PRECIPITINS AND THE ÆTIOLOGY OF SERUM SICKNESS.*

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SERUM disease is a reaction of the human body to parenteral administration of a foreign serum and may vary in severity within very wide limits. One of its features is the appearance of a cutaneous rash with varied characters and of different types. The majority of these are rarely seen, and urticaria or erythema multiforme either separately or combined form the greatest number of cases.† The eruption appears and disappears suddenly, and perhaps most cases show one or more recurrences. The conditions under which my cases were observed rendered it impossible to obtain figures on this point. The distribution of the rash may be localised to a very small area about the point of injection of the serum, or extend over practically the whole skin surface. Often it originates at the site of injection and subsequently spreads. Recurrences may vary in their distribution in exactly the same way as the original rash. Sometimes injection at one spot brings out a rash localised to the site of an earlier injection, the site of the last remaining free. In practically every case irritation (it may be almost unendurable), is a marked feature of the eruption. Urticaria is rarely seen alone; the weals vary in size from a few millimetres in diameter to the size of the palm of the hand with well-defined, irregular margin, and almost always surrounded by a zone of erythema. The latter is occasionally the only type of rash. Other symptoms may also occur, such as joint pains and enlargement and tenderness of the lymphatic glands. The temperature sometimes rises as much as 1° to 2° F. Finally dyspnoea may be present. The patient becomes acutely distressed, the face is congested, the chest rigid and fixed in deep inspiration and air enters the lungs only with difficulty. This condition also appears suddenly and disappears as suddenly; it may persist for half an hour. These accompanying phenomena may vary in severity up to true anaphy-

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† Thomson in two letters to the *Lancet*, 1917, i. p. 163, and 1917, ii. p. 837, insists on the frequency of a circinate erythema in serum disease. As he suggests in the second letter this may be commoner in children than in adults, thus accounting for its absence from this series. He further points out that circinate erythema appears at a longer time after injection and is more frequent after a previous urticaria. In the present series the rash was the first from which the patient had suffered, and owing to the rapidity with which evacuation was effected few or no cases of a second rash were observed.

lactic shock, so that it is impossible to draw any line between serum sickness and anaphylaxis.

Practically everyone has assumed that the two conditions are the same, and numerous theories have been elaborated to explain their occurrence. All of these are alike in postulating the presence in the patient's blood of antibodies to the serum injected, and the work described in this paper was undertaken with the object of determining if such is the case. One method of approaching the matter is along the lines laid down by Abderhalden, but the difficulties in the way of this were insuperable and consequently search was made for precipitins.

Some investigation of this matter had already been made by Longcope and Rackmann (1918¹) who demonstrated the presence of antibodies in the blood of persons suffering from serum sickness. Up to a point I have confirmed their findings, as will be seen later, in the course of a wider enquiry which will form the subject of a subsequent paper. Here precipitins alone will be considered.

The technique is the same as that employed by Longcope and Rackmann. From 40 to 80 c.c. of blood were drawn from a vein of a patient who presented or had presented signs of serum sickness. After standing overnight in the ice-chest the clear serum was pipetted off and put up against a series of dilutions of horse-serum (antitetanic serum—"A.T.S."—was employed in every case). The A.T.S. employed was always kept in an upright vessel which was not agitated in any way for at least a week before use. In this way a perfectly clear serum was obtained free from any suspended particles.

1. Nine tubes were put up in every experiment, eight of them containing 0.5 c.c. of undiluted serum from the patient, and in addition the first received 0.5 c.c. of undiluted A.T.S., the second 0.5 c.c. A.T.S. diluted 1 in 10 and so on, 1 in 100, 1 in 1000, 1 in 10,000, 1 in 100,000 up to 1 in 1,000,000 in the seventh. The eighth was a control containing patient's serum and 0.5 c.c. saline, and the ninth another control containing 0.5 c.c. horse-serum with an equal quantity of saline. Reversing this order, *i.e.*, using standard amount of undiluted A.T.S. and a series of dilutions of patient's serum did not prove satisfactory.

2. In most cases the patient's serum was inactivated by heat for ten minutes at 55° C. in a water-bath before being put up. Several experiments (but not enough to answer the question finally) were performed to determine whether fresh or inactivated serum gave the clearer results. The balance of evidence leans towards the latter. Out of nine sera tested five gave entirely negative results, neither fresh nor inactivated serum giving any precipitation at all. Of the other four one gave more marked precipitation when active.

Case S received two injections. A rash appeared six days after the first and about two hours before the second. The blood was drawn for examination five hours after the second.

