

SERUM SICKNESS IN RABBITS

II. PRECIPITINOGEN AND PRECIPITINS IN RELATION TO APPEARANCE OF THE REACTION

BY LLOYD JONES, PH.D., AND MOYER S. FLEISHER, M.D.

(From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine, St. Louis)

(Received for publication, October 16, 1931)

From the earliest days of the recognition of the syndrome of serum sickness in man, studies have been carried out in an effort to discover what relationship might exist between serum sickness and the demonstrable antigens and antibodies present in the affected individual. The evidence which has been gathered suggests the possibility of such relationship, but there exist a number of observations which apparently militate against sweeping or definite statements concerning this relationship.

We (1) have noted in rabbits the appearance of a reaction on the ears occurring usually 5 to 7 days after a single injection of a quantity of horse serum greater than 5 cc. pro kilo. The reaction is characterized by the presence of a more or less confluent erythema which may be morbilliform or scarlatinal, involving especially the lower third of the ears but frequently extending over the lower three-quarters of the ear. Frequently there is associated with this a more or less marked edema again especially evident or marked in the lower third of the ear. Such reactions were noted in 69 per cent of 103 animals. Limited reactions of erythema or edema were not considered as positive reactions since they corresponded in part with phenomena which were noted on the ears of normal animals. In 147 normal animals observed over periods of 2 weeks or longer, scattered discrete erythematous areas were noted in many cases and areas of edema involving the upper and middle portion of the ear were noted in thirty-five animals. The reactions of serum sickness as seen on the ears in rabbits differ distinctly from the reactions in normal animals both as to character and location.

It was naturally of interest to study in rabbits affected with serum sickness both the development of antibodies (precipitins) and the disappearance of the injected antigen with the view of determining what relationship might be demonstrable under these experimental conditions.

Method

In twenty-seven rabbits treated as described in the above mentioned earlier paper the precipitin production and the disappearance of horse serum from the circulating blood was studied. In a few animals tests for precipitins against horse serum were made before the injections, and in all these animals no precipitins could be demonstrated. In a number of animals blood was taken on the 1st or 2nd day after injection of horse serum, but in others blood was taken first on the 3rd or 4th day after injection. As a rule the examinations were made at 2 day intervals through the 8th or 9th day, and in a number of animals the examinations were made at the 10th, 11th, 12th, 14th, 15th or 18th days after injection. Since the majority of the animals showed serum sickness within the period including the 5th to 8th days, it was particularly the results observed within this time that were of interest.

Blood was taken from the heart for all tests. It was not possible to use the ear veins for obtaining specimens in view of the fact that the reactions of serum sickness were apparent in the ears. In titrating for the precipitins, the customary technic of using constant quantities of serum (in this case the serum of the rabbit under observation) and diminishing quantities of the horse serum antigen was followed: the readings of the results are therefore expressed in dilutions of the antigen which gave positive reactions with the rabbit serum. In titrating for the antigen (horse serum) found in the blood of rabbits used in the experiments, serum of rabbits which had been immunized against horse serum was used in a constant quantity, and the serum of the experimental animals diluted in the same gradations as used for the antigen in the precipitin tests, was added. The dilutions of the precipitinogens ran the scale from 1:10 to 1:20,960, the dilution being doubled in each successive tube. In all precipitinogen titrations, the antibody (serum of immunized rabbits) was diluted 1:5. In all the tests a parallel titration with known anti-horse precipitin serum and horse antigen was run.

Of fifteen rabbits injected intravenously, eight developed serum sickness and of twelve injected into the subscapular region ten developed serum sickness. No essential difference as regards either the precipitin or residual precipitinogen curves could be observed between these two groups, so that in further discussion we may consider together the two groups which were injected in these two different manners.

There are various possibilities which one might expect as demonstrative of the relationship of the precipitins or the content of precipitinogen to the reaction of serum sickness. The precipitins might show a distinct rise or increase at the time of the reaction, or the precipitins might show a suppression or delay in appearance in reacting animals as compared with non-reacting but injected animals. Or the precipitins, if previously present, might show a fall in titre at the time of reaction. Considering the precipitinogen, we might seek for evidence of a rapid fall of this substance in the blood of reacting animals, or an unusually slow disappearance of the injected antigen. There might be evident after, rather than before or during the period of reaction, changes in the content of either precipitin or precipitinogen in the blood of the reacting animals. Or finally, there might be observable in the reacting animals certain relations between the two agents which differed from those seen in non-reacting animals.

