PASSIVE SENSITIZATION WITH SYPHILITIC SERUM

By

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INTRODUCTION

Breinl (I) showed that the complement-fixing substance in syphilitic serum which is responsible for the Wassermann reaction can be bound by organ cells. This phenomenom together with the fact, that not only serum but cerebro-spinal fluid, milk and aqueous humor of the eye give a positive Wassermann reaction lead him to believe that the reaction is due not to a physico-chemical condition but to a true antigen-antibody reaction. This view is also supported by Wassermann (2).

In addition to the complement fixation test for syphilis flocculation reactions such as Sachs--Georgi (3) and Kahn (4) tests have been devised. In these reactions a macroscopic precipitate is formed with syphilitic serum and antigen in the absence of active complement.

Kolmer (5) and Wells (6), although not convinced of the presence of a true immune antibody as responsible for the Wassermann reaction believe that the fundamental mechanism of the Wassermann reaction and the flocculation reactions is identical. Wells (6) says: "The agglutination and pre-

cipitin reactions with formed elements and proteins in solution are colloidal reactions even though the chemical nature of the antibody in the serum is unknown. It is entirely reasonable to assume that in the Wassermann reaction the same antibody-like substance is operative as in the various flocculation reactions; that in the former the flocculation occurs which is invisible to the naked eye, but sometimes visible microscopically by the darkfield illumination method. In a general manner the tissue extracts yielding best results in the complement fixation reaction also prove most sensitive in the macroscopic flocculation reaction, e.g., the cholesterolized alcohlic extracts." Weil (7) concludes, from his work in which he passively sensitized animals by injections of a precipitate formed by precipitin and precipitinogen, that precipitin and sensitizer are identical.

Sherwood suggested that if the substance responsible for the Wasserman and flocculation reactions is a true immune antibody and precipitin and sensitizer are identical these might be demonstrated by passive sensitization with syphilitic serum and testing with Kahn antigen to produce anaphylactic shock.

Since the beginning of this work Milkovitch (8) by intravenous injection of a mixture of syphilitic serum, antigen and 10% solution of potassium di-hydrogen phosphate, obtained results which he believed to be clinical anaphylaxis in guinea pigs. The production of shock he believes is due to the increase of the positive ions produced by the potassium di-hydrogen phosphate and explains as follows: "The increase in positive ions is a necessary condition for union of antigen and antibodies to produce a precipitate, which throws a new light on the state of hypersensitiveness. The same antibodies are responsible for immunity or hypersensitiveness. A certain time is necessary for concentration of positive ions in the production of antibodies. If the second injection of antigen comes at the optimum concentration of positive ions shock is produced."

Falk and Caulfield (9), working with edestin and ovalbumin showed that the acuteness of anaphylactic shock was increased in both passively and actively sensitized guinea pigs if the sensitizing solution was of acid reaction and that the reaction is less severe the more alkaline the intoxicating dose.

The scope of this work is as follows:

1. A study of the nature of the substance responsible for the Wasserman reaction and flocculation reactions by attempting to passively sensitize animals with syphilitic serum and demonstrate anaphylaxis by (a) clinical manifestations with Kahn antigen of both acid and alkaline reactions, (b) smooth muscle reaction and (c) skin hypersensitiveness using the same antigens.

2. To check the work of Milkovitch on anaphylactic shock produced by injection of admixture of syphylitic serum antigen and 10% potassium di-hydrogen phosphate.

EXPERIMENTAL

Clinical Anaphylaxis

Technique

Antigen Chosen.

The antigen was prepared by one of the earlier methods suggested by Kahn (4) and used according to Procedure II. Although this method has been modified by Kahn it was chosen since the alcohol in the new antigen renders it unsuitable for smooth muscle work and animal inoculation. Furthermore the precipitate of the new Kahn antigen might be undesirable in this work.

Method of Preparation.

Three beef hearts were freed from fat and connective tissue, ground, dried in thin layers for 2 days, then ground to a powder. Of this powdered muscle 200 grams were placed in a 2 liter Erlenmeyer flask and 900 c.c. of ether added. The flask was thoroughly shaken and placed in the icebox over night. The ether was then decanted, 450 c. c. fresh ether added, shaken and again extracted in the icebox over night.