	Dilution of A.T.S. : 1 in						
	1.	10.	100.	1000.	10,000.	100,000.	1,000,000.
Fresh . . .	...	+	tr	tr	tr	0	...
Inactivated .	...	+	tr	0	0	0	...

The other three precipitated more strongly when inactive, in fact the fresh serum gave no precipitation as seen in the following figures which are typical :—

Case N received two (possibly three, see Table I.) injections ; a rash appeared five days after the penultimate injection and again about six hours after the last. The blood was taken three days after the last injection and gave precipitation as follows :—

	Dilution of Serum : 1 in						
	1.	10.	100.	1000.	10,000.	100,000.	1,000,000
Fresh . . .	0	0	0	0	0	0	0
Inactivated .	0	++	+	tr	tr	tr	0

3. The tubes containing the mixed sera were generally incubated at 37° C. for two hours and stood overnight at laboratory temperature the results being read next morning. Prolonging the incubation to four hours did not alter the readings to any appreciable extent. Further, incubation in a water-bath at 50° C. for one hour gave lower readings than those for two hours at 37° C., while for two hours in the same circumstances the figures were the same as, sometimes lower than, the latter.

4. The readings were made in the same way as for Dreyer's agglutination method, viz., against a dull black background by good artificial light.

5. The sera obtained from six men of good intelligence who stated that they had never at any time received any serum injection all gave negative results.

6. Inhibition zone. As with agglutinins a very definite inhibition zone is frequently revealed, and is illustrated in the three following examples :—

Case.	No. of Injections.	Blood taken days after last Injection.	Dilution of Serum : 1 in						
			1.	10.	100.	1000.	10,000.	100,000.	1,000,000.
Cg	10	3	0	tr	+	tr	0	0	0
Cn	3	1	0	tr	+	tr	tr	tr	0
Mk	2	8	0	tr	++	tr	0	0	0

It was observed also that only very rarely does precipitation occur when the undiluted sera are used.

7. It was contemplated to determine the presence of anti-horse-serum in these patients by means of the complement fixation reaction, but unfortunately circumstances made this impossible.

Observations were made on fifty-one men who had received a previous injection or injections of horse-serum, and the results are given in Table I.

TABLE I.

The following explains the nature of the data detailed in the numbered columns:—

1. An identification letter (heavy type *infra*).
2. The number of injections received.
3. The type of rash {
 - L. local urticaria appearing only in the immediate neighbourhood of the inoculation.
 - U. urticaria distributed over the whole or greater part of the body surface.
 - l. local erythema of similar distribution to L.
 - e. a widely distributed but sparse erythema.
 - E. erythema of similar distribution to U.
 - N.K. a rash of some sort may have appeared at some time but the fact is not known.
 - N. no rash.
4. The number of days intervening between the last injection and the appearance of the rash.
5. The highest dilution of serum giving clear precipitation.
6. The number of days intervening between that on which the precipitin content was estimated and the last injection of serum.
7. The corresponding period since the penultimate injection of serum. It will be noticed that in some cases the period amounts to months or even years; this is owing to the fact that the man had been previously wounded.
8. The corresponding interval since the appearance of the rash.

1.	2.	3.	4.	5.	6.	7.	8.	
A	2	c.	8	1 in 10,000	8	16	13/24	Intrathecal injection for cerebro-spinal fever.
Al	4	U.	4	0	4	380	1/12	
B	1	N.K.	7	1 in 100	59	...	...	
Bh	7	N.K.	?	0	40	44	?	Cerebro-spinal fever.
Bn	3	L.	2	0	3	9	1	
				0	3	9	7	
By	2	L.	5	0	6	210	1	
	3			1 in 100	3	9	4	
C	2	U.	7	0	8	8	1	
				1 in 1000	9	9	2	
				1 in 10,000	13	13	6	
Cg	10	L.	3	1 in 1000	3	210	1/3	
Cn	3	U.	1	1 in 100,000	1	600	1	
	4			0	5/24	9	8	
Cy	13	N.K.	?	0	84	90	?	
D	2	U.	1	0	7	14	6	
	3			1 in 100	1/4	7	6	
F	2	U.	4	0	5	240	1	
				1 in 100	8	240	4	Rash began locally and, later, spread. Very severe reaction.
G	1	U.	4	1 in 1	5	5	1	
				1 in 10,000	6	6	2	
				1 in 100	7	7	3	Severe reaction.
				1 in 10	11	11	7	
H	5	U.	9	1 in 10	2/24	11	2	
	6			1 in 1	4	15	6	A case of tetanus receiving intravenous, intramuscular or subcutaneous injections of A.T.S. at frequent intervals.
Ht	1	E.	5	1 in 100	7	...	2	
J	11	N.	...	1 in 1,000,000	1/4	...	...	
	17			1 in 100	1/4	...	...	

