TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN No. 45

JUNE, 1908

FURTHER STUDIES UPON ANAPHYLAXIS

By

M. J. ROSENAU
and
JOHN F. ANDERSON

WASHINGTON
GOVERNMENT PRINTING OFFICE
1908

ORGANIZATION OF HYGIENIC LABORATORY.

WALTER WYMAN, *Surgeon-General,*
United States Public Health and Marine-Hospital Service.

ADVISORY BOARD.

Major Walter D. McCaw, Surgeon, U. S. Army; Surgeon John F. Urie, U. S. Navy; Dr. A. D. Melvin, Chief of U. S. Bureau of Animal Industry, and Milton J. Rosenau, U. S. Public Health and Marine-Hospital Service, *ex officio*.

Prof. William H. Welch, Johns Hopkins University, Baltimore, Md.; Prof. Simon Flexner, Rockefeller Institute for Medical Research, New York; Prof. Victor C. Vaughan, University of Michigan, Ann Arbor, Mich.; Prof. William T. Sedgwick, Massachusetts Institute of Technology, Boston, Mass., and Prof. Frank F. Wesbrook, University of Minnesota, Minneapolis, Minn.

LABORATORY CORPS.

Director.—Surgeon Milton J. Rosenau.
Assistant director.—Passed Assistant Surgeon John F. Anderson.
Pharmacist.—Frank J. Herty, Ph. G.
Artist.—Leonard H. Wilder.
Acting librarian.—E. B. K. Foltz.

DIVISION OF PATHOLOGY AND BACTERIOLOGY.

Chief of division.—Surgeon Milton J. Rosenau.
Assistants.—Passed Assistant Surgeons John F. Anderson, L. L. Lumsden, A. M. Stimson, and Assistant Surgeon W. W. Miller.

DIVISION OF ZOOLOGY.

Chief of division.—Ch. Wardell Stiles, Ph. D.
Assistants.—Passed Assistant Surgeon Joseph Goldberger, Walter D. Cannon, M. D., and Charles Crane, B. S.

DIVISION OF PHARMACOLOGY.

Chief of division.—Reid Hunt, Ph. D., M. D.
Assistants.—Atherton Seidell, Ph. D., and René de M. Taveau, A. B.

DIVISION OF CHEMISTRY.

Chief of division.—Joseph H. Kastle, Ph. D.
Assistants.—Assistant Surgeon Norman Roberts and Elias Elvove, M. S.

CONTENTS.

FURTHER STUDIES UPON ANAPHYLAXIS.

By MILTON J. ROSENAU,
Surgeon, Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service,
and
JOHN F. ANDERSON,
Passed Assistant Surgeon, Assistant Director Hygienic Laboratory. U. S. Public Health and Marine-Hospital Service.

PART 1. THE PERIOD OF INCUBATION.

In our first publication[b] from a limited series of experiments we found the "period of incubation," or the time necessary to elapse between the first and second injection, to be about ten days. We here give the results of three series of tests in order to clear up the following interesting details concerning the period of incubation: (1) Does the hypersusceptibility come on abruptly or gradually? (2) Do guinea pigs sensitized in the brain have a shorter period of incubation than those sensitized subcutaneously? (3) Is the period of incubation constant or variable?

TABLE NO. 1.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1436	0.01 c. c. normal horse serum subcutaneously.	4	6 c. c. normal horse serum subcutaneously.	No symptoms.
1437	.....do.....	4	.....do.....	No symptoms.
1438	.....do.....	5	.....do.....	No symptoms.
1439	.....do.....	5	.....do.....	No symptoms.
1440	.....do.....	6	.....do.....	No symptoms.
1441	.....do.....	6	.....do.....	No symptoms.
1442	.....do.....	7	.....do.....	No symptoms.
1443	.....do.....	7	.....do.....	No symptoms.
1444	.....do.....	8	.....do.....	No symptoms.
1445	.....do.....	8	.....do.....	No symptoms.
1446	.....do.....	9	.....do.....	Slight symptoms.
1447	.....do.....	9	.....do.....	Slight symptoms.
1448	.....do.....	10	.....do.....	Marked symptoms.
1449	.....do.....	10	.....do.....	Marked symptoms.

[a] Manuscript submitted for publication May 5, 1908.

[b] Rosenau, M. J., and Anderson, John F.: A study of the cause of sudden death following the injection of horse serum. Hyg. Lab. Bul. No. 29, April, 1906, 95 p. Also Bul. No. 36, Further studies upon hypersusceptibility and immunity.

TABLE NO. 2.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1363	0.01 c. c. normal horse serum into brain.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1364	.....do.....	2	.....do.....	No symptoms.
1365	.....do.....	3	.....do.....	No symptoms.
1366	.....do.....	4	.....do.....	Suggestive.
1367	.....do.....	5	.....do.....	Suggestive.
1368	.....do.....	7	.....do.....	Died in 25 minutes.
1370	.....do.....	8	.....do.....	Suggestive.
1369	.....do.....	9	.....do.....	Slight symptoms.
1371	.....do.....	10	.....do.....	Dead in 35 minutes.
1372	.....do.....	10	6 c. c. normal horse serum subcutaneously.	Mild symptoms.

TABLE NO. 3.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1423	0.01 c. c. normal horse serum into brain.	4	6 c. c. normal horse serum subcutaneously.	No symptoms.
1424	.....do.....	4	.....do.....	No symptoms.
1425	.....do.....	5	.....do.....	No symptoms.
1426	.....do.....	5	.....do.....	No symptoms.
1428	.....do.....	6	.....do.....	No symptoms.
1429	.....do.....	6	.....do.....	No symptoms.
1427	.....do.....	7	.....do.....	No symptoms.
1430	.....do.....	7	.....do.....	Slight symptoms.
1431	.....do.....	8	.....do.....	Slight symptoms.
1432	.....do.....	8	.....do.....	Slight symptoms.
1433	.....do.....	9	.....do.....	Slight symptoms.
1434	.....do.....	9	.....do.....	Slight symptoms.
1435	.....do.....	10	.....do.....	Marked symptoms.

It will be seen from the above work that the period of incubation is about seven days or less in guinea pigs sensitized in the brain and about nine days in guinea pigs sensitized subcutaneously. So far as may be judged, it therefore appears that the period of incubation is somewhat shorter in guinea pigs sensitized in the brain than those sensitized subcutaneously. It also seems quite evident that the sensitization comes on somewhat gradually. Judged by our own results and the work of others, the period of incubation is quite constant.

Lewis[a] states that the incubation period is not to be considered as abruptly terminating at a given day. He says that he has made an animal quite sick by the intracardial injection of 2 c. c. of serum on the sixth day after a toxine-antitoxin mixture.

[a] Lewis, Paul A.: The induced susceptibility of the guinea pig to the toxic action of the blood serum of the horse. Journ. Exper. Med., vol. 10, No. 1, Jan. 1, 1908, p. 1.

RELATION OF THE AMOUNT OF THE SENSITIZING DOSE TO THE TIME INTERVAL.

It seemed to us at one time in our work that animals injected with a large quantity of horse serum at the first injection had a more prolonged period of incubation or were less sensitive than guinea pigs given the usual minute amounts. We conjectured that this might have been due to the slow absorption of the serum, thus having somewhat the immunizing effect of repeated injections. We therefore prepared four series of guinea pigs: One sensitized with 0.01 c. c., a second with 0.1 c.c., a third with 1 c. c., and a fourth with 8 c. c. of normal horse serum. All of these animals were subsequently tested by intracerebral injections of the same normal horse serum.

So far as may be judged from this work, the period of incubation is not appreciably prolonged by a large sensitizing dose. Further, the animals sensitized with horse serum alone, remain so for a long period of time (245 days).

TABLE No. 4.—*Relation of amount to sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1148	0.01 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Marked symptoms.
1149	.....do.....	17	.....do.....	Dead in 5 minutes.
1150	.....do.....	24	.....do.....	Severe symptoms.
1151	.....do.....	38	.....do.....	Severe symptoms.
1152	.... do.....	110	.....do.....	Dead in 6 minutes.
1155	.....do.....	155	.....do.....	Very severe symptoms.
1157	.....do.....	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.
1158	0.1 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1159	.....do.....	17	.....do.....	Severe symptoms.
1160	.....do.....	24	.....do.....	Severe symptoms.
1161	.....do.....	38	.....do.....	Dead in 4 minutes.
1163	.....do.....	110	0.5 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
1164	.....do.....	155	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1165	1 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1166	.....do.....	17	.....do.....	Severe symptoms.
1167	.....do.....	24	.....do.....	Severe symptoms.
1168	.....do.....	24	.....do.....	Severe symptoms.
1169	.....do.....	110	.....do.....	Very severe symptoms.
1170	.....do.....	155	.....do.....	Very severe symptoms.
1162	.....do.....	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.

TABLE No. 4.—*Relation of amount to sensitization*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1172	8 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Severe symptoms.
1173	.....do.....	17	.....do.....	Severe symptoms.
1174	.....do.....	24	.....do.....	Severe symptoms.
1175	.....do.....	38	.....do.....	Severe symptoms.
1177	.....do.....	110	0.5 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1176	.....do.....	155	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1178	.....do.....	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.

PART 2.—THE SENSITIZING PRINCIPLE.

THE EFFECT OF HEAT UPON THE SENSITIZING ACTION OF HORSE SERUM.

In previous publications we reported that the toxic action of horse serum was not destroyed when the serum was heated to 60° C. for six hours, but is entirely destroyed at 100° C. in fifteen minutes. Further work on this subject leads us to conclude that the sensitizing effect of horse serum is gradually influenced by heat, and almost entirely disappears when the serum is heated to 100° C. for one hour. The pigs sensitized with small quantities of horse serum heated to 100° C. for one hour, when subsequently tested, developed very slight symptoms.

TABLE No. 5.—*Effect of heat upon sensitizing principle.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Heated to 60° C.			
1193	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 60° for 1 hour, subcutaneously.	25	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
	Heated to 70° C.			
1194	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 70° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Slight symptoms.
1195	.....do.....	25	0.25 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
	Heated to 80° C.			
1197	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 80° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Marked symptoms; died 2 days later.
1196	.....do.....	25	0.25 c. c. normal horse (roan) serum into brain.	Severe symptoms.
3242	.....do.....	39	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
3244	.....do.....	39	.....do.....	Severe symptoms.
3240	.....do.....	39	.....do.....	Severe symptoms.
	Heated to 90° C.			
1199	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 90° for 1 hour, subcutaneously.	25	.....do.....	No symptoms.
1198	.....do.....	25	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
3238	.....do.....	39	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 90 minutes.
3235	.....do.....	39	.....do.....	No symptoms.
3239	.....do.....	39	.....do.....	Marked symptoms.

TABLE No. 5.—*Effect of heat upon sensitizing principle*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Heated to 100° C.			
1200	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 100° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1201	.....do.....	25	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
1358	1 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 100° for 1 hour, subcutaneously.	35	0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
1359	.....do.....	35	.....do.....	Symptoms (?).
1360	.....do.....	35	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1361	.....do.....	35	6 c. c. normal horse (roan) serum subcutaneously.	Symptoms (?).
1362	.....do.....	35	.....do.....	Symptoms (?).

CAN GUINEA PIGS BE SENSITIZED IN THE BRAIN?

We were led to make the following experiments in view of a statement made by Besredka and Steinhardt [a] that guinea pigs were not sensitized when very small amounts are injected into the brain. Very minute amounts when injected into the brain in our experiments, as well as the few reported by Besredka and Steinhardt, proved negative.

However, guinea pigs may be readily sensitized by intracerebral injections, provided quantities of 0.0001 c. c. or more are used. We obtained negative results with sensitizing doses of 0.00001 c. c.

