and Fractions.—A single large lot of sterile normal serum without preservative was contributed by the Lilly Research Foundation. A portion of this was set aside to be used for the whole serum injections and the remainder was fractionated using the cold alcohol procedure of Cohn and his coworkers (12). Electrophoretic analyses of the fractions are given in Table I and were determined in phosphate buffer at pH 7.7, ionic strength 0.2, using a 2 per cent protein solution. Owing to limitations in quantity of horse serum available it was not possible to further purify these fractions to obtain pure albumin (fraction V) and gamma globulin (fraction II). It was realized that this would seriously interfere with the interpretation of serological tests. It was hoped, however, that the inclusion of the additional globulin fractions III and IV-3,4, the former low in albumin and the latter low in gamma globulin, would somewhat offset this disadvantage and would aid in interpreting the pathological lesions as well as the antemortem observations.

The original serum contained 6.5 per cent protein. Accordingly, each of the fractions was made up to this same concentration in 0.86 per cent saline and adjusted to about pH 7.5

with sodium bicarbonate. The whole serum and fractions V and IV-3,4 were filtered through a medium Berkefeld candle while fractions II and III required a coarse Mandler filter. In each instance the first 25 ml. of filtrate was discarded to avoid protein loss by adsorption. Specific gravity determinations before and after filtration revealed no appreciable change when this procedure was followed and all samples were found to be sterile.

Rabbits.—Rabbits of a mixed breed (Dutch, American blue, and domestic) were used. All were albinos with the exception of 8 which were distributed among the groups. Their weights averaged about 3.0 kg. They were caged singly and were fed Rockland rabbit pellets and water *ad libitum.*

Temperatures, Skin Tests, and Sedimentation Rates.—These were performed by methods which we have described previously (10, 13). The Arthus reactions at 24 hours were graded from 1 to 4 plus by ordinarily accepted criteria (10). There was no visible skin reaction to any of the five fractions injected intradermally into 3 normal rabbits not included in the experiment.

TABLE I

*Electrophoretic Components in Horse Serum and Fractions**

	Whole serum	Fraction II	Fraction III	Fraction IV-3,4	Fraction V
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Gamma globulin	20.2	89.6	28.0	2.6	—
Beta globulin	32.3	9.3	34.0	47.1	1.8
Alpha globulin	16.0	1.1	36.2	23.6	5.3
Albumin	31.5	—	2.0	26.8	88.9

* Analyses were determined in phosphate buffer at pH 7.7, ionic strength 0.2, using a 2 per cent protein solution.

Serological Studies.—The blood obtained for antigen and antibody determinations was allowed to clot and the serum was separated by centrifugation on the same day. It was then stored in the deep freeze at −20°C. until the end of the experiment when all titers were determined simultaneously.

Tests were carried out with each serum for the presence of the circulating serum fraction with which the animal had been injected. The antisera used for these tests were obtained by immunizing one rabbit with each of the appropriate serum fractions using the aluminum hydroxide depot method of Hektoen and Welker (14). All antisera had a titer of 1:640 or 1:1280 when they were used. According to the amount of precipitate formed when the antiserum was mixed with the three dilutions of serum (1:1, 1:10, 1:25) containing antigen, reactions were graded as 0, ±, +, or ++. For purposes of evaluating the data, the reciprocal of the highest dilution of serum showing precipitate was multiplied by the number of pluses and the product was considered to be the "titer" of antigen. Although this method of titration of antigen was complicated by the presence of cross-reactions which may have been responsible for some of the precipitate formed, nevertheless it seems probable that the tests give some approximation of the concentrations of the fractions of foreign protein circulating at any one time and thus the rate of removal of the antigens from the blood stream.

Antibody titers were determined using the collodion particle technique of Cannon and Marshall (15). All sera were inactivated at 56° for half an hour. Some difficulty was encountered in preparing smooth particle suspensions with fractions II and III. This was eliminated by adsorbing these particles for 30 minutes with 0.25 per cent antigen protein

while the particles were coated with the other three antigens in a 1.5 per cent solution for one hour. The coated particle suspensions were centrifuged and resuspended in 0.86 per cent saline before they were added to the serial dilutions of the sera. Although all the possible cross-reactions between the various fractions were titrated, only the titrations to the fractions injected are included in this report.

Pathological Studies.—All animals were sacrificed with air embolism and a standard set of tissue samples was obtained from identical areas of each animal immediately after death. These were fixed in Zenker-formalin solution and were trimmed so that blocks from corresponding tissues in different animals were approximately equal in size. The blocks from which sections were prepared were as follows: one longitudinal block through each of the four cardiac valves including adjacent cardiac muscle; hilum of right lung; mediastinum; liver; spleen and pancreas; stomach (anterior wall near lesser curvature); mesenteric nodes and superior mesenteric artery; left kidney; left adrenal; either left ovary and uterus or left testis; diaphragm; and femoral marrow. In addition, synovia and the site of the Arthus reactions were examined from some rabbits. All blocks were imbedded in Fisher's tissue mat and the sections were stained with Delafield's hematoxylin and aqueous eosin. Additional kidney sections were stained with Mallory's connective tissue stain.

In order to have a more objective and easily recorded means of grading the intensity and frequency of the arterial lesions, the following method was adopted: On the basis of previous experience, the arterial lesions were subdivided into two categories, acute and chronic arteritis. The number of times each kind of lesion occurred in one section from each of the standard set of blocks was counted and tabulated. As a basis for comparing involvement of various organs and tissues, the total number of arteries seen was counted in corresponding sections from each of 4 rabbits. The total number of arteries was found to vary between 180 and 250 in the heart, 21 and 34 in the pancreas, 20 and 38 in the kidney, and 20 and 40 in the stomach. Although there is obvious and unavoidable variation in number of arterial vessels occurring in corresponding sections, it appears that this method of tabulation gives a fairly objective and accurate method for comparing the frequency of occurrence of the lesions in each rabbit.