It must, however, be stated that when we compare the curves of either precipitins or precipitinogen in non-reacting animals with those occurring in the reacting rabbits it is impossible to demonstrate any regularly occurring differences between the two groups or to note in the rabbits with serum sickness any consistent relationship between either precipitin or precipitinogen and the occurrence of the reactions.

In the nine non-reacting animals we find that in two animals no antibody was demonstrable at 5 days, in two none at 6 days (in no animals were antibodies found at 4 days), in five animals precipitins were demonstrable at 6 days in a titre varying from 1:40 to 1:1280. After the 5th or 6th days there was usually a marked increase in the titre of the antibody, but there appeared to be no regularity in the quantitative increase during the next 2 day period, so that the titre varied between 1:160 and 1:5120 on the 7th and 8th days. On the subsequent days (10th to 18th) there was usually an increase of the titre, in some animals very marked and rapid, in others slower, and in some animals little or no increase in the titre after the 8th day. In a few animals there occurred a drop in the titre after the 8th day, followed by a rise in later periods.

The highest dilution of the serum of the injected non-reacting rabbit in which antigen was demonstrated showed also wide variations in individual animals in the first 5 days, but there is evident within this period a tendency for a fairly rapid fall in the concentration of the horse serum in the blood. In the period from the 5th to 8th days there seems to be quite regularly (with one exception) a slower disappearance of the antigen from the blood stream; a tendency to maintain

TABLE I
Precipitin and Precipitinogen Titer in Blood of Non-Reacting Rabbits Subsequent to Injection of Horse Serum

Rabbit No.	Days after injection...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
111	Precipitin		0		0		0		640		5,120				5,120				10,240
	Precipitinogen		2,560		640		320		80		160				0				0
112	Precipitin		0		0		80		5,120		5,120				5,120				10,240
	Precipitinogen		1,280		320		320		320		40				0				0
114	Precipitin		0		0		0		640		2,560				2,560				2,560
	Precipitinogen		2,560		1,280		320		320		40				20				0
115	Precipitin		0		0		40		1,280		5,120				1,280				2,560
	Precipitinogen		20,480		1,280		320		320		80				20				0
132	Precipitin		0		0		1,280		5,120		5,120				5,120				2,560
	Precipitinogen		640		320		160		160		80				20				0
156	Precipitin		0		0		0		1,280		5,120				10,240				10,240
	Precipitinogen		640		640		160		320		40				40				0
157	Precipitin		0		0		0		2,560		20,480				20,480				20,480
	Precipitinogen		640		320		160		160		10				0				0
300	Precipitin		0		0		80		160						20,480				20,480
	Precipitinogen		1,280		1,280		320		160		80				80				0
303	Precipitin		0		0		320		1,280						5,120				5,120
	Precipitinogen		640		640		320		320		40				40				0

The figures in the table represent the lowest dilution of antigen giving precipitin reactions and should be read as 1: (the figure given).

a constant level (in one animal there was even an increase between the 5th and 7th days) of the circulating antigen. In later periods the antigen disappears; in four of seven animals examined at 14 or 15 days and in all tested at 18 days (four) there appeared to be no antigen present. The residual antigen in the latter periods, however, varied considerably in the different animals.

It is evident that in the non-reacting animals no regularity can be noted in regard to time relations and titres of either precipitins or precipitinogens.

Considering now the rabbits which showed serum sickness we find essentially the same degree of variation in the curves of the titres of the two agents studied as we have just described above in the non-reacting rabbits. It is apparent that on this basis we can draw no difference.

In one animal (No. 131) serum sickness first appeared on the 3rd day after injection; blood taken on the 4th day showed as in other rabbits, no precipitins; in another rabbit, showing reaction on the 5th day (No. 305), no precipitins were evident on this same day; in a 3rd rabbit showing serum sickness on the 5th day (No. 113) no precipitins were demonstrable on either the 4th or 6th days, and in a fourth rabbit showing serum sickness on the 7th day (No. 154) no precipitins were found on this day.

