THE RELATION OF CIRCULATING ANTIBODIES TO SERUM DISEASE.

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Though all phases of anaphylaxis have been studied in animals, comparatively few systematic investigations have been made upon the condition as it occurs in man. Moreover, different species vary in their susceptibility to artificial sensitization of foreign proteins as well as in their reactions to a second injection of protein, and hence it is highly essential when opportunities arise to study in detail the problems of anaphylaxis in man.

In this connection the essential difference between man and the experimental animals is that the primary sensitizing dose of serum in animals is not followed by any obvious symptoms, whereas in man it frequently results in serum disease. The object of this investigation was to determine what relation, if any, existed between the formation of antibodies to horse serum and the course of serum disease.

During the last few years we have had an opportunity to study this problem in twenty-five individuals who, for therapeutic purposes, have received various quantities of antitoxic or antibacterial horse serum. Of the twenty-five cases, twenty-one suffered from pneumonia and received repeatedly large doses of antipneumococcus horse serum intravenously.[1] The smallest amount administered to one patient was 5 cc., the largest 630 cc., and the average amounts varied from 180 to 360 cc. Of the remaining four cases, one of meningitis received 40 cc. of antimeningococcus serum intraspinally, one

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[1] This serum was kindly furnished by Dr. Rufus Cole of the Hospital of The Rockefeller Institute for Medical Research and was an antiserum for Pneumococcus Type I.

case of tetanus received 22.5 cc. of antitetanus serum intraspinally, and two cases of diphtheria each received 7.5 cc. of the antitoxin subcutaneously.

To demonstrate the antisubstances to foreign protein in animals and occasionally in man, three methods have heretofore been employed: first, the test for precipitins; second, the demonstration of anaphylactin by the passive transfer of anaphylaxis by the blood serum of the test animals to a normal guinea pig; and third, the presence of a specific skin reaction. Since the classic studies of von Pirquet and Schick upon serum disease (1), a number of investigations have been made upon the relation that one or another of these reactions bears to the development of serum disease, but without definite results as regards their significance.

Hamburger and Moro (2), who first studied the precipitin reaction in children following injections of diphtheria antitoxin, found that the reaction appeared during serum disease, and as the precipitin increased in concentration in the blood the precipitinogen or horse serum decreased. They concluded that the interaction of precipitin and horse serum in the circulation produced a poisonous substance that was responsible for serum disease. Von Pirquet and Schick could find no relation between the presence of precipitins in the circulation and serum disease. Lemaire (3) confirmed the results of Hamburger and Moro, and stated, further, that precipitins were not demonstrable unless serum disease followed the injections of antitoxin. Wells (4), in a study of the precipitin content of the blood of children to whom diphtheria antitoxin had been administered, found precipitin in the circulation occasionally before the onset of serum disease. During serum disease precipitin was present in low concentration but rose rapidly in concentration towards the end of the illness to fall gradually thereafter and eventually disappear. Weil (5) has made similar observations.

Regarding the development of anaphylactin during serum disease, little is known. The isolated observations of Rosenau and Anderson (6), Anderson and Frost (7), Achard and Flandin (8), and Weil (9) show that anaphylactin to horse serum may be transferred passively from man to guinea pig. Novotny and Schick (10), however, succeeded in demonstrating anaphylactin in the blood of only two of twelve children that had received injections of horse serum. Grysez and Bernard (11), on the other hand, were able to sensitize guinea pigs passively to horse serum with the blood drawn from eleven children 5 to 243 days after the injection of horse serum.

Doerr and Russ (12), Doerr and Moldovan (13), and recently Weil (14) have brought forth evidence to show that the precipitating substance and anaphylactin are one and the same, but since it has also been shown both by Doerr and Moldovan and by Weil that serum may transmit passive anaphylaxis and yet

show no precipitating properties, it is necessary in any investigation upon the development of antibodies in serum to take into account the presence of both these reactions. It is possible, moreover, that the anaphylactic antibody which these observers find in the precipitate formed when antigen and antiserum are brought together may not be identical with the precipitate but absorbed by it during the process of precipitation. That protective antibodies may be carried down from antiserum by precipitates is well known,[2] and it is possible that the same may be true of anaphylactic antibody.