The characteristics of the arterial lesions were as follows: *Acute Arteritis:* This was a panarteritis, usually accompanied by a rather intense collection of inflammatory cells in the adventitia consisting of a mixture of polymorphonuclear cells and mononuclear cells, an infiltration of inflammatory cells into the media with or without fibrinoid necrosis, and sometimes an intimitis with marked edema and proliferation of the intima, narrowing of the lumen, and infiltration with mononuclear inflammatory cells. Similar lesions have been noted by several previous investigators. For examples see Figs. 2, 3, and 4 (10), Figs. 1 to 9 (18), Fig. 1 (8), Figs. 17 and 18 (17), and Fig. 10 (11). *Chronic Arteritis:* This consisted of foci of mononuclear cells the majority of which were large lymphocytes and macrophages with a few small lymphocytes in the adventitia of the arteries and with no evidence of acute inflammation. Examples of this type of lesion can be seen in Fig. 14 (18) and Fig. 6 (10).

The valvulitis noted was qualitatively similar to that described previously (13) and was graded from 1 to 4 plus depending upon the extent of the proliferative and inflammatory changes observed.

EXPERIMENTAL RESULTS

The data for each of the 2 identical experiments have been examined separately and in view of the relatively small numbers of animals studied there was very good agreement between corresponding groups in the 2 experiments. In the interest of simplicity and brevity the results will be expressed as averages

of the data obtained for all animals receiving a single horse serum fraction. In no instance did this combining of data alter the conclusions to be drawn.

Four rabbits died during the course of the experiment, 1 of anaphylaxis after the second injection of horse serum and 3 of epizootic diarrhea. These animals have not been included in the tabulated results. One of the rabbits delivered 6 fetuses on the 9th day after the first injection of fraction III. It

TABLE II

Averaged Data for Groups of Rabbits Receiving Various Horse Serum Fractions

Fraction injected	No. of animals	Antigen "titer"*			Antibody titer			Arthus‡ reaction			Sedimentation rate		Temperature change	
		1 wk.	2 wks.	3 wks.	1 wk.	2 wks.	3 wks.	1 wk.	2 wks.	3 wks.	2 wks.§	3 wks.‖	2 wks.¶	3 wks.**
											mm./2 hrs.	*mm./2 hrs.*	°C.	°C.
Horse serum	9	26	16	28	184	162	1191	0.9	2.0	1.2	11.1	62.4	+0.3	+0.5
Fraction V (albumin)	10	35‡‡	22	48	28	232	336	0.7	1.6	1.3	6.9	27.2	−0.1	+0.2
Fraction IV-3,4	9	33	1	6	355	266	853	0.8	2.0	2.5	6.1	45.3	+0.7	+0.8
Fraction III	10	19	0	5	384§§	228	744	2.1	1.5	2.0	9.2	33.5	+0.4	+0.3
Fraction II (gamma globulin)	8	19	13	25	230	400	360	0.8	0.6	0.9	4.8	17.2	+0.1	+0.3
Control	5	0	0	0	0	0	0	—	—	—	0.5	1.8	+0.1	+0.2

* For method of computing these "titers" see Materials and Methods.

‡ Average of the total number of pulses for each group.

§ Average of 4, 7, 10, and 14 day determinations.

‖ Average of 17, 19, and 22 day determinations.

¶ Average of 3, 6, and 9 day determinations compared with the average of 2 control period readings.

** Average of 16, 18, and 21 day determinations compared with the average of 2 control period readings.

‡‡ Average of determinations on only 9 rabbits.

§§ Average of determinations on only 5 rabbits.

is of interest that one of the embryos showed a striking acute arteritis of the pancreatic artery.

Antemortem Studies.—The averaged data of the antemortem observations for the 2 experiments are summarized in Table II. Here certain differences are apparent among the groups of rabbits receiving the various serum fractions.

The groups injected with fractions high in alpha and beta globulin, namely fraction IV-3,4 and fraction III, both showed low concentrations (titers) of circulating antigen at 2 and 3 weeks as compared to the groups receiving

whole serum, fraction V, or fraction II. Furthermore, of the various serum fractions injected, the average antibody levels at 3 weeks were about 2 times as high in the groups receiving fractions IV-3,4 and III as in the groups receiving fractions II and V. We interpret these findings to mean that these fractions containing predominantly alpha and beta globulins disappeared relatively quickly from the blood stream whereas the gamma globulin and albumin fractions were retained longer in the circulation resulting in less antibody being formed or a quicker removal of that which was liberated into the blood stream. The antigen titrations performed on the sera of the animals injected with whole serum seemed to correspond most closely with those of the animals receiving fractions II or V whereas the collodion particle tests for antibody in the group receiving whole serum resembled more closely the

TABLE III

Incidence of Pathological Findings in Groups of Rabbits Receiving Various Fractions of Horse Serum

Fraction injected	No. of rabbits	Acute arteritis	Chronic arteritis	Valvulitis	"Aschoff nodules"	Focal pericarditis	Glomerulitis
Whole horse serum	9	7	9	7	5	2	2
Fraction V (albumin)	10	6	9	9	2	0	3
Fraction IV-3,4	9	3	9	8	2	7	0
Fraction III	10	3	9	9	4	3	2
Fraction II (gamma globulin)	8	0	2	4	2	0	0
Total.....	46	19	38	37	15	12	7
Control group	5	0	0	0	0	0	0

results of the titration of the groups receiving fractions IV-3,4 and III. This suggests that when a mixed antigen is used, the titrations reflect the antigenic fraction or antibody present in highest concentration.