This process was repeated twice, making four ether extractions or until the supernatant

ether was free of coloring matter. The powdered muscle was spread on filter paper and dried until no odor of ether remained. It was then weighed and 5 c.c. of 95% alcohol added per gram of muscle (195 gm. muscle--975 c. c. alcohol.) Alcohol extraction was carried on in the icebox for 8 days and at room temperature one day. The alcohol was filtered off and to 500 c. c. of the alcoholic extract 2 grams (0.4%) of cholesterol were added. The extract was warmed in a water bath to dissolve the cholesterol and then filtered.

Titration of Antigen.

The antigen was titrated with saline 0.85%, Tyrode solution and a modified Tyrode solution of acid reaction given below. Varying amounts of saline were mixed with constant amounts of antigen by pouring saline into the antigen tube and rapidly pouring back and forth. The tubes showing precipitate were then centrifuged, the supernatant fluid poured off, thus getting rid of the alcohol and twice as much saline was added to each tube as antigen originally used. That dilution showing opalescence was chosen as the proportion of antigen and saline to be used.

The same procedure was followed replacing the saline with Tyrode solution and modified Tyrode solution of acid reaction.

Antigen used in testing the animals for sensitivety was made up in Tyrode solution and the modified acid solution. Antigen made up in salt solution was used only in setting up Kahn tests with serum.

Materials Used.

The syphilitic serum was obtained through the courtesy of Dr. Axtell of Lansing and Dr. Bennett of Leavenworth. About once a week, blood was received by mail in sterile bottles. Each bottle contained blood from several persons. The serum was removed and kept frozen in brine until used. Kahn tests by Procedure II and complement-fixation by Kolmer technique were run an all serum used.

Normal guinea pigs weighing approximately 180-350 grams were given intraperitoneal injections, some receiving one injection, others three injections on successive days. Serum was at first used unheated; but was found to be toxic and for later use was heated to 56 ^{o}C. for 15 minutes before injections.

Intracardial injections of antigen were used in testing for sensitization.

Tyrode solution was used as a diluent and also in the bath in studying the Dale Reaction. It was made up according to Sollman (10), with the exception of the quantity of calcium chloride of which one-half the regular amount was used to keep down irregular contrac-

tions in smooth muscle work as recommended by Stoland and Sherwood (11). The P_H of this solution was 8.0 - 8.2.

To obtain an antigen of greater acidity, the Tyrode solution was modified by replacing the sodium bicarbonate with sodium di-hydrogen phosphate 1.31%. This amount of di-hydrogen phosphate contains the same weight of sodium as that in the sodium bicarbonate required and therefore does not change the cation ratio. This gave a P_H of 5.4-5.6.

Five guinea pigs were injected intracardially with a mixture of 0.2 c.c. syphilitic serum, 0.2 c.c. antigen made up in modified Tyrode (P_H 5.8-6.0) and 0.1 c. c. KH_2PO_4 10%. The solutions were mixed in the syringe immediately before injection. Controls consisted of four guinea pigs injected with a mixture in which the syphilitic serum was replaced by normal serum. This is the technique used by Milkovitch (8).

Results of Clinical Experiments:Alkaline Antigen.

To determine the toxic effect of Kahn antigen of alkaline reaction guinea pigs were injected both intraperitoneally and intracardially with varying amounts of antigen made up in alkaline Tyrode solution. Amounts

up to 20 c. c. had no apparent affect when injected intraperitoneally. Smaller amounts were used for intracardial injections, also with negative results as shown in Table I.

Table II shows those guinea pigs which were injected with syphilitic serum and tested with alkaline antigen.

Table I

Toxic Effect for Guinea Pigs of Kahn Antigen made up in Alkaline Tyrode Solution

Guinea Pig No.	Wt. gms.	Dose cc.	Method of injection	Results	No. of days lived	Autopsy
1	250	20	intraperi-toneal	Neg.	26	negative
2	250	10	"	"	16	----
3	240	5	"	"	17	----
4	240	2	intracard-ial	"	29	negative
5	240	2	"	"	72	negative
6	240	1	"	"	16	----
7	240	4	"	"	15	----