The occurrence of the specific skin reaction during and after serum disease has been observed by Hamburger and Pollak (15), Michiels (16), and Cowie (17). Michiels found that the reaction could rarely be obtained during serum sickness though it appeared shortly after the cessation of the illness. Cowie, by making repeated intradermal inoculations of 0.5 cc. of horse serum in children who had received immunizing doses of diphtheria antitoxin, obtained a positive skin reaction in one case on the 4th day, in seven cases on the 5th day, and in nine cases between the 6th and 10th days after the injection of antitoxin.

In the present investigation the three methods of study already discussed have been employed, though in only four cases have they all been applied to the same individual. In order to determine the time of appearance and the relation of these reactions to serum disease, observations were made at short intervals before, during, and after serum disease in all cases. The curves of precipitin formation were studied in fourteen cases, the development of anaphylactin in fifteen, and the appearance of the skin reaction in fifteen cases.

The precipitin reaction was carried out as follows: To 0.5 cc. of horse serum undiluted and in dilutions up to 1: 100,000 in isotonic salt solution, the patient's serum was added in quantities of 0.5 cc. As controls horse serum was titrated against anti-horse rabbit serum and normal human serum in similar amounts, while sheep serum and beef serum were titrated against the patient's serum. The tubes were kept at 37°C. for 1 hour and then allowed to stand over night in the ice box, when the final reading was made.

In attempts to transmit anaphylactin from one species to another cognizance must be taken of the fact demonstrated by Weil (18) and by Lewis (19) that when two different antigens or foreign proteins are simultaneously injected into an animal the protein in excess

[2] For a discussion of this subject see Gay, F. P., and Chickering, H. T., *J. Exp. Med.*, 1915, xxi, 389.

may prevent either active or passive sensitization to the other protein. It has also been repeatedly observed in attempts to transmit anaphylaxis passively that small amounts of serum will give positive results when the experiment fails with larger quantities. These facts soon became obvious in the present investigation and, therefore, in each experiment a series of guinea pigs was injected with varying quantities of the patients' serum.

The blood drawn from the patient was centrifugalized immediately, the serum separated, heated at 56°C. for 30 minutes, and injected intraperitoneally into four to eight guinea pigs in doses varying from 0.1 to 3 cc. In the majority of instances four doses were employed: 0.1 cc., 0.5 cc., 1 cc., and 3 cc. After an interval of 18 to 30 hours an intravenous injection of 0.5 cc. of horse serum was given to each guinea pig. Definite symptoms of anaphylactic shock were required before the result was termed positive.

To test the skin reaction 0.02 to 0.05 cc. of 1:10 and 1:100 dilutions of horse serum was injected intradermally by means of a specially graduated glass syringe. In the normal individual these minute quantities of serum may occasionally give rise to an immediate reaction. This consists in the appearance within 30 seconds to 1 minute of a red areola 1 to 3 cm. in diameter which rapidly fades within the next 5 minutes and is succeeded by a white indurated wheal 5 to 7 mm. in diameter. This may persist for 15 to 20 minutes. Since the same reaction may be obtained with various fluids, such as isotonic salt solution and water, it was considered by us as a traumatic reaction. In the individual sensitized to horse serum the traumatic reaction may or may not occur. Under any circumstances the true reaction makes its first appearance in 10 to 15 minutes. At this time the original wheal rapidly grows, becomes firm, yellowish in color, often itches, and at the same time a red areola spreads from the margin of the wheal until after 20 to 30 minutes the wheal reaches 15 to 20 mm. in diameter and the areola 30 to 50 mm. in diameter. Within half an hour the reaction begins to fade and after 1 to $1\frac{1}{2}$ hours subsides.

To follow the development of this reaction intradermal injections of diluted horse serum were always made before the therapeutic injections of horse serum and at frequent intervals afterwards until

strongly positive reactions were obtained. As controls, sheep and beef serum in the same quantities and isotonic salt solution were employed.

Of the twenty-five cases which were studied twenty-one developed serum disease that was manifest by such characteristic symptoms as enlargment of lymph nodes, skin eruptions, edema, fever, arthralgia, and enlargement of the spleen. The disease varied from a mild form (Case 11) to a severe relapsing form extending over a period of 3 weeks (Cases 1 and 2). In three cases there were no evidences of serum disease (Cases 12, 14, and 23), and in one patient (Case 13) who received three intraspinal injections of tetanus antitoxin, there were no other signs than slight enlargement of the lymph nodes.