In several rabbits no precipitins were shown on the day before the appearance of the reaction (Nos. 116, 135, 159, 326, 304) but on the 2nd day of the reaction precipitins varying in titre from 1:160 to 1:640 were evident.

In a number of rabbits (Nos. 160, 162, 321) precipitins were found in titre varying from 1:20 to 1:320 either 1 or 2 days before the appearance of the reactions. From these observations it would seem that the appearance of the reaction cannot be correlated with either the appearance or non-appearance in the blood of precipitins.

We find the precipitin titre on the days of the reactions varying from 0 to 1:2560 and fairly evenly distributed through this range.

On the days subsequent to the reaction, in most cases 1 or 2 days after the last day of evidence of serum sickness, the titre of the precipitins seems to correspond quite well with the titres noted in non-reacting rabbits, and shows neither an unusual increase nor a retardation in the rise usually noted at these days.

In the later periods following the 8th day after injection, the variations in the individual reacting animals are like those noted in the other injected rabbits.

Generally the fall of the precipitinogen in the reacting animals is similar to that noted in other rabbits; a fairly rapid but irregular tendency towards a diminution within the first 5 days; a tendency towards a slower fall between the 5th and 8th days, and a more or less rapid fall towards complete disappearance at 14 to 18 days. Certainly there is no evidence that the antigen disappears more rapidly

TABLE II
Precipitin and Precipitinogen Titre in the Blood of Rabbits Showing Serum Sickness
 The days within the blocks are the days the reactions were present

Rabbit No.	Days after injection.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
113	Precipitin		0		0				640		1,280				10,240				5,120
	Precipitinogen		1,280		1,280		160		160		20				0				0
116	Precipitin		0		0		0		160		2,560				1,280				2,560
	Precipitinogen		2,560		1,280		640		320		80				40				0
131	Precipitin				0		160		2,560		10,240				5,120				
	Precipitinogen				1,280		640		640		320				80				
135	Precipitin				0		640		5,120		2,560				5,120				
	Precipitinogen				2,560		640		640		160				80				
154	Precipitin		0		0		0		0										
	Precipitinogen		640		1,280		320		320										
159	Precipitin		0		0		320		1,280										
	Precipitinogen		320		1,280		160		320										
160	Precipitin		0		0		320		2,560										
	Precipitinogen		1,280		1,280		320		320										
162	Precipitin		0		0		160		1,280										
	Precipitinogen		1,280		1,280		640		640										

319	Precipitin Precipitinogen	0 1,280	0 640	640 160	2,560 160						
320	Precipitin Precipitinogen	0 1,280	0 640	80 320	640 320						
321	Precipitin Precipitinogen	0 640	20 320	640 160	10,240 160						
325	Precipitin Precipitinogen	0 640	0 1,280	640 640	1,280 320						
326	Precipitin Precipitinogen	0 640	0 320	640 160	1,280 160						
327	Precipitin Precipitinogen	0 640	80 320	640 320	2,560 160						
301	Precipitin Precipitinogen	0 1,280	0 1,280	80 320	640 320	10,240 0					
302	Precipitin Precipitinogen	0 640	0 640	320 640	2,560 320	10,240 10					
304	Precipitin Precipitinogen	0 640	0 640	320 320	5,120 160	10,240 0					
305	Precipitin Precipitinogen	0 640	0 640	320 320	1,280 80	5,120 20					

The figures in the table represent the lowest dilution of antigen giving precipitin reactions and should be read as 1: (the figure given).

in the reacting animals (between the 12th and 15th days) than in the non-reacting ones; the suggestion is rather that the reverse occurs, but probably these differences evident here are within the limits of experimental variations. In one animal only (No. 159) is there evident any tendency towards a rise of the antigen titre subsequent to and in this case during the appearance of the reaction. In one case also (No. 113) is there apparent a marked fall of the titre after the appearance of the reaction. However, similar appearances may be noted at similar periods after injections in non-reacting animals.