TABLE No. 6.—*Intracerebral sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1202	0.25 c. c. normal horse (roan) serum into brain.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Severe symptoms.
1203	.....do.....	25	0.25 c. c. same serum into brain..	Marked symptoms.
1204	0.1 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Mild symptoms.
1205	.....do.....	25	0.25 c. c. same serum into brain..	Marked symptoms.
1207	0.01 c.c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Severe symptoms.
1206	.....do.....	25	0.25 c. c. same serum into brain..	Marked symptoms.
1208	0.001 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Severe symptoms.
1209	.....do.....	25	0.25 c. c. same serum into brain..	Slight symptoms.
1210	0.0001 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Marked symptoms
1211	.....do.....	25	0.25 c. c. same serum into brain..	Slight symptoms.
1213	0.00001 c.c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	No symptoms.
1212	.....do.....	25	0.25 c. c. same serum into brain..	No symptoms.

[a] Ann. de l'Inst. Pasteur, vol. 21, 1907, p. 384.

GUINEA PIGS MAY BE SENSITIZED IN THE EYE.

So far as may be judged from the following experiment, it appears that normal horse serum dropped upon the eye of a guinea pig may sensitize it to a second injection of horse serum given after an interval of seventeen days.

TABLE No. 7.—*Ocular sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1340	1 drop normal horse serum into each eye.	17	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
1341	.....do.....	28	6 c. c. normal horse (roan) serum subcutaneously.	No symptoms.
1342	.....do.....	28	.....do.....	No symptoms.

ATTEMPTS TO SENSITIZE GUINEA PIGS WITH PURE PROTEINS.

We have assumed that it is a protein substance in the horse serum, egg-white, milk, vegetable extracts, etc., that we have tested which sensitizes guinea pigs and poisons them at the second injection. The substances used by us of course have a very complex composition, so that it became desirable to use solutions of pure proteins for these tests. We are indebted to Dr. Lafayette B. Mendel, New Haven, Conn., for a quantity of edestin and excelsin, which are protein substances obtained in a chemically pure crystalline state. Our tests with these two particular proteins resulted negatively, due probably to the difficulty of obtaining a satisfactory solution of them.

TABLE No. 8.—*Pure protein.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1393	0.1 gm. edestin subcutaneously...	21	3 c. c. 1 per cent solution edestin in 10 per cent NaCl subcutaneously.	No symptoms.
1394	0.01 gm. edestin subcutaneously..	21	.....do.....	No symptoms.
1395	0.001 gm. edestin subcutaneously.	21	.....do.....	No symptoms.
1396	0.1 gm. edestin subcutaneously...	22	6 c. c. 1 per cent solution edestin in 10 per cent NaCl subcutaneously.	No symptoms.
1397	0.01 gm. edestin subcutaneously..	22	.....do.....	No symptoms.
1398	0.001 gm. edestin subcutaneously.	22	.....do.....	No symptoms.
1399	0.1 gm. excelsin subcutaneously...	21	3 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	Symptoms(?).
1400	0.01 gm. excelsin subcutaneously..	21	.....do.....	Symptoms(?).
1401	0.001 gm. excelsin subcutaneously	21	4 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	Symptoms(?).
1402	0.1 gm. excelsin subcutaneously...	22	6 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	No symptoms.
1403	0.01 gm. excelsin subcutaneously..	22	.....do.....	No symptoms.

THE EFFECT OF BLOOD SERUM AND BRAIN OF SENSITIZED GUINEA PIGS UPON THE SENSITIZING SUBSTANCES.

Normal horse serum was mixed with an equal volume of the blood serum of a sensitive guinea pig and allowed to stand twenty-four hours at room temperature before the mixture was injected into a normal guinea pig.

Normal horse serum was also mixed with the brain substance of a sensitive guinea pig, well rubbed up in a mortar, and allowed to stand at room temperature twenty-four hours. The extract, strained through gauze, was injected subcutaneously into normal guinea pigs.

After a proper period of incubation these animals were tested and it was found that the anaphylactic phenomenon was in no wise modified by these procedures.

TABLE No. 9.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1406	1 c. c. mixture of serum of G. P. 9558 +normal horse (roan) serum, equal parts, subcutaneously.	19	0.2 c. c. normal horse (Teddy) serum into brain.	Severe symptoms.
1407	.....do.....	19	.....do.....	Severe symptoms.
1405	1 c. c. mixture of brain of G. P. 9558+normal horse (roan) serum, equal parts, subcutaneously.	19	.....do.....	Severe symptoms.
1408	.....do.....	19	.....do.....	Severe symptoms.

Guinea pig No. 9558 received a subcutaneous injection of .25 c. c. of diphtheria toxine No. 7 + 1/340 c. c. antitoxic serum (Welc. 839) 61 days before being bled.

PART 3.—THE TOXIC PRINCIPLE.

THE EFFECT OF HEAT UPON THE TOXICITY OF HORSE SERUM.

It will be seen from the following tables that normal horse serum may be heated to 90° C. for one hour and still remain slightly toxic when injected into a sensitized guinea pig. Its toxicity, however, is evidently markedly affected. Heating to 70° C. for one hour does not seem to appreciably diminish its poisonous properties, but it appears to be affected at 80° for one hour. At 100° for one hour the toxicity apparently disappears.

Blood serum of course can not be heated to these high degrees without coagulation, and it is therefore necessary to dilute it in the proportion of one part of blood serum to three parts of distilled water. This dilution may then be heated to a high temperature without producing any visible change other than a slight opalescence.

It appears that there is a slight difference between the sensitizing and toxic principles in horse serum so far as resistance to heat is concerned. Serum heated to 100° C. for one hour retains some power of sensitization, but seems to lose its toxicity when given at the second injection. This difference may be more apparent than real, for exceedingly minute amounts are sufficient to sensitize guinea pigs, while a very large quantity of weakened serum would be necessary to produce symptoms. It must be remembered that in our experiments 20 c. c. of the dilution represents but 5 c. c. of the serum.

These facts must be considered in drawing conclusions from work upon split proteins, fractional precipitation, or other methods to isolate the sensitizing substance in pure form. A very minute amount of the original protein substance in horse serum clinging to the globulins or other substances modified by chemical methods might be sufficient to sensitize guinea pigs, whereas it would require very large amounts of such a modified protein to poison a sensitive animal.

TABLE No. 10.—*The effect of heat upon the toxic principle.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Heated to 70° C. 1 hour.	
531	0.0006 c. c. tetanus toxine A+0.22 c. c. antitoxic horse serum (Ehrl.).	67	0.25 c. c. normal horse (roan) serum diluted 1:3, heated, concentrated, then injected into brain.	Marked symptoms.
533	0.0006 c. c. tetanus toxine A+0.24 c. c. antitoxic horse serum (Ehrl.).	67	.....do.....	Marked symptoms.
8496	0.24 c. c. toxine No. 42+1/500 c. c. antitoxic horse serum (Cutter 1858).	69	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	Dead in 40 minutes.
8495	.....do.....	69	.....do.....	Dead in 32 minutes.
			Heated to 80° C. 1 hour.	
8380	0.24 c. c. toxine No. 42+1/240 c. c. antitoxic horse serum (PD 08043).	54	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	Very severe symptoms.
8494	0.24 c. c. toxine No. 42+1/300 c. c. antitoxic horse serum (Cutter 1858).	42	.....do.....	Slight symptoms.
8857	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Hubbert).	67	.....do.....	Slight symptoms.
8886	0.245 c. c. toxine No. 42+1/150 c. c. antitoxic horse serum (Alex. A245).	67	.....do.....	Severe symptoms.
8890	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Natl. VI, 15).	67	.....do.....	No symptons.
8883	0.245 c. c. toxine No. 42+1/440 c. c. antitoxic horse serum (Alex. A262).	67	.....do.....	No symptoms.
8872	0.245 c. c. toxine No. 42+1/660 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do.....	No symptoms.
			Heated to 90° C. 1 hour.	
8860	0.245 c. c. toxine No. 42+1/240 c. c. antitoxic horse serum (Str. 61S).	67	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
8880	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Mul. 2321).	67	.....do.....	Severe symptoms.
8878	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Mul. 2350).	67	.....do.....	Mild symptoms.
8880	0.245 c. c. toxine No. 42+1/250 c. c. antitoxic horse serum (Natl. VI, 15).	67	.....do.....	Slight symptoms.
8873	0.245 c. c. toxine No. 42+1/800 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do.....	Slight symptoms.

TABLE No. 10.—*The effect of heat upon the toxic principle*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Heated to 100° C. 1 hour.	
9260	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 38B).	42	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
9218	0.23 c. c. toxine No. 7+1/1100 c. c. antitoxic horse serum (Ldrl. 21C).	42	10 c. c. same dilution (=2.5 c. c. serum) subcutaneously.	No symptoms.
9215	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (Ldrl. 21).	42	0.25 c. c. same dilution (=2.5 c. c. serum) into brain.	No symptoms.
9210	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 19B).	42	.....do.....	No symptoms.
9343	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Ldrl. 16F).	96	20 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
			Next day 6 c. c. unheated normal horse (Frank) serum intraperitoneally.	Severe symptoms.
9344	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 16F).	96	20 c. c. dilution heated.....	No symptoms.
			Next day 6 c. c. unheated (Frank) serum.	Severe symptoms.

DOES HORSE SERUM DECREASE IN TOXICITY WITH AGE?

Besredka[a] states that freshly drawn horse serum is more toxic than the same serum thirty days old. Our own results are not in harmony with this conclusion, as may be seen from the following series.

TABLE No. 11.—*Effect of age upon toxicity of horse serum.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Serum 2 days old.	
8895	0.245 c. c. toxine No. 42+1/180 c. c. antitoxic horse serum (Cutter) subcutaneously.	67	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 60 minutes.
8897	0.254 c. c. toxine No. 42+1/250 c. c. antitoxic horse serum (Cutter).	67	.....do.....	Dead in 14 hours.
8871	0.245 c. c. toxine No. 42+1/500 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do.....	Dead in 2 hours.
8896	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Cutter).	67	.....do.....	Very severe symptoms.
8858	0.245 c. c. toxine No. 42+1/300 c. c. antitoxic horse serum (Hubbert).	67	.....do.....	Dead in 50 minutes.

TABLE No. 11.—*Effect of age upon toxicity of horse serum*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Same serum 15 days old.	
8856	0.245 c. c. toxine No. 42+1/330 c. c. antitoxic horse serum (Hubbert)	112	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 40 minutes.
8859	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Hubbert).	112	.....do.....	Dead in 40 mmutes.
8860	0.245 c. c. toxine No. 42+1/330 c. c. antitoxic horse serum (Hubbert).	112	.....do.....	Very severe symptoms.
			Same serum 40 days old.	
9221	0.123 c. c. toxine No. 7+1/420 c. c. antitoxic horse serum (Alex. A283).	40	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 25 minutes.
9200	0.123 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (Ldrl. 19).	40	.....do.....	Dead in 27 minutes.
9257	0.123 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Ldrl. 30).	40	.....do.....	Very severe symptoms.
			Same serum 68 days old.	
9370	0.23 c. c. toxine No. 7+1/1120 c. c. antitoxic horse serum (NY 306).	61	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 45 minutes.
9447	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (PD 08033).	61	.....do.....	Dead in 85 minutes.
9375	0.23 c. c. toxine No. 7+1/1060 c. c. antitoxic horse serum (NY 306).	61	.....do.....	Severe symptoms.
			Same serum 91 days old.	
9460	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 11C).	72	6 c. c. normal horse (roan) serum subcutaneously.	Very severe symptoms.
9462	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 15B).	72	.....do.....	Dead in 40 minutes.
9465	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 13).	72	.....do.....	Dead in 90 minutes.
			Same serum 118 days old.	
9158	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Cutter 19734).	141	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 50 minutes.
9633	0.23 c. c. toxine No. 7+1/620 c. c. antitoxic horse serum (Alex. A249).	111	.....do.....	Dead in 40 minutes.
9636	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Alex. A249).	111	.....do.....	Dead in 45 minutes.

THE EFFECT OF VARIOUS CHEMICAL SUBSTANCES UPON THE TOXICITY OF HORSE SERUM.

Further attempts were made to influence the toxic action of horse serum by treating the guinea pig with various chemicals.

In the following series the sensitized animals were given a subcutaneous injection of various substances the day before the second injection of serum. No favorable influence upon the anaphylactic state was obtained by the substances used, namely, pancreatin, potassium oxalate, pepsin, sodium sulphate, magnesium sulphate, peptone, calcium chlorate, and calcium acetate.