Precipitins.

Of the fourteen cases in which the serum was tested for precipitins, twelve developed serum disease. In all but one of these, precipitins could be demonstrated on one and usually several occasions. Once having made their appearance in the serum they persisted, as a rule, for days and often weeks. In one patient who received 520 cc. of horse serum intravenously in divided doses precipitins could be demonstrated on the 34th day after their first appearance in such concentration that 0.5 cc. of the patient's serum formed a precipitate with 0.5 cc. of a 1: 100,000 dilution of horse serum. The serum from another patient who received 630 cc. of horse serum in divided doses contained precipitins for 58 days. In most instances the patient was discharged from the hospital before the precipitin reaction had disappeared. In two cases precipitins were demonstrated on one occasion only.

In the two patients who escaped serum disease, precipitins could not be demonstrated in the blood at any time. One patient received 300 cc. of horse serum in divided doses, the other 240 cc.

Anaphylactin.

Of the fifteen patients whose blood was tested for anaphylactin, twelve developed serum disease. With the serum of all but one of the latter patients it was possible on one or more occasions to

transmit anaphylaxis passively to guinea pigs. As a rule, the presence of anaphylactin in the circulation was of short duration and positive reactions could be obtained only once or twice. A typical example is shown in the protocol in Table I.

In one instance, however, positive reactions were obtained on five

TABLE I.

Passive Transfer of Anaphylaxis for Horse Serum from Patient to Guinea Pigs.

W. B.; lobar pneumonia. Feb. 6, 1916. 80 cc. of antipneumococcus horse serum intravenously.

Feb. 7. 80 cc. of antipneumococcus horse serum intravenously.

Feb. 17. Onset of serum disease continuing until Feb. 26.

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.	
	gm.	*1916*			*cc.*	*cc.*			
1	357	Feb. 11	5		0.5	0.5	26 hrs.	+ Recovery.	+
2	348	" 11	5		0.5	0.5	26 "	No symptoms.	
3	300	" 11	5		3.0	0.5	26 "	+ Recovery.	
4	205	" 11	5		3.0	0.5	26 "	No symptoms.	
5	Positive control. Jan. 12. 0.1 cc. of horse serum intravenously.					0.5	31 days.	+++ Dead in 3 min.	
6	195	Feb. 17	11	½	0.1	0.5	27½ hrs.	No symptoms.	−
7	204	" 17	11	½	0.5	0.5	27½ "	" "	
8	200	" 17	11	½	1.0	0.5	27½ "	" "	
9	210	" 17	11	½	3.0	0.5	27½ "	" "	
10	420	Positive control. Nov. 18, 1915. 0.25 cc. of horse serum intravenously.				0.5	92 days.	+++ Dead in 3 min.	
11	235	Feb. 21	15	4	0.1	0.5	28 hrs.	No symptoms.	++
12	210	" 21	15	4	0.5	0.5	28 "	" "	
13	183	" 21	15	4	1.0	0.5	28 "	++ Very slow recovery.	
14	217	" 21	15	4	3.0	0.5	28 "	+++ Dead in 12 hrs.	
15	299	Feb. 27	21	10	0.1	0.5	25 hrs.	No symptoms.	−
16	357	" 27	21	10	0.5	0.5	25 "	" "	
17	348	" 27	21	10	1.0	0.5	25 "	" "	
18	310	" 27	21	10	3.0	0.5	25 "	" "	
19	Positive control.					0.5		++ Recovery.	

TABLE II.

Passive Transfer of Anaphylaxis for Horse Serum from Patient to Guinea Pigs.

W. N.; age 12 years; meningitis. Dec. 11, 1915. Intraspinal injection of 20 cc. of antimeningococcus serum.

Dec. 12. Intraspinal injection of 20 cc. of antimeningococcus serum.

Dec. 14. Onset of serum disease continuing until Dec. 22.