It is not possible either to point out any particular or constant relationship existing between the titres of antigen and antibody found in the blood at the time of appearance of serum sickness in these rabbits. We can find all sorts of relationship; high antigen titre and low antibody titre; low antigen and high antibody, and the titres of the two agents very nearly equal.

DISCUSSION

In one of the earliest publications of von Pirquet and Schick (2) concerning serum sickness they demonstrate certain relations between the appearance of precipitins and the reactions of this phenomenon, and state that precipitins appear after serum sickness has begun. They are apparently of the opinion that the precipitins never appeared before the symptoms of serum sickness. In the majority of cases precipitins appeared in the blood on the 8th or 9th day after injection. Von Pirquet (3) however bases his explanation of the physiology of the reaction of serum sickness and other allergic phenomena largely upon the relationship of antigen and antibody within the affected individual. Hamburger and Moro (4) who studied the precipitin and precipitinogen in the blood of patients showing serum sickness after injection of Moser's antiscarlatinal serum, state that precipitins appear 2 days after the appearance of serum sickness—namely on the 16th and 14th days respectively. The antigen disappeared from the blood at about the 23rd day (about 7 days after serum sickness) or sometimes the antigen persisted for 16 or more days after the reaction. They found that in rabbits injected with 0.5 to 10 cc. of horse serum pro kilo, precipitins appeared at 8 days (none were found at 6 days) after injection and the horse serum tended to disappear at about the 9th day. These authors did not assume a definite relationship between antigen and antibody on one hand and the reaction on the other hand. Dehne and Hamburger (5) studying the presence of the antigen by means of titration of the residual tetanus antitoxin in the blood of human beings injected subcutaneously found a slow increase of antitoxin in the blood up to the 2nd or 3rd day—subsequently the antitoxin remained constant up to the 8th day, when there occurred a marked fall until the injected antibody disappeared in the 3rd week. These authors conclude that serum sickness and fall of antitoxin coincide so that

serum sickness follows shortly after or at the time of the fall of injected antigen, while the precipitins appear after the reaction. Hamburger and Pollak (6) studying the incubation period, tested for the appearance of skin reactions to horse serum in individuals previously injected with this antigen. Hypersensitiveness appeared at the 5th or 6th days usually, and there was a gradual, not an explosive appearance of hypersensitiveness; in relation to this early appearance of altered reactivity of the injected individual they quote the statement of Pollak and Mautner (7) that often only a few days after a first injection of serum there appear temporary exanthemata. Cowie (8) made observations similar to those of Hamburger and Pollak, excepting that in a few individuals he found the skin reactions to appear on the 2nd and 4th days after injection.

Wells (9) studying the precipitins in individuals injected with antisera, found no influence of age, sex or weight; he believed, however, that the titre increased irregularly but not constantly with the amount of the injected serum. He found precipitins appearing as early as the 4th day, even in a titre of 1:2560, and often as high as 1:1280 on the 5th day. In one case the precipitin titre fell during serum sickness, rising again after disappearance of the reaction; in two additional cases there appeared a rise of the precipitin titre after the reaction. Wells believed that serum sickness is connected with a union of the precipitin element with some other element in some manner not apparent or understood, and that the disappearance of the symptoms of serum sickness is associated with the liberation of the precipitin.

Longcope and Rackemann (10) studied precipitins, anaphylactins and the appearance of skin sensitiveness in persons injected with various antisera. Sensitiveness to skin reactions could not be correlated with serum sickness, although reactions to the intracutaneously injected horse serum appeared earlier in subjects who developed serum sickness than in those who did not. Anaphylactins were demonstrated in eleven of twelve persons developing serum sickness, but in none of those which did not; these antibodies were demonstrated only when serum sickness was well advanced or about to terminate. The precipitins were also demonstrable in the individuals who developed serum sickness but not in injected persons who did not develop the phenomenon. In the persons with serum sickness precipitins did not appear until 3 or 4 days after the onset of serum sickness and then rose rapidly. These authors consider serum sickness to be definitely associable with precipitin and precipitinogen, but assume that reactions in the cells must play an important rôle, due possibly to the presence of precipitins in the cells in the early period.