TABLE NO. 12.—*Effect of various chemicals.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
8545	0.24 c. c. toxine 42+1/640 c. c. antitoxic horse serum (Alex. A249).	32	1 gm. pancreatin; next day 0.5 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
8553	0.24 c. c. toxine 42+1/680 c. c. antitoxic horse serum (Alex. A249).	32	1 gm. potassium oxalate; next day serum as above.	Dead in 5 minutes.
8552	0.24 c. c. toxine 42+1/680 c. c. antitoxic horse serum (Alex. A249).	32	0.5 gm. pepsin; next day serum as above.	Dead in 2 minutes.
8532	0.24 c. c. toxine 42+1/620 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. sodium sulphate; next day serum as above.	Dead in 3 minutes.
8534	0.24 c. c. toxine 42+1/600 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. magnesium sulphate; next day serum as above.	Dead in 2 minutes.
8389	0.24 c. c. toxine 42+1/370 c. c. antitoxic horse serum (PD 08033).	32	0.2 gm. peptone; next day serum as above.	Dead in 2 minutes.
8498	0.24 c. c. toxine 42+1/260 c. c. antitoxic horse serum (Cutter 1838).	32	0.1 gm. calcium chlorate; next day serum as above.	Dead in 2 minutes.
8535	0.24 c. c. toxine 42+1/600 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. calcium acetate; next day serum as above.	Dead in 3 minutes.

IODINE.

Obermayer and Pick[a] found that when the aromatic radicals of a protein are combined with various substances the protein loses the power to produce precipitins of closely limited specificity for the original species. Their results suggest that the aromatic groups of the molecule are most closely related to the species specificity.[b] This indicates that the striking specificity of proteins of different species depends upon the aromatic groups of the protein molecule,

[a] Obermayer, Fr., and Pick, E. P.: Ueber die chemischen Grundlagen der Arteigenschaften der Eiweisskörper. Wiener klin. Woch., vol. 19, 1906, p. 327.

[b] Wells, H. Gideon: The present status of our knowledge of the chemistry of the processes of immunity. Arch. Internal Med., vol. 1, No. 2, February 15, 1908, p. 262.

and Vaughan has found evidence that the toxicity of the proteins depends upon these same groups.

Fleischmann[a] also found that tryptic digestion destroys this characteristic species specificity.

We made several tests to determine the effect of iodine upon the toxic action of horse serum, and it so turned out in the preliminary experiments that the symptoms appeared to be profoundly modified, in the sense that they were either delayed or inhibited. We therefore tested a large number of guinea pigs to determine this point, but found that so far as the toxicity of horse serum was concerned at the second injection it is not appreciably modified by the iodine.

In the following series the iodine was added to horse serum in the proportion of 1.5 gm. per 25 c. c. of serum and 3 gm. potassium iodide to aid solution.

TABLE No. 13.—*Iodine added to serum.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result
9826	0.23 c. c. toxine No. 7+1.250 c. c. antitoxic horse serum (P. I. Bur.).	42	6 c. c. normal horse Frank serum and iodine subcutaneously.	Dead in 50 minutes.
9837	0.23 c. c. toxine No. 7+1.210 c. c. antitoxic horse serum (Swiss USA).	42	.....do.....	Dead in 80 minutes.
9830	0.23 c. c. toxine No. 7+1.390 c. c. antitoxic horse serum (Mul. 2539).	42	.....do.....	Severe symptoms.
9832A	0.23 c. c. toxine No. 7+1.450 c. c. antitoxic horse serum (Mul. 2539).	42	.....do.....	Slight symptoms.
725	0.0004 c. c. tetanus toxine A+0.0075 c. c. antitetanic serum (Standard).	42	.....do.....	Mild symptoms.
9773	0.23 c. c. toxine No. 7+1.320 c. c. antitoxic horse serum Strn. 1351).	53	6 c. c. normal horse serum (Teddy and iodine subcutaneously.	Dead in 65 minutes.
9713	0.23 c. c. toxine No. 7+1.410 c. c. antitoxic horse serum Scher. II).	66	.....do.....	Slight symptoms.
9743	0.23 c. c. toxine No. 7+1.170 c. c. antitoxic horse serum (Strn. 1110).	53	.....do.....	Slight symptoms.
9182	0.23 c. c. toxine No. 7+1.140 c. c. antitoxic horse serum (Mul. 2352).	103	6 c. c. normal horse (roan serum and iodine intraperitoneally.	Dead in 30 minutes.
9187	0.23 c. c. toxine No. 7+1.320 c. c. antitoxic horse serum (Mul. 2362).	103	.....do.....	Dead in 45 minutes.
9189	0.23 c. c. toxine No. 7+1.300 c. c. antitoxic horse serum (Mul. 2423).	103	.....do.....	Dead in 15 minutes.
9753	0.23 c. c. toxine No. 7+1.300 c. c. antitoxic horse serum (Strn. 1110).	53	6 c. c. normal horse (roan serum and iodine subcutaneously.	Mild symptoms.

[a] Zeit. f. klin. Med., vol. 59, 1906, p. 515.

TABLE No. 13.—*Iodine added to serum*—Continued.

G.P. No.	First injection.	Interval in days.	Second injection.	Result.
9730	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Strn. 1110).	53	6 c. c. normal horse (roan) serum and iodine subcutaneously.	Mild symptoms.
8551	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Alex. A249).	237	.....do.....	Mild symptoms.
9554	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Well. 7L841).	73	.....do.....	Dead in 80 minutes.

TABLE No. 14.—*Iodine injected separately.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9782	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (5 c. c. Gram's solution) subcutaneously; next day 0.2 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
9752	0.23 c. c. toxine No. 7+1/180 c. c. antitoxic horse serum (Strn. 1110).	53	Iodine (3 c. c. Gram's solution) subcutaneously; 1 hour later 6 c. c. normal horse (Teddy) serum intraperitoneally.	Marked symptoms.
9772	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (6 c. c. Gram's solution) subcutaneously; 1 hour later normal horse serum (Teddy) intraperitoneally.	Marked symptoms.
9657	0.23 c. c. toxine No. 7+1/340 c. c. antitoxic horse serum (PD 08022).	64	Iodine (5 c. c. Gram's solution) subcutaneously; 1 hour later 0.2 c. c. normal horse (roan) serum into brain.	Dead in 3 minutes.
9781	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (5 c. c. Gram's solution); 1 hour later 0.1 c. c. same horse serum into brain.	Dead in 3 minutes.

TABLE No. 15.—*Controls for tables Nos. 13 and 14.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9836	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Swiss).	42	6 c. c. normal horse serum (Frank) subcutaneously.	Very severe symptoms.
9832	0.23 c. c. toxine No. 7+1/570 c. c. antitoxic horse serum (Mul. 2539).	42	.....do.....	Very severe symptoms.
9773	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Strn. 1351).	52	6 c. c. normal horse serum (Teddy) subcutaneously.	Severe symptoms.
9770	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Strn. 1351).	52	.....do.....	Dead in 40 minutes.
9780	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Strn. 1351).	52	.....do.....	Dead in 60 minutes.

NITRITES.

A few further tests were made to determine the relation of methemaglobin producing substances, such as the nitrites, upon the symptoms. The following two guinea pigs were given a subcutaneous injection of sodium nitrite. In thirty minutes the exposed mucous membranes appeared distinctly blue, and the guinea pigs were then tested for susceptibility.

Controls showed that the quantity of nitrite used was not sufficient in itself to kill the guinea pigs.

TABLE No. 16.—*Sodium nitrite.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9173	0.23 c. c. toxine No. 7+1/360 c. c. antitoxic horse serum (Mul. 2439).	106	0.03 gm. sodium nitrite; 30 minutes later 1 c. c. normal horse (roan) serum intraperitoneally.	Dead in 15 minutes.
9168	0.23 c. c. toxine No. 7+1/520 c. c. antitoxic horse serum (Mem. Inst.).	106	0.025 gm. sodium nitrite; 30 minutes later 1 c. c. normal horse (roan) serum subcutaneously.	Dead in 60 minutes

ETHER.

Besredka [a] reported some interesting observations concerning the prevention of anaphylaxis by ether narcosis. He stated that if sensitive guinea pigs are etherized to the stage of complete relaxation, and while in this state injected intracerebrally with normal horse serum and the administration of ether continued a short while, (if the narcosis is well conducted) the animals continue to sleep after the injection, and at the end of about half an hour awake without presenting the least symptoms of anaphylaxis. If the guinea pig is tested on the following day it will be found to be immune.

Of eight guinea pigs upon which we tried this experiment with ether seven died, we believe, from the effects of the second injection of horse serum. It is our belief that pig No. 9581, which recovered, had masked symptoms while under the influence of the ether and probably would not have died anyhow, for we have a certain number of recoveries from the intracerebral injections of 0.2 c. c. of horse serum. It is true, however, that the narcosis masks the symptoms.

[a] Annales de l'Institut Pasteur, Dec. 25, 1907, vol. 21, no. 12, p. 957.

The difference in our results may be accounted for by the difference in toxicity of the French and the American serums, or by differences in susceptibility of the animals used.

TABLE No. 17.—*Ether.*

G. P. No.	Treatment.	Result.
9419	0.23 c. c. toxine No. 7+1/440 c. c. antitoxic horse serum (Alex. A276). 55 days later tested as follows: The animal was first trephined, then deeply narcotized with ether, and in the stage of complete relaxation injected with 0.25 c. c. antitoxic horse serum (Mul. 2295) into the brain. The etherization was continued several minutes longer.	Died.
9376	0.23 c. c. toxine No. 7+1/1060 c. c. antitoxic horse serum (NY 306). 62 days later etherized and tested as above with 0.1 c. c. same serum.	Died.
9374	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (NY 306). 62 days later etherized and tested as above with 0.25 c. c. same serum.	Died.
9175	0.23 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Mul. 2439). 106 days later etherized and tested with 0.2 c. c. normal horse (roan) serum into brain.	Died in 5 minutes.
9174	0.23 c. c. toxine No. 7+1/450 c. c. antitoxic horse serum (Mul. 2429). 106 days later etherized and tested with 0.2 c. c. normal horse (roan) serum into brain.	Died in 5 minutes.
9678	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. A245.) 73 days later etherized and tested with 0.2 c. c. normal horse (Frank) serum into brain.	Died in 10 minutes.
9581	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Alex. A245). 73 days later etherized and tested with 0.2 c. c. normal horse (Frank) serum into brain.	Recovered.
	Next day 6 c. c. normal horse (Frank) serum intraperitoneally.....	No symptoms.
9582	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Alex. A245). 65 days later etherized and tested with 0.2 c. c. antitoxic horse serum (Lyons) into brain.	Died in few minutes.

ATTEMPTS TO NEUTRALIZE HORSE SERUM WITH THE BRAIN AND BLOOD OF SENSITIZED GUINEA PIGS.

We made further attempts to neutralize the toxic action of horse serum by first treating it with sensitized guinea pig serum and also with the brain substance of sensitized guinea pigs. These attempts all proved futile.

In the following experiments normal horse serum was mixed with equal parts of sensitized guinea pig serum, and kept at room temperature twenty-four hours.

The brain of a sensitive guinea pig was removed under aseptic precautions, mixed with about 20 c. c. of normal horse serum, ground up in a mortar, kept at room temperature for twenty-four hours, and then strained.

TABLE No. 18.—*Action of guinea pig serum and brain.*

G. P. No.	First injection.	Interval in days.	Second injection	Result.
9337	0.23 c. c. toxine No. 7+1/1020 c. c. antitoxic horse serum (NY 305).	96	0.2 c. c. mixture of normal horse (roan) and sensitized guinea pig (9558) serum into brain.	Dead in 4 minutes.
9340	0.23 c. c. toxine No. 7+1/1000 c. c. antitoxic horse serum (NY 305).	96	0.1 c. c. mixture of normal horse (roan) and sensitized guinea pig (9558) serum into brain.	Very severe symptoms.
9336	0.23 c. c. toxine No. 7+1/1040 c. c. antitoxic horse serum (NY 305).	96	0.2 c. c. mixture of normal horse (roan) serum and brain of sensitized guinea pig (9558) into brain.	Dead in 5 minutes.
9338	0.23 c. c. toxine No. 7+1/1020 c. c. antitoxic horse serum (NY 305).	96	0.1 c. c. mixture of normal horse (roan) serum and brain of sensitized guinea pig (9558) into brain.	Marked symptoms.