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.
	gm.	*1915*			*cc.*	*cc.*	*hrs.*	
20	218	Dec. 16	5	2	0.1	0.5	26	No symptoms.
21	234	" 16	5	2	0.1	0.5	26	" "
22	235	" 16	5	2	0.25	0.5	26	" "
23	219	" 16	5	2	0.25	0.5	26	" "
24	267	Dec. 18	7	4	0.15	0.5	39	No symptoms.
25	302	" 18	7	4	0.20	0.5	39	" "
26	242	Dec. 21	10	7	0.1	0.5	22	+++ Recovery.
27	197	" 21	10	7	0.1	0.5	22	+++ "
28	182	" 21	10	7	1.0	0.5	22	+++ "
29	216	" 21	10	7	1.0	0.5	22	+++ "
		1916						
30	205	Jan. 3	23	20	0.1	0.5	22	+(?) Recovery.
31	245	" 3	23	20	0.5	0.5	22	++++ Dead in 4 min.
32	170	" 3	23	20	1.0	0.5	22	++++ " " 2 "
33	250	" 3	23	20	3.0	0.5	22	No symptoms.
34	250	Positive control. Nov. 18, 1915. 0.3 cc. of horse serum intravenously.				0.5		++++ Dead in 2 min.
35	257	Jan. 18	38	35	0.1	0.5	20	No symptoms.
36	286	" 18	38	35	0.5	0.5	20	" "
37	227	" 18	38	35	1.0	0.5	20	+(?) Recovery.
38	297	" 18	38	35	1.0	0.5	20	+(?) "
39	305	" 18	38	35	2.5	0.5	20	+++ "
40	236	" 18	38	35	3.0	0.5	20	++++ Dead in 8 min.
41	335	Positive control. Dec. 7, 1915. 0.5 cc. of horse serum intravenously.				0.5		++++ " " 2 "
42	247	Feb. 2	53	50	0.1	0.5	20	No symptoms.
43	234	" 2	53	50	0.1	0.5	20	" "
44	265	" 2	53	50	1.0	0.5	20	++ Recovery.
45	218	" 2	53	50	3.0	0.5	20	+++ "

TABLE II—*Concluded.*

Guinea pig No.	Weight.	Date of patient's bleeding.	Day after injection of serum.	Day after onset of serum disease.	Amount of patient's serum.	Amount of horse serum.	Time after sensitization.	Result.
	gm.	1916			*cc.*	*cc.*	*hrs.*	
46	199	Feb. 21	72	69	0.1	0.5	28	No symptoms.
47	252	" 21	72	69	0.5	0.5	28	+ Recovery.
48	202	" 21	72	69	1.0	0.5	28	++ "
49	188	" 21	72	69	3.0	0.5	28	+++ "
50	302	Mar. 9	89	86	0.5	0.5	22	No symptoms.
51	270	" 9	89	86	1.0	0.5	22	" "
52	315	" 9	89	86	3.0	0.5	22	" "
53	Positive control. Jan. 2. 0.5 cc. of horse serum intravenously.					0.5		++++ Dead in 4 min.

occasions from the 10th to the 72nd day after the administration of 40 cc. of horse serum intraspinally. Table II gives the protocols of these experiments.

In three cases the injections of serum were not followed by serum disease. Anaphylactin could never be demonstrated in the serum drawn repeatedly from these patients.

Skin Reactions.

In fifteen cases repeated skin reactions were made and in all of these a positive response was sooner or later observed. The skin reactions, unlike the temporary presence of precipitins and the fleeting reaction for anaphylactin, having once appeared, persisted and increased in intensity as long as the patients could be observed. The reaction was also observed in three patients who did not develop serum disease and whose blood serum showed no precipitin or anaphylactic antibody for horse serum. In these three cases the sensitiveness of the skin was much delayed in its onset. In the twelve cases that suffered from serum disease, the hypersensitiveness of the skin became evident on the 7th to the 15th day after the first injection of serum, whereas in these three patients who did not develop serum disease the first evidence of skin hypersensitiveness was observed in one on the 17th day, in one on the 33rd day, and in one on the 50th day, after the first injection of serum.

From these results it is evident that in the cases which we have studied precipitins and anaphylactic antibodies were much more likely to be found in the blood serum of patients who suffered from serum disease following the injections of horse serum than in patients who escaped serum disease. In the latter instances precipitins and anaphylactic antibodies were not observed. The skin reaction, however, made its appearance irrespective of the amount of serum administered or the method of administration and appeared whether or not serum disease developed.