As judged by the results of the Arthus reactions the greatest degree of tissue hypersensitivity was present in the groups receiving fraction III or IV-3,4. Similarly, these groups showed greater elevations in sedimentation rates following the second injections and tended to show greater rises in body temperatures after each injection.

These findings would suggest that greater tissue reactivity is correlated with higher antibody titers and relatively quick removal of the antigenic material from the blood stream.

Pathological Studies.—Table III summarizes the main types of pathological lesions found in the various groups of rabbits and tabulates the number of rabbits reacting in each group. Chronic arteritis and valvulitis were the lesions

most consistently observed in the majority of the rabbits. Acute arteritis occurred in more than half of the animals receiving whole horse serum and the albumin fraction but the incidence of this type of lesion was lower in the other three groups. "Aschoff nodules" and focal pericarditis were seen most frequently in the groups receiving fractions high in alpha and beta globulins. Focal necrotizing glomerulitis was seen in only a few animals in the groups receiving whole horse serum and fractions V and III. The incidence of all types of lesions was significantly lower in the group receiving gamma globulin.

Arteritis.—Table IV presents a study of the frequency with which acute and chronic arteritis occurred in the various groups of rabbits. It is apparent that the group receiving albumin showed acute arteritis about 3 times as frequently as the group receiving whole horse serum. This is consistent with the recent

TABLE IV

Frequency of Arteritis in Groups of Rabbits Receiving Various Fractions of Horse Serum

Fraction injected	No. of rabbits	Sum of lesions counted in "standard sections"		Average lesions per rabbit	
		Acute arteritis	Chronic arteritis	Acute arteritis	Chronic arteritis
Whole horse serum	9	30	77	3.3	8.6
Fraction V (albumin)	10	91	87	9.1	8.7
Fraction IV-3,4	9	6	72	0.7	8.0
Fraction III	10	10	51	1.0	5.1
Fraction II (gamma globulin)	8	0	7	0.0	0.9
Control group	5	0	0	0	0

observation (11) that rabbits receiving beef albumin show predominantly arterial lesions. However, the animals injected with fraction IV-3,4 which contains almost as much albumin as whole horse serum showed very infrequent acute arterial lesions.

Chronic arteritis was noted with an almost equal frequency in all groups with the exception of that receiving gamma globulin. This finding suggests that arterial lesions are initiated by alpha and/or beta globulin as well as by albumin since it seems improbable that the small quantity of albumin contaminating fraction III would have led to the large number of lesions found in these rabbits. Since the difference between acute and chronic arteritis is probably only one of duration, one might expect that the second injection of fractions IV-3,4 and III would result in an equal number of acute arterial lesions as would follow the second injection of whole horse serum or albumin. It is possible that the low titer of circulating antigen found at 2 and 3 weeks in the rabbits of the groups receiving fractions IV-3,4 and III (Table II) indicates that humoral as well as cellular relationships are important in the initiation of

these lesions. It would appear that with suitable antigens acute arteritis is more likely to develop under conditions in which antigen and antibody are present simultaneously in the blood stream in relatively high concentrations.

Table V summarizes the distribution of the total arterial lesions counted in the "standard sections" of tissues from each rabbit. When the number of lesions counted is compared with the number of arteries in each section of tissue, it is evident that arteritis occurs with about equal frequency in the heart, pancreas, stomach, region of the mesenteric lymph nodes, and kidney. The incidence in the remaining tissues (hilum of lung, mediastinum, liver, spleen, adrenal, ovary and uterus or testis, diaphragm, and femoral marrow) is much lower. The higher incidence of lesions seen in this latter group of tissues in the animals receiving the albumin fraction is for the most part ac-

TABLE V

Distribution of Arterial Lesions in Groups of Rabbits Receiving Various Fractions of Horse Serum

Fraction injected	Average No. of arteries with arteritis per rabbit					
	Heart	Pancreas	Stomach	Mesenertic lymph nodes	Kidney	All other tissues
Whole serum	8.3	0.4	1.0	0.7	0.3	0.8
Fraction V	10.1	0.8	2.0	3.4	0.4	2.9
Fraction IV-3,4	5.4	0.0	0.4	0.7	0.3	1.2
Fraction III	3.6	0.1	0.7	0.1	0.2	0.5
Fraction II	0.9	0.0	0.0	0.0	0.0	0.0
Average No. of arteries per tissue	212	24	28	21	29	>500

counted for by two animals in this group one of which showed many lesions in the liver and the other a high incidence of lesions in the uterus.

Valvulitis.—Table VI summarizes the distribution and intensity of the valvular lesions observed in the various groups of rabbits. In a few instances, sections of the pulmonic and tricuspid valves did not pass through the valve itself so that observations on these valves are missing. Valvulitis occurred most frequently in the mitral valves no matter which fraction was injected, even including the gamma globulin fraction with which the total incidence of valvulitis was low. The intensity of involvement of this valve was highest in the albumin and whole horse serum group. The aortic valve was the next most frequently involved. The incidence of valvulitis was much lower in the pulmonic and tricuspid valves. With the exception of the gamma globulin group, the distribution of valvulitis was similar in all groups and approximately the same number of rabbits in each group showed lesions. It appears that albumin and alpha and/or beta globulin initiated the majority of the valvular lesions ob-

served. A comparison of the distribution of the valvular lesions observed in all the groups of rabbits and those noted in human beings with chronic rheumatic heart disease (16) shows a striking similarity.