Table II

Syphilitic Serum Followed by Alkaline Kahn Antigen

No.	Wt. gms.	cc. serum injected	Incubation time	cc. ant. injected	Symptoms	Temp. change °F.	No. days lived	Autopsy
113	320	9.0	6 hrs.	2.5	negative	0	---	----
119	300	9.0	6 hrs.	2.5	"	-1.2	---	----
120	315	9.0	6 hrs.	2.5	"	-3.0	---	----
105	210	9.0	12 hrs.	2.5	shivering	+0.4	7	negative
123	300	9.0	12 "	3.0	negative	0	---	----
124	250	9.0	12 "	3.0	negative	0	---	----
43	300	4.0	1 day	2.5	"		55	----
44	173	2.0	1 "	2.0	weak, increased resp., hair rough, defecation		31	negative
45	320	3.0	1 "	3.0	weak, restless	-3.8	21	negative
106	215	9.0	1 "	2.5	negative	0	1	negative
114	240	8.5	1 "	2.5	negative	+0.2	1	----
8	300	2.5	2 "	2.5	negative		1	cardiac hemorrhage
10	300	2.5	2 "	2.0	restless, weak		34	abdominal hemorrhage congestion
9	300	2.5	2 "	2.0	sneezed		1	cardiac hemorrhage
46	260	2.5	2 "	3.0	negative	+0.6	37	negative
22	240	4.0	3 "	4.0	negative		28	negative
23	240	4.0	3 "	4.0	negative anaphy.		½ hr.	cardiac hemorrhage
11	300	2.5	4 "	2.0	shivering, weak, hair rough, restless		13	abdominal hemorrhage adhesions congestion
12	300	2.5	4 "	2.5	"		27	"
13	250	2.0	4 "	4.0	negative		+ 62	----
14	250	2.0	4 "	4.0	" anaphy.		1 hr.	cardiac hem.
15	250	2.0	4 "	3.0	weak, hair rough, resp. deep		15	abdominal hemorrhage
24	240	4.0	5 "	3.5	neg. anaphy.		14 '	cardiac hem.
25	240	4.0	5 "	4.0	negative		66	----
47	320	4.0	6 "	4.0	"		+ 60	----
48	160	4.0	6 "	2.0	restless, weak		3	negative

(Cont'd p. 11a)

Table II (Cont'd)

No.	Wt. gms.	cc. serum injected	Incubation time	cc. ant. injected	Symptoms	Temp. change °F.	No. days lived	Autopsy
49	305	4.0	6 days	4.0	restless, neg.		+ 60	----
50	268	4.0	6 "	2.0	restless, shivering		+ 60	----
51	305	4.0	6 "	4.0	restless		15	abdominal hemorrhage adhesions congestion
109	240	8.0	7 "	2.5	negative	+0.8	23	----
32	385	4.0	8 "	4.0	" anaphy.		10 min.	abdominal cardiac hemorrhages
35	305	4.0	8 "	4.0	negative		14	negative
26	225	4.0	21 "	4.0	restless shivering		11	abdominal hemorrhage
28	245	4.0	21 "	4.0	negative		10	negative
112	235	8.0	21 "	2.5	"	+0.2	4	----

It will be observed from a perusal of Table II that 13 pigs exhibited one or more of the following symptoms: weakness, restlessness and shivering, subsequent to the injection of antigen. A possibility that such symptoms might be due to a non-specific reaction of antigen and serum of previous injections, suggested that a series of guinea pigs be injected with normal human serum and tested with antigen. This data is summarized in Table III.

Table III
Normal Serum Followed by Alkaline Kahn Antigen

Guinea Pig No.	cc. whole blood	Incubation Time in days	cc. antigen injected	Symptoms	no. days lived	Autopsy
16	3.0	2	1.5	negative	62	negative
17	3.0	2	2.5	"	20	abdominal hemorrhage
18	4.0 serum	2	3.0	"	81	negative
19	2.5	5	4.0	"	13	----
20	2.5	5	4.0	weak	1	clot on heart
21	2.5	5	4.0	negative	10	abdominal hemorrhage

Toxicity of Unheated Serum

1. Normal Serum

Two of the series in Table III and seven of Table II on autopsy showed a hemorrhagic condition of the peritoneal cavity, which was not observed in animals from the series summarized in Table I which received only antigen. This might be due to a combined action of serum

and antigen or serum alone. The results of guinea pigs injected intraperitoneally with unheated normal serum are given in Table IV. Autopsy at death showed two with abdominal hemorrhage and three with negative findings.

Table IV

Toxic Effect of Normal Serum

Guinea Pig No.	cc. serum injected	days lived	Autopsy
30	3.5	30	abdominal hemorrhages
31	3.5	28	abdominal hemorrhages
37	4.0	48	negative
38	4.0	11	negative
42	4.0	15 (killed)	negative

2. Syphilitic Serum

Another series were given unheated syphilitic serum. As shown in Table V, one animal on autopsy showed a hemorrhagic condition of the abdomen.