MacKenzie and Leake (11) studied both precipitin and precipitinogen. In a group of subjects showing severe serum sickness, they found the precipitin rising to the maximum near the time the symptoms disappeared, while the antigen fell with the cessation of symptoms. In a group of non-reacting injected individuals no precipitins or very weakly active ones appeared, and the antigen was retained in the blood stream longer than in the first group. A third group of individuals

who showed mild symptoms developed only scanty precipitins, and the antigen disappeared slowly. One individual who developed serum sickness showed a high precipitin titre at the end of the period of serum sickness, but the antigen persisted for a longer period in the blood stream. MacKenzie and Leake, like Longcope and Rackemann and Wells, were therefore of the belief that serum sickness could be correlated with the antigen-antibody relation with the body.

Tuft and Ramsdell (12-14) in a series of articles studied antibody formation subsequent to injection of horse serum. In the last one of this series they found that antibodies appeared both before and after the serum sickness or sometimes none appeared. They noted no regularity, and reached the conclusion that no clear relationship could be demonstrated between antibodies and the basic mechanism of serum sickness.

As the foregoing shows, the evidence regarding the relationship of antibody and antigen to the appearance of serum sickness in man is by no means uniform or definite. In our studies of the antigen and antibody curves in rabbits developing serum sickness, we find much the same irregularities which Tuft and Ramsdell report, and essentially no similarities to the results which Longcope and Rackemann and MacKenzie and Leake have noted. It does not appear possible to point out any relationship between the precipitin production, the disappearance of the antigen and the reaction of serum sickness in the rabbits we studied.

As discussed previously, the incubation period in serum sickness in rabbits is shorter than in man; it also appears that both the appearance of precipitins and disappearance of antigen take place more rapidly in rabbits than in man. These latter facts may account for the earlier appearance of serum sickness in rabbits; we do not imply that this is as cause and effect, but simply that it is possible that the immunological phenomena of appearance of precipitins and disappearance of antigens, like the incubation period for serum sickness, both occur more rapidly in rabbits. They might be considered as two parallel phenomena, which, however, do not regularly or constantly occur at the same rate in different individuals.

CONCLUSIONS

1. It has not been possible to demonstrate in rabbits affected with serum sickness any constant temporal relationship between precipitin and precipitinogen in the blood on one hand and of occurrence of serum sickness on the other hand.

2. It has not been possible to demonstrate any differences between the precipitin and precipitinogen curves in injected rabbits which develop serum sickness compared with injected rabbits which did not develop serum sickness.

3. There is therefore no evidence that we can directly associate the occurrence of serum sickness with the production or appearance of precipitins.

REFERENCES

1. Fleisher, M. S., and Jones, L., *J. Exp. Med.*, 1931, **54**, 597.
2. von Pirquet, C., and Schick, B., *Wien. klin. Woch.*, 1903, **16**, 1244.
3. von Pirquet, C. E., *Arch. Int. Med.*, 1911, **7**, 269, 383.
4. Hamburger, F., and Moro, E., *Wien. klin. Woch.*, 1903, **16**, 445.
5. Dehne, R., and Hamburger, F., *Wien. klin. Woch.*, 1907, **20**, 817.
6. Hamburger, F., and Pollak, R., *Wien. klin. Woch.*, 1910, **23**, 1161.
7. Pollak, R., and Mautner, B., *Wien. klin. Woch.*, 1910, **23**, 926.
8. Cowie, D. M., *Am. J. Dis. Child.*, 1914, **7**, 253.
9. Wells, C. W., *J. Infect. Dis.*, 1915, **16**, 63.
10. Longcope, W. T., and Rackemann, F. M., *J. Exp. Med.*, 1918, **27**, 341.
11. MacKenzie, G. M., and Leake, W. H., *J. Exp. Med.*, 1921, **33**, 601.
12. Tuft, L., and Ramsdell, S. G., *J. Exp. Med.*, 1929, **50**, 431.
13. Tuft, L., and Ramsdell, S. G., *J. Immunol.*, 1929, **16**, 411.
14. Tuft, L., and Ramsdell, S. G., *J. Immunol.*, 1929, **17**, 539.