TABLE I.—continued.

1.	2.	3.	4.	5.	6.	7.	8.	
K	27	U.	2/24	0	1	8	1	17 injections given for wounds received in 1915, 1916, 1917, and 1918; the rest for dysentery in 1917, exact number not known but about 10.
L	2	N.K.	?	0	30	30	?	
M	1	N.	...	0	3	...	...	
Md	3	U.	6	0	7	120	1	
				0	10	120	4	
Me	4	N.	...	0	35	58	...	Intrathecal injections for cerebro-spinal fever.
Mk	2	U.	6	0	3	750	1	
				1 in 1000	14	750	8	
My	3	U.	4	0	7	480	3	
N	2	L.	5					
	3	U.	1/4	1 in 100,000	1/2	7	1/4	
				1 in 100,000	1	8	1	
O	2	U.	1	1 in 1000	6	13	15	
P	4 (36)	L.	1/2	1 in 100	5	13	4	
				0	1	7	1	
				1 in 100	5	11	4	Wounded on two previous occasions, but did not remember whether he received 1 or 2 injections each time.
Ph	2	N.K.	?	0	930	930	?	
Pn	4	N.K.	?	0	360	360	?	
Pr	3	U.	3	0	3	10	1/4	
Pt	5	U.	1	1 in 100	1	540	1	
				1 in 100	7	540	6	
R	2	U.	5	1 in 100	8	180	3	
Rg	3	E.	6	0	9	1110	3	
Rl	3	U.	6	1 in 1000	6	810	1/3	Severe reaction.
Ro	4	N.	...	0	90	90	...	
Rs	2	U.	5	1 in 1000	7	14	2	
	3			1 in 1000	1/4	14	2	
S	2	e.	5	0	6	520	1	
Sm	2	N.K.	?	0	90	90	?	
Sp	1	U.	6					
	2			1 in 10,000	1/4	6	1/4	Second injection given while rash was still out. Rash disappeared and recurred frequently.
T	2?	L.	4 (3)	1 in 10	4 (3?)	4 (3)	1/12	
				1 in 100	5 (24)	5 (24)	1	
				1 in 100	6 (25)	6 (25)	2	
	3?			1 in 1000	4	12	8	
						(211)		
Th	3	U.	1	0	2	8	1	The man's statement and his Field Medical Card differed as to the number of injections of A.T.S.
Tn	3	U.	4	1 in 100,000	6	510	2	
Tr	4	U.	6	0	6	360	1/6	
	5			0	2	8	2	
V	4	N.K.	4	0	56	57	52	Injections intrathecal for cerebro-spinal fever.
Vs	1	N.K.	?	0	90	90	?	
W	1	L.	6	0	7	...	1	
	2			1 in 100	2	10	4	

TABLE I.—*continued.*

1.	2.	3.	4.	5.	6.	7.	8.	
We	5	N.	...	0	38	40	...	Injections intra- theical for cerebro- spinal fever.
Wh	2	U.	6	0	7	840	1	
	3			0	4	12	6	
Wm	2	U.	4	1 in 100	4	11	10/24	
WnP	2	e.	3	0	4	330	1	
WnS	3	N.	...	0	1	14	...	
Wr	3	U.	3	1 in 1000 1 in 1000	4 7	11 14	1 4	

Note.—Although on many occasions the patient's serum was put up against a series of batches of horse-serum, in every case the change or absence of precipitation noted in the above table is that which occurred with *one* batch common to each series of experiments. For example, the first serum from Case C was put up against batch No. D798 only, the second against that batch and six others, the third against the same and eight others, but the results given are those for D798 alone.