Table V

Toxic Effect of Syphilitic Serum

No.	cc. serum injected	Days lived	Autopsy
27	4.0	11	abdominal hemorrhage
29	4.0	4	negative
33	4.0	7 (killed)	"
34	4.0	31	"
36 A	4.0	7 (")	"
61	4.0	1	"
62	2.5	2	"
63	2.5	1	"
64	2.5	2	"
65	3.0	1	"
66	3.0	1	"
67	2.5	1	"
68	7.2	16	"
69	3.0	9	"

This series includes those animals which died in a short time (1 or 2 days) after injection of syphilitic serum and were intended for testing with antigen. All received one injection of unheated serum except No. 68, which received three injections of heated serum. This apparent toxicity of unheated serum lead to the use of heated serum for all animals after that time.

Acid Antigen

A series of three animals, Table VI, were used for testing the effect of Kahn antigen made up in modified Tyrode solution of acid reaction. Amounts up to 4 cc. by intracardial injection gave no gross symptoms. A drop in temperature of one degree was noted with one animal.

Table VI

Toxic Effect for Guinea Pigs of Kahn Antigen made up in Modified Tyrode Solution of Acid Reaction

Guinea Pig No.	Wt. gms.	Dose cc.	Method of injection	Symptoms	Temperature change °F	No. days lived	Autopsy
98	230	4.0	intra-cardial	negative	none	---	-----
99	210	3.5	"	negative	-1°	28	no apparent cause of death
100	250	3.5	"	negative	none	44	-----

Acid Antigen and Syphilitic Serum

Table VII shows those guinea pigs tested with

acid antigen after sensitization with syphilitic serum. All were injected on three successive days with heated serum. The amount of antigen used was 2.5 cc.

Table VII

Syphilitic Serum followed by Acid Kahn Antigen

No.	Wt. gms.	Total cc. ser. injected	Incubation time	cc. antigen	Symptoms	Temp. change °F.	Days lived
121	285	9	6 hr.	2.5	negative	0	11
122	310	9	6 hr.	2.5	"	-0.6	over 21
125	270	9	12 "	2.5	"	-1.0	"
126	305	9	12 "	2.5	died in ½ hr. after ant. injection	--	(8 min.)
127	330	9	1 day	2.5	"	--	(28 min.)
128	340	9	1 "	2.5	negative	0	over 21
115	205	8.5	2 "	2.5	"	0	"
116	250	8.5	2 "	2.5	"	+0.1	8
117	215	8.5	4 "	2.5	"	+0.2	10
118	250	8.5	4 "	2.5	"	0	over 21
107	240	8.0	7 "	2.5	"	+0.4	25
108	210	8.0	7 "	2.5	"	0	over 21
110	290	8.0	21 "	2.5	coughed rolled from side to side	-1.6	"
110	290	8.0	21 "	2.5	negative	-1.9	"

It will be observed from a perusal of Table VII that no symptoms were observed in any of the animals except No. 110 which coughed and rolled from side to side, then became quiet within five minutes. A drop of 1.6° F. in temperature was noted. Three animals also showed a slight drop in temperature, but no gross symptoms.

Results of Injections of Syphilitic Serum, Antigen and KH_2PO_4 (10%)

Of the five animals injected with mixtures of syphilitic serum, antigen and potassium di-hydrogen phosphate (10%) two were definitely negative as shown in Table VIII. One exhitited such symptoms as weakness, difficult breathing, shivering, excitement, gasping, scratched nose, then recovered in 55 minutes. The remaining two began gasping before the injection was completed, one died immediately, the other within 5 minutes, following symptoms of difficult breathing and excitement. On

Table VIII

Guinea Pigs Injected with Syphilitic Serum, Antigen and Potassium Di-Hydrogen Phosphate (10%)

Guinea Pig No.	Wt. gms.	Injection dose	Symptoms	Autppsy
101	230	0.2 cc. syphilitic Ser. 0.2 cc. acid antigen 0.2 cc. KH_2PO_4 (10%)	Difficult breathing, weak, shivers, excitement, heart irregular, gasps scratches nose	lived over 30 days
102'	215	"	negative	lived over 60 days
129	260	"	gasping, dies	lung collapsed
130	320	"	" "	"
131	260	"	negative	lived over 4 da

autopsy the lungs were collapsed, heart normal, no clot found within the heart, no hemorrhage in the pericardium, apparently no intravascular clotting in the pulmonary circuit or vena cava and no abdominal hemorrhage. Pig No. 102 in the preceding table showed a drop in temperature of 0.4° F. The others showed no temperature change.