PART 4.—SPECIFICITY.

SPECIFIC NATURE OF "ANAPHYLACTIN."

In a previous publication[a] on the specific nature of anaphylaxis we demonstrated that guinea pigs may be in a condition of anaphylaxis to three protein substances at the same time. Hypersusceptibility to each protein is manifested by a second injection of the corresponding protein. The three reactions may be obtained in the same guinea pig within a short space of time and are as distinct and specific as three separate infectious diseases.

We now bring forward experimental data proving that the substance in the blood serum of sensitized guinea pigs, known as anaphylactin, is also specific in the same sense. In the following table it will be seen that by transferring the blood serum of guinea pigs sensitized to horse serum, egg white, and milk three separate and distinct reactions were obtained in the guinea pig into which this serum was transferred:

TABLE No. 19.—*The specific nature of anaphylactin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9619	0.23 c. c. toxine No 7+1/600 c. c. antitoxic horse serum (Alex. A249) subcutaneously. 56 days later 1 c. c. cow's milk subcutaneously. 1 day later 1 c. c. egg white subcutaneously. 22 days later bled; blood defibrinated centrifuged, and serum used to inject G. P. No. 9619A.			
9619A	7 c. c. serum of G. P. 9619 subcutaneously.	2	6 c. c. milk intraperitoneally....	Mild symptoms.
			2 days later 0.2 c. c. normal horse (Teddy) serum into brain.	Dead in 5 minutes.
9620	Same treatment as G. P. No. 9619.			
9620A	8 c. c. serum of G. P. 9620 subcutaneously.	2	6 c. c. milk intraperitoneally....	Slight symptoms.
			2 days later 3 c. c. egg white intraperitoneally.	Mild symptoms.
			2 hours later 0.2 c. c. normal horse (Teddy) serum into brain.	Dead in 4 minutes.
9621	0.23 c. c. toxine No. 7+1/580 c. c. antitoxic horse serum (Alex. A249). Subsequent treatment same as G. P. 9619.			
9621A	6.5 c. c. serum of G. P. 9621 subcutaneously.	2	6 c. c. milk intraperitoneally....	Slight symptoms.
			2 days later 6 c. c. saturated solution egg white intraperitoneally.	Marked symptoms.
			2 hours later 0.2 c. c. normal horse (Teddy) serum into brain.	Very severe symptoms.
9624	0.23 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (Alex. A249). Subsequent treatment same as G. P. 9619.			
9624A	7 c. c. serum of G. P. 9624 subcutaneously.	2	6 c. c. milk intraperitoneally....	Slight symptoms.
			2 days later 0.2 c. c. normal horse (Teddy) serum into brain.	Dead in 5 minutes.

[a] Rosenau, M. J., and Anderson, John F.: The specific nature of anaphylaxis. Jour. Infec. Dis., vol. 4, No. 4, Nov., 1907, pp. 552–557.

EFFECT OF IODINE UPON SPECIFICITY.

In the following series we not only tested guinea pigs treated with horse serum to find out whether they react after an interval to chicken serum, and vice versa, but also to ascertain whether the presence of iodine in the sensitizing dose modifies the specific nature of the reaction. The results plainly show that under the conditions of the experiment the iodine had no modifying action upon the power of these serums to sensitize against a second injection of homologous serum and also had no power to impair the specific nature of the phenomenon.

TABLE No. 20.—*Effect of iodine on specificity.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1613	1 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	0.2 c. c. chicken serum into brain.	Severe symptoms.
1604	.....do.....	19	.....do.....	Severe symptoms.
1615	2 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	0.2 c. c. normal horse (Teddy) serum into brain.	No symptoms.
1616	.....do.....	19	.....do.....	No symptoms.
1617	1 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	.....do.....	No symptoms.
1618	2 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.....	Severe symptoms.
1619	1 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.....	Dead in 4 minutes
1620	.....do.....	19	0.2 c. c. chicken serum into brain.	No symptoms.
1621	2 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.....	No symptoms.
1622	1 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.....	No symptoms.

RELATION BETWEEN MILKS OF VARIOUS SPECIES.

HUMAN VERSUS COW MILK.

Eight guinea pigs were sensitized by the subcutaneous injection of 1 c. c. of human milk. After an appropriate interval they were tested with cow's milk without response.

A short time later they were again tested with human milk. This time most of them showed severe symptoms.

This series again indicates not only the specific nature of the anaphylactic reaction, but suggests differences between the protein matter in human and in cow's milk.

TABLE No. 21.—*Human versus cow milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1375	1 c. c. human milk subcutaneously.	37	6 c. c. cow's milk intraperitoneally. 2 days later 10 c. c. human milk subcutaneously.	No symptoms. No symptoms(?).
1376	.....do.....	37	6 c. c. cow's milk subcutaneously... 4 hours later 10 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1377	.....do.....	37	6 c. c. cow's milk subcutaneously... 4 hours later 10 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1378	.....do.....	37	6 c. c. cow's milk intraperitoneally.. 2 days later 10 c. c. human milk intraperitoneally.	No symptoms. No symptoms(?).
1401	.....do.....	24	6 c. c. cow's milk intraperitoneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1402	.....do.....	24	6 c. c. cow's milk intraperitoneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1403	.....do.....	24	6 c. c. cow's milk intraperitoneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1404	.....do.....	24	6 c. c. cow's milk intrapertioneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.

TABLE No. 22.—*Sheep milk versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1450	1 c. c. sheep's milk subcutaneously.	26	6 c. c. cow's milk subcutaneously.	No symptoms.
1451	.....do.....	26	.....do.....	No symptoms.
1452	.....do.....	26	6 c. c. cow's milk intraperitoneally.	Marked symptoms.
1453	.....do.....	26	.....do.....	Severe symptoms.
1454	.....do.....	26	.....do.....	Slight symptoms.
1455	.....do.....	26	.....do.....	Dead in 25 minutes.

TABLE No. 23.—*Dog milk versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1600	0.5 c. c. dog milk subcutaneously...	17	6 c. c. cow's milk intraperitoneally.	No symptoms.
1601	.....do.....	17	.....do.....	No symptoms.
1602	.....do.....	17	.....do.....	No symptoms.
1603	1 c. c. dog milk subcutaneously.....	17	.....do.....	No symptoms.
1604	.....do.....	17	.....do.....	No symptoms.
1605	.....do.....	19	.....do.....	No symptoms.
1606	.....do.....	19	.....do.....	No symptoms.
1607	.....do.....	19	.....do.....	No symptoms.

In the following series the dog milk was first treated with iodine and potassium iodide before it was injected subcutaneously.

TABLE No. 24.—*Dog milk+iodine versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1608	0.5 c. c. dog milk+iodine subcutaneously.	17	6 c. c. cow's milk intraperitoneally.	No symptoms.
1609	.....do.....	17	.....do.....	No symptoms.
1610	.....do.....	17	.....do.....	No symptoms.
1611	1 c. c. dog milk+iodine subcutaneously.	17	.....do.....	No symptoms.
1612	.....do.....	17	.....do.....	No symptoms.

RELATION BETWEEN EGG ALBUMENS OF VARIOUS SPECIES.

TABLE No. 25.—*Hen egg white versus duck egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1408	1 c. c. saturated solution hen egg white in salt solution (0.85) subcutaneously.	21	10 c. c. saturated solution duck egg white in salt solution (0.85) intraperitoneally.	Mild symptoms.
1409	.....do.....	21	.....do.....	Mild symptoms.
1410	.....do.....	21	.....do.....	Mild symptoms.
1411	.....do.....	21	.....do.....	No symptoms.

TABLE No. 26.—*Duck egg white versus hen egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1413	1 c. c. saturated solution duck egg white in salt solution (0.85) subcutaneously.	21	10 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Dead in 30 minutes.
1414	.....do.....	21	.....do.....	Dead in 35 minutes.
1415	.....do.....	21	.....do.....	Very severe symptoms.
1416	.....do.....	21	.....do.....	Very severe symptoms.
1417	.....do.....	21	.....do.....	Very severe symptoms.

TABLE No. 27.—*Guinea hen egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2040	1 c. c. saturated solution guinea hen egg white in salt solution (0.85) subcutaneously.	20	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Dead in 13 minutes.
2041	.....do.....	20	.....do.....	Very severe symptoms.
2042	.....do.....	20	.....do.....	Very severe symptoms.
2043	.....do.....	20	6 c. c. saturated solution of crane egg white in salt solution (0.85) intraperitoneally.	Marked symptoms.
2044	.....do.....	20	.....do.....	Marked symptoms.

NOTE.—The crane egg used in these experiments was from a Demoiselle crane and kindly furnished us by Dr. Frank Baker.

TABLE No. 28.—*Pigeon egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2035	1 c. c. saturated solution pigeon egg white in salt solution (0.85) subcutaneously.	21	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	No symptoms.
2036	.....do.....	21	.....do.....	No symptoms.
2037	.....do.....	21	.....do.....	No symptoms.
2038	.....do.....	21	6 c. c. saturated solution of crane egg white in solt solution (0.85) intraperitoneally.	Very severe symptoms.
2039	.....do.....	21	.....do.....	Marked symptoms.

TABLE No. 29.—*Goose egg white versus hen and turkey egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2030	1 c.c. saturated solution goose egg white in salt solution (0.85) subcutaneously.	38	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Marked symptoms.
2031	.....do.....	38	.....do.....	Very severe symptoms.
2032	.....do.....	38	.....do.....	Marked symptoms.
2033	.....do.....	38	6 c. c. saturated salt solution turkey egg white in salt solution (0.85) intraperitoneally.	Very severe symptoms.
2034	.....do.....	38	.....do.....	Severe symptoms.

TABLE No. 30.—*Turkey egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2025	1 c. c. saturated solution turkey egg white in salt solution (0.85) subcutaneously.	35	6 c. c. saturated solution of hen egg white in salt solution (0.85) intraperitoneally.	Very severe symptoms.
2026	.....do.....	35	.....do.....	Dead in 70 minutes.
2027	.....do.....	35	.....do.....	Dead in 70 minutes
2028	.....do.....	35	6 c. c. saturated solution of crane egg white in salt solution (0.85) intraperitoneally.	Severe symptoms.
2029	.....do.....	35	.....do.....	Severe symptoms.
2030	.....do.....	35	6 c. c. saturated solution of turkey egg white in salt solution (0.85) intraperitoneally.	Dead in 20 minutes.

PART 5.—ANAPHYLACTIN.

THE PRESENCE OF ANAPHYLACTIN DURING THE PERIOD OF INCUBATION.

It is of some interest to determine just when the substance called *anaphylactin* by Gay and Southard appears in the blood of a sensitized guinea pig, particularly whether its presence may be demonstrated during the period of incubation.

A series of guinea pigs was therefore sensitized by the subcutaneous injection of 1/100 c. c. of normal horse serum. On each succeeding day two guinea pigs of this series were bled and the serum obtained by whipping and centrifugation. This serum was then injected into normal pigs. In twenty-four hours these pigs were tested by the injection of 6 c. c. of horse serum intraperitoneally. In the latter part of the series forty-eight hours were allowed to elapse before the pigs were tested.

It will be seen from Tables 31 and 32, which give our work on this factor in detail, that no indication of anaphylactin appeared in the blood of sensitized guinea pigs until the tenth day.

TABLE No. 31.—*Anaphylactin during the period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1343A	2 c. c. subcutaneously serum G. P. 1343, which was bled *1 day* after subcutaneous injection of 0.01 c. c. normal horse serum.	1	0.25 c. c. normal horse serum into brain.	No symptoms.
1343B	.....do.....	1	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1344A	4 c. c. serum G. P. 1344, which was bled *2 days* after injection of 0.01 c. c. normal horse serum.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1345A	3 c. c. serum G. P. 1345, which was bled *3 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	No symptoms.
1345B	.. ..do.....	1	.....do.....	No symptoms.
1346A	2.5 c. c. serum G. P. 1346, which was bled *4 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	No symptoms.
1346B	.....do.....	1	.....do.....	No symptoms.