"Aschoff Nodules."—Lesions with most of the characteristics of Aschoff bodies were present infrequently in 15 of the rabbits. Of these, 5 animals had received whole horse serum, 2 had received fraction V, 2 fraction IV-3,4, 4 fraction III, and 2 fraction II (Table III). These lesions consisted of focal subacute and chronic periarterial inflammatory changes which were usually

TABLE VI

Distribution, Incidence, and Intensity of Valvular Lesions in Groups of Rabbits Receiving Various Horse Serum Fractions

Fraction injected	Aortic			Mitral			Tricuspid			Pulmonic		
	Number observed	Number with lesions	Intensity*	Number observed	Number with lesions	Intensity*	Number observed	Number with lesions	Intensity*	Number observed	Number with lesions	Intensity*
Whole serum	9	7	10	9	7	20	9	3	5	6	2	2
Fraction V	10	8	14	10	8	19	10	1	1	10	3	3
Fraction IV-3,4	9	7	13	9	7	11	8	0	0	6	1	1
Fraction III	10	8	11	10	9	16	10	1	1	10	1	1
Fraction II	8	1	1	8	4	4	8	0	0	7	0	0
Total	46	31	49	46	35	70	45	5	7	39	7	7
Per cent of rabbit valves with lesions	67.4			76.1			9.0			18.0		
Per cent of human valves with lesions (16)	63.2			73.1			10.0			2.0		

* Average of the total number of pluses for each group when the intensity of the valvulitis was graded from 1 to 4 plus.

fusiform in shape and localized to one or both poles of the adventitia of the small branches of the coronary arteries. They were made up principally of large mononuclear cells of the Aschoff type with occasional multinucleated giant cells, lymphocytes, and rare polymorphonuclear leucocytes. These lesions had the basic cellular structure of Aschoff bodies but they seldom showed significant fibrinoid degeneration of collagen and they were never seen in the abundance which often characterizes acute rheumatic fever (Figs. 1 and 2).

Pericarditis.—Pericarditis has not been a consistent finding of previous observers studying rabbit serum disease although it has been mentioned by Ehrich *et al.* (17). We found focal pericarditis consisting of infiltration by mononuclear cells, especially about the base of the heart in 12 of the animals.

It was especially frequent in the animals receiving fraction IV-3,4, seven of which showed this change to a greater or lesser degree. In no instance was any fibrinous exudation present (Figs. 3 and 4).

Moycarditis.—Areas of necrosis of varying size and similar to those described previously (13) were noted in the right ventricle of about one-half of the rabbits in each group. These lesions are apparently similar to those described by Longcope (19), Apitz (20), and more recently by Ehrich (17).

Synovitis.—A moderate amount of proliferation of the synovial membrane was noted in some of the joint capsules of the knee joints. It was rarely accompanied by mild mononuclear cell infiltration. This change was seen in 2 of 9 synoviae examined from rabbits receiving fraction V, in 2 of 5 rabbits receiving fraction IV, in 2 of 7 rabbits receiving fraction III, in 1 of 4 rabbits receiving fraction II, and in none of 4 rabbits receiving whole horse serum.

Bone Marrow Changes.—Acute necrosis, scarring or marked atrophy of the bone marrow, a finding reported in a previous study (10) was present in 4 of the animals receiving whole serum and in 1 each of the animals receiving fractions V and III. Although it seems probable that these were focal infarcts caused by interruption of the arterial supply, their pathogenesis or significance cannot be stated with certainty at present.

Glomerulitis.—The occurrence of acute and chronic arteritis in the kidney has already been summarized (Table V). Most of the other renal changes were limited to the glomeruli and consisted of two types of lesions. We observed a slight diffuse glomerular swelling accompanied by a variable thickening of the basement membrane and questionable proliferation of the endothelial cells in 3 of the 8 rabbits injected with fraction II (gamma globulin fraction), in 4 of the 10 rabbits injected with fraction III, and in 1 of the 10 rabbits injected with fraction V (Figs. 5 and 6). This lesion was qualitatively similar to that described by Rich and Gregory (18) and more recently produced by Hawn and Janeway (11) and More and Waugh (21) by injections of purified bovine gamma globulin. However, this change appears to have been much less marked in our animals. Several factors may have contributed to this decreased intensity of glomerular inflammation. More and Waugh (21) and also Hawn and Janeway (11) gave their animals considerably larger injections of gamma globulin, and employed beef globulin instead of horse globulin. Furthermore, these observers noted the most marked lesions about a week after the initial injection of gamma globulin. Although our animals were all sacrificed 1 week after the second injection and 3 weeks after the first injection of the various fractions, it is possible that these diffuse glomerular lesions might have been more marked earlier. However, we found no evidence of the healing or healed lesions described by Hawn and Janeway 2 to 4 weeks after 1 injection of gamma globulin.

The second type of glomerular lesion which we observed was a focal acute necrotizing glomerulitis characterized by fibrinoid necrosis and infiltrations of acute inflammatory cells. Some glomeruli showed healing by focal fibrosis

and tuft adhesions. These lesions never involved more than 10 per cent of the glomeruli and were not found in animals receiving gamma globulin. Instead the highest incidence and most conspicuous lesions were found in the rabbits injected with fraction V. The animals which showed this lesion were usually ones with generalized arteritis and in many instances when only part of the glomerular tuft was involved the lesion appeared to center about the afferent arteriole (Figs. 7 and 8). In our opinion the lesion may be initiated by constriction and either necrosis or thrombosis of the afferent arteriole at the point where it enters the glomerular tuft. In 2 of the rabbits showing this type of focal glomerulitis there was also striking proliferation of the juxtaglomerular apparatus similar to that described previously (10).