In those cases where no precipitation at all was obtained it may be that this was due merely to the strain of antigen used, and that had some other strain been employed precipitation would then have occurred. This possibility was, however, guarded against as far as possible. When the work was commenced the variability of the antigen was not suspected, consequently no precautions of this nature were taken and the first nine negative sera were put up against one batch only. Thereafter (with regard to negative sera *only*) this is true of one further case; in two other cases two batches were used for each; in three cases three batches; in two four, in three five, in three six, in three seven, in three eight, and in six ten. Further, in examining the protocols of the several experiments it is found that no antigen always gives a positive result with a positive serum, but that certain antigens more frequently give a positive result than others. Thus when D798 was put up against each of 36 positive cases it gave a positive result in 31, i.e., 86 per cent.; similarly D743 put up against each of 10 positive sera gave 8 positive results or 80 per cent. In this way five batches (I., II., III., IV., and V.) are found which gave positive results with 75 per cent. or more of positive sera, so that if a patient's serum be put up against one of these and gives a negative reaction it appears probable that this is a correct result, and this probability increases with the number of these antigens against which the serum is tested. Of the 36 negative sera given in the table, five were put up against batches I., II., and III., nine against II. alone, five against II. and III., two against II., III., and V., three against II. and V., four against II., III, IV., and V., six against III., one against IV., and one against V. Owing to the pressure of other work, the impossibility of obtaining more than a few c.c. of several of the batches of antigen, and the fact that each step in the investigation had to be taken before the next became visible, it was impossible to make all the series uniform with each other, or entirely to eliminate the possibility of overlooking the existence of precipitins in some cases where they were in reality present; but it seems justifiable to assume that in all cases the probability is in favour of the correctness of the finding recorded, while in more than half the cases the probability is high.

Fifty-one cases have been examined 76 times and precipitins were found in twenty-five of these 39 times and was absent from the others on 37 occasions. It will be seen, however, that some of the cases quoted did not present any symptoms of serum sickness and were examined for the purpose of determining if precipitins could still be found months or years after the last injection of serum. Eliminating these cases, out

of thirty-five suffering from serum sickness the blood of twenty-four contained precipitins to horse-serum. Nothing in the character of the rash affords any indication as to the presence or absence of precipitins—any type of rash may or may not be accompanied by precipitins in the serum while, with or without any rash, precipitins may or may not be present. Nor is there any time relation between these two phenomena, and there is after the outbreak of the rash no period during which precipitins are more likely or less likely to be found than at any other. Apparently human serum never contains “normal” precipitins to horse-serum, these only appearing after administration of the foreign serum. One such injection may be sufficient for this purpose, or a series of injections may be necessary. If in considering the cases described, attention be given only to the relation between the time of the last dose of A.T.S. and the precipitin content of the blood, it is impossible to obtain any idea of what is taking place, but if the time since the penultimate injection be also taken into account the result is different. It is then possible to get a more or less clear view of what is going on; in fact it is found necessary to realise that the production of precipitins may be completely independent of the final injection in such cases as these. Bearing this in mind the following deductions seem justified from the tabulated facts. After one injection precipitins may be found after 5 to 8 days (Cases W, G, H, Sp), and may persist for at least 59 days (Case B). In one case they were absent on the 3rd day (M) and in another (W) still absent on the 7th. A second injection on the 6th day in one case (Sp) and on the 7th in another (W) did not cause their disappearance. Although one injection may fail to call them forth a second may do so, when they make their appearance about the 7th to the 14th days after the last dose (F, W, C). Thereafter they may rise to a maximum in 5 to 9 days and then again fall, often fairly rapidly (N). Generally, it seems, they completely disappear, for although in three cases they were still found at 59 days (B), 18 months (Pt), and 20 months (Cn) after the last injection, in twelve other cases no precipitin could be found at times varying from 38 days to 2 years 7 months. If a person whose blood already contains precipitins receive a further dose of the antigen the former may immediately diminish or disappear (H, Cn); but they probably soon return again, for it is not found that they are absent after a prolonged series of injections (Cg). Nor does this result always ensue—the amount of precipitin may remain unchanged or even increase immediately following upon such further injection (Rs, T, D).

It is then impossible to establish any connection between serum sickness and circulating precipitins in the patient. This is further borne out by the fact that men whose blood contains these antibodies are in no way more likely to suffer from serum disease than those whose blood is destitute of them. A number of cases was observed from this

point of view, and not one showed any symptom attributable to serum disease.

With very few exceptions the antigen was administered by the subcutaneous route. In two only was the intravenous employed (Cy, J)—the former receiving two injections by this route, and 84 days after the last no precipitins could be found. In no case except J (*vide infra*) was the intramuscular route taken. Six others, all cases of cerebro-spinal fever, received the serum intrathecally (one also received two intravenous injections and has been mentioned above, Case Cy). Five of these examined 38 to 90 days after the last administration of serum showed no precipitin in their blood, the sixth 59 days after the end of treatment still gave precipitation in dilution of 1 in 100. Most of the cases of this series had received only prophylactic doses of A.T.S., so that the amount of serum varied between about 3 c.c. (500 units) and 10 c.c. (1500 units). One (J, *vide infra*) suffered from tetanus and therefore had much larger doses, one (K) suffered from dysentery with the same result, and two had had diphtheria in the past, but it was impossible to discover the amounts of serum administered. Only those of the first class were observed in sufficient number to give any data as to the effects of dosage, and within the limits stated the quantity of serum injected does not seem in any way to affect the production of precipitins—a single injection of any quantity may cause their appearance in the blood, a combination of any two quantities may have the same effect, a subsequent injection of any quantity may first remove the precipitins already formed but they probably return again later. Nor has the spacing of the doses any discoverable effect.