TABLE No. 31.—*Anaphylactin during the period of incubation*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1347A	3 c. c. serum G. P. 1347, which was bled *5 days* after injection of 0.01 c. c. normal horse serum.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1347B	.....do.....	1	.....do.....	No symptoms.
1348A	5 c. c. serum G. P. 1348, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	No symptoms.
1348B	.....do.....	1	.....do.....	No symptoms.
1349A	4 c. c. serum G. P. 1349, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	No symptoms.
1350A	5 c. c. serum G. P. 1350, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	No symptoms.
1351A	6 c. c. serum G. P. 1351, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	No symptoms.
1353A	5 c. c. serum G. P. 1353, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	Marked symptoms.
1354A	6.5 c. c. serum G. P. 1354, which was bled *11 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	Slight symptoms.
1355A	6.5 c. c. serum G. P. 1355, which was bled *12 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	Slight symptoms.
1355B	1 c. c. same serum.....	2	.....do.....	Marked symptoms.
1356A	3 c. c. serum G. P. 1356, which was bled *13 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	No symptoms.
1356B	2.5 same serum.....	2	.....do.....	Mild symptoms.
1357A	3 c. c. serum G. P. 1357, which was bled *14 days* after injection of 0.01 c. c. normal horse serum.	1	.....do.....	Mild symptoms.
1357B	2.5 same serum.....	3	.....do.....	Severe symptoms.

The first indication of anaphylactin appeared in the above series on the tenth day, that is, just about the time necessary to render guinea pigs sensitive. It is also evident that forty-eight hours is a better interval than twenty-four for the purpose of demonstrating the presence of anaphylactin in guinea-pig serum.

Our interest in this subject led us to sensitize another series of guinea pigs with 1/100 c. c. normal horse serum subcutaneously, but to test for the presence of anaphylactin by injections into the brain.

TABLE No. 32.—*Anaphylactin during the period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1380A	6 c. c. subcutaneously serum G. P. 1380, which was bled *5 days* after injection of 0.01 c. c. normal horse serum.	2	0.2 c. c. normal horse serum into brain.	No symptoms.
1381A	4 c. c. intraperitoneally serum G. P. 1381, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	No symptoms.
1382A	4 c. c. intraperitoneally serum G. P. 1382, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	No symptoms.
1383A	5 c. c. intraperitoneally serum G. P. 1383, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	No symptoms.
1384A	5 c. c. intraperitoneally serum G. P. 1384, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	No symptoms.
1385A	5 c. c. intraperitoneally serum G. P. 1385, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	No symptoms.
1386A	5 c. c. intraperitoneally serum G. P. 1386, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	No symptoms.
1387A	5 c. c. intraperitoneally serum G. P. 1387, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	Slight symptoms.
1388A	5 c. c. intraperitoneally serum G. P. 1388, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	Slight symptoms.
1389A	6 c. c. intraperitoneally serum G. P. 1389, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	Marked symptoms.
1390A	3.5 c. c. intraperitoneally serum G. P. 1390, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	Mild symptoms.
1391A	5 c. c. intraperitoneally serum G. P. 1391, which was bled *11 days* after injection of 0.01 c. c. normal horse serum.	2	.....do.....	Mild symptoms.
1391B	Hemaglobin intraperitoneally of G. P. 1391.	2	.....do.....	No symptoms.
1391C	Red corpuscles of G. P. 1391 intraperitoneally.	2	.....do.....	No symptoms.

In the above series the anaphylactin appeared in the blood of sensitized guinea pigs on the ninth day.

ANAPHYLACTIN IN MAN AND OTHER ANIMALS.

The presence of anaphylactin having been demonstrated in the blood serum of sensitized guinea pigs, it is interesting to know whether man and other animals that have received a previous injection of horse serum also contain a similar substance.

So far as may be judged from the following limited experiments upon the subject, we have been unable to demonstrate a similar property in the blood serum of man, the monkey, rabbit, and the cat when tested upon guinea pigs.

TABLE No. 33.—*Anaphylactin in man, monkey, rabbit, and cat.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Man.			
1310	1 c. c. normal human serum (CWC) subcutaneously.	44	6 c. c. normal horse (roan) serum intraperitoneally.	Slight symptoms.
280	5 c. c. human serum (LLL). 7 years prior this man had been injected with 12 c. c. antitoxic horse serum.	1¾	.....do.....	No symptoms.
1311	0.4 c. c. human serum (JWT). 1 year prior this man had been injected with 5,000 units antitoxic horse serum.	34	.....do.....	Slight symptoms.
1312	1.5 human serum (WWM). 2½ years prior this man had been injected with 1,500 units antitoxic horse serum.	36	.....do.....	Marked symptoms.
1313	0.5 c. c. human serum (MJR). 3 months prior this man had been injected with 10 c. c. antitetanic horse serum.	36	.....do.....	Mild symptoms.
1314	1.5 c. c. human serum (MJR). 3 months prior this man had been injected with 10 c. c. antitetanic horse serum.	36	.....do.....	Slight symptoms.
1379	10 c. c. human serum (WWM). 2½ years prior this man had been injected with 1,500 units antitoxic horse serum.	2½	0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
	Monkey.			
290	3.5 c. c. monkey (Billy) serum. 2 years prior this monkey had been injected with 2 (?) c. c. normal horse serum.	1⅝	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
290A	3 c. c. monkey (Billy) serum. 2 years prior this monkey had been injected with 2 (?) c. c. normal horse serum.	1⅝	.....do.....	No symptoms.

TABLE No. 33.—*Anaphylactin in man, monkey, rabbit, and cat*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Cat.			
285	3 c. c. cat serum. 41 days prior this cat had been injected with 2 c. c. normal horse serum.	15/6	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
	Rabbit.			
1377	8 c. c. rabbit serum. This rabbit had been frequently injected with normal horse serum.	2½	.....do.....	No symptoms.
1378	.....do.....	2½	0.2 c. c. normal horse (roan) serum into brain.	No symptoms

It will be noticed that a few of these guinea pigs showed symptoms, but in every case an interval of at least two weeks elapsed between the first and the second injections. The slight reactions obtained were probably those which occur when serums of different species are used at the first and the second injections (heterologous serums, see Hyg. Lab. Bull. 29, p. 55, and Bull. 36, p. 25).

ANAPHYLACTIN IN IMMUNE GUINEA PIGS.

We submit some further work bearing upon the question whether guinea pigs "immunized" against the phenomenon of anaphylaxis are in a refractory state or have returned to the normal or are really immune.

We first made a few experiments to determine whether guinea pigs immunized by repeated injections of small quantities of horse serum contain anaphylactin in their blood. In this series the guinea pigs received ten injections of 2 c. c. normal horse serum subcutaneously covering a period of seventeen days. Eighteen days following the last injection the pigs were first tested for immunity and then bled.

It developed from the following six guinea pigs that ten repeated subcutaneous injections (of 2 c. c. each) during the course of seventeen days were not sufficient to completely immunize the guinea pigs, for those tested developed slight symptoms. Indications of anaphylactin were demonstrated in the blood serum of five of the six pigs of this series. However, it must be noted that when bled these animals were certainly immune from the last treatment, as we and others have shown that when a sensitive guinea pig responds to a second injection immunity is quickly established. (See G. P. No. 671, Table 36.)

TABLE No. 34.—*Anaphylactin in immune guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1370A	6 c. c. serum G. P. 1370, which had received 10 subcutaneous injections 2 c. c. normal horse serum in a period of 17 days.	2	6 c. c. normal horse serum intraperitoneally.	Mild symptoms.
1373A	6 c. c. serum G. P. 1373, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	Mild symptoms.
1369A	6 c. c. serum G. P. 1369, which had same treatment as 1370.	2	6 c. c. normal horse serum intraperitoneally.	Marked symptoms.
1371A	6 c. c. serum G. P. 1371, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	Slight symptoms.
1374A	6 c. c. serum G. P. 1374, which had same treatment as 1370.	2	6 c. c. normal horse serum intraperitoneally.	Marked symptoms.
1372A	6 c. c. serum G. P. 1372, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	No symptoms.
671	0.0006 c. c. toxin A+1/1538 c. c. antitoxic horse serum (PD 09755).	55	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Very severe symptoms.
			Next day 0.2 c. c. normal horse (roan) serum into brain. 30 minutes later bled for serum.	No symptoms.
671A	5 c. c. serum of G. P. 671T intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms.
666	0.0006 c. c. toxin A + .0002 gm. antitetanic serum (Tizzoni).	55	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally. Next day bled for serum.	Very severe symptoms.
666A	6 c. c. serum of G. P. 666T intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms

The following series of guinea pigs is a more conclusive test that anaphylactin may be demonstrated in the blood serum of immune guinea pigs.

Guinea pigs were first immunized by repeated subcutaneous injections of 5 c. c. normal horse serum. The pigs received from 6 to 12 such injections, amounting to 30 to 60 c. c. About eight months after this treatment the pigs were tested by intracerebral injections, to which some responded with slight symptoms. Two of them showed no symptoms at all. While all the pigs were not completely immunized by the first treatment of subcutaneous injections, they were certainly rendered immune by the second treatment, given eight months later. Two hours after the second treatment the guinea pigs were bled, the blood centrifugated, the clear serum pipetted off and injected subcutaneously into normal guinea pigs. These normal pigs so treated were tested two days later by intracerebral injections, and all of them showed symptoms.

TABLE No. 35.—*Anaphylactin in immune guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1180	6 injections, each 5 c. c. normal horse (roan) serum subcutaneously, covering period of 18 days.	249 after last injection	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms
1180A	9 c. c. serum G. P. 1180 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1181	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	No symptoms.
1181A	8 c. c. serum G. P. 1181 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1182	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1182A	8 c. c. serum G. P. 1182 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1183	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1183A	12 c. c. serum G. P. 1183 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1186	7 injections, each 5 c. c. normal horse (roan) serum, in 21 days.	246	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1186A	8 c. c. serum G. P. 1186 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1187	8 injections, each 5 c c. normal horse (roan) serum, in 25 days	242	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	No symptoms.
1187A	9 c. c. serum G. P. 1187 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1188	9 injections, each 5 c. c. normal horse (roan) serum, in 28 days.	239	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1188A	8 c. c. serum G. P. 1188 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1189	10 injections, each 5 c. c. normal horse (roan) serum, in 32 days.	235	0.2 c. c. normal horse (roan) serum into brain. 2 hours latter bled for serum.	Slight symptoms.
1189A	5 c. c. serum G. P. 1189 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1190	11 injections, each 5 c. c. normal horse (roan) serum, in 35 days.	232	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Mild symptoms.
1190A	9 c. c. serum G. P. 1190 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1191	12 injections, each 5 c. c. normal horse (roan) serum, in 39 days.	228	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1191A	9 c. c. serum G. P. 1191 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.

It is therefore plain that anaphylactin does exist in the blood serum of immune guinea pigs.

We add the following examples showing that the presence of anaphylactin may not always be demonstrated. In these experiments the blood serum was collected in one of two ways. From guinea pigs Nos. 1000 to 1007, inclusive, the blood was drawn from the heart, allowed to clot at room temperature, and the serum pipetted off next morning. In guinea pigs Nos. 1138 to 1147, inclusive, the blood was drawn from the large vessels of the neck, defibrinated by whipping, centrifugated, and the clear supernatant serum used to inject into the normal guinea pig.

Perhaps the long interval may account for the negative results.