The Relation of Antibody Formation to Serum Disease.

Further study was made in an attempt to determine whether there existed any direct relation between the formation of circulating antibodies and serum disease, and, if so, what this relation might be.

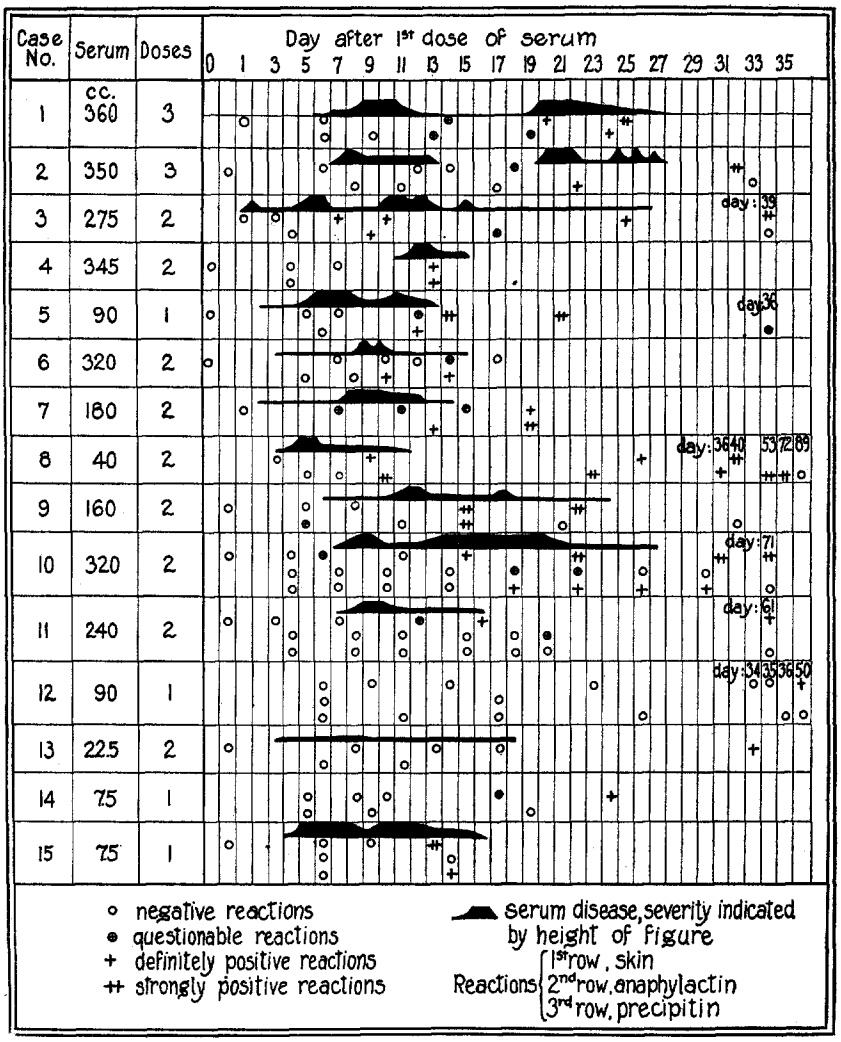
From the time that Hamburger and Moro demonstrated the presence of precipitin for horse serum in the blood of patients with serum disease, the question has constantly been raised as to whether these circulating antibodies play a part in the production of this illness. Von Pirquet and Schick were of the opinion that the illness depended upon the union of antibody and antigen either in the circulation or body cells and that the onset depended upon the proper balancing of these substances so that they might unite effectively to form a poison.

The problem may be viewed from three standpoints: (1) The formation of circulating antibodies may be totally independent of the serum disease. (2) The union of circulating antibodies and circulating antigen may produce a poison which gives rise to serum disease. (3) Antibodies may appear in the circulation as the result of a violent tissue reaction which manifests itself as serum disease, under which circumstances the antibodies might be protective rather than injurious.

The first proposition seems highly improbable, for otherwise one would expect antibodies to appear in the serum irrespective of serum disease, which was not true in this series of observations.