Control Animals.—Careful study of the tissues from the 5 rabbits which served as controls for these experiments failed to reveal any arteritis, myocarditis, valvulitis, pericarditis, or glomerulitis like that seen in the animals injected with horse serum or its various fractions. Furthermore, none of the non-specific myocardidites which have been frequently reported in some strains of rabbits was observed. This confirms previous experience with this breed of rabbits which is singularly free from the usual rabbit diseases. The absence of complicating lesions in this and other control series strengthens the conclusion that the cardiovascular and renal lesions described above are the result of injection of foreign serum or its fractions.

Correlation of the Antemortem Observations and the Pathological Findings

Antemortem studies on the rabbits receiving the albumin fraction (fraction V) showed a reaction, as judged by the sedimentation rate, Arthus reaction, temperature response, and antibody titer, of a low order compared to that of the animals receiving whole serum or the alpha and beta globulin fractions. Yet this group demonstrated far more acute arterial lesions and a roughly comparable number of chronic arterial and valvular lesions. On the other hand, the antemortem observations of the animals receiving the gamma globulin fraction (fraction II) showed little evidence of reaction to the foreign protein but the pathological findings in this group were also minimal. This demonstrates that on a group basis the antemortem observations which we made have little correlation with the severity of the lesions present at death. Furthermore a thorough comparison of the extent of each of these reactions as well as the antigen and antibody titers with the severity of the various pathological processes in individual animals by means of suitable spot graphs revealed only one instance of fairly consistent correlation, no matter how the data were treated (acute lesions *vs.* observations after the second injection, chronic lesions *vs.* observations after the first injection, total lesions *vs.* all observations, etc.). There was a definite trend for the rabbits with the greatest number of arterial lesions to have shown the greatest rise in sedimentation rate in the group receiving whole serum, an observation which we have noted previously

(10). On the other hand we found no correlation between the antibody level or the temperature rise and the incidence of cardiovascular lesions in any group. This finding does not support our previous impression (10) that there is a gross correlation between antibody response or temperature elevation and the extent and degree of arteritis following large doses of whole horse serum. At present it seems unlikely that any of the antemortem observations which we have recorded could serve as a reliable indication of the frequency and intensity of the developing lesions in rabbits given various horse serum fractions.

Further study of the combined antemortem and morphologic data reveals one other point of possible significance. The total findings in the groups receiving either fraction III or fraction IV-3,4 are very similar to the clinical and pathological picture in acute rheumatic fever. When the data for these groups are viewed as a whole it is apparent that here one finds a higher incidence of pancarditis (valvulitis, myocarditis with Aschoff-like lesions, and pericarditis), frequent generalized "chronic" arterial lesions which would be compatible with those found in a rheumatic subject, and a relatively low incidence of acute arteritis (Table III). Furthermore these groups showed, in general, a greater elevation in sedimentation rate, a greater temperature rise, and a greater tissue hypersensitivity than the groups receiving other partially purified serum fractions (Table II). This pattern of reaction simulates the clinical state in rheumatic fever and when combined with the autopsy findings presents a more convincing counterpart of the human disease than that seen following whole serum injections.

DISCUSSION

The purpose of these experiments was to study the reactions of rabbits to large doses of several of the partially purified fractions of horse serum. It was hoped by this means to find some more or less quantitative correlation of the antemortem observations and the postmortem findings which might extend our understanding of the pathogenesis of the lesions of rabbit serum disease. Neither the specific reactions of circulating levels of antigen or antibody or the skin hypersensitivity (Arthus reaction) nor the non-specific reactions of temperature elevation or rise in sedimentation rate have yielded an evident correlation with the degree of pathological change at death when the data for individual animals are examined. Perhaps the antigens are still too impure or the measurements too crude. It would seem more likely, however, that the major obstacle is the difficulty in assaying tissue antigen and antibody relationships in the intact animal.

Hawn and Janeway (11) as well as Ehrich, Seifter, and Forman (17) have recently stated that the lesions of serum disease most likely result from selective localization of antigens in certain tissues of the body. Thus far, however, experiments in this laboratory have shown no evident selective localization of labeled horse serum in the tissues of normal or sensitized rabbits (22).

Furthermore, if the endothelium is the site of the local anaphylactic reaction as suggested by Rich and Gregory (18), it is difficult to understand the widespread involvement of arteries and endocardium and the almost complete absence of lesions in the veins and capillaries. One must conclude, it seems, that our knowledge of the immunologic pathogenesis of these lesions is still quite vague.

The results of these experiments suggest that at least two factors operate in the initiation of arterial lesions when 2 large injections of the antigenic protein are employed. One is the nature of the antigen and the other the rate at which it leaves the blood stream. Although both albumin (fraction V) and gamma globulin (fraction II) were found to leave the circulation relatively slowly as compared to the fractions high in alpha and beta globulin (fractions III and IV-3,4) only the reinjection of fraction V was followed by a high incidence of acute necrotizing arteritis. On the other hand the reinjection of fraction III and fraction IV-3,4 at a time when very low levels of circulating antigen could be demonstrated resulted in relatively little acute arteritis.