TABLE No. 36.—*Anaphylactin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1000	4 c. c. normal guinea pig serum subcutaneously.	26	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1001	1.5 c. c. serum G. P. 8038, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No.9+1/540 c. c. antitoxic horse serum (NY 13C).	26	.....do.....	No symptoms.
1002	2 c. c. serum G. P. 8048, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9+1/360 c. c. antitoxic horse serum (Welc.).	26	.....do.....	No symptoms.
1003	3.5 c. c. same serum.....	26	.....do.....	No symptoms.
1004	4 c. c. serum G. P. 8051, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/400 antitoxic horse serum (Welc.).	26	.....do.....	No symptoms.
1005	4 c. c. serum G. P. 8045, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/560 c. c. antitoxic horse serum (Welc.).	26	.....do.....	No symptoms.
1006	4 c. c. same serum.....	26	.....do.....	No symptoms.
1007	6 c. c. serum G. P. 8050, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/320 c. c. antitoxic horse serum (Welc.).	26	.....do.....	No symptoms.
1138	1.5 c. c. serum G. P. 8128, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	16	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
1139	3.5 c. c. same serum.....	16	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1140	1.5 c. c. serum G. P. 8127, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.

TABLE No. 36.—*Anaphylactin*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1141	4 c. c. same serum.....	19	5 c. c. normal horse serum intraperitoneally.	Slight symptoms.
1142	1.5 c. c. serum G. P. 8003, which had been subcutaneously injected with 0.24 c. c. toxine No. 9 + 1/420 c. c. antitoxic horse serum (NY 1013) and bled 99 days afterwards.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.
1143	20 c. c. same serum.....	19	5 c. c. normal horse serum intraperitoneally.	Slight symptoms.
1144	1.5 c. c. serum G. P. 8126, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.
1145	3 c. c. same serum.....	19	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1146	4 c. c. serum G. P. 7846, which had been subcutaneously injected with 0.24 c. c. toxine No. 9 + 1/310 c. c. antitoxic horse serum (Alex. spl. 192) and bled 139 days afterwards.	19	0.25 c. c. normal horse serum into brain.	Very severe symptoms.
1147	1.5 c. c. same serum.....	19	.....do.....	Severe symptoms.

TABLE No. 37.—*Anaphylactin—Miscellaneous.*

G.P. No.	First injection.	Interval in days.	Second injection.	Result.
696T	0.0006 c. c. tetanus toxine A + 0.0025 c. c. antitoxic horse serum (PI).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
695T	0.0006 c. c. tetanus toxine A + 0.002 c. c. antitoxic horse serum (PI).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
711T	0.0006 c. c. tetanus toxine A + 0.0075 c. c. antitoxic horse serum (Standard T2).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
	Half an hour later above 3 pigs bled: blood mixed, defibrinated, and 12 c. c. of serum obtained.			
1392	12 c. c. blood serum of above 3 guinea pigs intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms.

PART 6.—MECHANISM.

LESIONS.

Gay and Southard,[a] 1907, found in guinea pigs dying from a second injection of serum, and in those which had severe symptoms and were later chloroformed, lesions which are interpreted as explaining the mechanism of anaphylaxis. They state that "the study of the histopathology of this serum disease shows us that we have to deal with an intricate cell reaction demonstrable by definite cell lesions." Considerable hemorrhages, rather definitely localized, are the characteristic gross lesions. The hemorrhages may be in one or several organs, gastric hemorrhages being especially frequent. Microscopically there are, in addition to the naked-eye hemorrhages, minute interstitial and oozing hemorrhages. They also found fttay changes in voluntary muscle fiber, heart muscle fiber, and in nerve fiber.

That the conjestion and dilatation of the blood vessels found in the abdominal cavity and the hemorrhages upon the mucosa of the stomach are not characteristic of death due to anaphylaxis is evident from the fact that we have found that in violent death produced by large subcutaneous injections of chloral cyanhydrin or hydrocyanic acid there are somewhat similar congestions and hemorrhages.[b] Further, we have lately had the opportunity to examine a guinea pig whose death was caused by suffocation in an atmosphere of carbon dioxid. In the stomach and lungs of this guinea pig lesions were found that, so far as the congestion and hemorrhages are concerned, were somewhat similar to those described in guinea pigs dying from a second injection of horse serum.

We were especially struck by the fact that the macroscopic congestions and hemorrhages may be absent in guinea pigs poisoned by intracerebral injections.

Further, this congestion and dilatation of the vessels of the abdominal cavity is well known to occur in shock and other states.

We were also unable to confirm Gay and Southard's findings in regard to the fatty changes.

---

[a] Journ. Med. Research, May, 1907, p. 143.

[b] Rosenau, M. J., and Anderson, John F.: A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing on experimental gastric ulcer. Journ. Infec. Dis., vol. 4, No. 1, Jan., 1907, p. 1–7.

We studied a large number of pigs in which death occurred within thirty minutes of the second injection of serum; also, a moderate number which were killed by chloroform or otherwise from one to four hours after the second injection.

We are indebted to our colleague Dr. W. W. Miller, United States Public Health and Marine-Hospital Service, for the following studies upon the post-mortem appearances and pathology of the tissues of guinea pigs dead of anaphylaxis.

The most noticeable lesion to the naked eye is the marked dilatation of the small veins and capillaries of the body, but most noticeable in those of the abdominal viscera. Associated with this in about 25 per cent of the cases are hemorrhages in the mucosa of the stomach and, more rarely, of the intestines. Minute hemorrhages 1 mm. in size are occasionally observed on the surface of the lungs; no hemorrhages of the heart muscle, spleen, pericardium, or striped muscle, as described by Gay and Southard, have been seen.

For microscopic study the tissues from 16 guinea pigs were utilized. These pigs had received the second dose of serum in three ways, viz, by subcutaneous, intraperitoneal, and intracranial injection. Material was selected from all the viscera and from the striped muscles and nervous systems. Particular attention was given to the study of tissues for the fatty changes described by Gay and Southard. For this purpose sections made with the freezing microtome were used to a great extent, as it is generally recognized that fresh material is superior to that prepared and sectioned in the customary way in celloidin and paraffin, although it must be admitted, as Gay and Southard contend, that such preparations are not as permanent or suitable for micro-photographic purposes. For a general study of microscopic changes tissues were fixed in 10 per cent formalin, in formalin and alcohol (5 and 85 per cent), and Zenker.

For fatty degeneration, fresh tissue and tissue fixed for twenty-four hours in 5 to 10 per cent watery solution of formalin was used and sectioned with the freezing microtome; for nerve tissue, formalin 5 to 10 per cent, Orth's fluid, and Müller's fluid.

As a stain for general purposes, hematoxylin and eosin were used.

For staining fat, Marchi's method was carried out as follows: Fixation in 10 per cent formalin and Müller's fluid six to ten days, in Marchi's mixture six to ten days; kept throughout in the dark; then washed twenty-three hours in running water; hardened in alcohol and ether celloidin as quickly as possible. Clove oil celloidin was not used, as it causes general blackening.

For frozen sections of tissue fixed in 10 per cent formalin for twenty-four hours, the admirable method so highly recommended by Schmörl was used, viz, sections placed in Marchi fluid in closed vessels in the

paraffin oven for one-half to one hour, washed quickly in water, and mounted in glycerin or dehydrated over night in alcohol and mounted in balsam.

As controls in this work, tissue was used from normal pigs, from a pig dead of puerperal sepsis with marked fatty changes in liver and kidney, and from pigs killed with diphtheria toxine. Sati's method of osmization was also used with some of the specimens.

Fresh and formalinized tissue was stained by the admirable method of Torrain Smith (nile blue sulphate); also, Sudan III.

Results.—In sections stained for general study the sole difference from normal tissue consists in the marked dilatation of the veins and capillaries, especially of the stomach and intestines, accompanied by extravasation of blood at points where the vessels are ruptured. The thin-walled veins of the mucosa of the stomach are often greatly distended. In the few instances where a ruptured point in the vessel was seen in section the extravasation of blood was from the portion of the vein nearest the inner surface of the mucosa. The veins of the submucosa participated in the dilatation, but were not so markedly enlarged as the veins and capillaries of the mucosa. No evidence of general giving way of the capillaries with extravasation of blood into the tissues was noted.

The small veins and capillaries of the intestines, kidney, heart, and muscles were found distended, but not nearly to the extent found in the stomach wall. No hemorrhages were observed in the heart walls or in the liver and striped muscles.

As regards fatty changes in the lining endothelium of the blood vessels, in the gastric mucosa or striped muscles, none was observed, although carefully sought for. The focal fatty changes described by Gay and Southard were not found. Neither were the "nodal" changes in peripheral nerves made out.

We find, then, that congestion and sometimes hemorrhage take place in guinea pigs dead of anaphylaxis, but these lesions are not specific. We were unable to demonstrate the fatty lesions and know, further, that they occur in other states. We are therefore unable to confirm the observations of Gay and Southard along these lines, and believe that these changes do not explain the mechanism of anaphylaxis.

PART 7.—RELATION OF TOXIC ACTION UPON GUINEA PIGS TO SERUM THERAPY.

THE RELATION OF SERUM ANAPHYLAXIS IN THE GUINEA PIG TO SERUM THERAPY.

Besredka and Steinhart[a] were the first to point out that the second injection may be given into the brain of guinea pigs. When a small quantity of horse serum is injected into the brain of a sensitized guinea pig the symptoms appear promptly and often with great violence, and death is a common result.

Besredka[b] believes that intracerebral injections may be used as a measure for the toxicity of therapeutic serums. He states that, measured in this way, different serums show a wide gamut of toxicity, the fatal dose varying from $\frac{1}{4}$ to $\frac{1}{1\,2\,8}$ c. c. He believes that this toxicity resides in the serum and not in the cellular elements; further, that the serums of horses living under apparently the same conditions have about the same toxicity, individual variations being rare and of little importance. He concludes that, in a general way, all serums that incite in guinea pigs grave anaphylactic phenomena in doses of $\frac{1}{1\,6}$ to $\frac{1}{2\,0}$ c. c. and, *a priori*, above this amount should be considered toxic.

We doubt whether there is a relation between the toxicity of serums as tested upon guinea pigs in this way and their power to produce the serum disease or collapse or sudden death in man. The unfortunate accidents, such as collapse and occasional death, depend more upon the sensitization of the individual than upon the so-called toxicity of the serum used.

Fortunately we were able to obtain two antidiphtheric serums which had been used in two cases of sudden death.

Case No. 1.—Serum No. 2277. Reported by Dr. S. N. Wiley, Norristown, Pa., Journ. Am. Med. Assn., vol. 50, Jan. 11, 1908, p. 137. Mr. E. W., aged 34 years, splendid physique, best of health. Prophylactic

[a] Besredka and Steinhart, Ann. de l'Inst. Pasteur, 1907, vol. 21, p. 117.

[b] Ann. de l'Inst. Pasteur, 1907, vol. 21, p. 777.

injection of 1,000 units antidiphtheric serum. Site of inoculation 4 inches above Poupart's ligament. Within two minutes had violent symptoms—anxious expression, itching, burning, labored breathing; lips, face, and neck swollen and red; paralysis; convulsions. Died within five minutes of injection.

The toxicity of this serum, i. e., another package of the same laboratory number, was tested upon the following series of guinea pigs:

TABLE No. 38.—*Toxicity of serum No. 2277.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9147	0.23 c. c. toxine No. 7+1,420 c. c. antitoxic horse serum (PD. 09913).	97	0.1 c. c. antitoxic horse serum (2277) into brain.	Dead in 7 minutes.
9131	0.23 c. c. toxine No. 7+1,250 c. c. antitoxic horse serum (Natl. V. 24).	97	.....do.....	Dead in 8 minutes.
9136	0.23 c. c. toxine No. 7+1,300 c. c. antitoxic horse serum (Natl. V. 24.)	97	0.05 c. c. antitoxic horse serum (2277) into brain.	Dead in 35 minutes.
9151	0.23 c. c. toxine No. 7+1,380 c. c. antitoxic horse serum (PD. 09491).	97	.....do.....	Very severe symptoms.
9161	0.23 c. c. toxine No. 7+1 320 c. c. antitoxic horse serum (Mem. C20).	67	2 c. c. antitoxic horse serum (2277) intraperitoneally.	Dead in 3 hours.
9170	0.23 c. c. toxine No. 7+1 330 c. c. antitoxic horse serum (Mul. 2438).	67	2 c. c. antitoxic horse serum (2277) subcutaneously.	Very severe symptoms.