Normal Serum Control Using Antigen and KH_2PO_4 (10%)

Table IX shows controls of normal serum substituted for syphilitic serum. Two showed no symptoms, with slight rise in temperature. One showed signs of weakness, gasped several times, then became quiet and recovered within fifteen minutes. The fourth jumped about and fell from side to side, then became quiet after five minutes.

Table IX

Guinea Pigs Injected with Normal Serum, Antigen and Potassium Di-hydrogen Phosphate (10%)

Guinea Pig No.	Wt. gms.	Injection dose	Symptoms	Temp. change °F.	No. days lived
103	260	0.2 cc. Normal serum 0.2 cc. acid antigen 0.1 cc. KH_2PO_4 (10%)	Weakness, gasping	-0.2	64
104	240	"	Negative	+0.4	over 14
132	303	"	Jumps about, falls backward several times.	+0.6	over 14
			Negative	+1.0	over 14

SMOOTH MUSCLE WORK

Technic

Since one of the criteria for true anaphylaxis is "demonstration of typical reactions in virgin guinea pig uterine strips", quoting Wells (12), it was planned to use this reaction together with the clinical reaction in testing for sensitization.

Normal virgin guinea pigs weighing from 180-260 grams were used.

Injections of syphilitic serum were made as for clinical anaphylaxis.

In testing the uterine strips the animals were killed, the abdomen opened, and the horns removed and placed in the muscle warmer. They were attached by means of ligatures to the rod in the muscle warmer and to a heart lever, which recorded the contractions on a smoked drum. The two arms of the lever were in the ratio of 2:1 so that the tracings had twice the value of the muscle reactions. The bath was kept at a constant temperature of 38 C. Oxygen was allowed to bubble slowly through the bath. A syphon was used for removing solutions from the muscle warmer. Pituitary extract was used to test the horns for viability.

A bath of 25 cc. volume was used throughout. Uterine strips tested with acid antigen were suspended in an acid bath. Where alkaline antigen was used the bath

was also alkaline. The right and left horns were used alternately for the acid and alkaline antigen.

Results of Smooth Muscle Work

Alkaline Antigen

1. Toxic Dose. Before testing uterine strips for sensitivity the maximal non-toxic dose of antigen for uterine horns was determined. These results are shown in Table X. Antigen amounts from 1.5 cc. to 10 cc. were negative with the exceptions of no. 59 in which both horns contracted upon the addition of 10 cc. of antigen to the bath, No. 55 in which the left horn contracted slightly with 5 cc. of antigen, the right horn being negative, and No. 54 in which the right horn did not react to 4 cc. of antigen, but the left horn gradually contracted three and one-half minutes after the addition of 4 cc. of antigen. This was considered to be due to some factor other than the presence of antigen, and 4 cc. was taken as a safe quantity for use as a testing dose. This large an amount was desired so that the antigen might not be too dilute, also the ratio of antigen to the bath (25 cc.) was approximately that of the antigen to serum in the Kahn test.

Table X

Toxic Effect of Alkaline Kahn Antigen on Excised Uterine Horns of Guinea Pigs

No.	Wt. gms.	Horn	cc. Antigen	Result
51A	210	right	2-1.5	negative
		left	-----	horn dead
52A	200	left	2 - 3	negative
		right	2 - 3	"
53A	212	left	4	"
		right	4	"
54	220	left	4	" ---(gradual rise
				2½ min. after
		right	4	" ant. was added)
55	220	left	5	slight rise
		right	5	negative
56	215	left	5	"
		right	5	"
57	210	left	5	"
		right	5	"
58	206	left	5	"
		right	5	"
59	210	left	10	rise
		right	10	"
60	200	left	8 and 10	negative
		right	8 and 10	"

2. Results of Tests for Sensitization. Table XI shows those horns tested with alkaline antigen after sensitization with syphilitic serum.

Of the thirty-seven horns tested all were negative with the possible exception of No. 75 in which the right horn gave a gradual contraction. The horn did not respond to a desensitizing dose but unfortunately was not tested for viability. The left horn from the same animal gave no response to the same amount of antigen but contracted when pituitrin was added.