Although the plan of these experiments was considerably different from that of Hawn and Janeway's the results are in accord with theirs in several respects. Two injections of horse serum fraction V, high in albumin, resulted in a high incidence of acute and chronic arteritis as did the single injection of bovine albumin in their rabbits. Furthermore, the injection of purified bovine gamma globulin was followed by relatively few cardiovascular lesions in their rabbits and 2 injections of horse serum fraction II, containing predominantly gamma globulin, led to the production of the lowest incidence of arterial and cardiac lesions of any of the fractions injected. In addition it would appear as Hawn and Janeway suggested, that other fractions in addition to albumin may initiate the arterial lesions. Employing highly purified bovine albumin Hawn and Janeway noted a lower incidence of arteritis in the animals receiving albumin than in those receiving whole serum. They proposed that perhaps the alpha and beta globulin fractions would be more antigenic. In these experiments animals receiving fraction III, which contains only 2.0 per cent albumin, showed as high an incidence of chronic arterial lesions and almost as many lesions per rabbit as those receiving fraction V, composed of about 90 per cent albumin. This suggests that reaction to the alpha and beta globulin may be responsible for some of the arteritis.

The results of these experiments differ from those of Hawn and Janeway in three respects. Using the ring test for precipitins, they found that circulating antibody to bovine albumin was not present in 15 rabbits tested at 2 weeks and was present in only 2 of 10 rabbits tested at 3 weeks after the injection of albumin. With the somewhat more sensitive collodion particle technique for demonstrating precipitins, it is apparent that all rabbits receiving fraction V developed humoral antibodies in titers of 1:80 or greater at 14 days after the first injection. Indeed low concentrations of antibody were demonstrable in

all these rabbits 7 days after the first injection. Thus we were unable to confirm their correlation of the presence or absence of circulating antibody and the development of lesions. Nor could we demonstrate a correlation of the frequency or intensity of either the acute or chronic lesions with the quantity of antigen or antibody present at any time following injection. However, since the rabbits were all sacrificed at the end of the experiment it is more difficult to make this type of comparison critically than it was in Hawn and Janeway's experiments, in which groups of animals were sacrificed at weekly intervals. These results do support the conclusion that antibody to serum albumin was liberated into the blood stream more slowly than that to any of the other serum fractions.

A second point of variance from the results of Hawn and Janeway's experiments concerns the rate of disappearance of the gamma globulin fraction from the blood stream. With pure gamma globulin they did not detect any of this antigen in the blood stream 2 weeks after the first injection. Our tests, performed similarly, revealed a moderate quantity of fraction II still circulating 2 weeks after the first injection. This difference cannot be accounted for by the 10 per cent of alpha and beta globulins contaminating our gamma globulin since these fractions left the circulation even faster than fraction II.

The third difference between these results and those of previous investigators was the frequency and type of lesion seen after the injection of the gamma globulin fraction (fraction II). Hawn and Janeway (11) and more recently More and Waugh (21) have reported striking glomerular lesions in the majority of rabbits following somewhat larger single injections of pure bovine gamma globulin. In our experiments, in which 2 injections of less pure equine gamma globulin were employed, the diffuse glomerular lesions seen 1 week after the second injection were present in only 3 of 8 rabbits and were quite mild. It is apparent that further experiments with larger doses of pure horse gamma globulin and earlier sacrificing of the animals are necessary before these differences can be explained.

On the basis of our findings it seems probable that there are two types of glomerular lesions following the injections of foreign serum as Ehrich *et al.* (17) have recently claimed. One is a focal necrotizing glomerulitis which is found in rabbits receiving any one of several of the serum fractions and the other a diffuse transient glomerular lesion which occurs relatively soon and particularly after the injection of the serum globulins. Our experiments add no evidence concerning the pathogenesis of this latter lesion which More and Waugh believe is due to platelet thrombi.

In general, our histological findings do not justify the conclusion reached by Hawn and Janeway that hypersensitivity to serum gamma globulin is much more readily elicited than to bovine serum albumin. Indeed we found a lower incidence of almost every type of lesion in the group receiving this serum fraction.

In masterful reviews of the evidence for an allergic mechanism in rheumatic fever both Rich (5) and more recently Waksman (23) have summarized the many observations responsible for this opinion.

These experimental results add two points to the long list of similarities between rabbit serum disease and human rheumatic fever. They demonstrate that the distribution of valvular lesions is similar in the two disorders and that a pancarditis with many points of similarity to human rheumatic pancarditis is rather frequently seen following the injection of the foreign alpha and beta globulin fractions. The reactions to these fractions show additional similarities to the clinical and pathological picture of human rheumatic disease. Acute arteritis is relatively infrequent and the animals show greater elevation of sedimentation rates, greater temperature rises, and more marked skin hypersensitivity than those receiving other serum fractions.

It seems appropriate to suggest a possible pathogenesis of rheumatic fever which might explain these similarities between serum disease, a reaction to relatively large quantities of circulating soluble foreign protein, and rheumatic fever, a state which in most instances seems to follow the focal infection of the susceptible individual with beta hemolytic streptococcus. It seems logical to ask whether certain products of the hemolytic streptococcus may not alter one or more of the patient's serum proteins, so that they become antigenic. This would result in a situation in the susceptible rheumatic subject very similar to that in the patient who develops serum disease in that a circulating antigen would be present at a time when antibody was being liberated into the blood stream. This might be expected to lead to similar lesions. Of the possible streptococcal substances which could theoretically alter normal serum protein, streptolysin S and fibrinolysin would seem to deserve special investigation (24, 25). Although streptolysin S is a poor antigen, in the susceptible host it might lead to unusual tissue hypersensitivity.

In a subsequent paper (26) the results of injecting large doses of streptococcal filtrates prepared by growing group A hemolytic streptococci isolated from the throats of rheumatic subjects in almost pure rabbit serum and in such a way as to preserve streptolysin S will be reported in detail. Our findings, although interesting, are not yet conclusive as to the validity of this hypothesis.