Case No. 2.—Serum No. 2295. Reported by Dr. H. F. Gillette, Cuba, N. Y., Journ. Am. Med. Assn., vol. 50, Jan. 4, 1908, p. 40. Mr. B., 52 years old. Had asthma and bronchial catarrh. Urine and heart normal. Rheumatic attack fifteen years prior. Coughed and raised plenty of sputum. Injection of 2,000 units antitoxic serum under left scapula. Prickling sensation in chest and neck; labored breathing; pulse regular and full. Seized with tonic spasm. Died within five minutes after injection.

The serum, i. e., another package of the same laboratory number, used in this case was tested for toxicity upon the following series of guinea pigs:

TABLE No. 39.—*Toxicity of serum No. 2295.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9135	0.23 c. c. toxine No. 7+1/370 c. c. antitoxic horse serum (Natl. V. 24).	97	0.1 c. c. antitoxic horse serum (2295) into brain.	Dead in 20 minutes.
9133	0.23 c. c. toxine No. 7+1/370 c. c. antitoxic horse serum (Natl. V. 24).	97	0.05 c. c. antitoxic horse serum (2295) into brain.	Dead in 22 minutes.
9132	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Natl. V. 24).	97	.....do.....	Dead in 11 minutes.
9373 m	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (NY. 306).	97	.....do.....	Severe symptoms.
9171	0.23 c. c. toxine No. 7+1/410 c. c. antitoxic horse serum (Mul. 2438).	67	1 c. c. antitoxic horse serum (2295) subcutaneously.	Dead in 90 minutes.
9164	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Mem. C23).	67	1 c. c. antitoxic horse serum (2295) intraperitoneally.	Very severe symptoms.

It has interested us very much to find that these two cases, and also others that have come to our notice, were in asthmatics. In our first publication we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man would seem to lend weight to this hypothesis. The knowledge of the fact that the injection of horse serum into some asthmatics may be attended with danger should be considered in the use of antitoxin.

In order to determine the comparative toxicity of the above two serums (Nos. 2277 and 2295); we submit the following experiments showing the toxicity of serums which have been largely used in human therapy without untoward effects.

The following serums (Nos. 2364, 2369, and 2442) were kindly sent us by Dr. A. P. Hitchens, who states "he has had no report whatever concerning any untoward effect resulting from the use of either of these numbers:"

No. 2364 is antitoxic serum.

No. 2369 is antitoxic globulin.

No. 2442 is antitoxic serum.

TABLE No. 40.—*Comparative toxicity of other serums (Nos. 2442, 2369, and 2364.)*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9936	0.23 c. c. toxine No. 7+1/260 c. c. antitoxic horse serum (Alex. 192).	47	0.05 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 4 minutes.
		47	.....do.....	Dead in 5 minutes.
9943	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Alex. 192.)	47	0.1 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 3 minutes.
9937	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. 192).			
9941	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. 192).	47	0.05 c. c. antitoxic horse serum (Mul. 2369) into brain.	Dead in 8 minutes.
9939	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Alex. 192).	47	.....do.....	Dead in 5 minutes.
9933	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Alex. 192).	47	0.1 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 3 minutes.
9945	0.23 c. c. toxine No. 7+1/180 c. c. antitoxic horse serum (Alex. 192).	47	0.05. c. c. antitoxic horse serum (Mul. 2364) into brain.	Marked symptoms
9946	.....do.....	47	.....do.....	Dead in 12 minutes
9934	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Alex. 192).	47	0.1 c. c. antitoxic horse serum (Mul. 2364) into brain.	Dead in 3 minutes.
9942	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. 192).	47	.....do.....	Marked symptoms

The following diphtheria antitoxic horse serum (123A) was kindly sent us by Dr. William H. Park, who reports that it is of moderate strength and has given very good results in the hospital.

TABLE No. 41.—*Comparative toxicity of serum No. 123A.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9245	0.23 c. c. toxine No. 7+1/480 c. c. antitoxic horse serum (Ldrl. 11) subcutaneously.	131	0.05 c. c. antitoxic horse serum (Park 123A) into brain.	Very severe symptoms.
9251	0.23 c. c. toxine No. 7+1/1000 c. c. antitoxic horse serum (Ldrl. 212) subcutaneously.	131	.....do.....	Very severe symptoms.
9242	0.23 c. c. toxine No. 7+1/360 c. c. antitoxic horse serum (Mul. 2377) subcutaneously.	131	.....do.....	Very severe symptoms.
8759	0.245 c. c. toxine No. 42+1/320 c. c. antitoxic horse serum (Strn. 1429) subcutaneously.	232	0.1 c. c. antitoxic horse serum (Park 123A) into brain.	Very severe symptoms.
9253	0.23 c. c. toxine No. 7+1/1500 c. c. antitoxic horse serum (Ldrl. 21B) subcutaneously.	131	.....do.....	Dead in 10 minutes.
9239	0.23 c. c. toxine No. 7+1/800 c. c. antitoxic horse serum (Ldrl. 57) subcutaneously.	131	0.2 c. c. antitoxic horse serum (Park 123A) into brain.	Dead in 5 minutes.

The following two serums were kindly furnished by Dr. E. M. Houghton, and were extensively used in human therapy, but no complaints were received concerning them:

TABLE No. 42.—*Comparative toxicity of serum No. 08725C.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9635	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	0.05 c. c. antitoxic horse serum (PD 08725C) into the brain.	Very severe symptoms.
9644	0.23 c. c. toxine No. 7+1/680 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do.....	Very severe symptoms.
9645	0.23 c. c. toxine No. 7+1/660 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do.....	Dead in 4 minutes.
9632	0.23 c. c. toxine No. 7+1/640 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	0.05 c. c. antitoxic horse serum (PD 09043C) into the brain.	Dead in 8 minutes.
9646	0.23 c. c. toxine No. 7+1/660 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do.....	Dead in 4 minutes.
9648	0.23 c. c. toxine No. 7+1/640 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do.....	Dead in 15 minutes

As a control, the following serums—some of them French antidiphtheric and some normal horse serums—are given for comparison:

TABLE No. 43.—*Comparative toxicity of French and other serums.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
532	0.0006 c. c. tetanus toxine A+0.23 c. c. antitoxic horse serum (Ehrlich standard).	65	0.25 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
8497	0.24 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Cutter 1828).	18	.....do.....	Very severe symptoms.
8359	0.24 c. c. toxine No. 9+1/1260 c. c. antitoxic horse serum (N. Y. 305).	35	.....do.....	Dead in 5 minutes.
9550	0.23 c. c. toxine No. 7+1/240 c. c. antitoxic horse serum (PD 07635).	62	0.2 c. c. antistreptococcic serum (Past. Inst.) into brain.	Dead in 3 minutes.
9557	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Welc. 2L839).	62	.....do.....	Marked symptoms.
9572	0.23 c. c. toxine No. 7+1/260 c. c. antitoxic horse serum (Alex. A245).	62	.....do.....	Dead in 4 minutes.
9580	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. A245).	65	0.2 c. c. antitoxic horse serum (Lyons) into brain.	Dead in 3 minutes.

TABLE No. 43.—*Comparative toxicity of French and other serums*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9552	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 58A).	65	0.2 c. c. antitoxic horse serum (Lyons) into brain.	Dead in 3 minutes.
9590	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. A245).	65	.....do.....	Dead in 4 minutes.

It therefore seems plain that the serums which do not produce untoward symptoms when injected into man, are quite as toxic upon sensitized guinea pigs, as the serums which have been followed by serious symptoms when injected into man. We believe the difference lies in the susceptibility of the individual and not in the toxicity of the serum.

PART 8.—THE EFFECT OF REPEATED INJECTIONS.

THE IMMUNIZING ACTION OF REPEATED INJECTIONS.

We found it desirable to obtain further data upon the effects of repeated injections of horse serum into guinea pigs in order to answer the question whether guinea pigs may be definitely immunized by repeated injections.

It will be seen by the following table that 10 injections of 2 c. c. of serum subcutaneously was not sufficient to render guinea pigs entirely immune. These four guinea pigs all showed mild symptoms when tested intraperitoneally fifteen or seventeen days after the last injection.

Reference to Tables No. 34, 35, 36, pages 36, 37, 38, shows the same results.

TABLE No. 44.—*The immunizing action of repeated injections.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1371	10 injections, each 2 c. c., normal horse (roan) serum, subcutaneously, covering a period of 17 days.	15	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
1369	.....do.....	17	.....do.....	Mild symptoms.
1372	.....do.....	17	.....do.....	Mild symptoms.
1374	.....do.....	17	.....do.....	Mild symptoms.

THE SENSITIZING ACTION OF REPEATED INJECTIONS OF SMALL AMOUNTS.

The following table shows that the repeated injections of small amounts are apparently as potent in sensitizing guinea pigs as one small injection.

TABLE No. 45.—*Sensitizing action of repeated injections of small amounts.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1364	5 injections, each 0.001 c. c., normal horse (roan) serum, subcutaneously, covering a period of 8 days.	[a]23	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 40 minutes.
1365	.....do.....	23	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 70 minutes.
1366	.....do.....	23	.....do.....	Dead in 75 minutes.
1367	.....do.....	23	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.
1368	.....do.....	23	0.2 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
1363	.....do.....	23	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.

[a] From last injection.

THE EFFECT OF REPEATED SMALL INJECTIONS UPON SENSITIVE GUINEA PIGS.

We were interested in determining whether repeated small injections would immunize sensitive guinea pigs without the production of anaphylactic symptoms. For this purpose we injected a series of guinea pigs subcutaneously with normal serum in amounts too small to produce apparent symptoms and repeated the injections daily for twenty days. One series received 0.001 c. c. subcutaneously (total 0.02 c. c.) the other series 0.01 c. c. subcutaneously (total 0.2 c. c.). It will be seen from the following table that these repeated injections had little, if any, effect upon the susceptibility of these guinea pigs.

TABLE NO. 46.—*Effect of repeated small injections upon sensitive guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9716	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Schr. II). 48 days later 20 subcutaneous injections, each 0.001 c. c., normal horse (Frank) serum daily.	[a]2	0.2 c. c. normal horse (Teddy) serum into brain.	Very severe symptoms.
9673	0.23 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.001 c. c. daily.	2	.....do.....	Marked symptoms.
9678	0.23 c. c. toxine No. 7+1/520 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.001 c. c. daily.	2	.....do.....	Marked symptoms.
9714	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Schr. II). 48 days later 20 injections 0.01 c. c. daily.	2	.....do.....	Dead in 5 minutes.
9715	.....do.....	2	.....do.....	Marked symptoms.
9677	0.23 c. c. toxine No. 7+1/520 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.01 c. c. daily.	2	.....do.....	Marked symptoms.
9155	0.23 c. c. toxine No. 7+1/200 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 120 minutes.
9156	0.23 c. c. toxine No. 7+1/240 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	0.2 c. c. normal horse (roan) serum into brain.	Dead in 7 minutes.
9252	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Schr. II). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 90 minutes.

[a] After last injection.

TABLE No. 46.—*Effect of repeated small injections upon sensitive guinea pigs*—Con.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9186	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Mul. 2362). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.
9157	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum subcutaneously.	Slight symptoms.

ATTEMPTS TO IMMUNIZE SENSITIVE GUINEA PIGS BY REPEATED INJECTIONS OF HEATED SERUM.

In the following series sensitized guinea pigs were injected with 1 c. c. of normal horse serum heated to 100° C. for one hour. The serum was first diluted in the proportion of 1 part to 3 of water to prevent coagulation. The 1 c. c. of the mixture injected into the guinea pigs represents, therefore, 0.25 c. c. of horse serum. The pigs received from 20 to 25 injections subcutaneously. They therefore received a total amount of 5 to 6 c. c. of normal horse serum. It is evident from the table that these injections failed to materially modify the susceptibility of the guinea pigs.