If the union of circulating antibody and circulating antigen were the sole cause of serum disease, one should be able to obtain evidence of the presence of these substances in the circulation immedi-

ately before or at the onset of serum disease. Such a demonstration has not been possible in these experiments. In only one instance (Table I) was a questionably positive result for anaphylactin obtained



TEXT-FIG. 1. The relation between the appearance of anaphylactin and precipitin in the circulation and of skin hypersensitiveness to serum disease in fifteen patients.

on the day previous to the onset of serum disease, and in no instance were precipitins present in the circulation before or at the time of onset of serum disease. Text-fig. 1 indicates the relation between the course of serum disease and the appearance of anaphylactin in the circulation.

It will be seen that blood serum drawn from the patients during the early stages of serum disease did not transmit anaphylaxis passively to guinea pigs. As a rule, anaphylactin was not demonstrable until the disease was well advanced or indeed about to terminate. This is particularly noticeable in Cases 1, 2, and 10, in whom relapses or recrudescences occurred. The blood from Case 1 did not give strongly positive results until the close of the relapse, which was 18 days after the onset of the disease. In Case 2 anaphylactin appeared 5 days before the close of the relapse and in Case 10 towards the subsidence of the recrudescence.

Table III is a composite table which shows even more strikingly

TABLE III.

The Relation between the Onset and Subsidence of Serum Disease and the Appearance of Anaphylactin in the Blood and of Hypersensitiveness of the Skin to Horse Serum, in Twelve Cases.

Days after 1st injection of horse serum	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	38	50
Onset of serum disease.....	1			2	1	1	4	1			2																		
Anaphylactin first demonstrated in blood serum..									1	2		1	3*		1			1		1†		1		1					
Recovery from serum disease.											2	1	1		2	2				1						1	2		
First positive skin reaction..							1		1			2	2	2	2			1	1				1‡					1‡	1‡

The numerals indicate the number of cases.

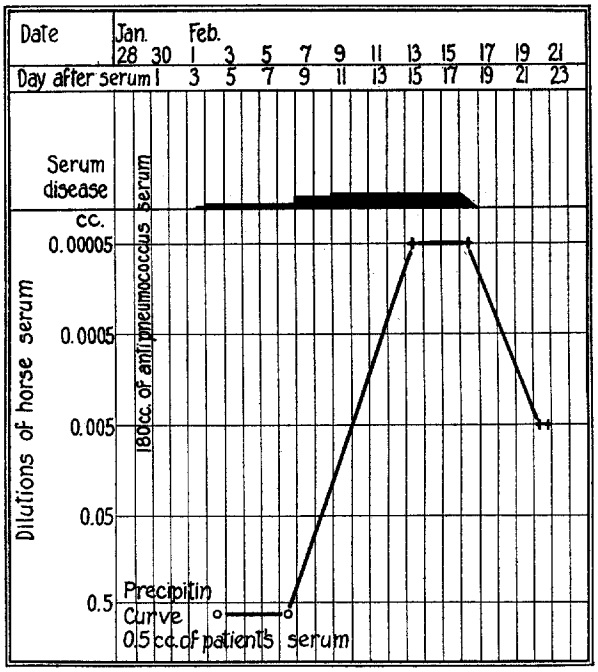
* One experiment doubtful but positive on 24th day.

† Doubtful reaction.

‡ No serum disease.

the average time relation between the appearance of anaphylactin and the course of serum disease. In Table III the average onset of serum disease came 4 to 8 days after the injection of serum, whereas anaphylactin makes its appearance 10 to 15 days after the injection of serum, and is more closely associated with the subsidence of serum sickness than with its onset.

Much the same relation was found to exist between the time of



TEXT-FIG. 2. The time relation of precipitin formation to serum disease. W. V. Type I pneumonia. 180 cc. of serum in two doses, January 29, 1917.

appearance of precipitin in the blood and the course of serum sickness in eleven cases that were studied. Precipitins were not demonstrable in the patient's blood serum until 3 or 4 days after the onset of serum sickness. After this time they rose rapidly in concentration to reach their maximum at the time of recovery. After recovery they diminished either rapidly or gradually in concentration. Text-fig. 2 is constructed from a typical protocol.

TABLE IV.