Several of the cases are of sufficient interest to deserve special mention. J received a compound fracture of the left humerus and left leg by gunshot wounds on 27/7/18. He received 500 units A.T.S. the same evening and a similar dose on 3/8/18. He had never before received any serum treatment. On 5/8/18 he first noticed twitching of the left arm but did not make any complaint then. The condition became steadily worse until he reported the matter on the morning 8/8/18. At that time the twitching was almost ceaseless; there was no sweating, insomnia, nor dysphagia. Thereafter he received on twenty-four occasions, between that date and 28/8/18 in all 352,000 units of serum chiefly by the intramuscular route. On 10/8/18 twitching of the limb was still marked but no worse. He was sweating profusely. The first specimen of blood was taken at 12.45 noon. On the 19/8/18 the second specimen was taken. He completely recovered. He never at any time presented symptoms of anaphylaxis or serum disease.

Cases Rl, H, G, Pt, Ht, Rg, Wh, and My all suffered from symptoms which might be called mild anaphylaxis, but I prefer to call severe serum sickness. In every case the first evidence of sickness

was the outcrop of an intense urticaria with erythema involving practically the whole body surface, the face then became puffy, the conjunctivæ injected, and the eyelids and lips swollen—once the swelling of the former was such that the patient was unable to see at all. There was sometimes dryness of the throat and a feeling that the throat is “closed,” so that swallowing is painful and difficult. Some experience nausea or even vomit. Dyspnœa was present twice, the difficulty being with inspiration. The puffiness may involve only the face, but other parts, *e.g.*, prepuce, extremities, etc., may also exhibit the same condition. The lymphatic glands may enlarge and become somewhat tender and the joints a little painful. Even in these cases where there is marked reaction the presence of precipitin is not essential, for although the first five quoted were found to contain them in greater or less degree in the last three none could be demonstrated.

One other point of interest already referred to in the Note to Table I. remains to be mentioned, *viz.*, the variability of the precipitin reaction with different strains of antigen. As already described A.T.S. was always used as the antigen, but by using different batches differing results were obtained. The A.T.S. used was supplied by Burroughs, Wellcome & Co., in phials of about 10 c.c. (1500 units) with one exception—one phial of about 10 c.c. supplied by the Lederle Antitoxic Laboratories, U.S.A. This last was tested against ten samples of human serum, and no precipitation was ever obtained although six of these sera precipitated one or more of the B. W. & Co. strains. No uniformity could be determined in the action of the various batches of A.T.S. For example, if two batches of A.T.S. are precipitated by a given human serum a second human serum may precipitate one of them without precipitating the other, as is seen in the following experiments:—

Batch No.	Serum from Patient No.	Dilution of Serum : 1 in						
		1.	10.	100.	1000.	10,000.	100,000.	1,000,000.
D780	C	...	sl	++	tr	0	0	0
	J	0	++	+	0	0	0	0
D741	C	...	tr	++	tr	tr	0	0
	J	0	+	+	0	0	0	0
D732	C	...	0	++	0	0	0	0
	J	tr	++	tr	0	0	0	0
LAB183	C	...	0	0	0	0	0	0
	J	0	0	0	0	0	0	0
D694	C	...	0	0	tr	0	0	0
	J	0	0	0	0	0	0	0
D798	C	...	tr	++	++	0	0	0
	J	0	++	+	0	0	0	0
D711	C	...	+	++	+	0	0	0
	J	0	++	tr	0	0	0	0
D740	C	...	tr	+	0	0	0	0
	J	0	++	tr	0	0	0	0
D772	C	...	0	+	0	0	0	0
	J	tr	++	tr	0	0	0	0

From the same results it is seen that the titre is different for different batches, and there is suggested the possibility that the liability to serum disease and anaphylaxis may vary in a similar way. Investigations along these lines are being carried out.

CONCLUSIONS.

1. Serum disease is sometimes accompanied by the presence of precipitins in the blood of the patient, but the disease has no demonstrable relation with them.
2. The factors involved in the production of precipitins in human beings are the same as for experimental animals.
3. The antigenic properties of horse-serum vary with the source of the serum.

REFERENCE.

1. LONGCOPE AND RACKMANN *Journ. Exp. Med.*, New York (1918), vol. xxvii, p. 341.