SUMMARY AND CONCLUSIONS

Five groups of 10 rabbits each were injected intravenously 2 times at 15 day intervals with either whole horse serum or one of its cold alcohol-precipitated fractions. Suitable serological and general observations were made at appropriate intervals before and after each injection. All animals were sacrificed on the 22nd day of the experiment. A study of the antemortem and pathological findings led to the following conclusions.

1. Allergic arteritis, valvulitis, and to a lesser degree, focal pericarditis,

Aschoff-like nodules, and glomerulitis can be produced by several of the cold alcohol-precipitated fractions of horse serum as well as by whole serum.

2. Most of the acute arteritis was seen in rabbits receiving fraction V (albumin). These rabbits showed the largest amounts of circulating antigen, low antibody titers, low tissue sensitivity, and slight elevation in sedimentation rate and temperature.

3. There was a high incidence of chronic arteritis in the rabbits receiving fraction III which is almost devoid of albumin, suggesting that the alpha and beta globulins in addition to albumin may produce arteritis.

4. A state most nearly resembling that of acute rheumatic fever was produced by either fractions III or IV-3,4 (alpha and beta globulins). Pancarditis (pericarditis, Aschoff-like lesions, and valvulitis) was found relatively frequently. Many of the rabbits developed a high sedimentation rate, elevated temperature, and high tissue sensitivity, but little acute arteritis was found in this group.

5. Gamma globulin (fraction II) produced little reaction either in the antemortem determinations or histopathologically.

6. Glomerulitis of an acute necrotizing type was seen in a few rabbits without particular correlation to the fraction injected.

7. The frequency of involvement of heart valves in rabbit serum disease follows a pattern very similar to that of rheumatic heart disesae.

8. Attempts to correlate antemortem observations and pathological findings either on a group basis or for individual animals failed.

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EXPLANATION OF PLATES

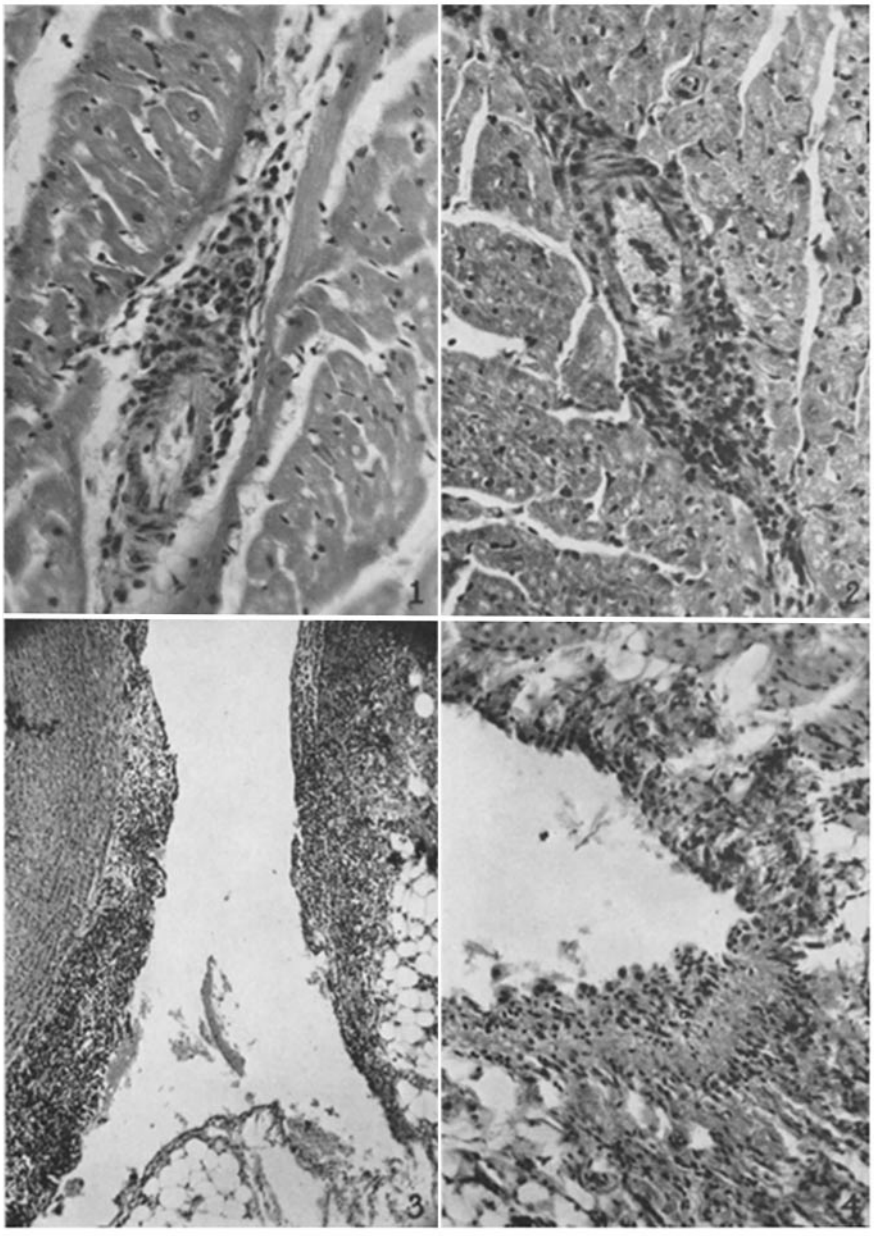
PLATE 40

FIG. 1. Aschoff-like lesion in adventitia of small coronary artery of rabbit which had received 2 injections of 0.65 gm. per kg. of horse serum fraction III 2 weeks apart and was sacrificed on the 22nd day. × 225.