Table XI

Sensitized Uterine Horns Tested in an Alkaline Bath with Alkaline Kahn Antigen

No.	Wt. gms.	No. of injections	Total cc. serum	Serum heated 56° C. 15 min.	Horn	Incubation time	Results
80	205	3	9.0	+	R	6 hrs.	negative
81	240	3	9.0	+	R	"	"
78	200	3	7.4	+	L	9 "	"
52	210	1	3.0	-	R	12 "	"
52	210	1	3.0	-	L	12 "	horn dead
75	205	3	6.5	+	R	12 "	doubtful
75	205	3	6.5	+	L	12 "	negative
76	193	2	4.0	+	R	12 "	"
76	193	2	4.0	+	L	12 "	"
79	205	3	7.4	+	R	12 "	"
82	200	3	9.0	+	L	12 hrs.	"
83	200	3	9.0	+	L	12 "	"
73	180	1	3.0	-	R	16 "	"
84	200	3	9.0	+	R	1 day	"
53	220	1	3.0	-	R	1 "	"
70	250	1	4.0	-	R	1 "	"
70	250	1	4.0	-	L	1 "	"
72	188	2	4.5	-	L	1 "	"
74	200	3	6.5	+	L	1 "	"
74	200	3	6.5	+	R	1 "	"
77	225	3	8.4	+	L	1"	"
71	290	1	4.0	-	R	2 "	"
93	205	3	8.5	+	R	2 "	"
71	290	1	4.0	-	L	2 "	"
94	250	3	8.5	+	R	4 "	"
95	165	3	8.0	+	L	4 "	"
96	285	3	8.0	+	R	4 "	"
36A	205	1	4.0	-	R	7 "	"
36A	205	1	4.0	-	L	7 "	"
85	230	3	8.0	+	R	7"	"
86	215	3	8.0	+	L	7 "	"
97	260	3	8.5	+	L	7 "	"
87	275	3	8.0	+	R	21 "	"
88	300	3	8.0	+	R	21 "	"
89	235	3	8.0	+	L	21 "	"
90	215	3	8.0	+	L	21 "	"

Acid Antigen.

1. Acidity produced by addition of KH_2PO_4. Two methods of obtaining an acid antigen were tried--the first, intended for comparison with the clinical work of Milkovitch (8) is as follows: One part of potassium di-hydrogen phosphate (10%) was added to four parts of alkaline antigen. But as indicated in Table XII, the minimal toxic dose was as low as 2 cc. of antigen and 0.5 cc. of KH_2PO_4(10%).

Table XII

Toxic Effect of Alkaline Kahn Antigen and KH_2PO_4 (10%) On Excised Uterine Horns

Guinea Pig No.	Wt. gms.	Horn	cc. antigen	+ cc. KH_2PO_4(10%)	Result
71	290	R	3.0	0.75	Large contractions
71	290	L	3.0	0.75	Sharp rise
72	188	R	2.4	0.6	Negative
72	188	L	3.2	0.8	Negative
73	180	R	1.0	0.25	Negative
73	180	L	2.0	0.4	Negative
74	200	R	1.0	0.25	Negative
74	200	L	2.0	0.5	Negative
75	---	L	4.0	1.0	Sharp rise
76	---	R	2.0	0.5	Rise
76	---	L	2.0	0.5	Rise

2. Acidity produced by Replacing Carbonates with Acid Phosphates. Since it was desirable to use a larger amount of antigen, this second method was used. The Tyrode solution was modified to give an acid reaction by replacing sodium bicarbonate with sodium di-hydwogen phosphate

(p. 8). This solution was then used as a bath in which the horns were tested with antigen made up in acid solution.

3. Non-Toxic Dose. To determine the toxic dose of acid antigen, five horns were tested with 8 cc. No reactions were obtained and a dose of 4 cc. of antigen was considered as safe to be used in testing for sensitization.

As shown in Table XIII the twenty horns thus tested gave negative reactions.

Table XIII

Guinea Pig No.	Wt. gms.	No. of injections	cc. Serum	Horn	Incubation	Result
80	205	3	9.0	L	6 hours	negative
81	240	3	9.0	L	6 "	"
82	200	3	9.0	R	12 "	"
83	200	3	9.0	R	12 "	"
91	165	3	8.5	L	18 "	"
91	165	3	8.5	R	18 "	"
84	200	3	9.0	L	1 day	"
92	160	3	8.5	R	2 "	"
92	160	3	8.5	L	2 "	"
93	205	3	8.5	L	4 "	"
94	250	3	8.5	L	4 "	"
95	165	3	8.0	R	4 "	"
96	185	3	8.0	L	4 "	"
85	230	3	8.0	R	7 "	"
86	215	3	8.0	R	7 "	"
97	260	3	8.5	R	7 "	"
87	275	3	8.0	L	21 "	"
88	300	3	8.0	L	21 "	"
89	235	3	8.0	R	21 "	"
90	215	3	8.0	R	21 "	"

4. Control of Effect of Acid Bath on Schultz-Dale Reaction. No increase in irritability or irregular contractions were observed with the usedof an acid bath instead of

an alkaline bath.