The Relation between the Onset and Subsidence of Serum Disease and the Appearance of Precipitins in the Circulation in Eleven Cases.

Days after injection of horse serum.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Onset of serum disease.....	1		1	2	2	1	1	1	1	1																							
Appearance of precipitins....									1	1	1	2	1	1	1	1		1	1														
Recovery from serum disease.		1										1	1		2		1		1	1	1					1							1

The numerals indicate the number of cases.

Precipitins were present in low concentration in the blood serum of three patients, in whom the serum sickness ran a protracted course, from the end of the 1st week to a period near the close of the disease when they rose rapidly in concentration. Wells and Weil have both made similar observations. On several occasions fever, which is often a late symptom of serum disease, was associated with the presence of precipitins in the circulation.

The composite Table IV, from the protocols of eleven cases, shows definitely the tendency for the appearance of precipitins in the circulation to precede immediately recovery from serum sickness.

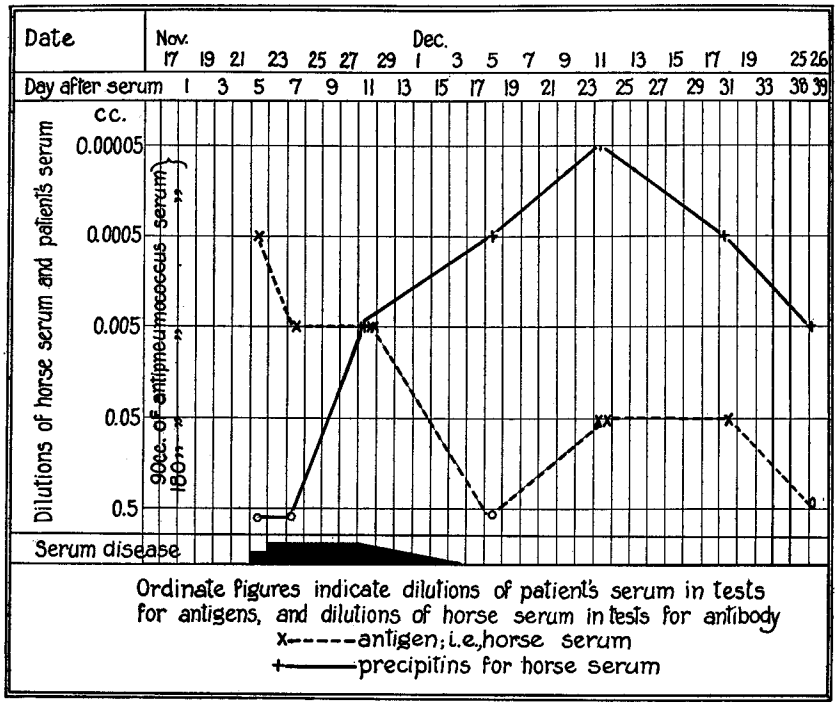
The relation of the appearance of the skin reaction to the course of serum disease in the cases that we have studied is made evident in Text-fig. 1. This reaction manifested itself only when serum disease was well advanced or had subsided, but since the skin became hypersensitive in all individuals who had received injections of horse serum and persisted over long periods of time, it seems probable that this reaction has a different significance from that of precipitin and anaphylactin formation, for the expulsion of these antibodies into the circulation is transient in character and definitely associated with recovery from serum disease, whereas the altered reaction of the skin is persistent and probably represents a changed characteristic of cells rather than a product of altered tissue reactions.

Our observations indicate a close relation between the appearance of antibodies for horse serum in the circulation and the termination of serum sickness. In no instance did antibodies appear either before or during the early stages of the disease, and in a few patients whose serum sickness was of unusually long duration there was a definite delay in the appearance of antibodies in the circulation. Further studies that have been made suggest that the appearance of antibodies in the circulation in great concentration is not only related to the termination of serum sickness but actually assists in bringing this about.

Early in the observations it became evident that circulating antibody to horse serum did not make its appearance unless it was preceded by a severe reaction in the tissue cells; namely, serum sickness. It seems highly improbable, therefore, that serum sickness depends upon a union of antigen and antibody in the circulation, but rather

that such a union, if it takes place, must do so, as Weil and others have frequently emphasized, within the cells of the body.