FIG. 2. Periarterial granuloma of the Aschoff type in the myocardium of a rabbit which had received 2 injections of 0.65 gm. per kg. of horse serum fraction IV-3,4 two weeks apart and was sacrificed on the 22nd day. × 225.

FIG. 3. Intense subacute pericarditis at the base of the heart and about the adventitia of the aorta in a rabbit which had been injected twice with 0.65 gm. per kg. of horse serum fraction III 2 weeks apart and was sacrificed on the 22nd day. × 55.

FIG. 4. Focal pericardial inflammation consisting of infiltration by mononuclear cells and fibrinoid degeneration of collagen in a rabbit which had received 2 intravenous injections of 0.65 gm. per kg. of horse serum fraction IV-3,4 two weeks apart and was sacrificed on the 22nd day. × 125.



(Wissler *et al.*: Cardiovascular and renal lesions produced by serum)

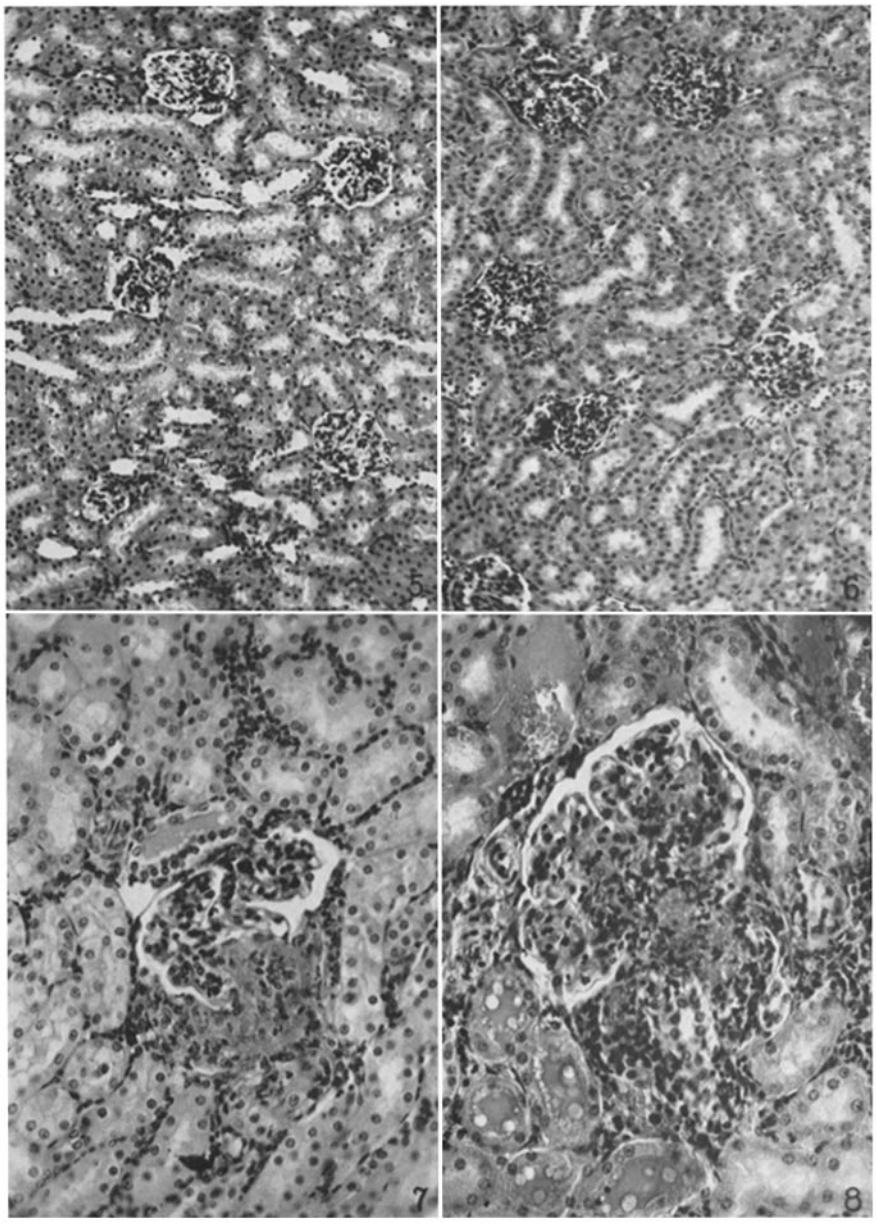
PLATE 41

FIG. 5. Low power view of renal cortex from a control rabbit showing the typical appearance of the normal glomeruli. × 125.

FIG. 6. Low power view of renal cortex from a rabbit which had received 2 injections of 0.65 gm. per kg. of horse serum fraction II 2 weeks apart and was sacrificed on the 22nd day. Note the presence of mild diffuse swelling and increased cellularity of the glomerular tufts. × 125.

FIG. 7. Focal necrotizing glomerulitis observed in a rabbit which had received 2 injections of sterile normal horse serum (0.65 gm. per kg.) 2 weeks apart and was sacrificed on the 22nd day. Note the focal fibrinoid necrosis of the glomerular tuft at a point near the entry of the afferent arterioles. × 225.

FIG. 8. Focal necrotizing glomerulitis found in a rabbit which had received 2 injections of sterile normal horse serum (0.65 gm. per kg.) 2 weeks apart and was sacrificed on the 22nd day. Here there is more marked glomerulitis consisting of infiltration of the swollen glomerular tuft with many lymphocytes and a few polymorphonuclear leucocytes. × 225.



(Wissler *et al.*: Cardiovascular and renal lesions produced by serum)