A virgin guinea pig was passively sensitized to sheep serum. One horn was suspended in an acid bath, the other in an alkaline bath. Both horns reacted to sheep serum.

SKIN TESTS

Technic

Since the Arthus phenomenon is considered a criterion of sensitization and since Opie ( ) feels that a precipitate is responsible for the reaction, the demonstration of sensitization by the use of intracutaneous tests was attempted in addition to clinical anaphylaxis and uterine horn reactions.

Serum and antigen used were the same as given under clinical anaphylaxis.

Rabbits, white or white with markings, were used. The abdomen was shaved free of hair and sterilized with alcohol at the points of injection, then the alcohol was removed with sterile saline.

Serum injections consisted of 0.5 cc. intracutaneously. After varying lengths of time, 0.4 cc. or 0.7 cc. of antigen of either acid or alkaline reaction was injected into the previously serum-inoculated area.

Controls consisted of syphilitic and normal serum, heated to 56degrees C. for 15 minutes, and unheated, and acid and alkaline antigen injected into separate areas.

Results of Skin Tests

1. Heated Syphilitic Serum followed by Alkaline Antigen.

Four areas tested with 0.4 cc. alkaline antigen after sensitization with 0.5 cc. heated syphilitic serum were negative after initial inflammation lasting for a few hours.

2. Heated Syphilitic Serum followed by Acid Antigen.

Four areas tested with 0.4 cc. acid antigen after sensitization with 0.5 cc. heated syphilitic serum were negative also after redness of one day.

3. Unheated syphilitic Serum followed by Alkaline Antigen.

Of six areas tested with alkaline antigen after sensitization with 0.5 cc. unheated syphilitic serum, three receiving 0.7 cc. antigen were negative, one tested with 0.7 cc. antigen was red, swollen and indurated for 3 days, and two (CI and CIII) tested with 0.4 cc. antigen showed white areas 1 cm. in diameter at the point of injection surrounded by a reddened ring 5 mm. across. The white centers gradually decreased until the next day when they measured 2.2 by 1.7 cm. The areas surrounding them were much redder than previously and were swollen and hard. This condition remained unchanged for 2 days when they showed diminution in size, inflammation and hardness. After 2 more days a scab formed in the center. The areas gradually decreased in size and were completely healed 10 days after the antigen injections.

4. Alkaline antigen.

Five controls of 0.7 cc. of alkaline antigen were negative.

5. Acid antigen.

Three controls of 0.7 cc. of acid antigen were negative.

6. Heated Syphilitic Serum.

One control of heated syphilitic serum was negative.

7. Unheated syphilitic Serum.

Four controls of unheated syphilitic serum were negative and one gave approximately the same reaction as CI and CIII described above but not so pronounced. This area lay between CI and CIII, the three being 2 cm. apart. A fourth area on the same rabbit injected with unheated syphilitic serum only, lying 3 cm. from CIII, measuring 2 by 1.5 cm. was red, swollen and hard for 2 days.

8. Heated Normal Serum.

Two controls of heated normal serum were negative.

9. Unheated Normal Serum.

Two controls of unheated normal serum gave reactions similar to the two areas (CI and CIII) injected with syphilitic serum and antigen. The reactions were slighter and disappeared within 5 days.

10. Heated Syphilitic Serum, Acid Antigen and KH_2PO_4 (10%).

Two areas each injected with 0.2 cc. heated syphilitic serum, 0.2 cc. acid antigen and 0.1 cc. KH_2PO_4 (10%) were negative.

DISCUSSION

As stated in the introduction it was thought that, if the substance responsible for the Wassermann reaction and flocculation reactions is a true immune antibody, animals could be passively sensitized with syphilitic serum and this sensitivity tested for by Kahn antigen. The work of Opie (13), who believes that a precipitate is formed in the tissues in anaphylaxis, and that of Weil (7) on the identity of precipitin and sensitizer offers sufficient reasons for this investigation. If the above be true then our findings should agree with those of Milkovitch (8).