It is, however, obvious that antigen must be present either in the circulation or within the cells in order to bring about the reaction which we recognize as serum disease. Repeated observations have been made by us and by others to show that precipitinogen, or horse



TEXT-FIG. 3. Balance between antigen and precipitin in the circulation, and their relation to the course of serum disease. M. B., female; age 32 years. Type I pneumonia. Total 270 cc. of horse serum.

serum, remains in the circulation during at least a part of serum disease, so that both precipitin and precipitinogen are present in the circulation during serum sickness and for a few days thereafter. Most observers state that the antigen disappears more rapidly than precipitin, which remains in the circulation for days or weeks after recovery from serum disease. We have made an attempt to deter-

mine, by the methods of precipitation and the production of active anaphylaxis in guinea pigs, the exact time of disappearance of antigen from the circulation and the relation of its disappearance to the concentration of antibodies and to the duration of serum sickness. These observations, together with some others upon the absorption of antigen, will be reported later. Repeated observations have shown, as a rule, that there is a rapid disappearance of antigen upon the appearance of antibodies in the circulation in great concentration, and that the disappearance of antigen or the rapid diminution of antigen follows the rapid rise of precipitin and is coincident with recovery from serum disease. Text-fig. 3 is the reconstruction of a typical series of protocols showing the relation between precipitinogen *i.e.* horse serum, and precipitin to the course of serum disease, and its termination.

DISCUSSION.

The experiments and observations recorded show that serum sickness is essentially dependent upon a reaction which takes place within the cells of the body and is probably dependent upon the formation of a toxic substance within these cells. Following this violent cellular reaction antibodies for horse serum are at first slowly and later rapidly extruded into the circulation in great concentration. As the antibodies rise in concentration the antigen rapidly disappears and coincident with this reversal in antibody-antigen content of the serum, serum sickness abates and the patient recovers. Should the antibody formation be slight in amount, the antigen may persist in large quantities in the circulation and the disease may be prolonged or may relapse.

We have shown that from the time of the primary injection of serum up to the onset of serum sickness, that is during the incubation period of this disease, the concentration of antigen in the circulation remains practically constant and antibodies to horse serum cannot be demonstrated. It seems highly probable, however, that during this period antisubstances are being formed within the cells and that at the time of onset of serum sickness, which is often explosive in character, there is a sudden union between the antibodies within the cells and circulating antigen which bathes the cells. The

course of serum disease depends at this time upon the production of antibody and the amount of available antigen. If antibody is formed in large amount and the quantity of antigen originally introduced is small, it is probable that the circulating antigen will rapidly be dispensed with and serum disease will be of short duration and mild in character. If, however, the amount of antigen originally injected is large and the formation of antibodies is slow and inefficient, it is highly probable that the available antigen will persist over long periods and that the serum disease will be severe in character and prolonged or relapsing in type. The production of circulating antibodies, therefore, seems a purely protective mechanism which is the method the body uses to destroy antigen, in this case horse serum. Since in serum disease the antigen is incapable of reproducing, the process can be accomplished, as a rule, rapidly and effectively, and recovery is prompt and complete.

Although we must always be cautious in drawing conclusions from analogies in biological experimentation, it is nevertheless admissible to suggest that these results may throw light on some of the methods by which the body rids itself of the injurious protein substances which represent antigen in an acute infectious disease.

CONCLUSIONS.

1. The injection of horse serum either in small or in large amounts in human beings is always followed sooner or later by the development of hypersensitiveness of the skin to subsequent injections of horse serum. For the development of this reaction serum disease is not essential.

2. The blood serum of most patients who suffer from an attack of serum disease following injections of horse serum shows anaphylactin and precipitin for horse serum.

3. Anaphylactin and precipitin cannot be demonstrated in the blood serum of patients treated with horse serum who do not later present symptoms of serum sickness.

4. The appearance of anaphylactin and precipitin precedes shortly recovery from the disease.

5. With the appearance in the serum of antibodies to horse serum in great concentration, the antigen rapidly diminishes or disappears.

6. It is probable that the extrusion of these antibodies into the circulation is the result and not the cause of serum sickness. Their presence serves to neutralize or destroy the antigen and thus determines the recovery from serum sickness.

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