TABLE No. 47.—*Effect of heated serum upon sensitive guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9197	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Schr. II). 62 days later 20 injections 1 c. c. daily normal horse (roan) serum heated 100° C. 1 hour, subcutaneously.	[a]10	0.2 c. c. normal horse (roan) serum into brain.	Dead in 10 minutes
9179	0.23 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Mul. 2443). 62 days later 22 injections 1 c. c. daily heated serum.	8	6 c. c. normal horse (roan) serum intraperitoneally.	Severe symptoms.
9180	0.23 c. c. toxine No. 7+1/680 c. c. antitoxic horse serum (Mul. 2443). 62 days later 25 injections 1 c. c. daily heated serum.	4	6 c. c. normal horse (roan) serum subcutaneously.	Marked symptoms.
9191	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Mul. 2358). 62 days later 24 injections 1 c. c. daily heated serum.	6	.....do.....	Very severe symptoms.

[a] After last injection.

PART 9.—THE RELATION OF ANAPHYLAXIS TO THE TOXEMIAS OF PREGNANCY.

The symptoms which cause puerperal eclampsia and the conditions under which it occurs suggest that anaphylaxis may explain some of the mystery of this state.

It occurred to us that either the blood or proteid substances in solution from the fetus or the placenta may first sensitize the mother. A subsequent introduction into the system of the mother of a similar substance may explain the convulsions and the symptoms which occur in a certain class of the toxemias of pregnancy.

"Through the establishment of the pathological anatomy of the condition a general agreement has been reached that puerperal eclampsia must be included among the diseases caused by toxic materials of unknown origin and nature."[a] A certain class of the toxemias of pregnancy are sometimes spoken of as reflex or neurotic origin.

There seems to be a fair agreement that the placenta must be the source of toxic material, especially as typical cases of eclampsia and pernicious vomiting have been observed in patients with hydatid mole, in which cases, of course, toxic matter of fetal origin could be eliminated. Furthermore, eclampsia may appear after the fetus has been removed. Much attention was therefore given to the hypothesis elaborated about four years ago by Veit, Weichardt, and others that through the entrance of placental cells into the circulation of the mother an intoxication was caused either by disintegration of the cells and the formation of toxic substances or in the development of antisubstances by the maternal organism.

In spite of much experimentation and discussion, however, no satisfactory conclusions have yet been reached concerning the validity of this hypothesis, and Martin has secured some very valuable evidence that at least in rabbits entrance of their own placental elements into the circulation in large amounts does not cause any serious disturbance. So far as we are aware, we are the first to suggest that certain of the toxemias of pregnancy may be a condition of hypersusceptibility.

Along these lines we first made a number of experiments to determine whether the fetal blood of the guinea pig could sensitize the mother guinea pig. We injected a number of female guinea pigs, both pregnant and not pregnant, with fetal blood, and after an appropriate interval gave them a second injection of the same material.

---

[a] From a recent discussion of the theories concerning the causes of the toxemias of pregnancy. Editorial in the Journal of the American Medical Association, vol. 50, No. 2, Jan. 11, 1908, p. 124.

All these experiments resulted negatively, which was anticipated from our previous studies upon the effect of homologous blood serums. This tends to confirm the clinical observations that the poisons causing the toxemias of pregnancy do not come from the fetus.

We then made a series of experiments upon female guinea pigs with placental extracts. The placenta (almost at full term) was ground up in a mortar and allowed to "autolyze" about an hour at room temperature, and some of the resulting extract was injected subcutaneously into female guinea pigs.

After an interval of twenty-two days the guinea pigs were again inoculated with a placental extract. This time the placenta was allowed to "autolyze" three hours in the incubator (37° C.). Five pigs were tested with this placental extract; three of them were given 6 c. c. into the peritoneum, two of these three showing pronounced symptoms of anaphylaxis. The remaining one showed slight symptoms. Six cubic centimeters of the same placental extract injected into the peritoneal cavity of two young normal guinea pigs as a control produced no apparent effect. The remaining pigs were injected with small quantities of the extract intracerebrally, with negative results.

These experiments were repeated with precisely similar results. Thus four more guinea pigs were sensitized with different quantities of guinea-pig placental extract, and after a period of twenty-two days were given a second injection of similar placental extract. The extract used at both the first and second injections in these four pigs was autolyzed three hours in the incubator, then strained through gauze. All four of them showed definite symptoms.

In a third experiment three guinea pigs were sensitized subcutaneously with placental extract (three hours at 37°), and after an interval of twenty-four days the second injection was given directly into the circulation by the intracardiac method. These three pigs showed severe and early symptoms, including convulsions. Three normal control guinea pigs treated in the same manner did not respond. The three pigs that responded to the second intracardiac injection were autopsied and found to have a fresh current-jelly clot in the pericardial sac. How much this hemorrhage, which is probably due to the puncture of superficial vessels of the heart, may account for the symptoms is doubtful. Further work along this line is in progress.

From this limited series it is evident that the mother guinea pig may be sensitized with the autolytic products of her own placenta. These experiments naturally suggest that there may be a certain relation between some cases of puerperal eclampsia and the phenomenon in the guinea pig which we are studying. Further studies along this line are now being made, especially to determine whether the liver lesions may thus be produced in the guinea pig and other animals.

PART 10.—MISCELLANY.

TIME.

The longest time that has elapsed between the first and the second injections in a guinea pig in our experiments has been two years two days (732 days). It therefore seems that guinea pigs once sensitized with mixtures of antitoxic horse serum and diphtheria toxine are sensitive throughout practically the remainder of their lives.

Time.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
4523	0.19 c. c. toxine No. 7+1 immunity unit antitoxic horse serum (B27).	732	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 60 minutes.

TABLE No. 48.—*Clam juice.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1650	0.1 c. c. clam extract subcutaneously.	127	6 c. c. clam extract subcutaneously.	Marked symptoms.
1651	.....do.....	127	.....do.....	Do.
1652	.....do.....	127	6 c. c. clam extract intraperitoneally.	Dead in 50 minutes.
1653	.....do.....	127	.....do.....	Marked symptoms.
1654	.....do.....	127	.....do.....	Do.

The above series shows that the protein matter of clams may sensitize and poison guinea pigs.

PART 11.—SUMMARY AND CONCLUSIONS.

The *period of incubation* of serum anaphylaxis is about seven days in guinea pigs sensitized in the brain and about nine days in guinea pigs sensitized subcutaneously. It also appears that the sensitization comes on somewhat gradually.

Judged by our results and the work of others, the period of incubation is quite constant.

It seems that the period of incubation is not appreciably prolonged by a large sensitizing dose.

Animals sensitized with horse serum alone remain so for a long period of time (at least 245 days). Guinea pigs sensitized with the toxine-antitoxin mixture remain sensitive throughout the remainder of their life (at least 732 days).

The *sensitizing principle* is gradually influenced by heat. It disappears almost entirely when horse serum is heated to 100° C. for one hour.

Guinea pigs may be sensitized by intracerebral injections, provided quantities of 0.000,1 c. c. or more are used. We obtained negative results with sensitizing doses of 0.000,01 c. c. into the brain.

Guinea pigs may be sensitized by dropping horse serum upon the eye.

The *toxic principle* in horse serum is gradually destroyed by heat.

A temperature of 70° C. for one hour does not seem appreciably to diminish the poisonous property of horse serum, but it seems to be affected at 80° C. for one hour. At 90° C. for one hour it still remains slightly toxic, but at 100° C. for one hour the toxicity apparently disappears.

The difference in the effect of heat upon the sensitizing and the toxic principle may be more apparent than real, for exceedingly minute amounts of serum will sensitize guinea pigs, while it would take a very large quantity of weakened serum to produce symptoms at the second injection.

The toxicity of horse serum does not appear to diminish with the age of the serum.

No favorable influence upon the anaphylactic state was obtained by injecting pancreatin, potassium oxalate, pepsin, sodium sulphate, magnesium sulphate, peptone, calcium chloride, and calcium acetate into guinea pigs the day before they were tested.

Iodine also apparently had no modifying effect upon serum anaphylaxis, whether dissolved in the serum or injected separately into the guinea pig.

Methemaglobin-producing substances, such as the nitrites, do not hinder anaphylaxis.

Ether narcosis masks the symptoms, but does not prevent the fatal issue of a second injection.

Further attempts to find free antibodies to neutralize the toxic action of horse serum by treating it with the sensitized guinea pig serum, and also with the brain substance of sensitized guinea pigs, proved negative.

The *specific nature of anaphylaxis* is further shown by various experiments. For example, guinea pigs sensitized with three separate proteins, viz, horse serum, egg white, and cow's milk, contain three separate anaphylactins in their blood.

Guinea pigs sensitized with human milk do not react to a second injection of cow's milk. This again indicates not only the specific nature of the anaphylactic reaction, but suggests differences between the protein matter of human and cow's milk.

Guinea pigs sensitized with sheep's milk react to a subsequent injection of cow's milk.

Guinea pigs sensitized with dog's milk do not react to a subsequent injection of cow's milk.

Guinea pigs sensitized with hen egg white react to a subsequent injection of duck egg white; and guinea pigs sensitized with duck egg white react to a subsequent injection of hen egg white. The results of similar studies with goose, guinea hen, crane, pigeon, and turkey egg albumin are recorded in the text.

The anaphylactic reaction in the guinea pig, therefore, seems to be specific in the sense that the precipitins are specific. That is, there is a group reaction in the proteins of allied species, but no reaction between the proteins of widely different species or between proteins of widely different origin.

A substance known as "*anaphylactin*" is present in the blood serum of sensitized guinea pigs. This substance is not present during the period of incubation.

We have been unable to demonstrate the presence of anaphylactin in the blood serum of man, the monkey, and the cat.

Anaphylactin is present in the blood serum of immune guinea pigs.

The mechanism of anaphylaxis.—We find that congestion and sometimes hemorrhages may be present in guinea pigs dead of anaphylaxis, but these lesions are not always apparent, and, furthermore, are not specific.

We were unable to demonstrate fatty lesions in guinea pigs dead of anaphylaxis, and know, further, they occur in other states.

We believe that these morphological alterations do not explain the mechanism of anaphylaxis. It is probable that the mechanism will not be unraveled until further light is shed upon the chemistry of protein metabolism.

Cases of sudden death in man.—Our experiments demonstrate that the horse serum used in cases followed by sudden death is no more toxic for guinea pigs than antitoxic horse serums used extensively in human therapy without untoward symptoms.

It is our belief that it is not the special toxicity of the horse serum, but the sensitization of the patient, which accounts for the collapse or sudden death sometimes following the injection of horse serum.

We are still unable to account for the ways in which man may be sensitized to a foreign protein. It seems perfectly plain, however, that man may be so sensitized.

In previous publications we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man seems to lend some weight to this hypothesis.

The knowledge of the fact that an injection of horse serum into some asthmatics may be attended with danger should be considered in the use of antitoxin.

The *repeated injections* of small amounts of horse serum sensitizes guinea pigs.

Repeated injections of large amounts render guinea pigs partially immune.

Repeated injections of small amounts of serum into sensitized guinea pigs have no appreciable effect.

Sensitized guinea pigs can not be immunized by repeated injections of heated serum (100° C. for one hour).

We suggest a possible relation between the *toxemias of pregnancy and anaphylaxis.*

Guinea pigs can not be sensitized with guinea-pig fetal blood. This shows that the fetal blood of the guinea pig does not contain an alien protein for the mother.

Guinea pigs may be sensitized and subsequently poisoned with guinea-pig placental extracts.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United Staes Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902, By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species Paragordius varius), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen of *Filaria restiformis* Leidy, 1880 = *Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations on two new parasitic trematode worms: *Homalogaster philippinensis* n. sp., *Agamodistomum nanus* n. sp., by Ch. Wardell Stiles and Joseph Goldberger. 4. A reexamination of the original specimen of *Tænia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

*No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar. Hosp. Serv., Wash., pp. ——.

MAILING LIST.

The Service will enter into exchange of publications with medical and scientific organizations, societies, laboratories, journals, and authors. ALL APPLICATIONS FOR THESE PUBLICATIONS SHOULD BE ADDRESSED TO THE "SURGEON-GENERAL, U. S. PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE, WASHINGTON, D. C.," EXCEPT THOSE MARKED (*).

The editions of the publications marked (*), available for distribution by the Surgeon-General of the Public Health and Marine-Hospital Service, have been exhausted. Copies may, however, be obtained from the Superintendent of Documents, Government Printing Office, Washington, D. C., who sells publications at cost, and to whom requests for publications thus marked should be made.

O