However, our results with injections of syphilitic serum, antigen and potassium di-hydrogen phosphate do not exactly confirm the results of Milkovitch. The lungs of our two guinea pigs (of five injected) which died immediately, on autopsy were completely collapsed. As the classical picture of anaphylaxis, and therefore a necessary criterion, is that of distended lungs at death, this cannot be called anaphylactic shock (Milkovitch did not describe autopsy findings, and therefore it cannot be said that he did not produce anaphylactic shock, however if the lungs were distended, as in anaphylaxis, proof that this was not due to mechanical obstruction of pulmonary arteries would be necessary) but in all probability was anaphylactoid in nature as described by Hanzlik and Karsner (14) who have shown that various colloids when administered intravenously give rise

to symptoms resembling those of anaphylaxis, but which they found due to injury to the respiratory or circulatory systems.

His interpretation that anaphylactic shock is brought about by the injection of antigen when the appropriate concentration of hydrogen ions is reached would indicate that there is a true antibody in syphilitic serum, which should passively sensitize animals to antigen, the positive ions being supplied with the antigen. This was not borne out by our findings as shown in Tables VII and XIII and on pages 27 and 28 where in testing with acid antigen, negative results were obtained with excised uterine horns, live animals and in the skin tests.

Since these experiments did not show sensitization, in view of the work of Falk and Caulfiend (9) it is to be expected that the same tests using an alkaline antigen would also be negative for anaphylaxis.

A glance at Table XI will show that this was found to be true of uterine horns tested. The results of skin tests (p. 27) with areas previously treated with heated syphilitic serum were definitely negative. Where unheated serum was used, the reactions although not indicating sensitization were of interest, since it appears that in two cases the effect of the presence of the serum was intensified by a later injection of antigen.

A perusal of Table II will indicate that no animal

injected with alkaline antigen as a shocking dose died immediately from anaphylactic shock, nor were there severe or moderate symptoms. The doubtful symptoms exhibited by nine animals and those which might be classed as slight symptoms are of interest. The following observations from Table II indicate that the above symptoms may not be true anaphylaxis. There is no correlation between the severity of symptoms, the amount of syphilitic serum per gram of body weight and the amount of antigen per gram of body weight. Weil (15) found in passive sensitization the minimal fatal dose of antigen to be in inverse relation to the previously sensitizing dose. Furthermore, there is no correlation between the severity of symptoms and the incubation period. The temperature changes are not sufficient to indicate anaphylaxis except in No. 45, Table II, with a drop of 3.8 degrees F.

In carrying out these experiments a maximum non-toxic dose of antigen was determined in every case. Stoland and Sherwood (11) have stressed the importance of this. They pointed out the great variation in reaction of animals of the same weight to a certain amount of protein.

A certain toxicity of human serum was observed as a perusal of Tables IV and V will show. In guinea pigs this toxicity was often manifested in abdominal hemorrhage. Not all sera used were toxic nor were all animals affected. Apparently the toxicity was diminished by heating to 56 degrees C. for 15 minutes.

SUMMARY AND CONCLUSIONS

1. Unheated human serum is toxic, to a certain degree for guinea pigs by intraperitoneal injection and for rabbits by intradermal injection. In guinea pigs this toxicity was commonly manifested by abdominal hemorrhage.
2. This toxicity is apparently decreased by heating to 56 degrees C. for fifteen minutes.
3. Kahn antigen, acid or alkaline, was not toxic for live animals in the doses used.
4. A variability in reaction to relatively large amounts of alkaline antigen was shown by excised uterine horns. Acid antigen was without effect in the amounts used.
5. Anaphylactoid phenomena were observed in some guinea pigs when antigen was injected intracardially after receiving syphilitic serum.
6. Death produced by intracardial injections of syphilitic serum, antigen and potassium di-hydrogen phosphate was not due to anaphylactic shock but was of anaphylactoid nature. This was indicated by the completely collapsed lungs.
7. Passive sensitization of guinea pigs with syphilitic serum was not demonstrated by the Schultz-Dale reaction by us with either acid or alkaline Kahn antigen.
8. Passive sensitization of guinea pigs with anti-sheep serum immune serum was demonstrated by the Schultz-Dale reaction using an acid bath.

9. Passive sensitization of rabbits with syphilitic serum was not demonstrated by the skin tests.
10. Our results do not confirm those obtained by Milkovitch by injections of syphilitic serum, antigen and potassium di-hydrogen phosphate.
11. Our results cannot be interpreted as true anaphylaxis, but suggest that either (1) a true antibody is not responsible for the Wassermann reaction and flocculation reactions or that (2) precipitin and sensitizer are not identical.

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